

Progress in research on energy and protein metabolism



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Editors:

W.B. Souffrant and C.C. Metges



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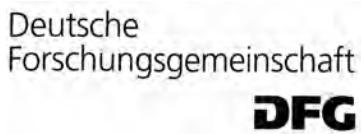
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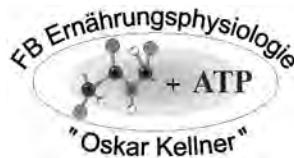
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Preface

In 2003, the dates for the International Symposia on Energy Metabolism in Animals and on Protein Metabolism and Nutrition coincide. Therefore, the international committees of both Symposia in agreement with the European Association of Animal Production decided to combine the 16th Symposium on Energy Metabolism in Animals and the 9th International Symposium on Protein and Nutrition and hold a joint meeting with both common and separate scientific sessions. This joint “Symposium on Energy and Protein Metabolism and Nutrition” is organized by the Research Unit of Nutritional Physiology “Oskar Kellner” of the Research Institute for the Biology of Farm Animals in Dummerstorf, Germany, on the 50th anniversary of the former “Oskar Kellner Institute” in Rostock.

Proteins and amino acids, their metabolism and phenotypic effects and different aspects of energy metabolism are the major themes of the present meeting. Special emphasis is placed on nutrition and gene expression, modelling and regulation of energy and protein metabolism and the microbiology of the gastro-intestinal tract.

These topics are the subject of most presentations throughout the meeting and give the current symposium its particular character. The organizing committee and scientific staff responsible for the programme of this symposium are delighted to have the opportunity to hold this joint 2003 symposium to celebrate the 50th anniversary of the former “Oskar Kellner Institute”. In fact, the science of animal nutrition has a long history in this region of Germany, particularly in ‘energy’ research, which has been influenced considerably by Oskar Kellner and Kurt Nehring. At present, in accordance with the spirit of this symposium, gut physiology and microbiology as well as regulatory and quantitative aspects of amino acid, protein and energy homeostasis have become the focus of interest for the researchers in Dummerstorf.

The organizers and contributing authors have worked hard to prepare the proceedings prior to the oral presentations, in order to provide an optimal basis for thorough discussions. Thanks are also due to organisations, foundations, institutions and companies for financial and other support.

This symposium is held in one of the most beautiful areas of Mecklenburg-Vorpommern, which we hope will contribute to making it another successful meeting in the continuing series of the ‘International Symposia on Energy Metabolism in Animals’ and the ‘International Symposia on Protein and Nutrition’.

Manfred Schwerin
Director of the Research Institute
for Biology of Farm Animals

50 years Oskar-Kellner-Institute -Research on energy and protein metabolism

W. Jentsch

Research Institute for the Biology of Farm Animals, Department of Nutritional Physiology „Oskar Kellner”, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

Summary

The history of the Oskar-Kellner-Institute in Rostock has its roots in the first German Agricultural Experimental Station in Leipzig-Möckern, Oskar Kellner's place of activities. In 1953 the Oskar-Kellner-Institute for Animal Nutrition, Leipzig-Möckern was set up. The foundation-stone for a new building of the institute in Rostock was laid in 1954. The new Oskar-Kellner-Institute with four new buildings was completed in 1960. The main building placed laboratories for analyses of feed quality, investigations on protein quality by chemical and microbiological amino acid analyses and animal tests for the estimation of biological value of proteins. In the respiration building 10 respiration units for farm animals and diverse units for rats, rabbits and poultry, were built up to study feed utilization and energy requirement of different animal categories with divergent productivities as well as the physiological background of energy metabolism. A third building served for feeding trials and forage preservation. The purpose of a fourth building was to analyze exponents using isotopes especially in studies on protein metabolism. Overall objective of the research was to establish the basis for developing a system of feed evaluation and feed requirement of animals with different productivity. The information obtained by feed analyses, energetic efficiency of feed utilization, protein quality, energy and protein requirement of animals etc. provided the fundamentals of the Rostock Feed Evaluation System, first edition in 1971. All divisions of the Oskar-Kellner-Institute and further institutes contributed to this book. The system has been continuously undergone improvement and completion up to date.

Keywords: Oskar-Kellner-Institute Rostock, history, nutritional research

Ladies and gentlemen!

Respectful to and deeply honoured by the International Organizing Committee's decision I present my paper on the occasion of the 50th anniversary of the Oskar-Kellner-Institute. I feel bound to say many thanks to the participants of the last symposium at Snekkersten, Danmark, 3 years ago for their vote to realize the 16th symposium on energy metabolism in animals - joint with that on protein metabolism - in Rostock-Warnemünde.

The history of the Oskar-Kellner-Institute (OKI) has its deepest roots in the first German Agricultural Experimental Station in Leipzig-Möckern, founded in 1852. Here Prof. Oskar Kellner carried out experiments with steers and established the Starch-equivalent System, a widely used feed evaluation system, based on net energy. Kellner was the head of this research station from 1892-1911. After the World War II, research on animal nutrition in Leipzig-Möckern was performed as a self-managing unit beside other agricultural institutions. By the way, at this place I began my practical and theoretical work in animal nutrition during my years of study as an auxiliary assistant. In the meantime research on animal nutrition got improvement in the Agricultural Experimental Station in Rostock, under the direction of Prof. Kurt Nehring. In 1953, thus 50 years ago, the Institute for Animal Nutrition Leipzig-Rostock was set up, named the Oskar-Kellner-Institute in Oskar Kellner's honour. Prof. Kurt Nehring received a call as director of this new institute.

Because of insufficient possibilities for experiments especially with farm animals Nehring applied in the Academy of Agriculture for the construction of a new institute of animal nutrition in Rostock.

Meanwhile Prof. R. Schiemann got permission to study the facilities for measuring energy metabolism in Copenhagen and Zürich. He often told about the grand support to his studies and sincere submission of details about respiration units and management. This was very important to prepare the proposition for designing the new institute and for the construction of respiration units. This cooperation resulted in friendships, which survived the hard time without possibilities to personal connections, especially between Schiemann and Dr. Grete Thorbek.

The foundation-stone for the new building of Animal Nutrition Research was laid in Rostock 1954, March 26. The main reasons for the choice of this location were the limited space in Leipzig-Möckern and the neighbourhood to the Research Institute of Animal Breeding at Dummerstorf as a base for close scientific cooperation in problems of animal nutrition regarding animal production and breeding.

Immediately after laying the foundation-stone the first stage of construction began and the main building was completed in December 1955. In January 1956 the laboratories could move from the Agricultural Experimental Station in Rostock to the new building (Figure 1). In this three-storied building different laboratories provided excellent conditions for feedstuff analyses, investigations on protein quality by chemical methods, animal tests and microbiological analyses. Here a relatively low number of offices for scientists existed, because Nehring succeeded in his opinion that scientists should work mainly in the lab with a desk for evaluation and interpretation of results and the preparation of publications. The respiration units for rabbits and rats were placed here provisionally for two years till the „respiration building“ was completed in 1957 for scientific studies on energy metabolism and physiology of digestion (Figure 2). This edifice had 3 floors too - cellar, raised ground-floor, upper story - and a large loft as storeroom for roughages. The centre of the raised ground-floor was equipped with 10 respiration chambers for farm animals - 4 for cattle, 4 for pigs and 2 for sheep. At both sides stables were placed; at one side for 16 cattle with facilities for digestion trials and collection of urine and at the other side facilities for pigs and sheep, 10 animals each with equipments for collection of faeces and urine too. Beside the respiration chambers the respiration pumps were situated in a separate room and upstairs in the middle of the house above the chambers the laboratories for gas gathering and analyses.

The so called „farm building“ had only a ground-floor with possibilities for forage preservation studies in experimental silos and for feeding trials with pigs and cattle (Figure 3). In this house a



Figure 1. Main building of the Oskar-Kellner- Institute for Animal Nutrition in Rostock, courtyard side.



Figure 2. Respiration building of the Oskar-Kellner- Institute for Animal Nutrition in Rostock, courtyard side.

well equipped workshop was installed too. The decision to integrate this installation was quite appropriate and of great importance and benefit regarding development, improvement and upkeep of equipments for research on energy and protein metabolism in animals. The fourth so-called „isotope building“ (Figure 4) was constructed with the intention of working with isotopes especially in protein metabolism research. A room intended for veterinary surgery did not get use there, because of availability of a veterinary clinic vis-a-vis for applications of fistulae, cannulae etc.. Most of them could be done directly in the experimental facilities.

According to the shortly described buildings the fields of research are already imaginable. The structural organization gives further insight into the scientific fields of the institute.



Figure 3. Farm building of the Oskar-Kellner- Institute for Animal Nutrition in Rostock, courtyard side.



Figure 4. Isotope building of the Oskar-Kellner- Institute for Animal Nutrition in Rostock, courtyard side.

There were following divisions:

1. Nutritional physiology, later: Protein metabolism research
2. Feed evaluation, later: Energy metabolism research
3. Feedstuff knowledge, later: Feed quality and preservation research
4. Forage harvest and preservation, later: Feed quality and preservation research

In 1963 a farm with about 1000 ha was attached to the institute. That resulted in several tasks and opportunities: Among others the establishment of an experimental station for field studies on forage cultivation and a second one for forage preservation and feeding trials were most essential. So several forage quality influencing provisions of cultivation and preservation could be examined systematically with regard to feed evaluation.

In 1970 the Research Centre for Animal Production Dummerstorf-Rostock was founded assembling hitherto 3 separate academy institutes: 1) Institute of Research on Animal Breeding Dummerstorf, 2) Oskar-Kellner-Institute for Animal Nutrition Rostock and 3) Institute of Animal Breeding and Keeping Clausberg. The OKI became a department of the research centre in maintenance of the name "Oskar Kellner", even though this name had to be borne unfortunately illegally, for about 12 years, opposite to the intention of the administration, that did not want to continue scientific tradition; however after that time naming turned to legality. In connection with the new organization the divisions cattle nutrition and pig nutrition localized to Dummerstorf were incorporated into the Department of Animal Nutrition "Oskar Kellner". These divisions retained their stand at Dummerstorf and were concerned with fundamentals of feeding. A more detailed description of the organization of the OKI shall not follow, but a review of fields of research shall be given.

The research on problems of protein quality can be referred to the development and improvement of techniques to analyze the amino acid (AA) composition of proteins. At the beginning, the analysis of composition of proteins was accomplished by ascending paper chromatography, later (since about 1960) by the ion exchange column-type chromatography. This technique allowed automation. Some AA remained to be analyzed microbiologically. Systematic studies of exogenous effects - as crops fertilizing, preservation or processing of feedstuffs on their AA content resulted on the one hand in an amino acids table of feedstuffs (Autorenkollektiv, 1981) respectively a feedstuff table (Nehring et al., 1972), on the other hand in recommendations of amino acid supply to monogastric farm animals (Autorenkollektiv, 1989). Practical consequences

were the development of a routine procedure concerning the AA supplying of farm animals on the base of their AA requirement, by combination of mixed feeds and of diets. The biological value of proteins was tested with Wistar-rats under standardized conditions and later with piglets and chickens by nitrogen and amino acid balance trials. Analytical progress was achieved by the use of full-automatic AA analysators and ^{15}N measuring instruments.

Beside research engaged in feedstuff and further development of analytics, research on animals was forced (in early 1970th) by introducing of essential techniques, such as experimental surgery in order to provide pigs with fistulae, catheters, re-entrant cannulae and ileorectal anastomoses. These techniques were necessary, because AA digestibility estimations by means of faeces collection are invalid, as AA destruction occurs in the large intestine. Farm animals are not capable of absorbing AA beyond the terminal ileum. Therefore the precaecal AA absorption was determined for a lot of protein feedstuffs using surgically prepared pigs, and the AA additivity was tested. Furthermore the AA and protein retention capacity and the efficiency of utilization were estimated in dependence on animals' age, body mass, sex and also AA and energy supply (Bock, 1989). For distinguishing the precaecal absorption from that in the whole digestive tract, intact and ileo-rectomized animals were compared in combination with studies on energetic utilization of several diets. These techniques allowed to examine AA absorption in sections of the digestive tract. Caecal infusion of ^{15}N labelled AA resulted in no absorption in the hindgut. For the improvement of feedstuff quality, increase in AA content and availability by plant breeding was initiated with practical success.

A lot of methodological developments and studies were performed in cooperation with the Kielanowski-Institute at Jabłonna near Warsaw, and with further institutes, e.g. for comparative controls of analytics. In the last decennium a cooperation was intensified with the Wageningen institutes in the Netherlands. Research on protein and energy metabolism was combined to obtain information about energy utilization for protein and fat energy retention (see below). Insights into the relationship between these variables provided their integration into compartment model, considering AA turnover or protein metabolism of whole animal body with the help of stable and radioactive isotopes and modelling of these processes (metabolic rates, flux rates etc.). Protein synthesis and breakdown rates were found to relate to multiples of protein retention with animal-specific effects. Attempts to quantify the energy costs factorially for the different processes did not yet succeed. I think with improved methods, such as labelling of different N and/or C containing compounds (extended since 1995) and continuous monitoring respiratory gas exchange combined with methods of N metabolism recording, this problem could be resolved by complex studies on protein and energy metabolism. The results seem to be important to get insight in the regulation of gene expression in different animal breeds or types. Altogether, the main results from protein research work were integrated in the Feed Evaluation System (collective of authors, 2003).

The division of energy metabolism research in the Leipzig institution was equipped with 3 respiration units for pigs and 2 with a treadmill for horses (until 1965) and in Rostock first of all 3 units for rabbits, 8 for rats - functioning to the principle of Haldane - and the 10 chambers for farm animals mentioned above (since 1957) (Figures 5 and 6) - functioning to the principle of Pettenkofer, with pumps for gas passage and devices of measuring gas composition. Later on 2 large chambers for groups of farm animal were built up (completed in 1976) with vacuum pump and flow meter and 2 chambers for small groups of poultry. The chambers for rabbits were reconstructed and thereafter 6 respiration units for fowl were available (since 1960). In the 4 respiration chambers for cattle an equipment for milking was installed (in 1967) and later on all 12 chambers for farm animals were insulated (around 1988) and the air-conditioning system was reconstructed. Thereupon the reconstruction enabled experiments in climatized chambers in a range from 3 to 35°C. Gas analysis was the most essential analytic method. Kellner and Fingerling worked with absorption of CO₂ at partial gas flow through BaOH and titration. In Rostock the Haldane units worked on the principle of total absorption of CO₂ in KOH followed by gravimetry.



Figure 5. Interior of a respiration chamber for cattle in Rostock.

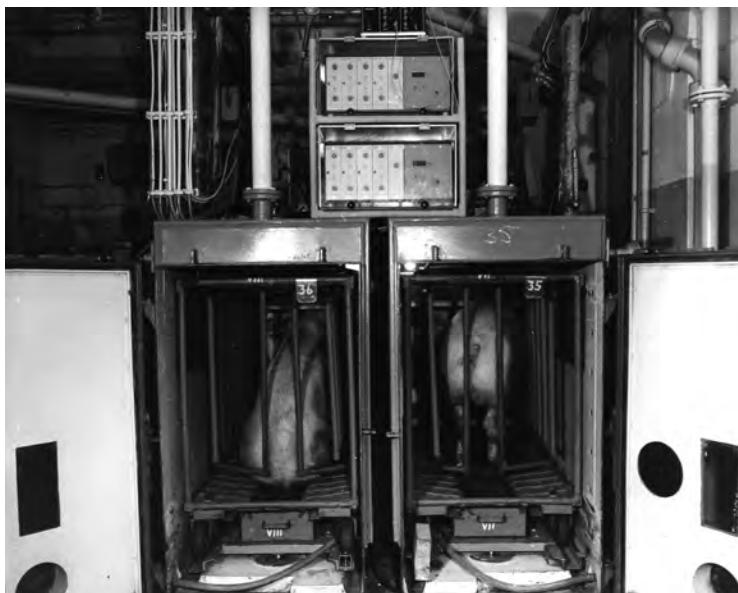


Figure 6. Interior of 2 respiration chambers for pigs in Rostock.

Volumetric gas analysis after absorption in KOH (CH_4 following burning) was used by the respiration units for farm animals, using two chambers for rabbits in pilot study. Since 1964 physical gas analysis (Hartmann and Braun, later Junkalor) was accomplished. The method was improved by an installation of a computer-controlled continuous gas analysis and records of the data (around 1987). This equipment was used for ten years parallel to the conventional method, dependent on the problems. Additional technical problems raised the question how to separate faeces and urine in female cattle. These were solved by "urinal" and chemical separation of the excrements in poultry. In female pigs bladder catheters were used. Parallel to almost all measurements of digestive and energy-metabolic processes, activity of rumen fermentation was

recorded; rumen fluid was collected by special esophagus probe. In some cases vein vessels were catheterized for bleeding and administration of drugs. Results of indirect calorimetry were compared with those of slaughter technique in rats, pigs, cocks and cattle. A second problem arose from the method of measuring the energy retention in dependence on metabolizable energy intake with rats, rabbits, pigs, sheep and cattle. Experimental data indicated that a linear correlation exists between intake and retention beyond the requirement of metabolizable energy for maintenance. Publications to methodical questions were compiled by Jentsch et al. (2000).

I would like to subdivide the research on energy metabolism in 4 periods. The first period was concerned with working up Kellner's and Fingerling's bequest. Kellner passed away abruptly by a cardiac infarct during a conference, so that not published manuscripts, compiled experimental results and original notes about experiments were existing. Diverse experimental data were also not processed by Fingerling. The same was applied to Fingerling's experimental results. The period of processing and publishing these data run parallel to the first stage of methodological developments (see above).

The second period involved experiments on energetic feed value of feedstuffs mainly from 1958 to 1970. In this time studies started on utilization efficiency of pure nutrients in rats, rabbits, pigs, sheep and cattle. Compared to results from Kellner there was agreement of net energy values for starch, protein and cellulose. Intake of oil by cattle yielded in a high decrease in digestibility of nutrients from the basal diet. In monogastric animals the data from pure nutrients, extended to disaccharides and monosaccharides, ethylalcohol, lactic acid agreed with calculations of the capacity to form ATP, with the exception of acetic acid because of additional need of 2 ATP-equivalents for activation of acetate. In model experiments with rats energy utilization efficiency of pure nutrients was measured at levels of energy supply below and above the maintenance requirement. Differences were observed between both levels in utilization of fat energy. Lower fat energy utilization at an energy level below maintenance agreed roughly with that for milk production (see below). This had to be considered in feed evaluation. Data from cattle agreed with those from sheep on the one hand. On the other hand results from monogastric animals were comparable using different species. Similar utilization efficiency of starch was later established in man, as opposed to other opinions, caused mainly by incorrect experimental design.

The nutritive potential of feedstuffs and their nutritional value was estimated in cattle, sheep, pig, rabbit and rat. Because of the special position of rabbits with regard to digestive physiology and their economic insignificance compared to fowl, this species was replaced by fowl, after completing the experiments with concentrates. Experiments with concentrates were followed by those with tuberous plants and roots as well with roughages. For the most parts, the results are published in Archives of Animal Nutrition and summarized by Schiemann et al. (1971). In this book the experimental methodology and analyses of data have been described too. Because of impossibility of testing the large number of feedstuffs experimentally and changes in the composition, especially of forages dependent on agro-technical provisions, the relations between net energy and digestible nutrients were estimated using a correlative approach. The derived equations provided the basis for the calculation of energetic feedstuff values. They were published in the edition of the GDR Feed Evaluation System (Autorenkollektiv, 1989) proposed to be named Rostock Feed Evaluation System in 1971, but this proposed term was not accepted by the government. After publishing the system, studies on energetic feedstuff values were continued to complete the Feed Evaluation System. The energetic values of a set of fresh and dried forages were compared using sheep to minimize the experimental costs. Values measured and those calculated by the estimation equation were compared to evaluate the validity of estimation. The results indicated that energy utilization efficiency of fresh and dried forages were in line. Similar comparisons were carried out using several types of green forage silages. Special experimental programs were developed to consider physical and chemical processing of different straws and the effect of particle size of dried forages on energy utilization in sheep. As a main result, energy digestibility of straw could be improved by its processing up to the net energy value when ruminal

fermentation processes were not impaired. In turn with higher pectin content of feedstuffs the estimated net energy value dropped, but due to problems of pectin analysis in feedstuff evaluation for practical use, pectin content was not considered particularly in the estimation of energy value of feedstuffs. In extensive experiments using pigs the effect of steam processing of potatoes and potato starch on net energy value was determined and an increase was observed that accounted for 25 %. The problem of energy utilization of starch in cattle was investigated in fistulated oxen (duodenal re-entrant fistula). The postruminal digestion of different starches amounted up to 15 % (maize starch compared with barley, wheat and potato starch). That means the impact of starch on utilization efficiency was found to be marginal in cattle, because of little effect on energy utilization of ruminally digested starch (65 %) compared with to postruminally digested starch (75 %).

In the third period research on energy metabolism was focused on energy requirement problems in laboratory and farm animals to develop these basis for energy resp. feed supply recommendations for animals with divergent productivity. In the first edition of the Feed Evaluation System, data for requirement referred to results from literature. Therefore investigation were initiated to fill purposefully the gaps . Model experiments with rats and chickens were carried out to determine energy expenditure for protein synthesis. One of the findings was that animals' age substantially influenced protein synthesis-related energy expenditure. In cooperation with the division of protein metabolism research experiments were accomplished with the aim to change the rate of protein synthesis and protein turnover. However, the results were too equivocally to describe exactly the relation between protein turnover and requirement of energy for protein retention. A lot of experiments were also carried out to study energy requirement for maintenance in dependence on a differing supply of nutrients. Most results agreed with the requirement of ATP, but there was also inconsistency, perhaps caused by thermoregulatory resp. compensatory effects. The influence of environmental temperature on heat production was studied intensively especially in chickens with different body mass. The experiments with farm animals on energy requirement intended an improvement of the Feed Evaluation System. The experimental series began with studies on cows (1967-1974). Aim of the studies was to determine the energy requirement for milk production. In detail, the following issues were addressed:

- Effect of nutrition level on energy digestibility and metabolizability
- Relations between capacity of performance, maintenance requirement and utilization efficiency of feed energy for milk formation
- Maintenance energy requirement in relation to milk yield
- Utilization of feed energy for milk synthesis in dependence on milk yield and lactation stage
- Utilization efficiency of metabolizable energy for milk formation depending on feed nutrients with particular consideration of feed fat (see above).
- Utilization of body energy for milk production
- Utilization of metabolizable energy for formation of conception products.

Most important results indicated that the energy requirement for maintenance and energy utilization for milk production was found to be independent on performance capacity. Moreover, the results revealed a linear relationship between energy supply for milk production and milk energy output. Consequently feed energy utilization for milk production (maintenance energy requirement excluded) was found to be similar in different lactation stages. Energy utilization for milk production and maintenance energy requirement in total increased with increasing milk yield. Factorial analysis of data allowed to assemble tables to calculate standards of the energy requirement. Feed fat energy utilization for milk energy production in relation to other nutrients in comparison with that for body fat energy production was found to be similar to feed fat energy utilization below maintenance level. Utilization efficiency of body energy for milk production amounted to 80 %. Results have been published, and a synopsis of publications with reviews of previous results can be found in a recent paper (Jentsch et al., 2001).

The energy requirement of laying hens with various performances was studied to determine the relationship between productivity and efficiencies of feed utilization. The chemical composition of eggs varied depending on egg mass, and egg energy content could be calculated from the chemical composition. Multiple regression analysis indicated a higher utilization efficiency of metabolizable energy for egg fat energy than for egg protein energy. Maintenance energy requirement was found independent of performance.

Parallel to the experimentally expensive investigations with dairy cows, less expensive experiments were accomplished with calves and fattening bulls. In this program for determination of energy requirement in dependence on energy supply, age and development of the animals and the structure of feeds, issues of feed evaluation were addressed. They included different whole plants of cereals, differently processed straw and silages. From 1975 to 1982, following the experiments with bulls, studies on heifer calves and heifers aimed to determine the effect of forages on N and energy balance. Animals received diets different in energy concentration by varying the ratio roughages : concentrates from 50:50 to 100:0. This induced divergence in animals' growth and in the age at first calving that varied between 18 and 30 months. The different growth rate was accompanied by covariations in body composition, N and energy balances. These responses provided a basis for factorial derivations of requirement recommendations in relation to the physiological state. Development stages were further characterized by monitoring ruminal fermentation as well as parturition and the body weight and size of new-born calves. Birth weight of calves did not depend strongly on mother's age and weight. Cows and heifers did not differ in energy utilization efficiency for fetal growth (Jentsch et al., 2001).

An extensive program over 4 1/2 years (1978-1983) addressed the task to deduce standards for energy requirements from measurements of energy and protein metabolism in pregnant and lactating sows with 9 experimental variants of energy supply and litter number, each from insemination to the end of the 4th lactation week. Experimental methods included the combination of indirect calorimetry and slaughter technique. Two large respiration chambers were available for suckling sows housed together with their standardized litters. Technical problems of recording milk yield and sampling milk specimens were resolved by hand-milking after oxytocin injection.

Issues addressed were:

- Energy and protein maintenance requirement
- Energy requirement for pregnancy
- Energy utilization for energy retention in maternal body
- Energy retention in conception products and reproductive organs
- Feed energy utilization for milk production
- Utilization of body energy for milk production
- Energy requirement for milk production
- Maintenance requirement of piglets
- Energy requirement for energy retention in piglets

Detailed information about changes in body composition through pregnancy and lactation, composition of conception products, prepartal and postpartal growth of piglets etc. were obtained in dependence on nutrition level.

Further studies on growing castrated pigs concerned energy requirements for maintenance and growth in particular for protein and fat deposition at different nutrition levels using various kinds of feedstuffs in the diets. These experiments provided information about the relations between digestion, utilization capacity and body composition. Animals were ileo-rectostomized to monitor the energy utilization efficiency of the nutrients absorbed precaecally or postileally. Similar experiments followed because of ambiguous experimental results reported hitherto. Applications of clenbuterol and recombinant porcine somatotropin increased gain and protein deposition, but decreased total energy and fat energy deposition. Drugs were used to induce high protein retention capacity in pigs at post-genetic level, but not as proposal for practical use. The experimental

periods 2 and 3 included some physiological aspects. This approach was emphasized in period 4 by studies on circulating hormones and metabolites. Special series of experiments served to determine sex-specific energy metabolism in pig and the energy requirement of young boars in combination with investigations on the glucose tolerance and changes in blood hormone pattern. These experimental summaries will render imaginable the intensive use of the experimental equipments during the year. The work was only interrupted in summertime for holiday. A highly motivated crew of technical staff was engaged in resolving the daily problems. This enabled us to lay the fundaments for compilation of the Feed Evaluation System (Autorenkollektiv, 1989) that based on measurement of the energy and protein metabolism requirement standards and considered results from literature. The validity of the recommendations for the use of the system in particular the requirement standards under farm conditions was examined in dairy farms and farms practiced the rearing of rams, mixed farming systems including milk production and rearing of bulls as well as rearing of heifers. Validation was the topic of three dissertations written between 1977 and 1984 to acquire the doctor degree. Conclusions were that the correct use of the system improved productivity.

The division of feed quality and preservation research was established (in 1979) by integrating two separate divisions, which existed since the foundation of the institute. One of both had to do services for the other divisions of the institute, first of all analyses of feedstuffs, faeces etc.. Research concerned an improvement of the Weende Feedstuff Analysis. A most early topic was to specify crude fibre by determination of cellulose, lignin, pentosane and hemicellulose. This program was ceased because of expensive analytical methods, which could not be used in routine laboratories for practical feedstuff analyses. In the combined division feed quality and preservation research, the goal turned toward the development of simply practicable methods for the estimation of starch and sugar, carbohydrates, that play the major role in energetic feed evaluation. These analytical methods provided the basis for calculation of a nitrogen free residue (NFR) instead of nitrogen free extractives (NFE) and for restructuring the equations for the estimation of feed value (collective of authors, 2003). That was an essential step of improving the Feed Evaluation System. Research on forage preservation was carried out systematically. A wide range of factors influencing preservation was tested in household glasses (about 2 l) and in experimental silos (6 m³ volume), which could be closed hermetically and exposed to series of environmental temperatures. Chemical analyses were performed with powerful techniques including high-pressure-liquid-chromatography and an automatic carbohydrate-analysator. The data from these analyses were prerequisite to substantially improve technologies for the production of silage and methods for predicting the forage fermentability, based on dry matter content, sugar content and buffer capacity of forages (Weißbach et al., 1974). The instantaneous quantification of forage fermentability enabled to control the fermentation process using different kinds and concentrations of preservatives. The development of a physiologically founded and experimentally tested model for the estimation of digestible nutrients (Weißbach et al., 1991) was highly important for practical evaluation of roughage (green forages, silages, hay etc.) on the base of digestible nutrients. Further issues addressed the preservation of humid feedstuffs, raw and steamed potatoes, preservation and processing of forages and cereals with urea (Schmidt et al., 1978) as well as determination of the mycoflora and development of evaluation schedules for the hygienic status of feedstuffs (Kwella & Weißbach, 1984). Since its foundation scientific strategy of the OKI was to directly apply findings of the basic research to practical agriculture, as shown in these and following examples. The division of cattle nutrition had its stand at Dummerstorf and was furnished with a building for investigation on cattle and laboratories equipped with automatic AA-analyser and spectro and flame photometers and other analytical techniques including those for experimental surgery, needed to install rumen and duodenal re-entrant fistulae. Principal issues addressed the feeding of ruminants in relation to performance and animal health. The investigations focused on the rumen and on the digestive physiology. One of the main findings was that straw processing, grinding and /or pelleting of roughage affected negatively ruminal fermentation and rumen mucosa.

Increased formation of keratin in the rumen papillae was observed by feeding ground roughage. Rumen parakeratosis reduced the rumen functions, impaired animal health and performance. Feeding trials with high-yielding cows resulted in feeding recommendations for cows with high performances, regarding interrelations between feeding and metabolic diseases, e.g. ketosis. Determination of acetone in milk was found to be of diagnostic value, working out proposals for prophylaxis of metabolic disorders. The studies on regulation of feed intake prosecuted the goal of higher intake of roughages. Experiments with heifers resulted in recommendations for feeding and rearing of calves and heifers. These recommendations included that heifers should be trained for high intake of roughages to enlarge the rumen (Piatkowski, 1987). Feeding sequence, feeding frequency and feeding of mixtures were found to be important for feed intake and ruminal fermentation. Methodical progress for investigations on the digestion of ruminal carbohydrates and proteins was achieved by the development of duodenal re-entrant cannulation in yielding cows. This improved the technique of collection of digesta to study destructive and synthetic processes in the rumen. Thereby protein digestion could be measured in single parts of the digestive tract. Studies on feed protein evaluation were conducted on the basis of estimating the duodenal protein passage. These experiments were also performed in calves with duodenal re-entrant canulae. The results provided proposals for feeding of calves, in particular milk feeding regarding frequency of feeding, processing of milk, and training of roughage intake. The effect of particle size of roughages, crude fibre content and structure on chewing and rumination activity was studied using different categories of ruminants to work out proposals for optimal portion of effective crude fibre in the diet. Cooperation with farms led to resolve problems in feeding, preservation of forages with ensiling additives, and processing of straw with NaOH. Cooperative research with other institutes of eastern countries concerned evaluation of non-protein N, leading to a complex of recommendations for the supply of nitrogen, depending on animals' performances. The scientific central results of the division entered a book (Piatkowski et al., 1990).

The division of pig nutrition had his stand at Dummerstorf too. Feeding trials were performed in reconstructed pig pens with sows, piglets and fattening pigs. Two field agencies allowed a direct contact with farms that had equipments for trials of feeding, digestion and N-balance. The activities were focused on three fields of research. They included topics of practical pig feeding and the task to transmit basic findings to practical use in pig keeping and housing, technology, breeding, reproduction and veterinary medicine. Special issues addressed problems in nutrition of weaners, gilts and pregnant sows. Early weaned piglets raised problems regarding microbially disturbed digestion. This initiated studies on the effect of microbes on digestive processes. Improvement was observed with supplementation of the diet with crude fibre. Addition of bran and other crude fibre-containing feedstuffs into starter diets protected from digestive disorders. Pregnant sows with little room to move were fed high crude fibre diets on the basis of milled straw or forage silages. This enabled a normal development of the body mass. These investigations were accompanied by studies on digestion in different sections of the digestive tract. An increase in postileal digestion was found to be associated with lower net energy concentration. These results, brought about by a cooperation with other divisions were important for feed evaluation. Further topics referred to protein metabolism, energy and lysine supply of sows.

Additional divisions dealt with problems of the planning economy of the GDR. The division of feed planning worked out planning standards for farms on the basis of feeding requirement standards, for diverse performances of animals. The recommendations are useful for calculating a balanced feeding. Planning computer programs were elaborated to organize feed production with regard to feed requirement. I am sure you have enough of assignments and institutional structures, but your attention may be refreshed by some general remarks.

The personal staff of the Oskar-Kellner-Institute developed as follows: In 1953 the institute had 46 co-workers, they increased to 170 in 1969 and to 240 in 1988.

What happened in 1989? Nobody could imagine the enormous extent of the political revolution, initiated by people of GDR, peaceful finished, supported by politicians, you know. Most of the

scientists were very glad about these great events, but the reasons were different. The most important point was that the Germans had now the possibility to come together. All the many positive aspects I cannot count up, but I would like to say the possibility to participate in international conferences belonged to the motives. Germany and the western countries became open to the public. But there were also frustrated hopes. All research institutes of the GDR were evaluated to reduce their number, thereby costs. Inquiries to institutes all over the world with similar objectives of research resulted in 25 letters of recommendations which paid tribute to the research of the Oskar-Kellner-Institute and expressed requests for continuing the research work. For me it is impossible to read the letters without emotion even 12 years later! Thanks for that and the support to survive! The letters affected not only the positive decision over the survival of the Oskar-Kellner-Institute, but also supported survival of other departments of the research centre Dummerstorf-Rostock, as to read in the evaluation protocol. After dissolution of the research centre (in 1991) the institutional refoundation (in 1993) was named Research Institute for the Biology of Farm Animals. One department of the new institute is the Department of Nutritional Physiology "Oskar Kellner". The refoundation was connected with a rigorous alteration in the scientific objectives. One of the most important decisions was to cease research on feed evaluation and determination of energy requirement. That was not understandable because no other institute has similar possibilities. Another decision was the liquidation of the division of feed quality research. However, feed evaluation and nutrition base upon founded knowledge of feedstuffs. One argument for the decision referred to research accomplished in other institutes. But research on feed with regard to knowledge of animal product quality and effects of feed on animal product quality nevertheless needs intensification.

Another consequence was a reduction of the personal staff from 240 to 55 people, a very hard decision! Thus several parts of the experimental equipment were impossible to use further. The institutional structure was altered to intensify integrated research. Therefore new topics of research after 1991 shall be described without any allocation to research teams.

The main approach of studies performed by the Oskar-Kellner-Institut had the character of analyses and descriptions of observation providing an immediate response of the organism to environmental changes with the focus on nutrition. This is valuable and central for providing some insights on what is possible. More recently, we addressed in addition the basic issue why organism respond the way that they do. This approach may allow predictions regarding novel conditions or a wider array of conditions, not only regarding the limited set of conditions we can observe in a given schedule. This may be helpful to better develop sustainable livestock production system. These livestock systems do not recently seem to be as productive in the short term as current systems, which are planned on the basis of maximum production, but they may be more productive over a longer period and produce less loss of biodiversity. In this context, a lot of questions are arising unanswered as yet. Farmers want livestock that utilize forage better because that increases production. Both natural and artificial selection foster that. However, high intake of metabolizable energy can impair productivity by reducing fertility as recently reported in high-merit dairy cows. Such problems require a quantifiable framework for predicting the feeding behaviour, nutrition, and performance of animals.

Therefore, research issues turned stronger towards questions such as:

Is there variation within and across breeds of cattle in the capacities of animals for processing high-quality and poor-quality feeds?

Are there differences in pathways of animals from breeds of cattle and other ruminants and non-ruminants for partitioning imbalanced nutrients?

Are there specific nutrients and nutritional conditions to modulate the expression of genes to productive advantage?

Which types of metabolic interactions are arising from changes in nutritional level and ambient temperatures?

Are there specific responses to nutritional changes dependent on the physiological state of animals?

We used the heat production and the heart frequency to refer to non-invasive measurements with the aim to throw light on the processes whereby nutrition affects whole body metabolism. In some experiments, invasive techniques were used to administer the thyroid hormone releasing peptide and corticotropin. These hypothalamic neuropeptides are released in response to nutritive changes and to a changed ambient temperature. Therefore, a general response was triggered to specific signals of the brain. The results indicated differences in the sensitivity to efferent signalling between breeds of cattle measured by thyroid hormones and cortisol, hormones known to be involved in the thermoregulation.

In turn, a change in afferent signalling by shearing induced thermoregulatory responses that were also different among breeds of cattle. This indicates there are divergencies across breeds in the regulation of heat production and the brain may play an important role. Therefore, experiments were conducted that tested molecular aspects of the thermoregulation in young bulls fed diets containing metabolizable energy restricted and ad libitum. Expression of genes encoding proteins that regulate cellular energy metabolism in the brain, cardiomyocytes, skeletal muscle and liver via vasoregulatory actions substantially responded to the nutritional level. The results regarding the intake of food are preliminary but suggest that nutritional conditions modulate the expression of genes involved in the regulation of feeding behaviour (Löhrke et al., 1999).

To investigate specifically a nutritive effect on gene expression, butyrate was infused into the rumen of oxen to simulate intake of a diet high in concentrate. As a pivotal result, infusion of butyrate cannot only cause growth of ruminal papillae but induce epithelial cell death that can exceed proliferation dependent on the feeding level. Epithelial cells from ruminal papillae, cultured without and with butyrate, produced 3β -hydroxybutyrate dependent on the butyrate dose in the medium. The increase in the oxidation of butyrate was associated with a change in the cellular system of defence against oxidative stress. Butyrate elevated the nitrotyrosine content of cellular proteins, indicative for oxidative stress. Butyrate induced proteins with a putative role in the defence of messy oxidation, including the prion protein. This suggests that there are specific responses to nutritional changes dependent on the physiological state of animals.

Experiments of feeding casein and soybean isolate with and without supplementation of amino acids detected interactions between the quality of protein fed growing pigs and metabolic response of cells from the liver and skeletal muscle. Partly persistent effects were observed on whole body metabolism, body composition, organs, tissues, and cells when the animals fed a diet deficient in essential amino acids, then with a diet with balanced amino acids. Imbalance induced up-regulation of proteolytical enzymes in liver suggesting mobilization of body's protein store to cope with dietary amino acid imbalance. This response persisted in part following intake a balanced diet. Thus, the results gave some insight into the induction of interactions at the level of cellular pathways.

Research on microbiology of digestive processes and interactions with mammalian cells from the digestive tract in monogastric mammals is ongoing. In laying hens, the impact of the environmental temperature, nutritive supply, lighting schedule and breeding line on energy and protein metabolism has been continued to detect possible interactions between these variables. The results allowed to work out recommendations for feeding based on the requirement of metabolizable energy, amino acids, and environmental conditions.

A sharp cut into the history of the Oskar-Kellner-Institute is the decision over moving to the stand at Dummerstorf. In 2001 the laboratories from the main building moved to a new building at Dummerstorf (Figure 7), used by the Department of Nutritional Physiology "Oskar Kellner". This modern equipment gives good conditions for research in combination with the new building for animal experiments, that included 4 respiration units (Figure 8 and 9). This building shall be usable in the last quarter of 2003. In consequence of these changes the great number of respiration units in Rostock cannot be used further-on. The main building found a new user. The respiration



Figure 7. The new laboratory building of the Department of Nutritional Physiology "Oskar Kellner" at Dummerstorf.



Figure 8. The new building for animal experiments.

building is a special construction, built to meet experimental unique demands. Therefore it may hardly be used for other purposes. That is a pity, but one can take comfort from the good new conditions at Dummerstorf with excellent experimental perspectives and one can be full of hope for success, if personal and research programs remain to be congruent at a high level.

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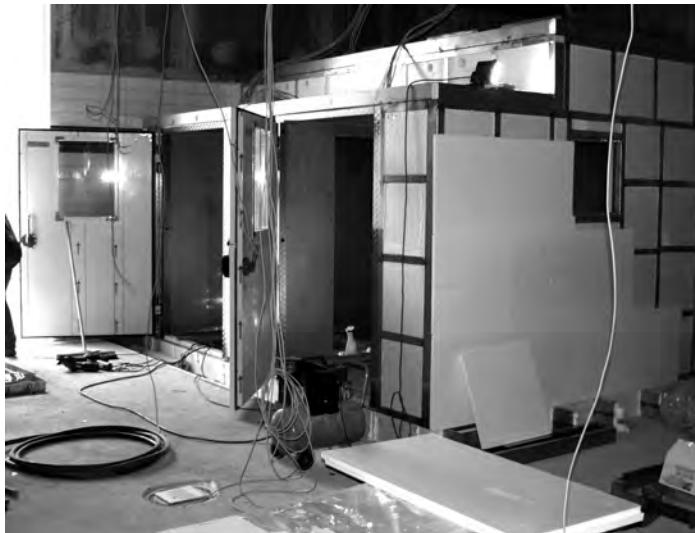


Figure 9. Respiration chambers under construction in the building, figure 8.

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Joint session 1

Genes and Nutrition

Amino acid regulation of genes expression

Julien Averous, Céline Jousse, Alain Bruhat, Christiane Deval, Sylvie Mordier & Pierre Fafournoux*

UNMP, INRA de Theix, 63122 Saint Genes Champenois, France

Summary

In mammals, the impact of nutrients on gene expression has become an important area of research. Because amino acids have multiple and important functions, their homeostasis has to be finely maintained. However, the amino acidemia can be affected by certain nutritional conditions or various forms of aggression. It follows that mammals have to adjust several of their physiological functions involved in the adaptation to amino acid availability by regulating expression of numerous genes. It has been shown that amino acids by themselves can modify the expression of target genes. However, the current understanding of amino acid-dependent control of gene expression has just started to emerge. This review focuses on the recent advances on mechanisms involved in the amino acids control of gene expression. Several examples discussed in this paper demonstrate that AA regulate genes expression at the level of transcription, mRNA stability and translation.

Keywords: amino acid, gene expression, C/EBP Homologous Protein, Asparagine Synthetase

Abbreviations

The following abbreviations are related to genes expressed in yeast: GCN2, GCN4. The meaning of these abbreviations is not related to the function of the encoded protein.

| | |
|---------------|---|
| AARE | Amino Acid Regulatory Element |
| APC | Anterior Piriform Cortex |
| AS | Asparagine Synthetase |
| ATF | Activating transcription Factor |
| b-ZIP | basic leucine Zipper |
| Cat-1 | Cationic Amino acid transporter -1 |
| C/EBP | CCAAT/Enhancer Binding Protein |
| CHOP | C/EBP Homologous Protein |
| eIF2 α | eucaryotic Initiation Factor 2 α |
| HRI | Heam-regulated eIF2a kinase |
| IGFBP-1 | Insulin like Growth Factor Binding Protein-1 |
| IRES | Internal Ribosome Entry Site |
| mGCN2 | mammalian GCN2 |
| NSRE-1 | Nutrient Sensing Response Element 1 |
| NSRE-2 | Nutrient Sensing Response Element 2 |
| NSRU | Nutrient Sensing Regulatory Unit |
| PERK | PKR-like ER kinase |
| PKR | Protein Kinase regulated by double stranded RNA |
| uORF | upstream Open Reading Frame |

Introduction

Mammals have the ability to adapt their own metabolic demand to survive in a variable and sometimes hostile environment. External stimuli to which they must be able to respond include thermal variations, rhythmic changes imposed by alteration of day and night, and the necessity to adjust to the intermittent intake of food. In addition, the animal itself provides internal metabolic variations, such as menstrual cycle or pregnancy in females and growth of tissues in young animals. All these external and internal factors demand metabolic responses, and associated regulatory mechanisms. Regulation of metabolism is achieved by mechanisms operating at the cellular level and also by coordinated actions between cells and tissues. These mechanisms involve the conditional regulation of specific genes in the presence or absence of appropriate nutrients. Control of gene expression by nutrient availability has been well documented in prokaryotes and lower eukaryotes. These organisms are able to adjust their metabolic capacity to variations in the nutrient supply by altering their pattern of gene expression. In multicellular organisms, the control of gene expression involves complex interactions of hormonal, neuronal and nutritional factors. Although not as widely appreciated, nutritional signals play an important role in controlling gene expression in mammals. It has been shown that major (carbohydrates, fatty acids, sterols) and minor (minerals, vitamins) dietary constituents participate in the regulation of gene expression (Towle 1995; Foufelle, et al. 1998; Pégorier 1998; Duplus, et al. 2000; Vaulont, et al. 2000; Grimaldi 2001). However, the mechanisms involved in the amino acid control of gene expression have just begun to be understood in mammalian cells (Kilberg, et al. 1994; Fafournoux, et al. 2000; Bruhat and Fafournoux 2001). This review summarizes recent work on the effect of amino acid availability in the regulation of biological functions. On the basis of the physiological concepts of amino acids homeostasis, we will discuss specific examples of the role of amino acids in the regulation of physiological functions, particularly focusing on the mechanisms involved in the amino acid regulation of gene expression.

Regulation of amino acid metabolism and homeostasis in the whole animal

Mammals are composed of a series of organs and tissues with different functions and different metabolic demands. As a consequence, the regulation of protein and amino acid metabolism in the whole animal is made up of the sum of the regulatory responses in all individual parts of the body and is achieved through a series of reactions that are both integrated and cooperative.

In addition, amino acids exhibit two important characteristics: 1) multicellular organisms are unable to synthesize all amino acids and 2) there are no important dispensable amino acid stores (in contrast with lipids or glucose). Consequently, when necessary, an organism has to hydrolyse protein (particularly muscle proteins) to produce free amino acids. This loss of protein will be at the expense of essential elements. Therefore, complex mechanisms that take into account these amino acid characteristics are needed for maintaining the free amino acid pools.

The size of the pool of each amino acid is the result of a balance between input and removal. The metabolic outlets for amino acids are protein synthesis and amino acid degradation whereas the inputs are *de novo* synthesis (for non-essential amino acids), protein breakdown and dietary intake. Changes in the rates of these systems lead to an adjustment in nitrogen balance. For example, amino acid homeostasis and protein metabolism can be altered in response to malnutrition (Baertl, et al. 1974; Jackson and Grimble 1990) and/or various forms of trauma (sepsis, fevers, thermal burns...) (Jeejeebhoy 1981; Jeevanandam, et al. 1984; Cynober 1989; Wolfe, et al. 1989; Ziegler, et al. 1994; Biolo, et al. 1997) with two major consequences: a large variation in the blood amino acid concentration and a negative nitrogen balance. In these situations, individuals have to adjust several physiological functions involved in the defense/adaptation to amino acid limitation by

regulating numerous genes. The specific role of amino acids in the adaptation to two different amino acids deficient diets will be considered.

Specific examples of the role of amino acids in the adaptation to protein deficiency

Protein undernutrition

Prolonged feeding on a low protein diet causes a fall in the plasma level of most essential amino acids. For example, leucine and methionine concentrations can be reduced from about $100-150\mu M$ and $18-30\mu M$ to $20\mu M$ and $5\mu M$, respectively, in plasma of children affected by kwashiorkor (Grimble and Whitehead 1970; Baertl, et al. 1974). It follows that individuals must adjust several physiological functions in order to adapt to this amino acid deficiency. In both children and young animals, the main consequences of feeding a low protein diet is the dramatic inhibition of growth. Straus et al (Straus, et al. 1993) demonstrated that growth inhibition was due to a striking overexpression of IGFBP-1, which binds the growth factors IGF1 and IGF2 and modulates their mitogenic and metabolic properties (Lee, et al. 1993). According to the literature (Lee, et al. 1993), IGFBP-1 expression is regulated by GH, insulin or glucose. However, the high IGFBP-1 levels associated with feeding a protein deficient diet cannot be explained by only these 3 factors. It has been demonstrated that a fall in amino acid concentration is directly responsible for IGFBP-1 induction (Straus, et al. 1993) (Jousse, et al. 1998). Therefore, amino acid limitation, as occurs during dietary protein deficiency, participates in the down-regulation of growth through the induction of IGFBP-1 expression.

Imbalanced diet

Because mammals cannot synthesize all of the amino acids, the diet must provide the remaining ones. Thus, in the event of a deficiency in one of the indispensable amino acid, the remaining amino acids are catabolized and lost and body protein are broken down to provide the limiting amino acid (Munro 1976). It follows that mammals (with the exception of the ruminants) need mechanisms that provide for selection of a balanced diet.

After eating an amino acid imbalanced diet, animals first recognise the amino acid deficiency and then develop a conditioned taste aversion. Recognition and anorexia resulting from an amino acid imbalanced diet takes place very rapidly (Rogers and Leung 1977); (Gietzen, et al. 1986). The mechanisms that underlie the recognition of protein quality must act by the way of the amino acids resulting from intestinal digestion of proteins. It has been observed that a marked decrease in the blood concentration of the limiting amino acid can become apparent as early as few hours after feeding an imbalanced diet. The anorectic response is correlated with a decreased concentration of the limiting amino acid in the plasma. Several lines of evidence suggest that the fall in the limiting amino acid concentration is detected in the brain. Dr Gietzen's laboratory demonstrated (Gietzen 1993; Gietzen 2000) that a specific brain area, the anterior piriform cortex (APC), can sense the amino acid concentration. This recognition phase is associated with localised decreases in the concentration of the limiting amino acid and with important changes in protein synthesis rate and gene expression. Subsequent to recognition of the deficiency, the second step, development of anorexia, involves another part of the brain.

These two examples demonstrate that a variation in blood amino acid concentration can activate, in target cells, several control processes that can specifically regulate the expression of target genes. Although the role of the amino acids, considered to be regulators of genes expression, is understood in only a few nutritional situations, recent progress has been made in understanding the mechanisms by which amino acid limitation controls the expression of several genes.

Amino acid control of gene expression

Genes up-regulated by amino acid

Genes that are specifically up-regulated in response to supra-physiological concentration of amino acids have been described. For example, a high concentration of L-tryptophan enhances the expression of collagenase and of tissue inhibitors of metalloproteinase. In rat hepatocytes, Na⁺-cotransported amino acids like glutamine, alanine or proline stimulate acetyl-coA carboxylase, glycogen synthetase and arginino succinate synthetase activity. It was demonstrated that the swelling resulting from the addition of amino acid could be involved in the regulation of gene expression (Watford 1990; Haussinger 1996; van Sluijters, et al. 2000), however, the molecular mechanisms involved in these processes are poorly understood.

Genes up-regulated by amino acid starvation

In mammalian cells, a few examples of specific mRNAs that are induced following amino acid deprivation have been reported (Straus 1994). Most of the molecular mechanisms involved in the amino acid regulation of gene expression have been obtained by studying the up-regulation of C/EBP homologous protein (CHOP), Asparagine synthetase (AS) and the cationic amino acid transporter (Cat-1) genes.

Molecular mechanisms involved in the regulation of gene expression by amino acid limitation

A Post-transcriptional regulation of genes expression by amino acid availability

For most of the amino acid regulated genes that have been studied, it has been shown that the mRNA is stabilized in response to an amino-acid depletion. However, the molecular mechanisms involved in this process have not been identified yet (Gong, et al. 1991; Bruhat, et al. 1997; Aulak, et al. 1999).

Recently, it was shown that the translation rate of specific genes could be regulated by amino acid availability. Hatzoglou and collaborators demonstrated that amino acid depletion initiates molecular events that specifically activate translation of the CAT-1 gene. They have shown the presence of an Internal Ribosome Entry Site (IRES) located within the 5'UTR of the Cat-1 mRNA (Fernandez, et al. 2000; Fernandez, et al. 2002). This IRES is involved in the amino acid control of translation of the Cat-1 transcript. Under conditions of amino acid starvation, translation from this IRES is stimulated whereas the cap-dependent protein synthesis is decreased. Another example of translation induced by amino acid starvation was recently reported for the branched-chain α -ketoacid dehydrogenase kinase, but the mechanism of translational control was not studied (Doering and Danner 2000). This mechanism of compensatory response allows translation of major proteins despite the inhibition of the cap-dependent translational apparatus.

B Transcriptional activation of mammalian genes by amino acid starvation

The molecular mechanisms involved in the amino acid control of gene transcription have been studied using the regulation of CHOP (C/EBP Homologous Protein) and AS (Asparagine Synthetase) expression by leucine or histidine availability as a model.

CHOP

CHOP is a stress-inducible nuclear protein that dimerizes with members of the C/EBP family of transcription factors (Ron and Habener 1992) and activates gene transcription (Ubeda, et al. 1999). The *CHOP* gene is regulated tightly by a wide variety of stresses in mammalian cells (Luethy and Holbrook 1992; Sylvester, et al. 1994) including many conditions that are known to induce an endoplasmic reticulum stress response (ERSR) (Wang, et al. 1996). The ESR, also known as the unfolded protein response (UPR), is an intracellular signaling pathway to remedy the accumulation of unfolded protein in the ER. Transcriptional control of *CHOP* by the ESR involves the binding of ATF-6 in the presence of NF-Y to the cis-acting ESR element (ERSE) located between nt -75 and -93 (Yoshida, et al. 2000).

Amino acid starvation regulates *CHOP* transcription through a specific amino acid response (AAR) pathway that is distinct from the ESR signaling cascade (Jousse, et al. 1999). Transcriptional control elements used by the AAR pathway are contained within nucleotides -313 to -295 of the *CHOP* promoter (Figure 1) (Bruhat, et al. 2000). This short sequence can regulate a basal promoter in response to starvation of several individual amino acids and therefore was called the AAR element (AARE). Mutations affecting a stretch of nine nucleotides (called the "AARE core"; 5'-ATTGCATCA-3') in the AARE result in a loss of amino acid responsiveness.

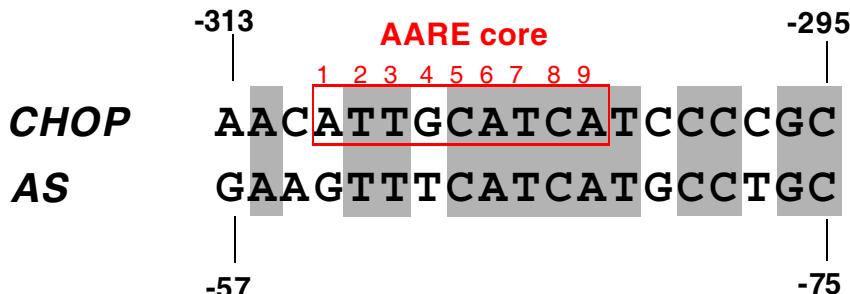


Figure 1. Sequence comparison of the *CHOP* AARE (-313 to -295) with the AS NSRE-1 (-57 to -75). Identical nucleotides are boxed in grey. The minimum *CHOP* AARE core sequence is boxed.

The sequence of the *CHOP* AARE region shows some homology with the specific binding sites of the C/EBP and ATF/CREB transcription factor families. We have shown that many transcription factors that belong to the ATF or C/EBP family have the ability to bind *in vitro* to the *CHOP* AARE. Among these factors, ATF-2 and ATF-4 are involved in the amino acid control of *CHOP* expression: when knock-out cell lines for these two proteins were tested, amino acid regulation of *CHOP* expression was abolished (Bruhat, et al. 2000)(submitted for publication). This work was enlarged to the regulation of other amino acid regulated genes and confirms that ATF-4 and ATF-2 are key components of the amino acid control of gene expression (Averous J, submitted for publication).

Asparagine synthetase

AS is expressed in most mammalian cells as a housekeeping enzyme responsible for the biosynthesis of asparagine from aspartate and glutamine (Andrulitis, et al. 1987). The level of AS mRNA increases not only in response to asparagine starvation but also after deprivation in several essential amino acids (Gong, et al. 1991; Hutson and 1994; Hutson, et al. 1997). Dr Kilberg's group has analyzed the regulation of the AS promoter by amino acid availability. They have characterized a Nutrient Sensing Regulatory Unit (NSRU) which includes two cis-acting elements termed Nutrient Sensing Response Elements (NSRE-1, NSRE-2) that are required to induce the

AS expression level by amino acid deprivation (Barbosa-Tessmann, et al. 2000). Gel shift experiments and over-expression of dominant negative mutants suggested that activation of the AS gene by either amino acid limitation or ERSR involves ATF-4 and C/EBP α binding to the NSRE-1 site (Baker, et al. 1993; Siu, et al. 2001; Siu, et al. 2002).

It appears that the CHOP AARE and the AS NSRE-1 sequences show some similarities (Figure 1). However, there are several lines of evidence suggesting that induction of *CHOP* and *AS* following amino acid starvation does not occur through a unique and common mechanism. (i) AS NSRE-1 and CHOP AARE share nucleotide sequence and functional similarities. However, the CHOP AARE can function alone whereas AS NSRE-1 is functionally weak by itself and requires the presence of NSRE-2 (Bruhat, et al. 2002) which amplify the amino acid response of NSRE-1. (ii) The region immediately following the *CHOP* AARE does not have a readily identifiable sequence that would correspond to NSRE-2. (iii) The amino acid specificity with regard to the degree of induction of these two genes is different (Jousse, et al. 2000). (iv) ATF2 or ATF4 inactivation abolishes CHOP induction by amino acid starvation whereas AS induction is reduced but not totally suppressed in cells devoid of ATF2 protein.

C Amino acid signaling pathway

Several studies suggest that mammalian cells have more than one amino acid signalling pathway (Jousse, et al. 2000; Bain, et al. 2002). However, the individual steps required for these pathways are not well understood. From ATF2 and ATF4 one can progress backwards up the signal transduction pathway to understand the individual steps required in the amino acid control of genes expression.

ATF-2 and the amino acid signalling pathways

ATF-2 contains a DNA binding domain consisting of a cluster of basic amino acids and a leucine zipper region (b-ZIP domain). Through its leucine zipper region, ATF-2 can form heterodimers with other b-ZIP proteins. It is likely that ATF-2 bind to the *CHOP* AARE as a protein complex with unknown transcription factor(s) and then activate transcription in response to amino acid starvation. It is well known that the transactivating capacity of ATF-2 is activated via phosphorylation of the N-terminal residues Thr-69, Thr-71 and Ser-90 (Gupta, et al. 1995; Livingstone, et al. 1995). There are several lines of evidence suggesting that ATF-2 phosphorylation belongs to the AAR pathway leading to the transcriptional activation of *CHOP* by amino acids: (i) Leucine starvation induces ATF-2 phosphorylation in human cell lines (submitted for publication) and (ii) an ATF-2 dominant negative mutant (Sano, et al. 1999), in which the three residues cannot be phosphorylated, inhibits the *CHOP* promoter activity enhanced by leucine starvation. (iii) Cotransfection of the ATF2 expression plasmid into ATF2-/- cells results in a partial rescue of amino acid inducibility of the *CHOP* promoter. Taken together these data suggest that the specific AAR pathway that leads to the transcriptional activation of *CHOP* may involve a phosphorylation of prebound ATF-2 rather than an increase in ATF-2 binding. However, the identity of the kinase(s) involved in ATF-2 phosphorylation by amino acid starvation remains to be discovered.

ATF-4 and the amino acid signaling pathways

The group of D. Ron has revealed a signalling pathway for regulating gene expression in mammals that is homologous to the well-characterized yeast general control response to amino acid deprivation (Harding, et al. 2000). Its components include (Figure 2) the mammalian homologue of the GCN2 kinase, the translation initiation factor eIF2 α and ATF-4.

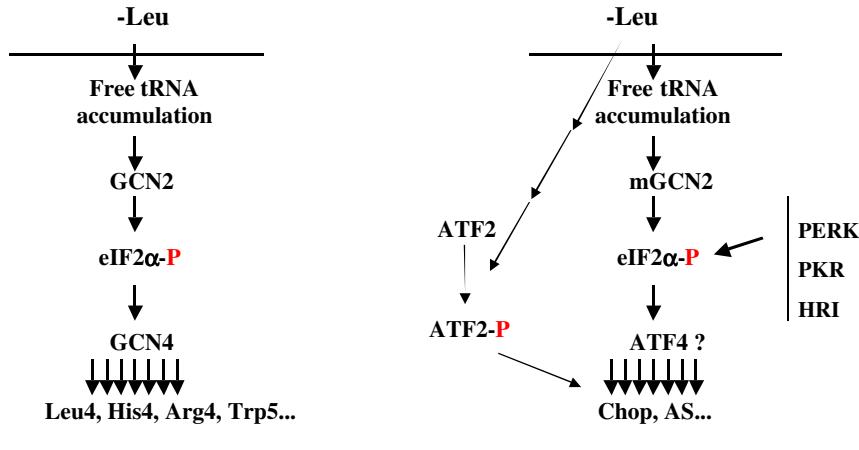


Figure 2. Comparison between the general control process of genes expression by amino acid availability in yeast and the amino acid regulation of CHOP and AS expression in mammals. The mammalian pathway appears to be more complex than the yeast pathway; for example, ATF-2 needs to be phosphorylated to allow CHOP induction in response to leucine starvation. In addition, eIF2 α can be phosphorylated by 4 different kinases (GCN2, PERK, PKR, and HRI).

In response to amino acid starvation, accumulation of free t-RNA activates the kinase activity of GCN2 that leads to phosphorylation of eIF2 α . Like GCN4 transcript, the ATF-4 mRNA contains uORFs in its 5'UTR that allow translation when the cap dependent translation is inhibited. The authors showed that GCN2 activation, phosphorylation of eIF2 α and translational activation of ATF-4 expression are necessary but not sufficient for the induction of CHOP expression in response to leucine starvation. These data are in good agreement with the analysis of the CHOP and AS promoter showing that ATF-4 can bind to the promoter sequences involved in the response to amino acid starvation.

These results show that increased gene transcription in response to amino acid starvation is linked to the translation of regulatory factors that depend on the presence of IRES or uORF in their messenger 5'-noncoding region. In addition, it appears that at least two different pathways that lead to ATF-2 phosphorylation and to ATF-4 expression are necessary to induce CHOP expression in response to one stimulus (amino acid starvation). However, we have no evidence that ATF-2 and ATF-4 form a dimer that binds the AARE sequence but they could be included in a larger regulatory protein complex. For example, it has been shown that ATF-2 interacts with at least two transcription factors (CP1 and NF1) in a large protein complex to regulate transcription of the fibronectin gene (Alonso, et al. 1996).

Conclusion

In mammals, the plasma amino acid concentration shows striking alterations as a function of nutritional or pathological conditions. Amino-acidemia can arise after a protein rich meal, whereas, under poor nutritional conditions, the organism can experience limitations in the supply of essential amino acids. In such a situation, an adaptive response takes place by adjusting several physiological functions involved in defence/adaptation to amino acid limitation. The cellular machinery compensates for a deficiency in amino acids by both (i) decreasing the cap-dependent protein translation initiation and the capacity for peptide chain elongation, and (ii) increasing the

rate of degradation of the resident proteins by induction of macroautophagy. These two processes contribute to the restoration of the free amino acid pool. At the same time, cells have the capacity to specifically increase both (i) translation of a set of proteins independently of the cap-dependent process, and (ii) transcription of specific genes.

The molecular mechanisms involved in the cellular response to amino acid availability have just begun to be discovered. Some components of the pathway(s) involved in the regulation of gene expression in response to amino acid limitation have been identified. However, the precise cascade of molecular events by which the cellular concentration of an individual amino acid regulates gene expression has not yet been identified.

Defining the molecular steps by which individual amino acids can regulate gene expression and protein turnover will be an important contribution to our understanding of metabolite control in mammalian cells. The molecular basis for gene regulation by dietary protein intake is important with respect to the regulation of physiological functions of individuals living under conditions of restricted or excessive food intake.

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Age dependent development of obesity under standard and high fat diet in extreme mouse models

G.A. Brockmann¹, S. Kuhla² & U. Renne³

¹ Research Institute for the Biology of Farm Animals, Department of Molecular Biology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

² Research Institute for the Biology of Farm Animals, Department of Nutritional Physiology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

³ Research Institute for the Biology of Farm Animals, Department of Genetics and Biometry, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

Summary

Obesity has become a major health risk factor worldwide. The tendency of gaining weight is influenced by the genetic predisposition and the environment. We used the high body weight selected mouse line DU6 and its inbred derivate DU6i (Research Institute for the Biology of Farm Animals in Dummerstorf, Germany) as polygenic models for growth and obesity research. In the present study, we have analysed effects of genotype-diet interaction on gaining weight. Therefore, 12 male and 12 female animals of the selection lines DU6 and its inbred derivate DU6i as well as the unselected control lines DUKsi and DBA/2 were fed either a standard low fat diet (5% crude fat) or a high fat diet (15.5% crude fat) over a period of 19 weeks, beginning at the age of three weeks when animals were weaned. Body weights were measured at the ages of 3, 6, 12, and 22 weeks. Body composition was analysed at the age of 22 weeks when animals were killed and necropsied. As expected, the responses to high fat diet were line dependent, indicating a strong genetic effect on energy partitioning. The two selection lines DU6 and DU6i and the control line DBA/2 responded a high fat diet by increased fat deposition. DUKsi animals had same weights under high and low fat diets. Females became significantly fatter than males under same feeding conditions. Fat deposition was higher at later ages. This indicates specific sex, genotype, and age effects on dietary fat intake, metabolism, heat production, or energy deposition.

Keywords: diet, obesity, genetic predisposition, mice

Introduction

The identification of factors controlling body weight and fatness in mice is of basic interest for a better understanding of growth regulation, nutrient turnover, and energy partitioning in humans and farm animals. Although rare cases of monogenic control of these traits are known, most growth and obesity related phenotypes are complex traits and genetically determined by many genes. Therefore, several inbred and selected mouse lines have been used as polygenic models for obesity research to access the molecular nature of such traits (rev. by Brockmann & Bevova, 2002).

The examination of gaining weight under different feeding conditions has shown that the responses to high-energy diets were different between various inbred mouse lines (rev. by West & York, 1998). So far, little has been known about response to dietary fat in growth-selected animals. Recently, a comparison between the selected and inbred “large” (LG/J) and “small” (SM/J) mouse lines has shown that SM/J mice were more responsive to increased fat diet than LG/J mice for growth after 10 weeks, while the growth from 3 to 10 weeks had a much greater response in the LG/J strain (Cheverud et al., 1999).

We used the high body weight selected mouse line DU6 and the inbred derivate DU6i as polygenic models for growth and obesity research. In this study, we analyzed the effect of high fat diet on weight gain in the two selection lines and in the control lines DUKsi and DBA/2. These mouse lines have been used repeatedly to map quantitative trait loci (QTLs) and to study the interaction between loci influencing growth and fat deposition (Brockmann et al. 1998, 2000).

Material and methods

The study was carried out on the mouse lines DU6 and DU6i as high body weight selected lines and DUKsi and DBA/2 as controls. DU6i is an inbred derivate from the selection line DU6. Line DUKsi is an inbred derivate of the line DUKs which was the unselected control of the selection experiment. Lines DU6, DU6i, and DUKsi descend from the same base population and were generated in the Research Institute for the Biology of Farm Animals, Dummerstorf, Germany (Schüler, 1985). The inbred line DBA/2 was bought from Harlan (Nederland, Horst). Animals were fed *ad libitum* with either a low fat (LF) or a high fat (HF) diet. The LF breeding diet contained 12.5 MJ/kg metabolic energy with an average content of 22.5% crude protein, 5.0% crude fat, 4.5% crude fibre, 6.5% crude ash, 13.5% water, 48.0% N-free extract, vitamins, trace elements, amino acids, and minerals. The HF diet contained 14.65 MJ/kg metabolic energy with an average content of 21.0% crude protein, 15.5% crude fat, 3.5% crude fibre, 5.5% crude ash, 10.0% water, 44.5.0% N-free extract, and other components as above (Altromin diet 1314, Germany).

Groups of 12 males and 12 females per mouse line were analysed in every diet group. Animals were weighed at the age of 3, 6, 12, and 22 weeks. Body composition was analysed at the age of 22 weeks when animals were killed and necropsied. The weights of quadriceps and the weights of the reproductive, perirenal, and inguinal fat depots were measured. The reproductive fat measured in males was the gonadal fat and in females the perimetrial fat. The ratio of the sum of the weights of all abdominal fat depots to body weight was defined as abdominal fat percentage. The weight of the quadriceps comprising *musculus rectus femoris*, *musculus vastus intermedialis*, *musculus vastus lateralis*, and *musculus vastus medialis* was recorded as representative of the muscle development.

Mice were housed in Makrolon-cages Type II (2-4 animals per cage), in a semi-barrier system under conventional conditions. The room temperature was between 22.4 and 22.7 °C, the humidity between 50 and 60 %.

Results

The selected and control mouse lines differed extremely in their responses to high fat diet (Figure 1). Mice of the two selected lines DU6 and DU6i increased their body weights in the period between 3 and 22 weeks of age by the factors 4.2 and 4.8 under LF diet, respectively, and by the factor 5.0 at HF diet. The selected lines had similar body weights at 22 weeks at LF diet. When fed a HF diet, animals of the selected lines DU6 and DU6i were on average 20.4 and 11 g heavier at the age of 22 weeks than animals fed the LF diet. The differences found between the two selected lines were significant in males ($\alpha < 0.001$), but not in females.

Among the unselected lines, DBA/2 was highly responsive to HF diet, while the line DUKs did not show additional body weight increase at HF diet. After feeding LF diet, the animals of lines DUKs and DBA/2 differed significantly by 7.4 g at the age of 22 weeks, while DBA/2 animals increased their body weights at HF diet and had similar body weights as DUKsi animals at 22 weeks.

After weaning at the age of three weeks, no sex specific differences in body weight were found in all feeding groups within lines, except in the LF diet group of DBA/2 mice where males were heavier than females ($\alpha < 0.001$). After weaning the sex dimorphism of body weight developed. Males became heavier than females. As a result of HF diet, females of the selection lines and the

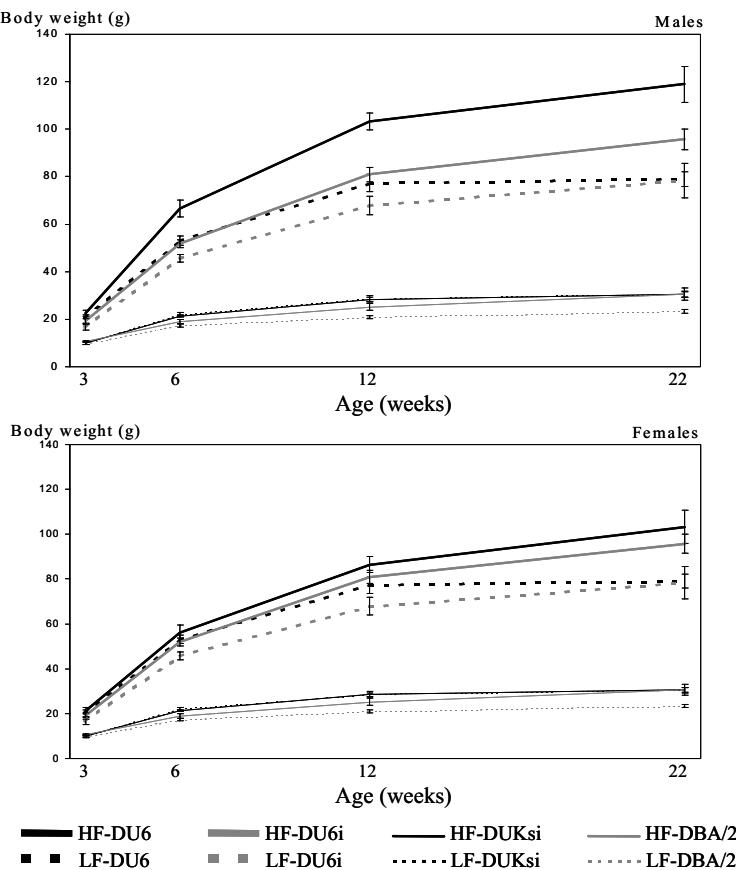


Figure 1. Age dependent increase of body weight in dependence of low (LF) and high fat (HF) diet in males and females of different selected and control mouse lines.

Table 1. Percentage of muscle and fat tissues at the age of 22 weeks after feeding a low (LF) or high fat (HF) diet over a period of 19 weeks.

| | Males | | | | | | Females | | | | | |
|-------|---------------------|------|-------|------------------|-------|-------|---------------------|------|-------|------------------|-------|-------|
| | Muscle ¹ | | | Fat ² | | | Muscle ¹ | | | Fat ² | | |
| | LF | HF | HF:LF | LF | HF | HF:LF | LF | HF | HF:LF | LF | HF | HF:LF |
| DU6 | 0.82 | 0.71 | 0.87 | 9.37 | 16.89 | 1.80 | 0.92 | 0.70 | 0.76 | 14.92 | 27.36 | 1.83 |
| DU6i | 1.05 | 0.96 | 0.91 | 7.12 | 13.27 | 1.87 | 1.03 | 0.83 | 0.81 | 18.90 | 26.48 | 1.40 |
| DUKsi | 1.28 | 1.21 | 0.95 | 2.41 | 3.20 | 1.32 | 1.31 | 1.25 | 0.95 | 2.48 | 2.82 | 1.14 |
| DBA/2 | 1.28 | 1.07 | 0.84 | 2.44 | 8.53 | 3.49 | 1.25 | 1.07 | 0.86 | 3.65 | 11.57 | 3.17 |

¹ Percentage of bide-sided quadriceps weights to body weight

² Percentage of the summed weights of the reproductive, perirenal, and inguinal fat pads to body weight

DBA/2 control line deposited faster fat than males, in particular in the period between 12 and 22 weeks. No significant body weight differences were found between males and females in these lines at the age of 22 weeks. The sex dimorphism was not influenced by the diet in HF diet resistant mice of the control line DUKsi.

The analysis of body composition at the age of 22 weeks supports the finding of interaction between diet and mouse line and diet and sex (Table 1). While the relative weights of muscle to body weight decreased, the percentage of fat increased at HF diet. The increase was highest in DBA/2 mice.

Discussion

Different responses to HF diet in the high body weight selected mouse lines DU6 and DU6i versus the control line DUKsi represent genetic differences between these lines. Obviously, selection for high body weight caused an enrichment of gene variants, which are sensitive to diet induced obesity, although the selection was carried out at standard LF diet. In the inbred line DUKsi gene variants are fixed which did not respond to HF diet in this study. The inbred line DBA/2 was highly responsive to dietary fat. This is in agreement with reports on genetic variation between various inbred lines in dietary obesity (West & York, 1998). Beside line differences, sex specific responses to HF diet were found. The increase of obesity was much higher in females than in males.

Recently, QTL studies in these mouse lines have shown that many genes with relatively small effects account for the genetic difference between these lines at LF diet (Brockmann et al. 1998). We expect also multiple genes, which are responsible for HF diet induced weight gain. QTL studies in crosses between these lines can also contribute to map genes, which are responsible for the sex dimorphism in the development of obesity in response to dietary fat. Therefore, the identified genetic differences in diet induced fat deposition can be used to elucidate the genetic and physiological causes of body weight control.

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Rainbow trout liver proteome - dietary manipulation and protein metabolism

S.A.M. Martin, O. Vilhelsson & D.F. Houlihan

School of Biological Sciences, University of Aberdeen, Aberdeen AB24 2TZ, UK

Summary

Changes in the expressed protein complement of rainbow trout livers have been studied using a proteomics approach. This report details the alterations of individual protein abundance in the livers of trout that have been subjected to dietary manipulations. These dietary changes have direct and indirect effects on protein turnover. Three experiments have been performed, 1) short term starvation, 2) addition of soy proteins to the diet, 3) use of plant protein as replacement of fish meal in the diet. For each experiment we have identified proteins that change in abundance and have used two programs, Mascot and MS-fit to identify proteins following trypsin digest fingerprinting.

Introduction

Proteomics describes the expressed protein complement of a genome, this varies between tissues and within tissues as results of metabolic and physiological changes.

High resolution two-dimensional protein gels, coupled with gel image analysis allows hundreds of protein to be monitored in parallel, permitting a global picture to emerge of changes in protein profile under different metabolic states. In addition to gel image analysis peptide mass mapping of trypsin digested protein spots by MALDI-TOF mass spectrometry can be used to identify particular proteins of interest. A rainbow trout proteome map is being developed in which all proteins are recorded in terms of molecular weight, isoelectric point (pI) and abundance. In the current report, we describe how the use of protein profiling in a non model organism, the rainbow trout to show that proteomics has potential to help us study cellular mechanisms that can alter as a result changes in dietary proteins.

Rainbow trout which are carnivorous fish require a high protein diet, when being raised in aquaculture, this protein is provided by fish meal which is produced from the harvest of wild fish. The sustainability and impact of such activities on world fisheries is questioned (Nylor et al., 2000), and thus a major goal for the continued growth and sustainability of the aquaculture industry is to increase the efficiency with which fish deposit ingested protein and secondly to replace as much of the fish meal derived protein with proteins from plant sources. Any alteration in the dietary intake can have down stream effects on the protein synthesis rates, protein degradation rates and the efficiency with which proteins are deposited as growth. In trout protein synthesis increases with protein consumption (Carter et al. 2001), however this increased synthesis rate does account for growth when the food consumption is similar (Carter & Houlihan 2001). Hence the rate of protein degradation may be key to understanding the regulation of protein deposition during growth. Changes in diet, amino acid composition of diets and antinutritional factors that can co purify from plants may all have effects on the protein metabolism in fish (Gomes et al., 1995). These effects may be manifested in a number of different biochemical pathways, and hence proteomics may help understand such changes.

Materials and methods

All rainbow trout were immature fish, and kept in fresh flowing water. For the starvation experiment fish were either starved for 14 days or fed a normal diet. Fish were fed a diet containing 30% proteins derived from soy, of 30% proteins derived from a number of different plant sources, The third experiment were fish fed either 100% plant derived proteins or normal fish meal diet. Following the dietary treatments fish were killed and the liver frozen -80°C until proteins were extracted. For details of protein extraction and 2 dimensional electrophoresis see Martin et al. (2001) for details. Proteins that were identified as up or down regulated were identified by trypsin digest fingerprinting followed by mass spectrometry. The peptide masses were used to search public access data bases using mascot or ms-fit programs.

Results and discussion

On all gels used for protein expression approximately 800 protein spots were analysed. A reference gel was constructed to which each experimental gel was matched so as spot numbering was consistent throughout all experiments. Protein spots were characterised by pI and molecular weight. The protein abundance of each spot was recorded once the gels had been normalised and

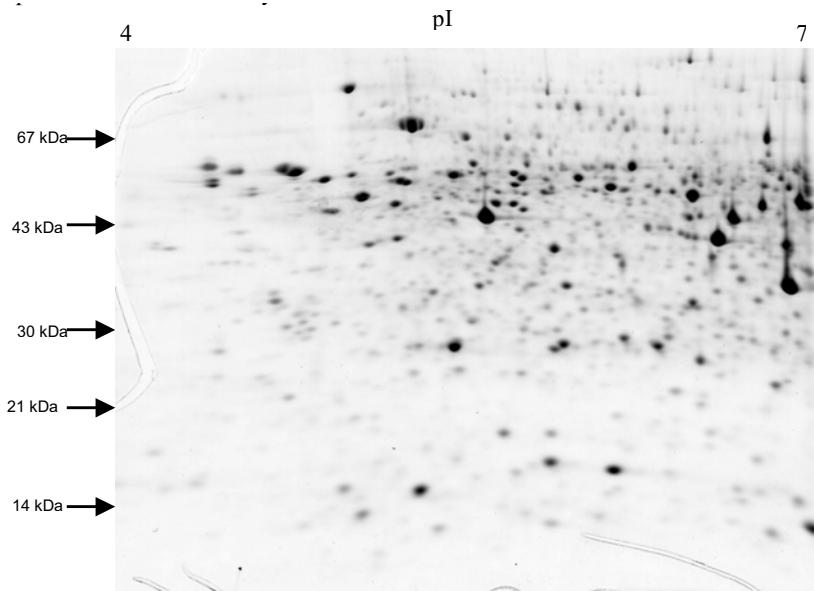


Figure 1. Representative 2 D gel of rainbow trout liver proteins. Proteins were separated by charge (pI 4-7), followed by size on a gradient 10-15% polyacrylamide gel. Approximately 800 protein spots were analysed for abundance on each gel.

Table 1. Numbers of differentially expressed proteins identified during the three experiments. These proteins were significantly different between control and experimental groups ($P>0.05$).

| | Food withdrawal | 30% Soy protein | 100% plant protein |
|-----------|-----------------|-----------------|--------------------|
| Increased | 16 | 12 | 22 |
| Decreased | 8 | 19 | 5 |

background removed, for each dietary trial. Three or five replicates were performed for each dietary treatment.

All proteins that are assigned as being up or down regulated could be observed on both control and experimental gels. Several proteins that were only present in one or two gels were not used in the analysis as these may have been the result of polymorphisms.

Selected protein spots were excised from the gels for protein identification using either mascot (Perkins et al. 1999) or ms Fit (Clauser et al 1999) programs for protein identification. The ms fit program allows the use of expressed sequence tag sequences to be searched. There are >170,000 EST sequences for salmonid fish, and recently this has greatly increased the identification of proteins. Thirty five differentially expressed protein have been positively identified, and an additional 44 proteins to help increase the trout proteome map. In total 85% of protein spots subjected to mass spectrometry were positively identified.

From the protein identities, different proteins were altered in abundance, cathepsin D, a lysosomal endopeptidase was increased in the starved fish, indicating increased catabolism. In those fish fed the diet that contained 30% protein derived from soy, stress proteins were increased in abundance and other enzymes such as Aldolase B which may reflect an increased energy demand in these fish. Many of the proteins altered in expression in the soy replacement diets may be a direct result of antinutritional factors co purified with the soy protein (Francis et al. 2001).

A variety of proteins including heat shock proteins, enzymes, fatty acid binding proteins and structural proteins were differentially regulated. The most likely reason for altered metabolism is the co purification from soy of antinutritional factors such as phytoestrogens, antigenic agents and many other compounds such as phorbol esters. Similar diverse alterations in gene expression have been shown in rats fed soy protein extracts (Iqbal et al. 2002). When fish meal protein was replaced with 100% protein derived from plants, heat shock proteins were not increased as was found with soy protein, however several subunits of the proteasome were found to be increased in

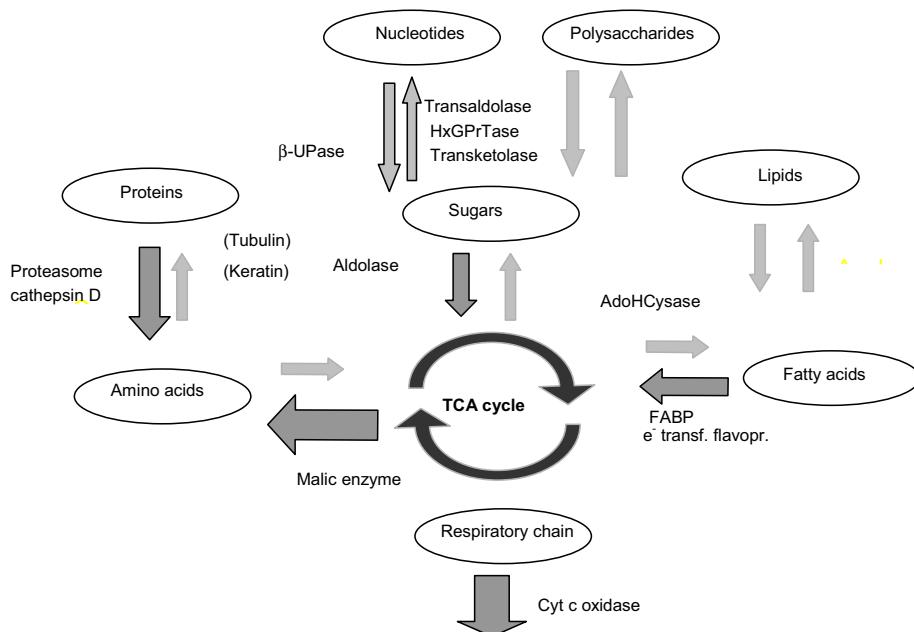


Figure 2 summarises the proteins that have been identified and a putative role in metabolic pathways are indicated.

abundance which may indicate changes in protein degradation, which could result in poorer deposition of synthesised proteins.

Although the primary aim of these experiments was to study metabolic pathways related to protein metabolism, it is clear that many interlinked effects are found. Of interest was that soy proteins had were shown to cause a difference in heat shock proteins and proteins involved in stress responses.

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Influence of IGF-I transgene and dietary CLA on efficiency of dietary energy and protein utilization in growing pigs

A.D. Mitchell & V.G. Pursel

USDA-ARS, Growth Biology Laboratory, Building 200, Beltsville, MD 20705 USA

Summary

The purpose of this study was to compare the efficiency of utilization of dietary energy and protein by control and IGF-I transgenic pigs in response to dietary conjugated linoleic acid (CLA). At 60 kg each pig was scanned by dual-energy X-ray absorptiometry (DXA) for body composition analysis, then placed on diets with a CP content of 182 g·kg⁻¹, a ME content of 13.8 MJ·kg⁻¹, and supplemented with either corn oil at 20 g·kg⁻¹ (CO diet) or CO at 10 g·kg⁻¹ plus CLA at 10 g·kg⁻¹ (CLA diet). Each pig was scanned again by DXA at 110 kg. Total body fat and protein deposition were based on the differences between the 60-kg and 110-kg DXA measurements of fat and lean. The efficiency of energy (k_g) deposition was higher ($P<0.05$) for the C-CO pigs compared to the C-CLA or T-CLA pigs, while the T-C pigs were intermediate. The k_g was higher ($P<0.05$) for pigs fed the CO diet compared to those fed the CLA diet. There was no difference in k_g based on genotype or sex. The efficiency of protein (PE) deposition was higher ($P<0.05$) in the T-CO and T-CLA pigs compared to the C-CO and C-CLA pigs; hence there was a significant genotype effect. There was also a significant sex effect (females > barrows), but no diet effect. These results demonstrate that the addition of CLA to the diet reduces the efficiency of energy deposition, however, inclusion of the IGF-I transgene into the genetic makeup improves the efficiency of protein deposition in pigs.

Keywords: pigs, efficiency, transgenic

Introduction

Conjugated linoleic acid (CLA), when added to the diets of mice and pigs reduces fat deposition and increases lean tissue mass (Park et al. 1997; Dugan et al. 1997). In mice the reduced fat deposition has been attributed to an increase in energy expenditure (West et al. 2000; Terpstra et al. 2002). However, an increase in energy expenditure was not observed in pigs and hamsters fed CLA (Muller et al. 2000; Boutheogourd et al., 2002). Pigs expressing the skeletal α -actin-hIGF-I transgene have been shown to deposit less fat and more lean than their littermate controls (Pursel et al. 2001). Thus, the objective of this study was to measure the efficiency of energy and protein accretion in control and IGF transgenic pigs when fed diets with or without CLA added.

Material and methods

Transgenic pigs were produced with a fusion gene composed of avian skeletal α -actin regulatory sequences and the cDNA encoding IGF-I (Figure 1). Transgenic (T) and sibling control (C) progeny were produced by mating two half-sib G-1 transgenic boars to non-transgenic gilts.

At 60 kg each pig was scanned by dual-energy X-ray absorptiometry (DXA) for body composition analysis, then placed on diets with a CP content of 182 g·kg⁻¹, a ME content of 13.8 MJ·kg⁻¹, and supplemented with either corn oil at 20 g·kg⁻¹ (CO diet) or CO at 10 g·kg⁻¹ plus CLA at 10 g·kg⁻¹ (CLA diet). Thus, the four genotype-diet combinations were: C-CO (n=25), C-CLA (n=25), T-CO (n=24), and T-CLA (n=23). Distributed among these were 54 females and 43 barrows. Total feed intake was measured for each pig individually. Each pig was scanned again by DXA at 110 kg.

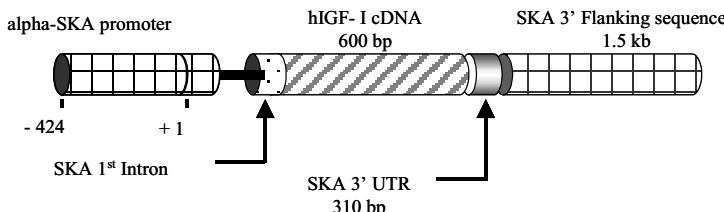


Figure 1. Schematic diagram of the avian skeletal α -actin-humanIGF-I fusion gene.

Total body fat and protein contents were calculated from the DXA readings using the following equations (Mitchell *et al.*, 2000): Fat (%) = 493.4 + (DXA R value · 348.8), Protein (g) = -1.062 + (0.2 · DXA Lean). Total body fat and protein deposition were based on the differences between the 60-kg and 110-kg DXA measurements of fat and lean. Energy deposition (RE_g) was calculated as the sum of fat deposition (RE_f) (39.6 MJ·kg⁻¹) and protein deposition (RE_p) (23.7 MJ·kg⁻¹).

Results and discussion

DXA analysis of body composition revealed that already at 60 kg the transgenic pigs had 19% less fat and 7% more protein than the control pigs (Table 1). These differences persisted to 110 kg and are similar to those reported previously (Pursel *et al.*, 2001). CLA supplementation resulted in a reduction in body fat (8.2%), only in the control pigs, and no effect on protein content.

The efficiency of energy (k_g) deposition (deposition/intake) was higher ($P < 0.05$) for the C-CO pigs compared to the C-CLA or T-CLA pigs, while the T-C pigs were intermediate (Figure 2). Overall, the k_g was higher for pigs fed the CO diet compared to those fed the CLA diet ($CO = 0.35, > CLA = 0.32, P < 0.05$). This is consistent with the CLA induced increase in energy expenditure that has been observed in mice (West *et al.* 2000; Terpstra *et al.* 2002). There was no difference in k_g based on genotype or sex.

There was a close relationship between k_g and the rate of fat accretion, independent of diet or genotype (Figure 3). Thus, according to this model, a more efficient utilization of energy allowed more energy to be available for fat deposition.

The efficiency of protein (PE) deposition (deposition/intake) was higher ($P < 0.05$) in the T-CO and T-CLA pigs compared to the C-CO and C-CLA pigs; hence there was a significant genotype effect (Figure 4). There was also a significant sex effect (females = 0.24, > barrows = 0.22, $P < 0.05$), but no diet (CLA) effect. There was a close relationship between the efficiency of protein accretion and the protein accretion rate (Figure 5). There were also close inverse relationships (not shown) between both the rate ($R^2 = 0.62$) and efficiency ($R^2 = 0.78$) of protein accretion and

Table 1. Body composition of pigs at 60 and 110 kg.

| Group | 60 kg (start) ¹ | | 110 kg (final) ¹ | |
|-------|----------------------------|--------------|-----------------------------|---------------|
| | Fat (kg) | Protein (kg) | Fat (kg) | Protein (kg) |
| C-CLA | 11.28 ± 1.40b | 8.57 ± 0.45a | 25.83 ± 3.52b | 15.10 ± 0.69a |
| C-CO | 11.29 ± 1.49b | 8.45 ± 0.46a | 28.15 ± 3.41c | 14.69 ± 0.75a |
| T-CLA | 9.21 ± 1.39a | 9.02 ± 0.44b | 22.39 ± 3.90a | 15.88 ± 0.75b |
| T-CO | 8.96 ± 1.42a | 9.23 ± 0.56b | 21.84 ± 4.40a | 15.74 ± 0.97b |

¹ Means ± SD within each column followed by a different letter were significantly different ($P < 0.05$).

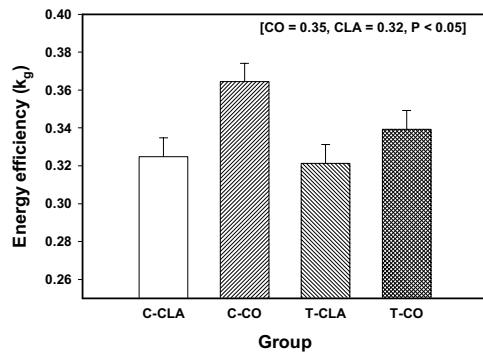


Figure 2. Effects of CLA and IGF-transgene on the efficiency of energy utilization in pigs.

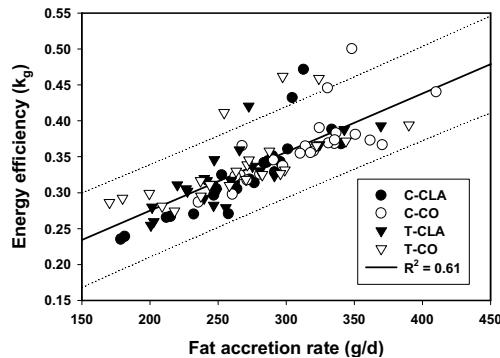


Figure 3. Relationship between the rate of fat accretion and the efficiency of energy utilization in pigs.

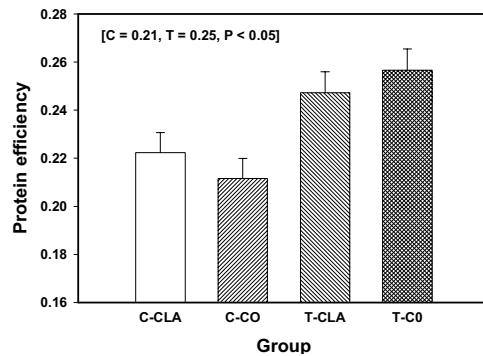


Figure 4. Effects of CLA and IGF-transgene on the efficiency of protein utilization in pigs.

the total energy intake during the growth of pigs from 60 to 110 kg. The reduction in efficiency of protein accretion with increasing energy intake is consistent with the results of Bikker (1994). These results demonstrate that while both the IGF transgene and CLA supplementation reduce the total body fat content in pigs, it appears that different mechanisms are involved and the effects do not appear to be additive. With CLA supplementation, the reduction in fat content was associated with a reduction in the efficiency of energy utilization, whereas, with the IGF transgene, it was associated with an increase in the efficiency of protein utilization and an increase in total body protein content. Total feed efficiency (G:F) improved in pigs expressing the IGF transgene ($C = 0.312$, $T = 0.338$, $P < 0.05$), but decreased slightly with CLA (CLA = 0.320, CO = 0.329, NS).

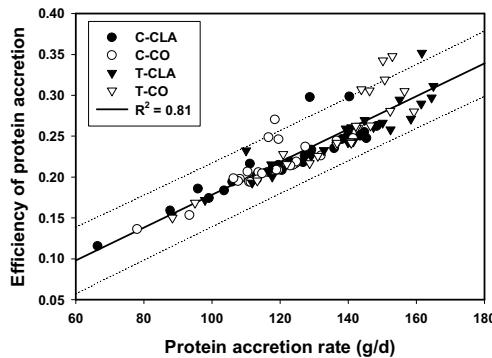


Figure 5. Relationship between the rate and efficiency of protein accretion in pigs.

Consequently, G:F improved in the following order: C-CLA < C-CO < T-CLA < T-CO. Although CLA produced a small (not significant) reduction in k_g in the transgenic pigs, at this time it is not clear why CLA failed to produce a reduction in fat content equivalent to that seen in control pigs. It is likely that the increase in protein accretion observed with the IGF transgene was achieved via a modulation in protein synthesis and breakdown as observed with somatotropin (pST) administration (Tomas et al., 1992). However, contrary to the effects of pST (Campbell et al., 1991), since the IGF transgene specifically targets skeletal muscle (Pursel et al., 2001), there may be little or no effect on maintenance energy requirement.

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Long-term dietary protein intake affects uncoupling protein mRNA gene expression in liver and muscle of rats

K.J. Petzke¹, M. Friedrich¹, C.C. Metges² & S. Klaus¹

¹ *German Institute of Human Nutrition, Arthur-Scheunert-Allee 155, 14558 Bergholz-Rehbrücke, Germany*

² *Research Institute for the Biology of Farm Animals, Department of Nutritional Physiology "Oscar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany*

Summary

An increased amino acid oxidation during high protein intake was hypothesized to contribute to enhanced mitochondrial oxygen radical generation resulting in oxidative stress. Thus, we measured markers of oxidative stress (liver lipid peroxide concentrations, protein carbonyl concentrations in plasma, total liver and blood glutathione concentration) in rats fed high protein diets. Groups of adult rats were adapted (18 wk) to diets containing either 13.8%, (AP), 25.7% (MP) or 51.3% (HP) casein. A fourth group received a HP diet lacking the antioxidative protection factor vitamin E in the vitamin mixture (HP-E) for comparison purposes.

Higher concentrations of plasma protein carbonyls and liver lipid peroxides were determined in rats fed the AP and the HP-E diets compared with those fed the MP and HP diets. Total glutathione concentrations in liver were significantly lower in rats fed the AP diet compared with MP, HP and HP-E groups. Therefore, a long-term intake of high protein diets did not necessarily result in oxidative stress. This led us to suggest that proton leak mediated by mitochondrial uncoupling proteins (UCP) might prevent oxidative stress during high protein intake. UCP2 mRNA expression in liver correlated positively with the dietary protein intake. Highest mean values were found in vitamin E deficient rats. In skeletal muscle mRNA expression of UCP2 was significantly lower in MP group as compared to AP and HP groups. UCP3 mRNA expression in skeletal muscle showed a similar pattern but was not significantly different between diet groups. A positive correlation was calculated between UCP2 mRNA expression in both liver and skeletal muscle and lipid peroxide concentrations in liver.

Our results indicate that UCP2 mRNA expression in liver is dependent on the level of dietary protein intake and provide evidence for a role of UCP2 in the control of reactive oxygen species during substrate oxidation and protection against dietary induced oxidative stress.

Keywords: *high protein diet, vitamin E, oxidative stress, uncoupling protein*

Introduction

Western diets provide about 1.5 g protein/kg/d which considerably exceeds the recommended intake for adults of 0.8 g/kg (GNS, 2000). Because there is no clear definition of the upper tolerable level of protein intake studies are needed to explore the effects of a high protein consumption. Undesirable metabolic effects have been already shown with about 1.6 times the recommended protein intake (Metges & Barth, 2000).

High dietary protein supply results in an increase in amino acid transport and oxidation (Petzke et al., 2000; Jean et al., 2001), which may contribute to mitochondrial generation of free radical oxygen species (ROS) associated with an increased oxidative stress (Petzke et al., 1999). Further, a lower food energy efficiency and a lower adipose tissue mass of rats after long term high protein intake as compared to an adequate protein intake suggests a reduced efficiency of ATP production (Petzke et al., 2000; Lacroix et al., 2002). UCPs have been shown to lower membrane potential

suggesting uncoupling activity of oxidative phosphorylation which enables a control of the liberation of ROS (Schrauwen & Hesselink, 2002). Therefore, UCPs might be involved in a high protein intake mediated oxidative stress. The aim of the present study was to explore the effect of high protein diets on markers of oxidative stress and whether UCPs mRNA expression in liver and skeletal muscle is altered in dependence of dietary protein supply.

Material and methods

Male adult Wistar rats (~230 g) were randomly assigned to experimental diets ($n = 10$) and were housed in a climate and light:dark cycle controlled room. The experimental diets (*ad libitum*) contained 13.8% (adequate protein; AP), 25.7% (medium protein; MP) or 51.3% (high protein; HP) casein which was isoenergetically exchanged by wheat starch (supplement: DL-methionine, 0.35 g/100g). A fourth group (HP-E) which consumed the HP diet but without vitamin E in the vitamin mixture was included in the experiment to subject one group of rats to defined oxidative stress conditions. After 18 wk of feeding the experimental diets a [1-¹³C]leucine infusion study was performed to determine postabsorptive leucine kinetic parameters (Petzke et al., 2000). Blood, liver and skeletal muscle (m. biceps femoris) samples were collected and stored in liquid nitrogen until analysis. Parameters of leucine kinetics, oxidative damage (lipid peroxide concentrations in liver measured as thiobarbituric acid-reacting substances, carbonyl concentrations in plasma proteins) reduced glutathione (GSH) and plasma free amino acid concentrations were determined essentially as described (Petzke et al., 2000).

Total RNA from individual tissues was extracted and analyzed by northern blotting (Boef et al., 2001). Differences between mean values were determined by ANOVA followed by the Newman-Keuls multiple range test. Pearson correlation coefficients were calculated to determine the relationship between selected parameters. Differences with $P < 0.05$ were considered statistically significant.

Results

Plasma protein carbonyl concentrations and liver lipid peroxide concentrations were higher in rats fed the AP and the HP-E diets as compared with those fed the MP and HP diets (Table 1). Total GSH concentrations in liver were significantly lower in rats fed the AP diet as compared with MP, HP and HP-E groups.

Rats fed high protein levels (MP, HP) had significantly higher UCP2 mRNA expression in liver than rats fed the AP diet. In contrast, in skeletal muscle the UCP2 mRNA expression was lowest in MP fed rats but was significantly higher both in AP and HP fed rats. A similar effect was observed for UCP3 mRNA expression in skeletal muscle but the mean values did not differ between the feeding groups ($P > 0.05$). For UCP2 in liver and skeletal muscle and UCP3 in skeletal muscle highest mean values of mRNA expression were observed in the HP-E group fed for 18 wk without vitamin E (Petzke et al., 2000).

A positive correlation was observed between liver UCP2 and leucine oxidation ($P < 0.000$). Further, positive correlations were obtained between UCP2 expression in liver and in skeletal muscle and liver lipid peroxide concentrations ($P < 0.033$ and $P < 0.002$, respectively). Further, we found a positive correlation between UCP mRNA levels and plasma protein carbonyl concentrations after feeding the experimental diets for only 1 wk (data not shown). No association occurred between UCP expressions and the plasma protein carbonyl concentrations after wk 15 of feeding the experimental diets.

Table 1. Liver lipid peroxide concentrations (TBARS), plasma protein carbonyl concentrations (PPCC), liver glutathione (GSH) concentration and arbitrary units (AU) of liver UCP2 and skeletal muscle UCP2 and UCP3 mRNA expression in rats fed for 18 wk diets with different protein concentrations (means \pm SD, $P < 0.05$). For diet composition see Material and methods.

| Diet | AP | MP | HP | HP-E |
|--------------------------|-------------------|-------------------|-------------------|-------------------|
| TBARS, nmol/g | 377 \pm 75 B | 264 \pm 71 A | 297 \pm 87 A | 497 \pm 149 C |
| PPCC, μ mol/g | 3.72 \pm 1.56 B | 2.43 \pm 1.06 A | 2.55 \pm 0.77 A | 3.75 \pm 1.68 B |
| GSH, μ mol/g | 4.44 \pm 0.63 A | 5.30 \pm 0.54 B | 5.38 \pm 0.71 B | 5.24 \pm 0.63 B |
| Liver UCP2, AU | 0.60 \pm 0.46 A | 0.89 \pm 0.30 B | 1.17 \pm 0.41 B | 1.90 \pm 0.33 C |
| Skeletal muscle UCP2, AU | 1.08 \pm 0.69 B | 0.63 \pm 0.28 A | 1.10 \pm 0.45 B | 1.36 \pm 0.43 B |
| Skeletal muscle UCP3, AU | 1.19 \pm 0.46 | 0.88 \pm 0.26 | 1.06 \pm 0.52 | 1.31 \pm 0.28 |

Discussion

UCP2 expression in liver reflected leucine and whole-body protein oxidation rates (Table 1, see also Petzke et al., 2000), indicating a relationship between amino acid oxidation and liver UCP2. A negative association was observed between UCP2 mRNA expression in liver and the occurrence of oxidative stress in long-term high protein diet (MP, HP) fed rats. On the other hand, after only 1 wk of feeding high protein diets (MP, HP) as compared to the AP diet, higher plasma protein carbonyl concentrations were determined suggesting an incomplete adaptation of ROS defense mechanisms. Therefore, we concluded that the levels of dietary protein intake can determinate ROS generation. However, there appears to be a difference between short and long-term exposure to high protein consumption. Nevertheless, our results confirm the suggested role of UCP2 in the regulation and limitation of ROS production (Schrauwen & Hesselink, 2002). This conclusion was also drawn from studies in UCP2 and UCP3 knockout mice showing that in knockout mice more ROS were generated in macrophages and mitochondria of muscle tissue, respectively (Arsenijevic et al., 2000; Vidal-Puig et al., 2000), and that markers of damage related to ROS were increased in skeletal muscle mitochondria from UCP3 knockout mice (Brand et al., 2002). We further observed a lower hepatic UCP2 mRNA expression in AP diet fed rats as compared to rats receiving diets MP and HP which coincided with an enhanced level of oxidative stress parameters as well as the reduction of hepatic GSH concentration, the latter being involved in ROS control and defense. These observations in AP rats are in contrast to our initial hypothesis according to which we anticipated higher levels of oxidative stress parameters as a consequence of an excessive dietary protein intake (Petzke et al., 1999). It is possible that the AP diet is inadequate with respect to supply indispensable amino acids to maintain oxidative stress defense. The MP diet provide a more adequate protein supply in rats than the AP diet.

The results obtained with the HP-E diet also show that deficiency of vitamin E, i.e. the lack of an oxidative stress protection factor led to an induced UCP mRNA expression in liver and muscle. Plasma tocopherol concentrations in rats fed the HP-E diet were only approximately 15% as compared with rats eating AP, MP or HP diets providing sufficient vitamin E (Petzke et al., 1999). A relation between dietary vitamin E supply and tissue UCP mRNA expression has not been shown before. But, different conditions of increased oxidative stress can lead to adaptive UCP2 overexpressions in several tissues (Pecqueur et al., 2001).

In conclusion, the level of dietary protein intake influenced UCP2 and UCP3 mRNA expression in liver and skeletal muscle of rats. UCP2 expression in liver reflects protein intake and oxidation as well as acute oxidative stress. UCP2 expression in muscle better correlates with oxidative stress after prolonged exposure. The associations of UCP2 gene expression with markers of oxidative stress support the role of UCPs in the control of ROS production.

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Gene nutrient interactions as a result of amino acid deficiency; effect on mRNAs associated with the cell cycle

William D. Rees, Mieke E. Prins & Susan M. Hay

Rowett Research Institute, Aberdeen, AB21 9SB Scotland UK

Summary

Cell growth is dependent on amino acids for both protein synthesis and the de novo synthesis of nucleotides. We have investigated the levels of the mRNAs for the cyclins and for the cyclin dependent kinase inhibitors (CDKI's) in cell cultures to identify marker genes associated with growth arrest. Cultures of embryonal carcinoma cells were chosen as a simple in vitro model of cells which both divided and underwent differentiation. Growth was inhibited in two different ways: Nucleotide synthesis was inhibited when an analogue of aspartic acid (PALA) was added to the medium. Protein synthesis was restricted by culture in a medium deficient in the amino acid lysine. Although these treatments restricted growth they did not affect the expression or the levels of the mRNAs for the principal cyclins although the trend was indicative of arrest at the G1/S checkpoint. PALA treatment decreased the mRNAs for p27 and p57. Lysine deficiency increased the mRNAs for the CDKI's p19, p27 and p57. These results show that it is possible to distinguish between different types of growth arrest by changes in the patterns of gene expression.

Keywords: *cell-growth, growth-arrest, apoptosis*

Introduction

The growth and differentiation of mammalian cells is dependent on the availability of amino acids. These are required for the synthesis of protein and as intermediates for biosynthesis of other products, in particular the synthesis of nucleotides. Interactions between nucleotide and protein metabolism play an important role during periods of rapid cell proliferation such as embryonic and fetal growth (Jackson 1991, Reeds et al 2000). Previously we have shown that the inhibition of protein and DNA synthesis results in markedly different patterns of gene expression (Fontanier-Razzaq et al 2002). Deficiencies in the nutrient supply will also interrupt the progression of the cell through the cell cycle and may alter the expression of the genes which regulate it. In this study we have investigated the effects of altered amino acid supply on the expression of some of these genes.

There are four separate stages in the division of a eukaryotic cell: G1, S, G2 and M. Progress through each of these stages is regulated by a group of proteins known as the cyclins (Johnson & Walker 1999). Each stage of the cell cycle is characterised by the appearance of the specific mRNAs coding for the appropriate cyclin protein. The cyclin proteins form complexes with another group of proteins, the cyclin dependent protein kinases (CDKs). This complex can then phosphorylate proteins to activate specific functions associated with each step in the cell cycle. When a quiescent non-dividing cell enters the first phase of the cell cycle (G1) it begins by expressing cyclin D1. This will peak in the early G1 phase then as the cycle progresses it is replaced by cyclin D3. The appearance of cyclin E at the end of G1 triggers the transition into S phase. Cyclin A accumulates at the start of S phase before being replaced with cyclin B1 as the cell enters M phase. Growth arrested cells may be stopped at particular checkpoints and be unable to progress to the next stage of the cell cycle. This will be reflected by a change in the relative expression of the cyclin mRNAs associated with that stage of the cell cycle.

By inhibiting the phosphorylation reactions catalysed by the cyclin/CDK complex, the cyclin dependent kinase inhibitors (CDKIs) also regulate progress through the cell cycle. These proteins are able to bind to the complex and inhibit the kinase activity. The CDKIs p19, p21, p27 and p57 have been shown to play important roles in response to growth inhibitory signals including contact inhibition, DNA damage and antimitogenic signals (Nakayama & Nakayama 1998). Therefore these genes are important markers of growth arrest.

Through an understanding of the patterns of gene expression it may be possible to identify the effects of amino acid deficiency on the growth and differentiation of tissues. Cultures of embryonal carcinoma cells were chosen as a simplified *in vitro* model system of cells which both divided and underwent differentiation. The growth-arrested cells were cultured either in the presence of phosphonacetyl L-aspartic acid (PALA) which inhibits the incorporation of aspartic acid into pyrimidine nucleotides or in amino acid deficient media. Previously we have used this system to investigate the expression of genes associated with growth arrest in response to amino acid deficiency (Fleming et al 1998, Fontanier-Razzaq et al 2002).

Material and methods

Cell culture

Mouse F 9 embryonal carcinoma cells (American Type Culture Collection, CRL-1720) were cultured in Dulbecco's Modified Eagles essential medium containing 10% fetal calf serum (Gibco, Paisley UK). Serum from the same batch was used for all experiments. Lysine deficient medium was prepared according to the same formula except that lysine was omitted. PALA was a generous gift from Dr J.Johnson, Drug Synthesis Branch, National Cancer Institute, Bethesda USA.

Northern analysis

Appropriate cDNA probes for the cyclins and CDKIs were obtained from the MRC Geneservice (Babraham, Cambridge. UK). The isolation of total RNA, its separation on agarose gels, transfer to a nylon membrane, probe labelling and hybridisation has been described previously (Fleming et al 1998). The blots were washed in 0.5 X SSC + 1% SDS at 65°C and quantified by imaging on a wire proportional counter (Packard Instant Imager). Errors in transfer were corrected by probing the blots for 18S ribosomal RNA.

Results

Two different treatments were used to inhibit the growth of F9 embryonal carcinoma cells in culture. In the first case cells were treated with 120 μ M PALA to inhibit the synthesis of nucleotides from amino acids, thus inhibiting DNA synthesis. In the second treatment cells were cultured in medium which was deficient in the amino acid lysine. Because lysine is primarily used for protein synthesis and has very little involvement in intermediary metabolism these changes must be the result of limitations in the amino acid supply. Both of these treatments inhibited cell growth by approximately 50% when compared to the control cells cultured in complete medium. (Fleming et al 1998). Samples of mRNA extracted from the cells were analysed by Northern blotting and the mRNA levels are expressed relative to those found in control cells. Thus a relative expression of one indicates that there is no change in the steady state level of the mRNA.

The relative changes in the mRNAs for the cyclins are shown in figure 1. All of the mRNAs showed bands of the anticipated sizes and strong signals were detected for cyclin D3, E and B1. The signals for cyclin D1 and A were weaker but still readily detected on the Northern blots. The steady state levels varied by no more than about 50% in response to the treatments and none of the changes were statistically significant.

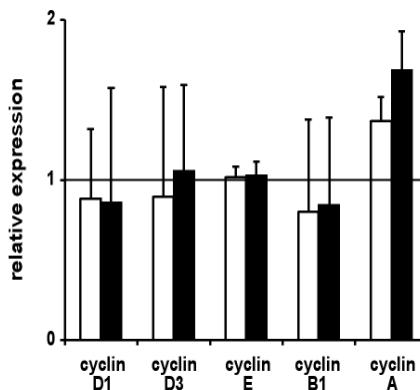


Figure 1. Changes in the expression of the cyclin mRNAs in F9 embryonal carcinoma cells treated with PALA (open bars) or grown in lysine deficient media (solid bars). The data are the mean of three estimations. Error bars = s.d.

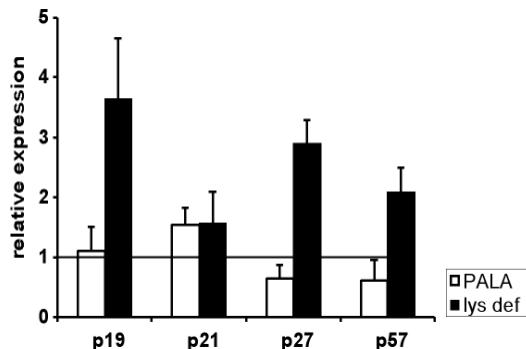


Figure 2. Changes in the relative expression of the Cip/Kip family of CDKI mRNAs in F9 embryonal carcinoma cells treated with PALA (open bars) or grown in lysine deficient media (solid bars). The data are the mean of three estimations. Error bars = s.d.

The relative expression of the mRNAs for p19, p21, p27 and p57 is shown in figure 2. Lysine deficiency increased the expression of p19 by approximately four fold relative to the control ($p<0.01$) whereas PALA treatment had no effect. The overall expression of p21 was low in both control and treated cells and showed no significant effect of treatment. Both p27 and p57 were highly expressed and they responded to the treatments in similar ways. Treating cells with PALA reduced the levels of both mRNAs to about half those in the control cells (both $p<0.01$). In contrast the levels were increased by approximately two to three times relative to the control in the cells cultured in lysine deficient media (both $p<0.01$).

Discussion

The changes in the steady state levels of the cyclin mRNAs suggests that restricting the synthesis of nucleotides or limiting protein synthesis has a similar effect on the distribution of cells within the cell cycle. If cells had become checked at a particular point in the cell cycle we would have anticipated that the relative expression of one cyclin would be increased while others are decreased. Although the data fail to show significant changes in the expression of the cyclins, the trend of a relative increase in cyclin A and a decrease in the others is indicative of the cells

becoming arrested briefly at the G1/S checkpoint. However, the change in expression is so small that it cannot be used in isolation to evaluate the effect of perturbations in the amino acid supply. Whilst the growth of mammalian cells is inhibited when either protein or DNA synthesis is disrupted, the mechanism and the response are different. Blocking the synthesis of pyrimidine nucleotides reduces the expression of the CDKIs along with a few other genes such as *gas5* (Fontanier-Razzaq et al 2002). In contrast to the small and generally downward trend in mRNA levels caused by the inhibition of DNA synthesis there are large increases in the steady state levels of the mRNAs for the CDKIs in lysine deficient cells. The changes in the expression of the CDKI genes is very similar to previous studies which have shown increases in the expression of a number of different genes including *gadd153*, (Fafournoux et al 2000), *gas5* (Fleming et al 1998), *Dmrt1* and several EST sequences (Fontanier-Razzaq et al 2002). This suggests that these genes are part of an active response to the stress caused by an imbalance in the amino acid supply. Many of these changes lead to the initiation of apoptosis, suggesting that these stress responses are a means of eliminating damaged cells. Changes in the relative expression of the CDKI genes may be useful in investigating possible mechanisms of growth restriction, particularly in complex systems such as the developing fetus.

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Diet and gene expression in broilers

R.W. Rosebrough, S.M. Poch, B.A. Russell & M.P. Richards

Growth Biology Laboratory, ANRI, USDA-ARS, BARC, Beltsville, MD 20705 USA

Summary

We determined the possible relationship between lipid metabolism and gene expression in chickens fed graded levels of dietary crude protein. Male, broiler chickens growing from 7 to 28 days of age were fed diets containing 12, 21 or 30% protein ad libitum. Another group of birds was fed on a regimen consisting of a daily change in the dietary protein level (12 or 30%). This latter group was further subdivided such that one-half of the birds received each level of protein on alternating days. In vitro lipogenesis and malic enzyme activity were inversely related to dietary protein levels (12 to 30%) and to acute changes from 12 to 30%. In contrast, expression of malic enzyme, fatty acid synthase and acetyl CoA carboxylase genes were constant over a dietary protein range of 12 to 21%, but decreased by feeding a 30% protein diet. Results of the present study demonstrate a continued role for protein in the regulation of broiler metabolism.

Keywords: *lipogenesis, protein, gene expression*

Introduction

Over a period of several years, we have studied the effects of dietary protein levels on various indices of lipid metabolism. Briefly, increased dietary protein decreases de novo lipogenesis and the activities of several associated enzymes. In contrast, increased dietary protein increases plasma thyroxine (T_4) and insulin-like growth factor I (IGF-I) while decreasing plasma triiodothyronine (T_3) and growth hormone. It may be possible to regulate gene expression anywhere from transcription to the actual enzyme protein. It should be emphasized that nutritional factors could regulate enzyme activity by any combination of factors impacting translation and post-translational events (Hesketh et al., 1998). The purposes of this experiment were to further study the metabolic effects of chronic or acute dietary protein treatments and to determine if changes the levels of mRNA for certain lipogenic enzymes related to changes in metabolic rates noted with various levels of dietary crude protein.

Materials and methods

Male broiler chickens growing from 7 to 28 days of age were fed diets containing 12, 21 or 30% protein (Table 1). In addition, another group of birds was fed on a regimen consisting of a daily change in the dietary protein level (12 or 30%). The latter treatment was continued for ten cycles. Additionally, This group was also subdivided such that one-half of the birds received each level of protein on an alternating day. This subdivision was necessary to account for possible daily variation in responses. Birds were selected on days 28 to 30, weighed, bled by cardiac puncture and sacrificed. There were six pen replicates for each dietary treatment.

Tissue handling for in vitro lipogenesis

Livers were rapidly excised into phosphate buffered saline, rinsed to removed debris and held at 2 C for later. Portions of each liver were also snap frozen in liquid N₂ and stored at -80 C for subsequent extraction of total RNA. Livers were then sliced and explants were incubated at 37 C

for 2 h in Hanks' balanced salts containing , 10 mM-HEPES and 10 mM-sodium[2-¹⁴C]acetate (166 MBq/mol). At the end of the stated incubation periods, the explants were placed in 10 mL of 2:1 chloroform: methanol for 18 h. The extracts were evaporated to dryness and dispersed in scintillation fluid. In vitro lipogenesis was expressed as (moles of acetate incorporated into lipids per g of tissue.

Enzyme activities

Liver tissues were also homogenized (1:10, wt/vol.) in 100 mM-HEPES (pH 7.5) 3.3 mM β-mercaptoethanol and centrifuged at 12,000 x g for 30 min. The supernatant fractions were kept at -80 C until analyzed for the activity of malic enzyme, isocitrate dehydrogenase and aspartate aminotransferase.

Lipogenic enzyme gene expression

Total RNA was isolated using the Tri-Reagent procedure and measured spectrophotometrically. RT reactions (50 µl) consisted: of 5 µg total RNA, 100 units MMLV reverse transcriptase (RNase H minus, point mutation), 40 units RNAsin, 1.0 mM of each dNTP, and 6 pmol random hexamer primers. Hot-started PCR was performed in separate 27.5 µl reactions containing: PCR buffer, 0.5 (fatty acid synthase, acetyl CoA-carboxylase) or 1.25 (malic enzyme) units of Platinum Taq DNA polymerase, 0.2 mM of each dNTP, 10 pmol each of each gene specific primer including a set for β-actin (see below), the internal standard. Each PCR was run as a duplex with primer sets added for a particular lipogenic enzyme and for β-actin. PCR thermal cycling parameters were as follows: 1 cycle 94 C for 2 min, followed by 30 cycles, 94 C for 30 sec, 58 C for 30 sec, and 72 C for 1 min with a final extension at 72 C for 8 min. RT-PCR produced dsDNA amplicons of 423, 431, 447 and 300 bp for fatty acid synthase, malic enzyme, acetyl-CoA carboxylase, and β-actin, respectively.

Capillary Electrophoresis (CE) - Aliquots (2 µl) of RT-PCR samples were diluted 1:100 with deionized water prior to analysis by CE. A P/ACE MDQ (Beckman Coulter, Inc., Fullerton, CA) configured for reversed polarity and equipped with an argon ion laser-induced fluorescence (LIF) detector was used to separate and detect the dsDNA amplicons. A (SIL-DNA capillary (J and W Scientific, Folsom, CA) with a 75 µm I.D., 0.075 µm film thickness, and length of 32 cm was used at 25°C. The dsDNA separation buffer was from Sigma (St. Louis, MO). EnhanCE™ dye (1 mg/ml stock in methanol) was added to the separation buffer to produce a final concentration of 0.5 µg/ml. Diluted RT-PCR samples were loaded by electrokinetic injection at 3.5 kV for 5-10 sec. Separations were performed at a field strength of 300 V/cm (8.1 kV) for 4.5 min. A lipogenic enzyme/β-actin peak area ratio was then calculated and used to compare tissue samples with respect to relative lipogenic enzyme gene expression activities. Ratio values are expressed as mean ± SEM

Results and discussion

Table 1 summarizes the effect of the dietary treatments on growth of the chickens in the present study. The 12% protein diet decreased ($P<0.01$) body weight, feed intake efficiency of feed utilization compared to the other three dietary treatments. The rotational protein-feeding regimen resulted in feed and protein efficiencies that were similar to feeding the 21% protein diet. In contrast, feeding the 30% protein diet resulted in the poorest ($P<0.01$) utilization of dietary protein. There were no significant differences in calculated carbohydrate intakes among the treatments.

Table 1. Effects of diets containing either 12, 21 or 30% crude protein on the growth of broiler chickens from 7 to 28-d^{1,2}.

| Variable | Dietary crude protein (%) | | | |
|--------------------------------------|---------------------------|-----------|-----------|-----------------------|
| | 12 | 21 | 30 | Rotation ³ |
| Body weight (g) | 721±25.9 | 1477±31.2 | 1559±27.3 | 1392±25.9 |
| Food eaten (g) | 1741±75.6 | 1973±45.1 | 1894±45.8 | 2113±53.8 |
| Carbohydrate intake ⁴ (g) | 1300±56.7 | 1323±30.2 | 1255±27.9 | 1358±38.3 |
| g gain/g food | 0.33±0.02 | 0.68±0.02 | 0.75±0.02 | 0.60±0.03 |
| g gain/g protein | 2.77±0.14 | 3.22±0.09 | 2.50±0.08 | 3.01±0.13 |

¹Birds were fed diets containing 12, 21 or 30% on an ad libitum basis or on a rotation consisting of a daily change in dietary protein (12 or 30%).

²Values are means ± SEM, n =6.

³ Calculated from the recorded intakes of both the 12 & 30%.

⁴ Derived from multiplying the calculated carbohydrate percentage by the total feed intake.

Table 2 depicts a rather precipitous decrease ($P<0.01$) in *in vitro* lipogenesis accompanying an increase in dietary protein. The daily change in protein also resulted in a decrease in lipogenesis as the birds were switched from 12 to 30% protein. Malic enzyme activity also paralleled the decrease in lipogenesis noted as dietary protein increased from 12% to 30%.

Table 2. Dietary protein and the activity of certain enzymes implicated in lipid regulation in the broiler.

| Diet ¹ | ME ² | ICD (NADP) ³ | AAT ⁴ | IVL ⁵ |
|-------------------|-----------------|-------------------------|------------------|------------------|
| 12% | 30.2±1.65 | 22.2±0.70 | 54.8±1.96 | 45.56±2.32 |
| 21% | 17.6±1.17 | 26.8±1.14 | 65.4±2.34 | 33.0±3.74 |
| 30% | 3.0±0.24 | 46.0±2.50 | 102.8±3.86 | 6.9±0.52 |
| r12% | 18.6±1.08 | 36.7±2.04 | 73.5±3.96 | 32.4±2.35 |
| r30% | 16.3±1.34 | 38.5±2.08 | 75.2±2.71 | 19.7±1.84 |

¹ Dietary protein level. r12% and r30% refer to birds fed alternating levels of protein with a switch occurring daily, ² Malic enzyme, ³ Isocitrate dehydrogenase, ⁴ Aspartate aminotransferase, ⁵ *In vitro* (IVL) lipogenesis

Table 3. Dietary protein and the expression of certain genes implicated in lipid regulation in the broiler.

| Diet ¹ | ME ² | FAS ³ | AcCBX ⁴ |
|-------------------|-----------------|------------------|--------------------|
| 12% | 0.46±0.03 | 1.84±0.18 | 1.03±0.11 |
| 21% | 0.46±0.03 | 1.97±0.18 | 1.04±0.01 |
| 30% | 0.12±0.03 | 1.03±0.18 | 0.83±0.06 |
| r12% | 0.58±0.03 | 2.84±0.18 | 1.38±0.09 |
| r30% | 0.10±0.03 | 1.23±0.18 | 0.88±0.09 |

¹Dietary protein level. r12% and r30% refer to birds fed alternating levels of protein with a switch occurring daily, ²Malic enzyme, ³ Fatty acid synthase, ⁴ Acetyl CoA carboxylase

Malic enzyme gene expression was very nearly identical in birds fed diets containing either 12 or 21% protein, but was less ($P<0.01$) in birds fed the 30% protein diet (Table 3). The daily switch in dietary protein from 12 to 30% resulted in a rapid decrease in gene expression. Acetyl CoA carboxylase gene expression was identical for birds fed 12 and 21% protein diets, but was decreased ($P<0.01$) by feeding the 30% protein diet. The decrease, was not as pronounced as the decrease in malic enzyme gene expression. The daily switch in dietary protein from 12 to 30% again, resulted in a rapid decrease ($P<0.01$) in gene expression.

The present experiment demonstrated that increasing dietary protein decreases lipogenesis as well as malic enzyme activity. Very early work described an inverse relationship between dietary protein and the subsequent rate of lipogenesis and that feeding increased and starvation decreased malic enzyme and fatty acid synthase gene expression. Later work (Ma *et al.*, 1990) reaffirmed that feeding fasted chickens would stimulate malic enzyme gene transcription. This study showed that this increase could occur in as little as 1.5 hr following refeeding. Semenkovich *et al.* (1993) reported that glucose availability could control fatty acid synthase mRNA levels without having any effect on transcription initiation. A more recent study (Lagarrigue *et al.*, 2000) showed that mRNA levels of genes coding for certain lipogenic enzymes were not greater in a line of chickens selected for high feed intake in spite of consistently larger abdominal fat pads. Previous suggestions had been made that lipogenic enzyme activities were regulated at transcriptional steps and, by inference, lipogenesis. It is interesting to note that reversible phosphorylation of acetyl CoA carboxylase dramatically changed fatty acid synthesis. Hesketh *et al.* (1998) reported that although nutrition could alter the amount of enzyme protein by transcriptional, post-transcriptional or translational events, increasing evidence indicated that the latter two events were likely candidates for nutritional events.

In summary, feeding chickens diets containing crude protein levels from 12 to 21% will decrease lipogenesis without affecting the expression of genes coding for enzymes involved in lipogenesis. An increase in crude protein to 30% will further decrease lipogenesis and the expression of genes coding for lipogenic enzymes. Rapid changes in dietary protein will change lipogenesis and lipogenic gene expression.

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Paternal genetics affects the regulation of blood glucose concentration

M.P. Tygesen¹, M.O. Nielsen¹, H. Ranvig², P. Nørgaard² & A.P. Harrison¹

¹ Department of Anatomy and Physiology, The Royal Veterinary and Agricultural University, Grønnegårdsvej 7, DK-1870 Frederiksberg C, Denmark

² Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Grønnegårdsvej 3, DK-1870 Frederiksberg C, Denmark

Summary

In a study of lambs born to ewes fed adequately throughout gestation, a glucose challenge test administered at day 155 *post partum*, resulted in a significantly elevated plasma glucose concentration 60 minutes after a challenge in lambs born to a ram with a high genetic potential for high muscle and low fat content compared to those born to a ram with a high genetic potential for daily live weight gain. No difference in plasma insulin concentration in response to the glucose challenge was found, suggesting that pancreas response and rate of insulin secretion are not different in these two groups of lambs. It is concluded that a high genetic potential for lean body mass predisposes to an impaired glucose tolerance in a direction similar to that associated with non insulin dependent diabetes mellitus.

Keywords: lambs, insulin, IGT

Introduction

Cohort studies have shown that malnutrition *in utero* affects metabolic control systems predisposing to type II diabetes and obesity (Phipps *et al.*, 1993; Barker, 1998). Indeed, the development of the hypothesis referred to as the “thrifty phenotype” states that adaptation to a period of undernutrition during foetal development ensures that the subsequent infant is nutritionally “thrifty” at birth, only predisposing to impaired glucose tolerance (IGT) and non-insulin-dependent diabetes mellitus (NIDDM) upon exposure to a better plane of nutrition later in life (Law, 1996). Since genetics may play a role in determining the set point of these metabolic control systems, we have investigated whether nutrition post-weaning and paternal genetics affects the outcome of a glucose challenge test in lambs born to sheep fed identically during pregnancy.

Materials and methods

Animals

A total of 17 female Shropshire lambs were born to either a ram with a high genetic potential for daily live weight gain (Zink; Identification no. 85769 - 1493) or high muscle and low fat content (Sul; Identification no. 8594 - 0294). Lamb birth weights were 3.9 ± 0.2 and 4.2 ± 0.1 kg, for the Zink and Sul paternal lines, respectively. Lambs were reared by the ewe until 63 days of age with *ad lib.* access to artificially dried grass (11.3 MJ ME/kg DM) and concentrate (12 MJ ME/kg DM), after which they were weaned and fed either a low or high concentrate diet supplemented with *ad libitum* artificially dried grass (11.3 MJ ME/kg DM); 0.7 and 0.9 MJ ME per kg LW^{0.75}, respectively.

Glucose and insulin measurements

Lambs were locally anaesthetised with lidocaine and a catheter was placed in a jugular vein. Plasma glucose concentration was measured in samples taken 5 min prior to an intravenous glucose challenge ($0.45 \text{ g glucose / kg LW}^{0.75}$; 2.8 M D-glucose), and 60 minutes subsequent to the challenge. Glucose concentration was determined on 50 μl sample volumes using a quantitative enzymatic microplate assay (Sigma Diagnostics KIT 510-DA, Denmark). Plasma insulin concentration was measured on samples taken 5 min prior to the intravenous glucose challenge and 10, 30 and 60 minutes subsequently. Insulin concentration was determined on 25 (1 sample volumes using a quantitative enzymatic microplate sheep insulin assay (DRG Instruments KIT EIA-2339, Germany).

Results

Growth rate

The mean daily live weight gain (0-155 days) for lambs born to Zink was $225 \pm 9 \text{ g/day}$, which was not significantly different from the value of $210 \pm 34 \text{ g/day}$ for lambs born to Sul.

Glucose

Dietary treatment post weaning had no effect on the regulation of blood glucose concentration. Moreover basal glucose concentrations were not different from those measured 60 minutes after a glucose challenge for lambs at 56 days of age regardless of their paternal background (see Table 1). After weaning and at an age of 155 days, basal glucose concentrations were significantly lower than those measured at day 56 regardless of paternal background ($P < 0.05$, paired t-test). However, the glucose concentration 60 minutes after a glucose challenge was significantly different from basal levels for lambs born to Sul but not Zink at 155 days of age ($P < 0.05$, paired t-test).

Table 1. Plasma glucose concentrations (mean \pm SE) before and after a glucose challenge in lambs from two paternal lines.

| Time from injection (min) | Day 56 | | Day 155 | |
|---------------------------|---------------|---------------|---------------|-----------------|
| | Zink | Sul | Zink | Sul |
| -5 | 4.6 ± 0.1 | 5.1 ± 0.1 | 3.8 ± 0.1 | 3.7 ± 0.1 |
| 60 | 4.8 ± 0.1 | 5.4 ± 0.1 | 3.7 ± 0.1 | 4.4 ± 0.1^1 |

¹Indicate significant difference from basal levels ($P < 0.05$, paired t-test).

Insulin

Plasma insulin values (see Figure 1), showed no significant effect of paternal genetics on the insulin concentration released in response to a glucose challenge and additionally, showed a very similar time response curve for lambs at both days 56 and 155 *post partum*.



Figure 1. Plasma insulin concentrations (mean \pm SE) before and after a glucose challenge in lambs from two paternal lines. Panel A: values measured at day 56 post partum, and Panel B: values measured at day 155 post partum.

Discussion and conclusions

It has been suggested that the incidence of chronic disease in later life stems from a period of undernutrition during foetal development, so-called “*in utero* programming” reflecting the long-term effects of reduced or impaired development of for example, the endocrine pancreas and other tissues (Phipps *et al.*, 1993). In the present study, lambs born to either Zink or Sul had very similar birth weights and postnatal rates of daily liveweight gain, however, despite these findings, results indicate that paternal genetics affects the lambs ability to respond to a glucose challenge. Data show that blood glucose concentration remains elevated (17% higher; $P<0.05$ paired t-test) 60 minutes after a glucose challenge compared to the pre-challenge level for Sul rather than Zink lambs. Such a response might have been due to differences in the circulating concentration of insulin released in response to the glucose challenge administered. However, measurements of plasma insulin concentration before and after a glucose challenge for both lamb groups, and at two different ages postnatally, show that this was not the case. It therefore seems likely that the difference in blood glucose concentration between Sul and Zink lambs 60 minutes after a challenge at day 155 is the result of diminished insulin receptor numbers and/or receptor function in all or some body organs or tissues.

The findings of the present study, in which nutritional status was not altered during foetal development, reveal that lambs born to rams with a high genetic potential for high muscle and low fat content (Sul), have a predisposition to IGT after weaning at, and around day 155 postnatally compared with lambs born to rams with a high genetic potential for daily liveweight gain (Zink). This gene-IGT interaction is perhaps not so surprising when examined in the light of recent data showing that individuals who are carriers for the Ala-allele of the codon 12 polymorphism of human peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene have a significantly improved whole-body sensitivity to insulin compared to non-carriers (Ek *et al.*, 2001).

It is concluded that a genetic predisposition towards a lean body mass changes the response to a glucose challenge in a direction similar to the insulin resistance observed in type II diabetes, an effect that is independent of nutrition after weaning.

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Joint session 2

Regulation and Modelling of Energy and Protein Status

Role of the liver in the regulation of energy and protein status

I. Ortigues-Marty¹, C. Obled², D. Dardevet² & I. Savary-Auzeloux¹

¹ Unité de Recherches sur les Herbivores, INRA, Theix, 63122 Saint Genès Champanelle, France

² Unité Nutrition et Métabolisme Protéique, INRA, Theix, 63122 Saint Genès Champanelle, France

Summary

This review addresses the role of the liver in the regulation of the nutrient balance in the peripheral tissues of economical interest. Examples are taken in healthy animals supplied with increasing amounts of nutrients or showing increasing nutrient requirements of their peripheral tissues as well as in pathological situations. The influence of the arterial-portal gradient of nutrient concentrations and of the neurohormonal regulations on hepatic and peripheral glucose and amino acid metabolism is discussed. This review also shows that when specific functions of the liver are stimulated (eg. immune function) or depressed (eg. leucine uptake), other hepatic functions and peripheral metabolism may be altered. Regulatory signals generated by the cytokines and probably by the oxidative stress are discussed. Vice versa, a number of situations have been identified in which peripheral metabolism impacts hepatic metabolism.

Keywords: liver, regulation, metabolism

Introduction

In productive farm animals, the energy and protein status is usually defined in terms of whole body energy and protein balance, which may be either positive with storage (growth, fattening, gestation) or export (lactation) of energy or protein, or negative with mobilisation of body energy or protein stores (undernutrition, sepsis). Peripheral tissues of economical interest (muscle, udder, uterus...) constitute targets towards which nutrients are directed. Positive whole body energy and protein status aims at getting positive nutrient balances at the level of those peripheral tissues. Inversely in case of negative balance some of those tissues, the muscle along with the adipose tissues, constitute a reserve of nutrients. The focus of this paper will be the regulation of the nutrient balance in the peripheral tissues of economical interest.

Nutrients fluxes to and from tissues are transported by blood and lymph. When considering the vascular routes followed by absorbed blood and lymph nutrients before being used by target tissues, the liver appears to be at a major metabolic crossroad. It receives all the blood draining the gastrointestinal tract which carries the absorbed hydrophilic nutrients, plus some arterial blood which carries the lipophilic nutrients absorbed via the intestinal lymph and nutrients mobilised or transformed in peripheral tissues. The liver has a key nutritional role of delivering nutrients to peripheral tissues where they are metabolised to support physiological functions (maintenance, growth, lactation, gestation ...). It synthesises large macromolecules from low molecular weight molecules absorbed through the digestive tract and appeared in the portal blood, and transforms molecules of arterial blood origin. As such, it is involved in glucose and lipid homeostasis and it is a central organ in the metabolism of amino acids. However besides these metabolic functions, the liver possesses a range of other basic functions 1) storage and filtration of blood, 2) secretion of bile into the gastrointestinal tract, 3) detoxification of endogenous metabolites or xenobiotics, and 4) secretion of most plasma proteins with a specific role as an immune organ.

Because of this large panel of functions, the liver has a high metabolic activity per unit tissue weight which is 10 to 20 fold higher than that of resting muscles (Ortigues & Doreau, 1995).

Despite the fact that it represents only 2-3% of body weight in ruminants, its energy expenditure represents approximately 25% of whole body energy expenditure in ruminants (Ortigues & Doreau, 1995) and 13% in preruminants (Ortigues *et al.*, 1995). Its high protein synthesis rate is also responsible for the fact that it contributes to 4-15% to whole body protein synthesis in ruminants (Lobley, 2003).

After a review of the anatomy of the liver for background knowledge, this paper will address a question generally asked by nutritionists of whether the liver plays a regulatory role in the metabolism of peripheral tissues. Examples will be taken from three distinct metabolic situations. First, in healthy animals supplied with increasing amounts of nutrients in order to improve the nutrient balance of peripheral tissues. Second, in healthy animals which show increasing nutrient requirements of their peripheral tissues. And third, in (sub)pathological situations where the whole organism is in negative energy and protein status. This non-exhaustive review attempts to identify new nutritional concepts developed in different metabolic areas and with different animal models, including humans, that could be useful to farm animal nutrition, and especially ruminant nutrition.

Physiological anatomy of the liver

This aspect has been thoroughly studied in laboratory animals. The basic functional unit of the liver is the liver lobule (Guyton, 1976), which is constructed around a central vein that empties into hepatic veins and hence into vena cava. The lobule itself is composed of many hepatic plates that radiate from the central vein. Each plate is two cells thick separated by bile canaliculi that empty into terminal bile ducts. In between hepatic plates lie hepatic venous sinusoids receiving blood from portal venules and hepatic arterioles. They are lined by non-parenchymal cells which are the endothelial cells (which present pores of different sizes and specific receptors, and which can then filter the blood), the Kupffer macrophage cells, the Stellate (or Ito or fat-storing) cells as well as the granular lymphocytes or pit cells. A narrow space exists between the non-parenchymal cells and the parenchymal cells (hepatocytes) called space of Disse, which is connected to the lymphatics. 72% of total liver volume is occupied by hepatocytes, 6% by non-parenchymal cells (one third of which being occupied by Kupffer cells), 15% by extracellular space and 7% by connective tissues and vessel lumina (Van Berk, 1982).

The liver receives neural input from sympathetic and parasympathetic nerves which form plexuses around the portal vein, the hepatic artery and the bile duct (Gardemann *et al.*, 1992). Innervation is associated with endothelial cells, spaces of Diss, the Kupffer cells, the fat-storing cells, hepatocytes, the different blood vessels, the gall bladder and bile ducts. It is involved in all major hepatic functions (Lautt, 1980; Friedman, 1982).

The liver architecture makes it necessary for any blood substance wanting to reach hepatocytes to first cross the endothelial lining. Substances such as plasma enzymes, endotoxins, insulin, glycoproteins, Vit A, cellular debris are first removed by non-parenchymal cells (Van Berk, 1982). The liver is thus supplied by blood which presents gradients of oxygen-, nutrient- and hormonal composition from the periportal to perivenous zones as well as different autonomic innervation which are responsible for a metabolic heterogeneity between hepatocytes and between non-parenchymal cells, and which led to the concept of the liver acinus (Jungermann & Katz, 1982; Gardemann *et al.*, 1992; Jungermann & Kietzmann, 1996).

Acini are defined based on the linear inflow of blood. They consist of three main zones : a periportal one which is supplied with oxygen-, substrate- and hormone-rich blood, a transitory zone and a perivenous zone which receives blood poor in oxygen, substrate and hormones but enriched in CO₂ and other substances (Jungermann & Kietzmann, 1996). Cells in each of these zones possess different structures, enzymatic equipment and functions. Mitochondria are predominant in the periportal zone, and with it the oxidative energy metabolism as well as the endergonic metabolic processes such as in particular gluconeogenic activity, transamination and amino acid degradation. Catabolism to acetyl-CoA, β-oxidation and ketogenesis are also

preferentially located in periportal zones (Katz, 1992). Metabolic functions in the perivenous zone deal with exergonic processes, independent of oxygen, such as the handling of excess carbohydrate with liponeogenesis, fatty acid esterification and lipoprotein formation or with the supply of glucose in case of insufficiency (glycolysis). Other processes such as glycogen formation and degradation as well as urea synthesis are distributed homogeneously throughout the liver acini but are regulated by substrate availability (gluconeogenic substrates, including amino acids, in periportal zone, and glucose and ammonia in perivenous zone) (Jungermann & Katz, 1982; Katz, 1992). Plasma protein synthesis and secretion are also randomly distributed over the liver parenchyma, forming a mosaic (Jungermann & Katz, 1982). Secretory and detoxification functions of hepatocytes are heterogeneously distributed. Uptake and synthesis of bile acids as well as secretion of bile are more active in periportal hepatocytes. Conversely rates of biotransformations (oxygenation, hydroxylation and reduction) of xenobiotics and conjugation with glucuronic acid or glutathione are higher in perivenous zone. On the other hand, conjugation with sulfuric acid takes place in periportal zones (Katz, 1992; Sastre *et al.*, 1992).

Hormones (insulin, glucagon, catecholamines, corticosteroids..) are degraded during their liver passage to different extents thereby inducing gradients of hormone concentrations and more precisely of ratios between hormones concentrations from periportal to perivenous areas which regulate hepatic metabolism (Jungermann & Kietzmann, 1996). The zonation of hormone receptors is not well known yet.

This metabolic heterogeneity is accompanied by zonal differences in the expression pattern of genes (Jungermann & Kietzmann, 1996). Noteworthy is the dynamic capacity of metabolic zonation of acini to adapt to long-lasting alterations of metabolic situations (Katz, 1992).

Although the specificities of ruminant liver would first have to be established, a number of questions arise from this metabolic heterogeneity. For example, how is insulin degradation by the endothelial cells regulated and what are the consequences on the insulin concentration gradient which may subsequently regulate some metabolic pathways within hepatocytes ?

Changes in nutrient supply to the liver, consequences on hepatic and peripheral tissue metabolism

In productive farm animals, nutrition aims at improving nutrient supply to and utilisation by peripheral tissues of economical interest. When increasing the plane of feeding or when supplementing the diet with nitrogen or energy, it is expected that the liver increases its net release of nutrients and that peripheral tissues increase their nutrient uptake to the same proportional extent. When considering tissue responses measured instantaneously or after a few days of adaptation, this is not always the case, indicating specific short or medium term regulation mechanisms.

Increased supply of glucose or glucose precursors

Hepatic metabolism and role of the portal signal

In terms of carbohydrate metabolism, the liver is considered to be the metabolic ‘glucostat’ of the organism. It regulates blood glucose levels via regulation of gluconeogenesis, glycogen synthesis, storage and mobilisation as well as via regulation of intermediate metabolite metabolism. In ruminants, glucose which is neosynthesised in the liver, is not always released by the liver proportionally to the amount of precursors taken up. In growing ruminants, the rise in net hepatic glucose release with level of feeding is definitely more pronounced below than above maintenance (Lapierre *et al.*, 2000). Above maintenance, no significant relationship was noted between the availability of propionate and net hepatic glucose release (Majdoub *et al.*, 2003) or glucose turnover (Ortigues-Marty I. *et al.*, unpublished). More striking, when looking at short term

metabolic changes, hepatic glucose metabolism remains remarkably stable pre- and postprandially (Van der Walt, 1978). Achievement of glucose homeostasis involves management of hepatic glycogen stores, which in growing ruminants can be calculated to represent approximately 15% of the daily whole body glucose flux.

The key glucostatic role of the liver has been studied with depth in monogastrics which depend for their glucose supply on dietary intake. In those species, glucose is taken up and stored as glycogen in hepatocytes during the post-prandial phase, and the liver is then mainly responsible for providing glucose to the other tissues during the post-absorptive period. Besides the role of insulin and glucagon in controlling hepatic glucose utilisation and glucose production, respectively, it has been shown that net hepatic glucose uptake in the conscious dog model was greatly enhanced in response to intra-portal glucose infusion when compared to peripheral glucose infusion (Adkins-Marshall *et al.*, 1990). This phenomenon was recorded with identical insulin, glucagon and hepatic glucose load, factors known to modify directly glucose uptake.

Based on these observations, it has been postulated that a “portal signal” occurred during the portal infusion of glucose and that this signal was initiated by a negative arterial-portal glucose gradient. A study carried out by Pagliassotti *et al.* (1991) demonstrated the relationship between the glucose arterial-portal gradient and the net hepatic glucose uptake. Interestingly, glucose uptake by the liver is enhanced by the physiological glucose arterial-portal gradient which can be reached in normal post-prandial states.

Glucose sensors have been found in the portal vein (Niijima, 1982) and respond to glucose by altering the afferent and efferent firing rate of the hepatic branch of the vagus nerve. Neurophysiological evidences are also consistent with the involvement of the acetylcholine and α adrenergic mediators in the signal elicited by the portal glucose sensors (DiCostanzo *et al.*, 2003).

When fed with a normal mixed meal, post-prandial net hepatic glucose uptake in conscious dogs was a lot less than would have been assumed based on the rise in insulin, portal glucose level and hepatic glucose load (Moore *et al.*, 1998). Despite the parallel rise in glucagon which may have reduced the net hepatic glucose uptake, it has been postulated that an interaction between macronutrients may occur when the later are delivered simultaneously into the portal vein. Indeed, more than 15 different amino acids have been shown to modify the afferent firing rate of the hepatic branch of the vagus nerve and sensors for amino acids have been identified in the hepatoportal region in the rat (Niijima *et al.*, 1995). Interestingly, depending on the amino acid, the activity of the vagus nerve differed. Alanine, arginine, histidine, leucine, lysine, serine, tryptophane, and valine increased vagal afferent discharge rate, whereas cysteine, glycine, isoleucine, methionine, phenylalanine, proline and threonine suppressed it. Furthermore, when amino acids were infused peripherally, in order to achieve the same amino acid hepatic load, the reduction of net hepatic glucose uptake was not seen (Moore *et al.*, 1999b). This was consistent with the presence of portal amino acid sensors which initiated a portal signal that modulated or competed with the signal generated by portal glucose infusion. Furthermore, as recorded with glucose, a negative arterial-portal gradient might be created to generate the portal amino acid signal; negative gradient only present during the portal infusion of amino acids.

It might be speculated that intraportal amino acid infusion, and the portal signal thus generated, not only modulated net hepatic glucose uptake but also modulated their own hepatic uptake and utilisation. This hypothesis has been tested by Moore *et al.* (1999a) and revealed that at similar amino acid hepatic load, portal infusion of amino acid enhanced the liver uptake of several amino acid infused when compared to peripheral amino acid infusion (glutamine, serine, alanine and glutamate). This study was limited to the gluconeogenic amino acids but it is tempting to generalize this observation for most of the amino acids. With a peripheral amino acid infusion, the net hepatic glucose and amino acid uptakes were very similar to the net hepatic glycogen deposition and lactate release. With the portal amino acid infusion, only 70% of their uptake could be accounted for by glycogen synthesis and lactate release. Thus, the fate of the 30% remaining

carbons remained unknown. Once again, it is very tempting to hypothesize that the portal signal, initiated by the amino acids, directed the amino acids to protein synthesis. In the last past years, it has been clearly demonstrated that some amino acids (i.e. branched chain amino acid and leucine particularly) enhanced protein synthesis by modulating intracellular signalling pathways directly linked to protein synthesis translation. Besides its role of substrate, the amino acids as “signal molecules” are becoming a real concern in the general understanding of protein metabolism regulation. Taken together, we are assuming that the hepatoportal amino acid sensors, in combination with a negative arterial-portal gradient, activate liver protein synthesis.

In ruminants, these homeostatic regulatory mechanisms and their time sequence may partly differ due to a greater relative stability of nutrient absorption over time, but would be worth investigating.

Interactions between liver and peripheral tissues

Our results obtained in growing ruminants with simultaneous measurements of net nutrient fluxes across the splanchnic tissues and the hindlimb showed some uncoupling between hepatic release of glucose and utilisation of glucose by peripheral tissues. When the supply of glucose precursors (propionate) was increased by supplementation, the partition of glucose utilisation among peripheral tissues seemed to be altered for the benefit of musculature despite unchanged net glucose release by the liver (Majdoub *et al.*, 2003). The signal responsible for this repartitioning was not identified. These effects could only be associated with large arterial-portal concentration differences in propionate as well as drastic increases in insulin secretion and hepatic removal.

The impact of nutrient supply on the responsiveness of tissues to hormones and the role of variations in arterial-portal concentration differences in the regulation of nutrient utilisation by peripheral tissues are starting to be explored in ruminants. They involve the neuro-hormonal regulations of metabolism. For example, half of the propionate-induced rise in insulin release in sheep is mediated via the parasympathetic nervous system (Sano *et al.*, 1993), which in laboratory animals seems to modulate muscle responsiveness to insulin (Xie & Lautt, 1996). However in ruminants, the influence of propionate on tissue sensitivity to insulin as assessed by glucose clamp techniques is not clear. Indeed, whole body-tissue sensitivity to insulin was greater for high-concentrate diets than for high-forage diets (Sano *et al.*, 1992), but it was reduced by supplementary propionate (Sano & Terashima, 1998).

Research on the neuro-hormonal regulations of the partition of nutrient utilisation among tissues is more advanced in laboratory animal models. The effect of the portal signal being established on the liver, it was questioned whether it could also affect other tissues. Galassetti *et al.* (1998) performed a study in dog in which the non hepatic glucose uptake and hindlimb glucose uptake were measured in the presence or absence of negative arterial-portal glucose gradient. In order to equalise glucose delivery to peripheral tissues whatever the glucose infusion route, a pancreatic clamp was performed to maintain insulin, glucagon and arterial glucose concentrations at fixed levels. This study revealed that the stimulation of the portal signal decreased by 40% glucose uptake by peripheral tissues. A similar inhibition was recorded by directly assessing muscle glucose uptake, demonstrating that muscle was the main site of this inhibition. As previously discussed, the stimulation of the portal signal increased hepatic glucose uptake and by consequence could decrease the availability of glucose for peripheral tissues. It was not the case in this study since arterial glucose concentrations were maintained constant and were not limiting for peripheral tissues. Interestingly, the portal signal decreased nonhepatic glucose uptake by an amount almost identical to the amount by which it increased net hepatic glucose uptake. Thus, it appears that the portal signal does not increase whole glucose clearance but directs glucose to the liver and away from the muscle. If amino acids are also involved the portal signal, it might be postulated that the same phenomenon occurs in the distribution of the amino acids during the post-prandial state.

Increased supply of amino acids

Net hepatic amino acid release, endogenous and exported protein metabolism

The liver has important functions in protein metabolism including replacement of structural hepatic proteins, deamination and interconversions among amino acids, ammonia detoxification and ureogenesis (connected to the regulation of blood pH), synthesis of active peptides, formation of all plasma proteins (mainly albumin) except gamma globulins.

Plasma proteins synthesised by the liver have various functions, which are recalled here. First, binding and transport of metals and biological active compounds (haptoglobin, hemopexin, $\alpha 1$ glycoprotein acid or albumin). Albumin is the major protein synthesized and secreted by the liver into blood. It is involved in the transport of numerous molecules and is important to maintain the oncotic pressure. Its synthesis amounts to 18 % of total liver protein synthesis in the sheep (Connell *et al.*, 1997). Its synthesis contributes for about 30-50% to the synthesis of total exported proteins in well-fed animals and humans (Von Allmen *et al.*, 1990; Lewandowski *et al.*, 1988). Second, a role in coagulation, fibrinolysis and tissue repair (fibrinogen, C-reactive protein or C3 complement). Synthesis of fibrinogen contributes only by 6.7 % to synthesis of exported proteins in humans (Obled C., unpublished). Third, functions of inhibition of proteinases released from phagocytic cells and thereby protection of the integrity of the host ($\alpha 1$ -antitrypsine, $\alpha 1$ -antichymotrypsine or $\alpha 2$ -macroglobulin). And finally, a role as modulator of the immune response either for its activation or its inhibition (C-reactive protein, fibrinogen, haptoglobin or $\alpha 1$ glycoprotein acid).

Among the active peptides synthesised by the liver, special notice should be given to glutathione. The liver is the main site of glutathione synthesis and export in the plasma and bile (Deneke & Fanburg 1989). The amount of glutathione excreted in the bile, 3-10 nmol/min.g of liver, is equivalent to 10-50% of its rate of hepatic synthesis in normal conditions in the rat (Yang & Hill, 2001; Pastor *et al.*, 2000; Hunter & Grindle, 1997).

Because of those various functions, relationships between amino acid supply to the liver and net hepatic release of amino acid are not straightforward. In some cases, for example when the intake level was increased by supplementation with both nitrogen and energy in ruminants (Savary-Auzeloux *et al.*, 2003b; Lapierre *et al.*, 2000; Tanigushi *et al.*, 1995; Huntington *et al.*, 1996), the net hepatic release of amino acids increased. No effect of the supplementation could generally be demonstrated on the net hepatic amino acid uptake (except with relatively high intake levels (Reynolds *et al.*, 1991)). Conversely, when nitrogen was supplemented alone in the form of casein, rumen undegradable protein (Guerino *et al.*, 1991; Bruckental *et al.*, 1997; Ferrell *et al.*, 2001), soyabean meal (Krehbiel *et al.*, 1998; Ferrell *et al.*, 2001) or of amino acids infused intramesenterally (Lobley *et al.*, 2001), the increased amino acid supply to the liver induced a rise in the net hepatic uptake of amino acids (from +30% to more than twice). This was also the case when energy was supplemented alone as cornstarch and dried molasses or intraruminal propionate infusion (Ferrell *et al.*, 1999; Savary-Auzeloux *et al.*, 2003a). In all those latter situations, the net hepatic release of amino acids was not or only marginally improved by supplementation.

Two mechanisms are usually considered to be responsible for those hepatic responses. One is dealing with peptide fluxes which have been little studied but which may be partly responsible for unaccounted amino acids. The other one deals the regulation of endogenous and exported protein metabolism. As for endogenous proteins, the protein weight in the liver varies rapidly when the intake is altered. In ruminants, the endogenous protein mass depends mainly on changes in protein degradation since fractional synthesis rate in the liver is not altered by intake (Lapierre *et al.*, 1999; Adams *et al.*, 2000).

As far as the exported proteins are concerned, their regulation is different from that of endogenous proteins. For instance, 3 day fasting strongly reduces the fractional synthesis (-28%) and the total amount (-22%) of exported albumin in ruminants (Connell *et al.*, 1997). Differences exist however

among specific proteins. As for albumin, its synthesis is increased by 30% in the post prandial state by comparison to the post absorptive state in humans (Hunter *et al.*, 1995). Albumin synthesis decreased by 50% in rats (Pain *et al.*, 1978) or in humans (Cayol *et al.*, 1996) fed protein deficient diets. Similar results are obtained after food restriction (Ruot *et al.*, 2002). By contrast the synthesis of fibrinogen, for example, was unaffected (Cayol *et al.*, 1996) or even increased (Jackson *et al.*, 2001) by protein restricted diets. It may be questioned whether responses of exported proteins may depend on their function, with proteins involved in the transport of nutrients being responsive to dietary changes at the opposite of proteins involved in non-nutritional functions.

Interactions between liver and peripheral tissues

Increased absorption of amino acids is usually associated with anabolism, especially in muscles. There are situations in rodents (Mosoni, 1995) and humans (Arnal, 1999) where postprandial protein anabolism is defective, such as during ageing. This defect has been proposed to be one of the mechanisms responsible for the loss of muscle mass during ageing. Amino acids play an important role in regulating muscle protein synthesis (May & Buse, 1989) and it has been assumed that during ageing, the availability of amino acids is affected. Boirie *et al.* (1997) showed in humans that the first-pass splanchnic uptake of leucine increases with age and may limit the availability of amino acids to the peripheral tissues. Volpi *et al.* (1999) confirmed this observation while showing that the delivery of phenylalanine to the tissues increased to the same extent in adult and elderly humans. Postprandial stimulation of muscle protein synthesis in rats originated mainly from absorbed amino acids because this stimulation was observed after feeding a high protein meal but not after an isoenergetic protein-free meal (Yoshizawa, 1998). Similar observations were made in humans (Bennet, 1989; Fryburg, 1995). Among the amino acids, leucine seems to play the major role. In one of our studies (Dardevet *et al.*, 2002), leucine supplementation had no additional effect on muscle protein synthesis in adults but totally restored its stimulation in old rats, and this occurred in both muscles studied, the soleus and gastrocnemius. Only leucine concentrations in plasma reached supraphysiological levels in both age groups (twice the control postprandial values) and confirmed that old rat muscles are less sensitive to the "leucine signal" than adults but are still able to respond when the concentrations of this amino acid are greatly increased.

If the decrease of muscle protein synthesis sensitivity to leucine is well demonstrated, it cannot be ruled out that the increase in the splanchnic extraction of amino acids or of selected amino acids may also play a role in the decrease of post-prandial muscle anabolism during ageing. Indeed, in conditions of normal feeding, the requirement in amino acids for the splanchnic area may lower the delivery of some amino acids to the peripheral tissues. This hypothesis has been verified by Papet *et al.* (2003) who measured albumin and plasma protein synthesis rates between adult and old rats. The absolute synthesis rate of albumin and other plasma proteins were 49 and 40% higher in old than in adult rats, respectively. The age-related increase in plasma protein absolute synthesis rate could contribute to the increased utilisation of dietary proteins by the splanchnic area and consequently to a potential decrease in the availability of amino acids to peripheral tissues in the old. In this physiological state (i.e ageing), it appears clearly that the decrease in post-prandial muscle anabolism results from combined effects of a decreased sensitivity of muscle protein synthesis to leucine but also of higher hepatic amino acid uptake. As recorded for the glucose, the liver plays an essential role in amino acid distribution and availability to muscle and may thus indirectly alter peripheral metabolism.

Increased nutrient requirements of peripheral tissues, consequences on hepatic metabolism

Increased glucose requirements

In ruminants, there is evidence that glucose requirements of peripheral tissues can modulate the net hepatic release of glucose at fixed intake. In lactating or pregnant ruminants, glucose turnover (supplied for 85-90% by hepatic glucose synthesis) is clearly affected by changes in glucose requirements (Bergman & Hogue, 1967; Chaiyabutr *et al.*, 1998). Beenink *et al.* (1972) reported very demonstrative results in dairy cows maintained on constant dry matter intake from 45 d pre- to 40 d postpartum: glucose entry rate increased in support of lactation.

In adult sheep, glucose turnover was better correlated with energy balance than with intake level, suggesting that the nutritional status of the animal had at least as much influence as the supply of glucose precursors (McNiven, 1984). Since muscles are important net utilisers of glucose (Hocquette *et al.*, 1998), indirect evidence can be found that any modifications in the stage of growth or in muscle growth may influence whole body glucose turnover (Ortigues-Marty I., unpublished results). Clear demonstration has been obtained when sheep were subjected to physical exercise (Pethick & Johnson, 1996)

Experimentally glucose requirements may be increased by creating an artificial drain for glucose, such as with a phlorizin treatment which induces urinary glucose excretion. In sheep (Overton *et al.*, 1999) and steers (Veenhuizen *et al.*, 1988) injection of phlorizin significantly increased glucose turnover rate at fixed intakes. This was accompanied by an increased efficiency of precursor use as generally noted when glucose requirements increase (Herbein *et al.*, 1978; Overton, 1998) and a reduction in glucose oxidation. Interestingly, both the effects of phlorizin injection and of precursor (propionate) supplementation on glucose turnover were additive in steers fed slightly above maintenance (Veenhuizen *et al.*, 1988). The adaptation mechanisms at the hepatic level probably involve a more efficient utilisation of glucose precursors (alanine, propionate) considering the general surplus in neoglucogenic precursor supply at limited intake (Armentano, 1992), and when the dietary supply of precursors is insufficient a greater contribution of amino acids mobilised from skeletal muscles (Overton *et al.*, 1999; Bell *et al.*, 2000).

Increased amino acid requirements

When amino acid requirements of peripheral tissues are increased by growth hormone or somatotropin treatment (Reynolds *et al.*, 1992; Bruckental *et al.*, 1997), the proportion of portal appearing amino acids that are taken up by the liver is not modified, similarly to what is observed when energy and protein supply are increased altogether (Lapierre *et al.*, 2000; Savary-Auzeloux *et al.*, 2003b). Additionally, when amino acid requirements of peripheral tissues are high, such as in growing or lactating animals, the more amino acids reaching the portal vein, the less urea being synthesized by the liver and the more amino acid being released by the splanchnic tissues (Lapierre *et al.*, 2000). This allows more protein to be synthesized in peripheral tissues, such as in muscle (Lapierre *et al.*, 1999) or in the udder (Lapierre *et al.*, 2002). Thus hepatic amino acid metabolism and especially removal of amino acid surplus appears to be modulated by amino acid requirements in peripheral tissues as discussed by Lobleby (2003).

Injury

An interesting situation, little studied in farm animal metabolism, is that of injury. Some generalities will first be reviewed before focusing on hepatic metabolism and liver-muscle interactions. Most of the work reported here has been conducted on humans or laboratory animal models.

Whole body metabolic responses

During injury, whatever the type of insult, the organism defends itself thanks to a non specific acute phase response which involves the activation of inflammatory cells. If this activation is well controlled, it leads to the clearance of the initial insult and recovery by appropriately down regulating the response mechanisms and stopping the process. However the balance between the inflammatory process and the down regulation response may be inappropriate leading to either chronic diseases or poor outcome (Hill & Hill, 1998).

Disease and injury are frequently accompanied by anorexia which leads to the mobilization of endogenous stores. They are also characterized by an hypermetabolic state, increased energy expenditure and increased utilisation of carbohydrate and lipid. One of the most dramatic effects of injury is protein loss. Injury results in an overall increase of protein metabolism where the increased whole body protein breakdown (25-127%) predominates over the increased whole body protein synthesis (16-47%) leading to a negative whole body protein balance as shown in inflammatory states or critical illness (Cayol *et al.*, 1995; Arnold *et al.*, 1993). In critically ill patients, up to 20% of body proteins is lost in 3 weeks, most of it during the first 10 days following injury, and about 70% of this protein loss comes from skeletal muscle (Plank & Hill, 2000).

Whatever the nature of the initial insult, a similar pattern of responses, globally known as the acute phase response, is noted. They will be presented as a clear sequence but in reality these processes act more or less simultaneously. The very early events take place at the vascular level with the interaction between endothelial cells and circulating white blood cells, and with the activation of these circulating cells, and in all tissues with the activation of the macrophages. Activation of inflammatory cells (macrophages, lymphocytes...) releases a cascade of mediators such as free radicals or cytokines which modulate the cellular metabolism.

The impact of free radicals on cellular metabolism is increasingly studied but the mechanisms involved in tissue peroxidation and the consequences on health, growth and breeding of farm animals remains to be more precisely investigated (Aurousseau, 2002). In humans peroxidation damages of proteins, nucleic acids and lipids seem to be involved in numerous diseases. In the case of the proteins, it has been hypothesised that peroxidation leads to alterations of their functional properties (Levine, 2002). Accumulation of oxidised proteins depends on subtle interactions between oxidative agents, presence of antioxidants and presence of other oxidised molecules (e.g. lipids). It also depends on the capacity of the cells to degrade them. In this respect, the inefficiency of the proteasome and of the lysosomes to degrade the oxidised proteins is now clear (Lee & Wei, 2001). Free radicals are also of major importance in the immune response. For instance, the liberation of reactive oxygen species leads to the activation of transcriptional factors such as Nuclear Factor kappa B (NFkB) which allow the transcription of many genes such as those of cytokines or acute phase proteins (Bellingan, 1999).

Among the other mediators involved in the acute phase response, cytokines play a very important role (Vilcek 1998). They are regulatory proteins, classically classified as pro- and anti-inflammatory cytokines even if this classification is too simplistic. For example cytokine interleukine-6 (IL-6) is pro-inflammatory since it stimulates numerous activities of the immune cells but it is also implicated in the production of acute phase proteins that limit the inflammatory process. In addition, IL-6 inhibits cytokines Tumor Necrosis Factor (TNF) and interleukine-1 (IL-1) production, thereby showing anti-inflammatory properties. Cytokines are produced by circulating white blood cells and tissue macrophages. They have mainly an autocrine and paracrine mode of action. The most characteristic features of cytokines are the redundancy and pleiotropy of their actions. For example both TNF and IL-1 are involved in anorexia and fever. TNF is able to increase the release of free fatty acids by adipocytes and to induce IL-1 and IL-6 production by monocytes and macrophages. This last point illustrates also that a cytokine may increase the production of another cytokine. Their actions can be modified by the presence of other active

agents such as other cytokines as well as hormones. For example glucocorticoids amplify the action of IL-6 on acute phase protein production (Marinkovic *et al.*, 1989).

Hepatic metabolism

The liver contains the largest number of macrophages in the body (Szabo *et al.*, 2002). Liver macrophages are the Kupffer cells. When increased quantities of foreign material, bacteria or endotoxins are present in the blood, the number of Kupffer cells in the sinusoids increases markedly. Kupffer cells synthesise pro-inflammatory cytokines upon activation due to phagocytosis or binding of compounds such as endotoxin. Other cells, such as the hepatic stellate cells can also produce cytokines, eg. Transforming Growth Factor beta, a growth factor which acts also as a potent immunomodulator. Released cytokines may stimulate hepatocytes and other non-parenchymal cells. Therefore, the liver is an important organ in the acute phase response due to the fact that it is a prominent source and target of cytokines.

In the perfused or *in vivo* liver submitted to an inflammatory challenge, protein synthesis is increased (Klasing & Austic, 1984, Breuillé *et al.*, 1998). The contribution of the liver to whole body protein synthesis more than doubled after infection (Breuillé *et al.*, 1994). This increase involved both non-exported and exported proteins with a greater stimulation of the synthesis of the exported proteins involved in the acute phase reaction (Vary & Kimball, 1992, Jahoor *et al.*, 1999). As for non-exported proteins, their synthesis is increased probably to sustain the increased enzymic activity in the liver and to compensate for the alteration in the functional properties of enzymes following peroxidative attacks from free radicals (e.g. glutamine synthetase; Levine, 2002). In those conditions, liver weight is maintained, contrary to expectations, despite reduced feed intake as shown in rats (Breuillé *et al.*, 1998).

As for exported proteins (e.g. fibrinogen, a1-glycoprotein and a2-macroglobulin), the increase in their synthesis is directly related to the anti-inflammatory response. The contribution of total plasma protein to whole body protein synthesis increased from 7.2 % in healthy volunteers to 15.6 % in polytrauma patients (Breuillé D, Obled C, Mansoor O, personal communication). It should be noted that the change in synthesis is usually far greater than the change in the plasma concentrations of those proteins, which is for example of the order of 140% and 28% respectively for fibrinogen in piglets after turpentine injection (Jahoor *et al.*, 1999). Moreover, the synthesis of albumin is enhanced in stress conditions while its plasma concentrations fall (Mansoor *et al.*, 1997). The magnitude of those responses depends on the level of intake, the stimulation of protein synthesis induced by lipopolysaccharide injection was lower in starved as compared to well-fed animals (Jepson, 1986). Another exported protein of importance is glutathione as it is the major intracellular antioxidant and it is synthesised in the liver for protection against the oxidative stress associated with sepsis (Malmezat *et al.*, 2000b). Glutathione is synthesised from cysteine which is itself formed in the liver mainly from methionine. In injury hepatic synthesis of cysteine from methionine is increased (Yu *et al.*, 1993; Malmezat *et al.*, 2000a) and cysteine catabolism is reduced (Malmezat *et al.*, 1998).

Increased synthesis of exported proteins implies increased liver requirements for specific amino acids. To cover those requirements, greater amounts of all amino acids are taken up, this leads to some wastage, some amino acids being then directed towards oxidation. Additionally, greater amounts of amino acids are used for hepatic gluconeogenesis. This results in a concomitant increase in urea synthesis, to remove the extra amino acid nitrogen extracted by the liver. In septic or burn patients, the rate of urea production is more than doubled (Shaw *et al.*, 1985; Yu *et al.*, 1995). Similar data were reported for liver urea production after surgery in pigs (Deutz *et al.*, 1992).

Besides the regulatory roles played by cytokines, catabolic hormones cortisol, glucagon and epinephrine are also involved, although regulatory mechanisms are complex and not clearly known yet. Glucocorticoids are probably the most important for the regulation of liver metabolism during

injury. However, alone or with the other stress hormones, they do not reproduce all the effects of injury since liver protein synthesis is increased but not that of secreted proteins (Pedersen *et al.*, 1989). Glucocorticoids alone are able to induce the secretion of some proteins such as α 1 glycoprotein acid but generally they act as a permissive or synergistic factor to the action of cytokines (Marinkovic *et al.*, 1989).

Interactions between liver and peripheral tissues

The net catabolism of muscle proteins mentioned earlier is the result of both decreased protein synthesis and sustained increased protein breakdown. Therefore protein metabolism is modified totally differently in muscles and in numerous organs, especially in the liver (Breuillé *et al.*, 1998). The net catabolism of muscle proteins allows to increase the supply of amino acids to visceral organs and immune cells. These amino acids are used for processes which are activated as part of the body's defence mechanisms such as specific liver protein synthesis, and for energy supply. The balance of amino acids needed for the synthesis of compounds and proteins involved in host defence may be different from that provided by muscle proteolysis, leading to an excessive mobilization of muscle proteins until the appropriate amount of the most limiting amino acid is reached (Reeds *et al.*, 1994). For example, increased hepatic cysteine requirements for glutathione synthesis implies increased methionine mobilisation from muscles in case of insufficient absorption. Similarly, Preston *et al.* (1998) estimated that the synthesis of 1g fibrinogen would require the degradation of 2.6 g muscle protein. Subsequently, other amino acids issued from muscle mobilisation which are provided in excess to the liver, have to be catabolised.

Signals for this muscle wasting to the benefit of hepatic synthesis of exported proteins involve cytokines which are produced by activated Kupffer cells and released into the blood such as those produced in other tissues. Cytokines are likely to induce perturbations in all tissues and particularly in muscle. Pro-inflammatory cytokines and particularly TNF have been shown to be involved in muscle wasting. Indeed inhibition of TNF production by administration of pentoxifyllin prior to infection in rats suppressed the increase in plasma TNF concentration but also decreased the production of IL-1 and to a lesser extent of IL-6. This prevented the loss of muscle mass and accelerated the recovery mainly because of a rapid normalisation of proteolysis (Breuillé *et al.*, 1999). However the precise role of each cytokine and their mechanisms of action are complex and not elucidated yet. For example, the ability of insulin to limit proteolysis is blunted 2 days after infection but pentoxifyllin administration maintained the responsiveness of protein degradation to insulin (Vary *et al.*, 1996). Moreover, glucocorticoids are also involved in muscle wasting since their inhibition inhibits the increased protein breakdown (Hall Angeras *et al.*, 1991).

Conclusions

The selected examples illustrate the fact that the liver plays a regulatory role in the metabolism of peripheral tissues. This role stems both from modifications in the magnitude of specific hepatic functions and from a range of neurohormonal regulatory mechanisms. Free nutrients released in the blood by the liver give only a partial picture of overall nutrient fluxes. In this respect the ability of the liver to partially and temporarily "store" nutrients either in its own tissues (protein, glycogen) or as circulating large macromolecules (eg. peptides, proteins) contributes to the regulation of peripheral metabolism. Interactions seem to exist between the different hepatic functions and it would be worth measuring the partition of hepatic nutrient use between those functions, in particular biliary secretion and detoxification not developed here besides those associated with immunity and plasma protein synthesis. The impact of the portal signal both on hepatic and peripheral glucose and amino acid metabolism appears to be a promising research area, similarly to the impact of oxidative stress and of cytokine regulations. The time sequence of those adaptation mechanisms would also be worth investigating. Vice versa modifications of

peripheral metabolism do influence hepatic metabolism probably via intricate regulations that would be worth exploring.

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A perspective on research and future of metabolic models of farm animals

John P. McNamara

Department of Animal Sciences, Washington State University, PO Box 646351, Pullman WA 99164-6351

Summary

Models are representations of reality. The fields of nutrition, metabolism and biomedicine have used models to aid in research and its' application since before World War II. A model may also be defined as an ordered way of describing knowledge of some 'real' system. Such models have been useful in ordering our knowledge into practical systems to describe nutrient requirements for agricultural animals. Our ability to describe metabolic transactions, and their resultant effect on nutrient requirements is critical to continued ability to raise food-producing animals in efficient ways around the globe. Models of increasing complexity, ever grounded in validated research data, will continue to improve our true knowledge. Only a model that can represent reality as closely as realistically possible will allow the level of complexity we need to truly answer these questions, to answer them in a way that respects the true complexity of the organisms that we are dealing with. The only way to eventually define reality is to have an ordered model approach, which slowly but surely, in a planned iterative fashion, asks complex questions and increases our knowledge with the clear answers we receive. A mechanistic, dynamic model of metabolism exists in the dairy cow (Molly, Baldwin 1995) and allows testing of complex hypotheses on the nutrition of the dairy cow. A major area that we still lack full understanding of is the metabolism in early lactation. A series of model challenges has determined that descriptions of basic processes (such as ion pumping, protein and fat turnover rates, increased metabolic costs associated with increased intake) in this model are inadequate. Milk production can be described very well from feed inputs. However, error in the biochemical transactions of viscera and muscle tissue result in excess energy accumulation in the adipose tissue. A hypothesis, based on the validated experimental evidence explicit in the model and on observations made since construction of the model, is that simulated rates of energy use in the viscera, due to the hormonal and nutrient intake changes that take place in early lactation, are too low in the model. A corollary hypothesis is that rates of energy use by the body, especially in protein turnover and associated metabolic costs is also too low in the model. Simulations increasing these energetic costs in the model resulted in realistic reductions in body fat compared to observed experimental data. These hypotheses become the framework for continued experimentation and incorporation of new information into the model. Work in all agricultural animals can benefit from this approach.

Keywords: lactation, mechanistic model, body fat, energy use, dairy cows

Introduction

Models have been useful in ordering our knowledge into practical systems to describe nutrient requirements for agricultural animals. It would not be far-fetched to say that almost all research into nutrition of farm animals since the 1900's began was used, directly or 'by default', to build, evaluate and improve models of nutrient requirements. It would also not be far-fetched to say that billions of US dollars, represented as conservation of resources and decreased costs of raising feed for livestock and of the labor for feeding of livestock, has been saved by application of these nutritional models.

Research and development of models of nutrient metabolism are no less important today than they have ever been. Our ability to describe metabolic transactions, and their resultant affect on nutrient requirements is critical to continue ability to raise food-producing animals in efficient ways around the globe. As our knowledge of nutrient metabolism has increased, several have taken the philosophy that it is in understanding the detail that we will improve our application of our knowledge, not in some noble attempt at ‘simplicity’ or ‘elegance’. Simple and elegant empirical systems have been adequate to date and will continue to have great utility. However as our knowledge of the true variation in genetic and environmental situations continues to increase, this author agrees with the philosophy that it is only through continuing to develop models of increasing complexity, ever grounded in validated research data, that we will continue to improve our true knowledge, wisdom and their application to feeding the world.

A modeling approach has and can continue to help: using model systems based on real-world knowledge, we can ask questions of the order: “What would happen if we increased protein solubility in the rumen by 10 % units on this diet? or: “What would the effect of changes in glucose concentration and progesterone and blood urea nitrogen have on the fertility and pregnancy rate of cattle?” We already do such calculations, or if we are sophisticated we use a spreadsheet. Yet only a model that can represent reality as closely as realistically possible will allow the level of complexity we need to truly answer these questions, to answer them in a way that respects the true complexity of the organisms that we are dealing with. Another point of view would be questions of the sort: what precision would we like to achieve, at what cost, and what are the practical limits to achieving that level of precision? Is it ‘good enough’ to understand and thus predict in a simple situation, how much ‘net energy’ and ‘crude protein’ are required to make a kilogram of milk? Or would we rather be able to describe the true biochemical interactions in all the organs of the cow that lead to that amount of net energy and crude protein for that milk, and in fact to be able to describe this for any cow in any situation? I personally know that we can do one ‘pretty well’ now, but also think we should continue to strive for the latter.

The only way to eventually define reality is to have an ordered model approach, which slowly but surely, in a planned iterative fashion, asks complex questions and increases our knowledge with the clear answers we receive. Thus, this type of approach clarifies and goes beyond the argument often made in what some refer to as ‘pure, hypothesis driven’ research, that is, “What is the primary cause of....? Or “What is the driving force for?” If we ‘change our attitude’ for just awhile, the answer hopefully becomes obvious when applied to the narrow field of biology that we study. There is one basic ultimate cause: the genome of the animal existing. There is one basic factor affecting the expression of the genome and that is the environment in which the ‘genome’ finds itself. Some of you may be thinking about now in the absurd obviousness of such a statement. But if we reflect for a moment, we may be able to break out of the ‘hypothesis driven’ rut, and realize that if we accept this level of simplicity, then it is straightforward to make objectives to study the detail! It is in defining the detail that we must excel, and research must continue to have the goal of defining the detail in the full context of the system under study.

One final set of reasons to continue to expand research in models has already been given by one much more capable than myself, so I will close the introduction with a list of reasons for using research models quoted directly from Chapter 1 of Modeling Ruminant Digestion and Metabolism (Baldwin, 1995):

Objectives in Research Modeling

- a. Integration of existing concepts and data in a format compatible with quantitative and dynamic analyses
- b. Reduction of conceptual difficulties in analyses of interactions among elements of complex systems
- c. Evaluation of concepts and data for adequacy in both the quantitative and dynamic domains
- d. Evaluation of alternative hypotheses for probably adequacy when current concepts are found to be inadequate, and identification of critical experiments and measurements

e. Estimation of parameter values not directly measurable and interpretation of new data.

There is no reason why animal agriculture should not be as integrated, quantitative and precise as physical and chemical sciences.

The example of lactation

One major challenge is in the description of nutrient use in dairy cattle. This is the example we will use in this paper, but there are also several models of lactation in the sow (McNamara and Boyd, 1999; McNamara and Pettigrew, 2002a, b; Pettigrew et al., 1992 a and b, and growth of several agricultural species (Black, et al., 1986; Oltjen, et al., 2000), in addition to the models of the US National Research Council (NRC, 2001). Late pregnancy and early lactation, recently called the “transition period” in dairy cattle, is a time of metabolic stress met with a coordinated response from the hormonal and neural systems of the cow. In order to manage the changes in ‘flux’ [or nutrient flow through the animal] there is a complex and redundant system of control factors, better known as hormones and neurotransmitters (McNamara, 1994; McNamara and Boyd, 1998). These systems are inextricably linked, and although we must use a reductionistic approach to identify key elements of each subsystem, that knowledge gained is useless unless coordinated in a fashion respectful of the total living organism. There are many examples of effects one can elicit *in vitro* which are completely opposite or at least never able to be measured *in vivo*. Technological limits aside, we must recognize that the living animal, the complete system, is the one true ‘reality’ and all research must continually be weighted against our best estimates of this reality.

In late pregnancy, the cow is faced with a 10 to 25 % increase in demand for glucose, fatty acids, amino acids and minerals, increasing over the course of 4 to 12 weeks, or a rate of change from 0.1 to 1 % units per day. At parturition, demand for all nutrients doubles within a few days and within a few weeks can be 3 to 5 times as high as in mid gestation. For a cow reaching 40 kg (88 lbs) of milk in 60 days after calving, pretty much an average cow presently, this is a rate of change in nutrient requirements of about 8.6 % per day! Not only is this a major challenge to the cow, it has proven to be an even greater challenge to scientists endeavoring to describe the system!

The daily changes in rates of metabolism to prepare for (in pregnancy), initiate (in early lactation) and maintain milk production over several months of times still provides a major challenge for dairy producers. It is a strong endorsement of the modeling approach that we do as good of a job as we already do. But if we are going to truly have a dynamic model system (see below), what will the error of our measurements need to be? Also, how will we need to design experiments in order to determine the ideal bio-mathematical equation forms and their parameters? If we continue to do short-term experiments with a measurement of error of 5 % points and then attempt to apply these results to a situation over much longer periods of time (6 months, 10 months?), how precise can we expect our estimates to be? It has been a saving grace that we can measure milk composition to with impressive precision (certainly less than 5 % measurement error, perhaps only 1 %). The error of some measures of feed composition may be as low as 1 %, some as high as 5 or 7 %. Yet over 300 days, what would be the net effect of a 1 % error in precision? A change of 10 % (in requirements) over 28 days is 0.36 % per day. If a 1000 lb beef steer is gaining 2 lbs per day, that is a change of 56 lbs, or 5.6 % in 28 days, or 0.20 % per day. Are any of our measurements this precise?

Now for those of you that have hung in here this far, you should be thinking, that is all well and good, but all we have to do is make measurements over several days (weeks, months) and then we can create equations with good precision. If we have several measurements, then the daily change is not that important. I agree, if our goal is to feed a pen of 300 beef steers to the average need for a given month or two, or average body weight, or average rate of gain. Similarly, if our goal is to feed a ‘fresh pen’ to the average for the first month of lactation (milk production, protein requirements, etc.) then that is fine. We already do that well. But if we truly want to understand the

real system, which is an animal with tremendous variation in rates of metabolism over short periods of time, then we must continue to strive for more detail at the biochemical level and more precision of measurements over shorter as well as longer periods of time.

During early lactation, the primary challenges to the cow include a severe shortage of glucose, amino acids, and major minerals such as calcium, phosphorous, sodium, chloride and potassium. The primary practical problems are allowing a very rapid increase in intake of a ration properly supplied and balanced with all nutrients. In addition, in the cow, is the troublesome problem of balancing the physical form of the diet to ensure sufficient intake, to discourage excess intake of energy and to supply the rumen ecosystem with inputs in the proper physico-chemical form to ensure the proper amount and balance of microorganisms. No Problem!

Actually, dairy nutritionists, veterinarians and managers have done an outstanding job in preparing cows for the demands of lactation. Our continued worldwide increase in dairy cattle efficiency with a large reduction in peri-parturient diseases is proof of that. With what we already understand we can do a good job of ensuring the health and welfare of the animal while allowing large quantities of milk production. Further improvement will happen as we understand in more detail the metabolic regulation in the animal during this time and the complex interactions between the physical and chemical form of the ration and the rumen microbial ecosystem. The challenge of proper nutrition must be met by continued increases in our quantitative understanding of the chemistry and utilization of a variety of plant cellulose, hemicellulose, lignin, other complex polysaccharides, starch and organic acids. It will require continued research into all aspects of amino acid utilization, from physical breakdown of feed in the rumen, to transport of amino acids across cell membranes, and utilization of amino acids for cellular metabolism, growth and regeneration in all organs of the body. Our focus should be primarily on those organs about which we still understand the least: those of the digestive tract and metabolism (liver primarily) and the body muscle. At present our ability to predict more precisely is most limited by our inadequate knowledge of what happens in the gut tissues, liver and body muscle during lactation, especially of how changes in metabolism in these organs change maintenance requirements (Baldwin, 1995; McNamara and Baldwin, 2000; Reynolds et al., 2003). Most importantly, continued improvement in nutrition will happen as we recognize that the use of any one nutrient is intrinsically linked with nutrition and metabolism of all compounds (why do you think we started feeding totally mixed rations?).

In early lactation, as the mammary gland demands 5, 10, 20 times more glucose than the brain uses in a day, there are definite, coordinated, homeostatic and homeorhetic endocrine and neural systems that are activated, de-activated, attenuated and/or enhanced to ensure that glucose supply to neural tissues remains adequate. We see the effects of these signals in rates of voluntary feed intake, in rates of lipogenesis and lipolysis in the adipose tissues, in proteolysis, protein synthesis and amino acid interconversions in muscle and liver, and in the increase in supply of glucose to the mammary gland.

As we understand the metabolic, hormonal and neural regulation that controls the use and interconversions of glucose, acetate, propionate, butyrate, amino acids and fatty acids we will improve our practical management. Even though our eventual focus should always be on the practical on-farm situation, we must remember our primary goal is to provide a quality, nutritious milk while ensuring the health and welfare of the cow, minimizing our use of natural resources and providing a reasonable profit to the producer. In order to meet this goal, we cannot ignore, in fact we need to focus more strongly on, the endocrine and neural regulation of gluconeogenesis, lipolysis and lipogenesis, amino acid interconversions and of feed intake. An excellent way to do this is in the continued development, testing, evaluation, and challenging with real data, of dynamic, mechanistic, metabolic models of metabolism in dairy cows.

Brief description of dynamic, mechanistic, metabolic models

Models have been in use for several decades to help engineers, physicians and scientists store massive amounts of information and describe structures, systems and processes, which, without the storage and calculation power of computers, is practically impossible. One could argue that nutrition as a science cannot progress on many fronts without computing capabilities and practical applications on the farm would be severely limited without the use of computers. Computers, numbers and math will not replace ‘experience and common sense’; they provide experience and sensible farmers serious tools with which to improve their lot and that of others.

Dairy nutritional models and modeling have been described in detail in many publications, major ones of which are listed in the readings sections. A model should have a clear objective, such as: “To help dairy nutritionists formulate and design effective and practical rations for lactating dairy cattle”; or “The model prepared in this publication was designed to provide practical, situation-specific information in a user-friendly format” (NRC, 2001) or, “Develop a dynamic, mechanistic model of digestion and metabolism in lactating dairy cows suitable for evaluation of hypotheses regarding underlying energetic relationships and patterns of nutrient use” (Baldwin, 1995). The U. S. National Research Council “Requirements of Animals” series have been using mathematical models since the 1940’s, improving and expanding them as data and knowledge increases. Many other models have been developed, proposed, evaluated, used and/or rejected over the years. Most likely the most widely known practical model of dairy cattle nutrition apart from the NRC publications is the Cornell Net Carbohydrate and Protein System, now developed into the Cornell-Penn-Miner Program for dairy management and nutrition (CPM-Dairy; Boston et al., 2000).

In the 1960’s, R. L. Baldwin, working with colleagues already well on the way to formulating the most successful nutritional model to date, the Net Energy System (Lofgreen and Garrett, 1968), began a program, eventually lasting more than 35 years, to “Develop a dynamic, mechanistic model of digestion and metabolism in lactating dairy cows suitable for evaluation of hypotheses regarding underlying energetic relationships and patterns of nutrient use” (Baldwin 1995). [The actual beginning did not have that specific objective, yet the end result was that was the primary objective met]. Dozens of experiments on ruminal and tissue processes of dairy cattle, and dozens of publications on experimental data and on model development have come from that effort, and there are many dairy nutritionists in the world who have built directly on or benefited in some way from that effort. Without a doubt the CPM-Dairy program sprang from these efforts, in fact many scientists contributing to this program were graduate students or colleagues of Dr. Baldwin. The United States NRC for Dairy and the commercial/university effort on CPM Dairy have without a doubt been shining examples of use of models to improve efficiency of animal production.

There are many different types of models, the type usually dependent on the objective. **Empirical** models, like empirical equations or empirical formulae, describe the system at the same level the user ‘uses’ or ‘sees’ it, but by that nature can not give much insight into ‘what is inside’ or, the ‘mechanism’ of action of the system. The NRC models for nutrient requirements are classic examples of good empirical equations. These equations are usually derived experimentally, in fact from many experiments, and are very useful. However, by their nature they cannot describe how the net energy for maintenance is really used by the tissues. Nor can they legitimately be used to extend beyond the boundaries within which the data were collected. Recognized deficiencies still exist with the NRC and CPM on rates of intakes now achieved by several dairy cattle, as we know that changes in efficiencies for digestion, maintenance, and productive functions, are not linear over the entire range. Introduction of some non-linear functions and more mechanistic elements have improved the workings of these models tremendously, but description of the ‘extremes’ is still inadequate. A more mechanistic model equation may include elements for quantity of liver, gastrointestinal tissue, muscle and fat, ascribing to each one an energy requirement, and then summing them to yield the energy requirement for the body. At the level of the organ, this is now

empirical, but at the level of the animal, it is mechanistic, as it helps explain the mechanism by which the body energy requirement was derived.

Another key characteristic of a model is how it describes change over time. A model that describes a process at one time, usually through an empirical equation as above is **static**. This is true even if the ‘time’ was a growth or lactation phase extending several months. That does not make the model ‘dynamic’; it just provides a static picture of a certain period of time. A dynamic model *integrates* change over time. Both are very useful, however, I argue strongly that only a dynamic model will help us truly improve our nutritional understanding from this point forward. This is primarily because the requirements of any animal for any one time or short time period are always partially a function of what has come before. The other key factor is that dynamic models using differential equations over time can describe the constant turnover functions that actually are ‘maintenance’, such as ion transport, protein turnover in the muscle and viscera, and triglyceride turnover in the adipose tissue. When one actually studies these functions, one gets an appreciation for just how important and variable they can be. For example, a change in muscle protein turnover of 10 %, which is at least a minimum increase in early lactation (McNamara and Baldwin, 2000; McNamara et al., 2003; Overton et al., 1998; Drackley et al., 2003) would increase energy for maintenance about 2 Mcal/d (see Baldwin 1995 for calculations and stoichiometry). Over 100 days, that is an error or 2000 mcal or about 28 kg of adipose tissue (assuming adipose tissue will grow relative to the energy ‘left over’ after other energetic costs).

The requirements in early lactation are clearly understood now to be a function in part of the situation the animal was in 60 [or 130, or 30...] days prior to lactation. The same can be noted for any time period in lactation—the state of the animal is a function of the previous conditions, and the requirements for any animal are a function of its state. A cow producing 50 kg of milk at 200 days of lactation with a body condition score of 1.5 (about 30 kg of body fat) has a different requirement for the total body than a cow producing 50 kg of the same quality milk but with a body condition score of 3 (about 65 kg of body fat), although the requirement for the milk output may be the same. Also, a cow producing 50 kg of milk at 15 days of lactation has a different set of requirements than a cow of the same weight making the same amount of milk at 150 days of lactation, because of the differences in metabolic rates in non-mammary tissues. Static models can incorporate some of these effects of time by adding more equations relating to previous condition, but they are still static—they cannot describe the process over time. A dynamic model usually requires some type of integration program using various integral calculus formulae, so they often scare off otherwise quite competent nutritionists. However, software packages available today do this all ‘behind the scenes’ and I can vouch that a fair nutritionist can use these programs just as easily as I use a spreadsheet, without being able to describe mathematically ‘how’ this is done. But it is simple to understand the time effects and changes.

Integration of nutrition, ruminal ecosystem and metabolism in a dairy cow model

Readers desiring an in-depth description of the model should read the works of Baldwin cited below. Text of the latest published version of the model is available upon request of the author of this paper, or directly from Dr. Baldwin. The model of Baldwin inputs chemical components of the diet: soluble carbohydrate, organic acids, pectin, lactic acid, lipid, starch, hemicellulose, cellulose, soluble protein, insoluble protein, non-protein nitrogen, lignin, soluble ash, insoluble ash and added fat. The model inputs also include feed acetate and butyrate for high silage diets, and urea. It also includes factors for the starch solubility, particle size and to calculate organic matter. All these data can be obtained either by analysis or readily calculated from tabular values of CP, RUP/RDP, NDF, ADF, and NSC (non-structural carbohydrates) or NFC (non-fiber carbohydrates). Something must also be known about the physical characteristics such as average particle length and starch solubility. Thus there is complete flexibility in the system. Although

this paper will concentrate on the processes of metabolism in the body, we still have some way to go in improving our nutritional terminology, concepts and application away from analytical terminology and towards true physical and chemical science terminology. Great strides are being made as most scientists working in this field recognize that limiting ourselves to the basis of analytical terminology prevents further quantitative understanding, especially on diets made up from 'non-traditional' feedstuffs and at intake rates of modern high-producing dairy cattle.

The model describes, at the request of the user, most of the practical feeding strategies and intake estimates, based on either single or multiple meals per day, a specified feeding rate (usually used for simulating research trials where intake is measured), feed based on 1 kg of feed intake for each 3 kg of milk, two different equations used by earlier NRC versions based on actual data from thousands of records (Ely or Mertens equations; see Baldwin, 1995), and several others. The point is if you have feed intake data you can simulate it. If you want to use basic accepted equations to describe intake, you can do that. The model can use any newer equation developed as well, it is as simple as including it in the model text.

Model descriptions of body processes

The majority of the equations in the model are contained in the rumen submodel. However, that is not necessarily where the majority of the inadequacies obtain. It is the bodily processes about which we still know less, not surprisingly, as the majority of ruminant nutritionists have spent their time studying the rumen. As the focus has changed somewhat in the last 2 decades we are at the point where we are beginning to know 'what we don't know'. Thus for purposes of this paper I will concentrate on the modeling of the chemical processes in the body, and will build a focus around glucose. This is also with a purpose, as it is glucose around which the major regulatory processes of the body have evolved. It is the brain and central nervous system that require glucose. This change in glucose flux has demonstrable and significant effects on the major turnover pathways for protein and fat, on other energetic costs of cell function (respiration, ion transport, protein synthesis), that in turn affect the amount of carbon and nitrogen available for body fat and protein synthesis. As this is where the major errors in the model appear to remain, that is where we will concentrate, trying to follow as close as possible to the general objectives of research models listed above.

Carbohydrate in the body which is metabolized for energy (or to make fat or lactose) eventually is converted to triose phosphates or glucose, or is used through the same metabolic pathways so for simplicities sake we can aggregate a lot of this. So for glucose use in the body:

$$Gl = upGl + AaGl1 + PrGl1 + LaGl1 + GyGl1 - GlLm - GlHyF - GlHyV - GlTpF - GlTpV - GlLaB - GlCd.$$

We sum the uptake of glucose, gluconeogenesis from amino acids (Aa), propionate (Pr), lactate (La) and glycerol (Gy) and the use of glucose for lactose (Lm), triose phosphates (Tp) [in the viscera (V) or body (B)], that used to make pentose phosphates (NADPH₂, Hy), lactate and that oxidized to carbon dioxide (Cd). If the loss from the pool exceeds the inputs into the pool, then gluconeogenesis from amino acids can supply the deficit. This in turn, reduces the amounts of amino acids circulating, and if uptake from the gut cannot maintain the pool, then proteolysis of muscle protein will increase and muscle protein synthesis will increase, allowing maintenance of amino acid supply. Thus, as in the cow, in this model, carbohydrate nutrition cannot be described without invoking amino acid nutrition.

In the transition cow, deficits of energy and glucose are met by two major processes: lipolysis to release free fatty acids that the cow can use for energy and milkfat, and proteolysis of proteins to amino acids for gluconeogenesis. It must be stressed that lipolysis is not only responding to the glucose lack but also to the need by the mammary gland for half of the milk fat. So some increase

in lipolysis is inevitable. Also, fatty acid release can only spare a limited amount of actual glucose, as most organs require some glucose for energy in addition to the mammary demand. Excess lipolysis is undesirable and if we can minimize the glucose lack we can reduce excess lipolysis. Supply of glucose in the model, as in the cow, also directly affects body fat and protein synthesis. For body fat synthesis, primarily from acetate we have the aggregate equation:

$$AcTs = VAcTs / (1.0 + KAcTs/cAc + KGIAcTs / (Ahor*cGl))$$

This shows us the key elements relating to glucose use and therefore nutrition: body fat synthesis is a function of genetics of the cow (V or maximal velocity; and K, sensitivity to substrate (McNamara, 1994); nutrition: acetate availability (Ac, circulating acetate, primarily from absorbed acetate) and glucose-which is a both a direct supplier of reducing equivalent (energy) for fat synthesis and a direct indicator of energy balance, represented in the model as "Ahor" or anabolic hormone. Thus, as glucose availability drops dramatically in early lactation (in relation to demand), as does acetate, the rate of body fat synthesis drops as well, basically to zero for several days (McNamara, 1994). In addition, the 'anabolic hormone' of the model is equal to: $cGl/rcGl$, or glucose concentration divided by reference glucose concentration at energy balance = 0. As glucose drops, so does anabolic hormone (just like insulin) and this further reduces the rate of body fat synthesis. The control of lipolysis, body fat release, is in the opposite direction. This is probably an over-simplification, as there are now ample data to suggest that rates of lipolysis can be dramatically fast even when total body fat is low, suggesting that the fractional breakdown rate is not a constant (suggested by the scaling to amount of body fat; McNamara and Baldwin, 2000; McNamara, 1994). Said otherwise, animals with very little body fat can still be releasing amounts far greater in absolute quantities than cows with more body fat). Thus this is another way in which the modeling process has focused our attention to areas known to be inadequate, and more importantly, provable to be inadequate in a strict quantitative fashion. Thus in the model, the increased milk production puts a drain on available glucose and acetate, food intake is not yet sufficient to meet the total needs, and the integrated system reduces the rate of body fat synthesis and increases the rate of body fat loss.

The principles and concepts outlined above for the interaction of carbohydrate and fat apply directly to the connection with glucose, amino acids and body protein synthesis and degradation. We have in the body the following summation for use of amino acids:

$$DAa = absAa + PbAaB + PvAa - AaPb - AaPv - AaPm - AaGl - SAPsAa - Aapreg,$$

Where amino acid use per day is the sum of absorbed amino acids, amino acids released from the body (Pb) and from the viscera (Pv) and amino acid use for body protein synthesis (AaPb), visceral protein synthesis, milk protein (Pm), gluconeogenesis (AaGl) saliva and pregnancy. As glucose supply decreases in early lactation, the only major source besides for propionate absorbed from the gut is amino acids residing in body proteins. Circulating blood proteins are broken down and the amino acids oxidized and converted to glucose, but that is a tiny percentage of the need. As the body viscera (gastrointestinal organs, liver, udder) usually grow or at least stay the same size in early lactation, no net glucose can be derived from proteolysis there. Thus that leaves body muscle protein as the major source of amino acids for glucose. Gluconeogenesis is represented in the published model as a summation of amino acid use:

$$AaGl = VAaGl * (EBW^{**0.75}) / (1.0 + KaaGl / cAa),$$

such that as amino acid concentration goes up, glucose synthesis does as well. Amino acid concentration increases because body protein synthesis: $(AaPb=VAaPb*Bdna / (1.0+KAaPb/(Ahor*cAa)))$ decreases more than does proteolysis ($PbAaB = kPbAa*Pb$). These

are a function of anabolic hormone as well, so as glucose decreases, anabolic hormone (insulin) decreases, and body protein synthesis decreases, leading to a net increase in release of amino acids, to supply glucose.

Challenging the model for improvement

In order to continue to improve this model, as any model, one must challenge the behavior of the model with data from experiments designed with the following broad null hypotheses: "Our knowledge is inadequate to describe the system adequately." The specifics are supplied by what the model does NOT do well. If a model fails to describe some aspect adequately, compared to what we already know or at least have some serious basis from chemical or physical principles to hypothesize how a system should act, then, we learn what we do not know. The next step is to design an experiment to determine the equation forms or parameter values of the model to improve it. Experiments designed over the last 18 years in our laboratory have had one over-riding goal of improving our models of metabolism of the cow or the sow (see McNamara and Baldwin, 2000; McNamara and Pettigrew, 2002a,b). I will give one brief example.

Several experiments done over the last decade, in many laboratories, strongly point to the description of body fat and protein use as the major inadequacy in this model, as in many other models of dairy cattle nutrition (Komaragiri and Erdman, 1998; McNamara and Baldwin; 2000; Sage et al, 2002; Citron et al., 2000; McNamara and Phillips, 2003; Overton et al., 1998, Drackley et al. 2003). We do a quite good job of describing milk production from nutrient inputs, or vice versa. However, in Molly, challenge experiments which measured variations due to genetic merit, dietary starch, fiber and protein composition in dairy cattle over the majority of the lactation cycle all demonstrated that the model accumulated too much body fat and to some extent, body protein. This was after careful and repetitive analysis of feed and milk chemistry and model behavior to allow us to say that the model was doing a very precise job of balancing the feed inputs and milk outputs. However, the model was 'balancing' these by accumulating error in the body pools. A detailed description of all the reasoning behind our eventual conclusions as to exactly where the errors were is beyond repeating in this paper, but is available in (McNamara and Baldwin, 2000; and in Baldwin, 2000).

The final conclusion, based on measurements done on muscle, adipose tissue, mammary gland and liver (in many labs, not only ours) was that the errors, or at least the majority of them, were not in the quantitative descriptions in the adipose tissue itself, but that the adipose tissue was receiving a greater quantity of nutrients than was actually observed. Rather, the model underestimates the true costs of maintenance-type functions in the lactating cow, resulting in accretion of more muscle and fat than would be expected or measured. In this context, the true biochemical costs include the increase energy use due to increased metabolic activity and turnover of protein and fat in the viscera and muscle. In the Net Energy scheme, these increased costs are not truly maintenance, but Heat Increment. Regardless of the methodological definition, the point is that these are truly increased energy costs that are not accounted for by milk and 'basic maintenance functions', but must be described and accounted for in any model.

It is some comfort after detailed analysis of the literature and examination of the other systems that in fact the same error appears to be common to the major dairy nutrition models. This is not a condemnation, nor even a criticism of other models, but is in fact a great finding in support of them: when different models made for different purposes show the same results (inadequacies) it is strong evidence for concluding that that is where the inadequacy in knowledge truly lies. The other two model systems are the Dairy NRC (2001) and the CPM dairy model (Boston et al., 2000;). They both do an adequate job of relating feed inputs to milk outputs; however, they both overestimate the amount of body condition or reserves that would obtain in the given situation. Recent versions of CPM Dairy have acknowledged this error and introduced terms to correct it (model software available at: <http://mail.vet.upenn.edu/~ejjancze/cpmbeta3.html>). However, the

problem still remains that it is a lack of knowledge that prevents us from truly describing the system accurately. Both of these systems partition energy into body reserves on what is ‘left over’ from intake minus milk production minus ‘maintenance’, and taking into account the energetic efficiencies of these functions. However, if the true use of energy for non-mammary functions is greater than what is described in the model presently as ‘maintenance’, then total energy use is too low and then body fat and/or protein accumulate too quickly. So using completely different types of models, but all constructed from basically the same available data, the same errors result. It must also be noted that these errors are very small, and would not be noticeable easily in static models such as the NRC or CPM, but would accumulate over time in a dynamic model, allowing a more ready recognition of the problem.

We conducted an experiment in early lactating cows (days -21 to 120 postpartum) and measured indirectly the body fat and protein of the animals (Citron et al., 2000; Phillips et al., 2003). After running simulations on individual cows, it was evident that the model did a very good job of describing milk production (predicted = $2.93 + .903 * \text{observed milk}$, $r^2 = .96$; Citron et al., 2001), however, it over-accumulated body fat and protein. For body fat at 180 days the equation was Predicted Fat = $180.1 + 0.58 * \text{Observed fat}$, $r^2 = .09$. The mean bias accounted for 97 % of the mean square prediction error. For protein in the body the equation was Predicted Protein = $-5.1 + .90 * \text{Observed Protein}$, $R^2 = .33$ for body protein overall, with mean bias making up 83 % of the MSPE. However, by day 120, this equation was Predicted Protein = $-86.5 + 1.75 * \text{Observed Protein}$, $r^2 = 0.45$. Thus, for body fat, the model has a large mean bias, suggesting that we do not understand the absolute amount of energy used, and for body protein, we do have an ‘average’ understanding of body protein use, but still cannot describe the time-curve of protein use adequately in early lactation.

Previous experiments and model challenges determined that the error was not in the equations describing body fat use. There are still errors in the model description of protein as noted above, but even with that acknowledgement, there is still evidence that errors in maintenance costs are at fault. These would include the rates of and energetic costs of body protein turnover, liver protein turnover, body fat turnover, and the increased costs of metabolism upon a large increase in feed intake. These are energetic costs, which increase during lactation. It is the inability of the model

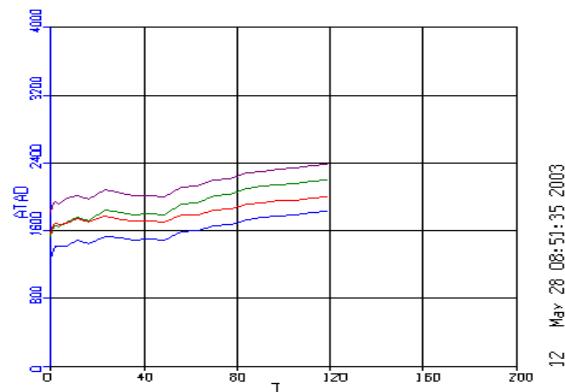


Figure 1. Simulated energy use by the cow in early lactation. Simulation of total moles of ATP used by body (muscle), viscera (all other organs except adipose) and adipose tissue by the cow during early lactation. Data are in moles of ATP. Bottom line is default simulation; second line from bottom is increased energy use in body; third line from bottom is increased energy use in viscera and top line is increased energy use in both. Energy use in body was increased by doubling the rate of metabolism; in viscera the factor increasing the energy use due to increased feed intake was increased.

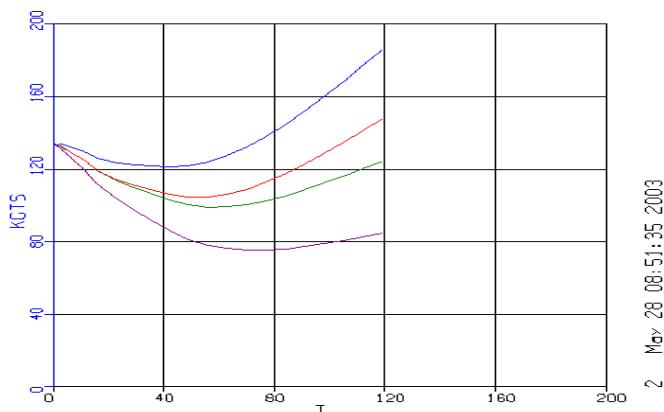


Figure 2. Simulated body fat content in early lactation. Kilograms of body fat simulated by the Molly Model. Top line is default simulation; second line from top is increased energy use in body; third line from top is increased energy use in viscera and bottom line is increased energy use in both. Output format is directly off of ACSL (Advanced Continuous Simulation Language, Aegis Technologies Group, Huntsville AL).

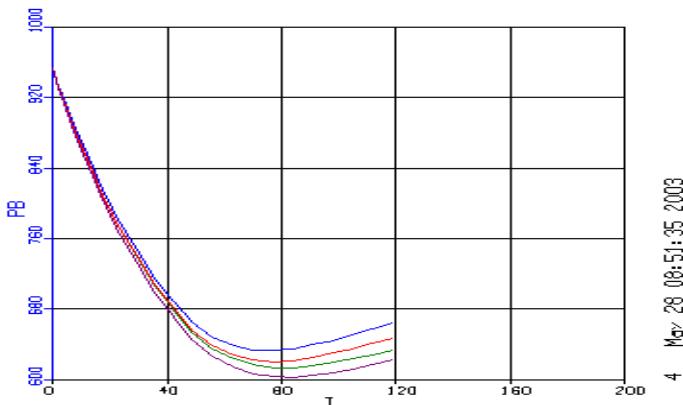


Figure 3. Simulated body protein content in early lactation. Moles of body (muscle) protein simulated by the Molly Model. Value for molecular weight is .11 kg/mole. Top line is default simulation; second line from top is increased energy use in body; third line from top is increased energy use in viscera and bottom line is increased energy use in both.

to describe the increased energetic costs of these pathways in lactating cows that results in the error in body fat and protein accretion. It also must be noted here that no other model can readily identify these errors, as this is the only available model that describes these processes dynamically, over time.

Based on our reasoning and findings from the previous and present challenges, we tested what would happen if we increased the energetic costs of the increased food intake on metabolic rates in the viscera, as is justified by recent data (Reynolds et al., 2003). We did find that we could correct for a large proportion of the error in body fat (Table 1; Figures 1 to 3). This then becomes a clear hypothesis to test with additional data from the literature or through continued study of these processes. It is only through the strict and thorough approach of using a mechanistic and dynamic system that we can pinpoint such specific areas in which to concentrate our future efforts.

Table 1. Observed and simulated use of energy in early lactation ending at d 120¹.

| Item | Observed | Default model | Increased visceral energy use | Increased Body energy use | Increase both body & viscera |
|---|----------|---------------|-------------------------------|---------------------------|------------------------------|
| Milk, kg/d | 40 | 40 | 39.8 | 39.8 | 39.8 |
| Body fat, kg | 80 | 186 | 125 | 149 | 85 |
| Body protein, kg | 78 | 73 | 70 | 71 | 68 |
| Total Energy use in Viscera, Moles ATP/d | | 1116 | 1589 | 1106 | 1575 |
| Total Energy use in Body, Moles ATP/d | | 433 | 420 | 665 | 649 |
| Total Energy use in Fat, Moles ATP/d | | 274.0 | 189.0 | 227.0 | 152.0 |
| Maint. Energy, MJ/d | 43.7 | 109.2 | 122.6 | 127.7 | 166.3 |
| Energy Balance, MJ/d | 4.6 | 54.2 | 28.1 | 41.6 | 16.2 |
| Blood Glucose, mM | 3.0 | 4.4 | 4.2 | 4.1 | 3.7 |

¹ Data are those for day 119 of lactation as measured or simulated, except for milk, which is the average of the 119 day period. The observed energy figures are determined from observed body weight and NRC (2001) calculations (0.08 Mcal/d * BW.75).

² The simulated Maintenance Energy figures are the totals of actual energy used by body, fat and viscera during metabolism, thus this is greater than the NRC maintenance figure.

³ The EB figure observed is Energy Intake - Milk Energy - Maint. Energy and is available for reserve growth. The EB figure simulated is the sum of body and visceral protein and body fat for that day.

Summary and implications

When one stands on the shoulders of giants, one is humbled and grateful that the horizon is closer in view. With the objective of eventually understanding everything that happens in an animal coming closer and closer to fruition, our true purpose of feeding the world efficiently throughout many geographical areas and for many centuries becomes closer. A quantitative and systems approach is the only way to continue upon this path. Reductionistic research, especially into the true functionality of the genome and physiological control of metabolism will be essential to improvement. In addition, continued efforts in simple, economical and effective chemical methodology for defining feedstuffs is a must. But over-riding all, it is the integration of knowledge, data, and concepts, into an organized representation of reality, a model that will achieve our goal. Some may disagree, but Mr. Armstrong stepping onto the moon must be symbolic of the ability of the human brain to use technology to integrate information. There was only one way to test the model: send off the rocket. Imagine how much more powerful, when we can overcome politics and other human frailties with a system that can ensure adequate food supply for all. That is our ultimate goal, and societal, industrial, and governmental support for the modeling approach to research is critical to achieve it.

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Effects of growth hormone and monensin on adipose tissue lipogenesis in transition cow

A. Ariel¹, J.E. Vallimont², S.S. Donkin³, A. Shamai⁴ & G.A. Varga²

¹ Hebrew University of Jerusalem, Israel

² Pennsylvania State University, University Park, PA, USA

³ Purdue University, West Lafayette, IN, USA

⁴ ARO, Bet Dagan, Israel

Summary

Twenty nine multiparous, Holstein dairy cows in late gestation were used to evaluate the effect of prepartum administration of monensin (M) and/or bST on prepartum and postpartum adipose tissue lipogenesis. During the prepartum period 300 mg of M was added to the total mixed ration, and 500 mg slow release bST was injected on d 28 and 14 prior to calving. Subcutaneous adipose tissue samples were taken at 3 weeks prepartum and 2 weeks postpartum. Rate of lipogenesis was assessed by measuring the incorporation of labeled acetate into total lipids of adipose tissue explants over 2-h period of incubation at 38 C. On the week of the adipose tissue biopsy, blood samples were collected and analyzed for insulin, growth hormone, IGF1, NEFA and glucose. In prepartum cows, lipogenesis was increased by bST treatment but not by M. Postpartum lipogenesis was about 200 fold lower than the prepartum values, and was not affected by M or by bST. In prepartum cows the level of NEFA tended to decrease by 20% in bST or M treated cows. Concentration of GH was elevated by 60% in bST treated cows. Level of insulin, IGF1 and glucose were similar among treatments. In postpartum cows DMI tended to increase by 11% in M treated cows. Levels of GH, insulin, and IGF1, NEFA and glucose were not affected by M and bST.

Keywords: bST, lipogenesis, monensin, transition cows

Introduction

Cows undergo many metabolic changes in preparation for parturition and lactation, to satisfy the augmented requirements of the fetus, uterus, and the mammary gland. Due to metabolic and fill constraints, DM intake by transition cows is limited (Grummer, 1995). To support the energy needs of these cows there is a marked adaptation in the adipose tissue, leading to reduction in lipogenesis and to a concomitant increase in lipolysis (Bell and Bauman, 1994).

The homeorhetic agent bST increases gluconeogenesis from propionate (Knapp et al., 1992), increases prepartum plasma glucose and decreases plasma NEFA and BHBA (Putnam et al., 1999), and decreases the rate of lipogenesis in adipose tissue of lactating dairy cows that are in a positive EB (Lanna et al., 1995). Monensin increases the molar proportion of propionate in the rumen (Bagg, 1997), and decreases plasma BHBA and NEFA (Duffield et al., 1998). In this study, the effects of M and bST administered to pre-calving dairy cows on adipose tissue lipogenesis before and after calving and on the concentrations of some circulating metabolic fuels and hormones, were determined

Materials and methods

Twenty nine multiparous, Holstein dairy cows were used. Cows were 4 weeks before calving, and remained in their groups until 9 weeks postpartum. During the prepartum period only, 300 mg of

M was topdressed daily into the total mixed ration, and 500 mg slow release bST (POSILAC) was injected on d 28 and 14 prior to calving. The pre calving diet contained 6.5 MJ of NEL per kg, 14% CP and 42% NDF, and the post calving diet contained 7.4 MJ of NEL per kg, 18% CP and 33% NDF. Feed was provided to ad libitum intake prepartum and postpartum. Subcutaneous adipose tissue samples (20 g) were taken at 3 weeks prepartum and 2 weeks postpartum. About 25 mg wet weight tissue slices in triplicates were incubated in 3 ml Krebs-Ringer bicarbonate buffer (pH 7.4) under 95% O₂ and 5% CO₂ atmosphere. Rate of lipogenesis was assessed by measuring the incorporation of labeled acetate into total lipids of explants over 2-h incubation at 38 C. A sample of each biopsy was used to determine the DNA content (Labarca and Paigan, 1980) per wet weight of adipose tissue. On the week of the adipose tissue biopsy, blood samples were collected about 3 hours post feeding. Samples were analyzed later for insulin, growth hormone, and IGF1. The concentrations of NEFA and glucose were determined by commercial kits. Data are presented as covariates of the respective prepartum or postpartum BCS.

Results

Content of DNA was not affected by treatment, and averaged 100 and 135 µg per g tissue in prepartum and postpartum cows, respectively. In prepartum cows, basal rate of lipogenesis averaged 50 nanomol of acetate incorporated per µg of DNA (Table 1). It was about 40% higher in the presence of 10 or 100-nug/ml of insulin. The bST treatment increased or tended to increase lipogenesis rate by about 60%, whereas M treatment did not affect lipogenesis rate. Postpartum lipogenesis was about 200 fold lower than prepartum values, and was not affected by M or by bST.

In prepartum cows DMI averaged 1.8% of body weight and was not affected by the treatments (Table 2). Level of NEFA averaged 100 µeq/L and tended to decrease by 20% in bST or M treated cows. Concentration of GH averaged 3.8 ng/ml and was elevated by 60% in bST treated cows. Level of insulin, IGF1 and glucose averaged 0.9 ng/ml, 12 ng/ml and 75 mg/dl, respectively and were similar among treatments. In postpartum cows, DMI averaged 2.9% of BW. DMI tended to increase by 11% in M treated cows. Levels of GH, insulin, IGF1, NEFA, and glucose averaged 6 ng/ml, 0.5 ng/ml, 6 ng/ml, 350 µeq/L, and 55 mg/dl, respectively, and were not affected by M and bST.

Table 1. Rate of lipogenesis (nmol/µg DNA-2h) in adipose tissue from prepartum and postpartum Holstein cows.

| | -bST | | +bST | | SEM | P< | | |
|-------------------|------|------|------|------|------|-------|-------|-------|
| | -Mon | +Mon | -Mon | +Mon | | bST | Mon | INT |
| Prepartum | | | | | | | | |
| 0 ng/ml insulin | 18 | 51 | 77 | 50 | 32 | 0.067 | 0.440 | 0.070 |
| 10 ng/ml insulin | 25 | 77 | 125 | 70 | 35 | 0.023 | 0.403 | 0.014 |
| 100 ng/ml insulin | 29 | 62 | 123 | 55 | 32 | 0.072 | 0.945 | 0.044 |
| Postpartum | | | | | | | | |
| 0 ng/ml insulin | 0.06 | 0.25 | 0.36 | 0.27 | 0.12 | 0.157 | 0.451 | 0.148 |
| 10 ng/ml insulin | 0.06 | 0.27 | 0.29 | 0.32 | 0.12 | 0.103 | 0.185 | 0.134 |
| 100 ng/ml insulin | 0.06 | 0.25 | 0.45 | 0.28 | 0.19 | 0.127 | 0.515 | 0.127 |

Table 2. Intake of DM and plasma level of GH, insulin IGF1, NEFA and glucose in prepartum and postpartum Holstein cows.

| | -bST | | +bST | | SEM | P< | | |
|-------------------|------|------|------|------|------|-------|-------|-------|
| | -Mon | +Mon | -Mon | +Mon | | bST | Mon | INT |
| Prepartum | | | | | | | | |
| DMI, % of BW | 1.61 | 1.85 | 1.79 | 1.94 | 0.10 | 0.249 | 0.106 | 0.716 |
| GH, ng/ml | 2.03 | 3.38 | 4.14 | 5.83 | 0.84 | 0.028 | 0.130 | 0.861 |
| Insulin, ng/ml | 0.92 | 0.83 | 1.02 | 0.92 | 0.08 | 0.284 | 0.309 | 0.951 |
| IGF1, ng/ml | 13.2 | 10.7 | 10.7 | 12.1 | 1.0 | 0.575 | 0.595 | 0.075 |
| NEFA, µEq/L | 124 | 105 | 108 | 76 | 11 | 0.073 | 0.064 | 0.661 |
| Glucose, mg/dl | 74.2 | 75.5 | 74.4 | 78.2 | 3.3 | 0.684 | 0.484 | 0.739 |
| Postpartum | | | | | | | | |
| DMI, % of BW | 2.49 | 3.03 | 3.00 | 3.19 | 0.17 | 0.122 | 0.079 | 0.395 |
| GH, ng/ml | 5.03 | 6.30 | 5.11 | 7.61 | 1.12 | 0.623 | 0.191 | 0.685 |
| Insulin, ng/ml | 0.51 | 0.40 | 0.47 | 0.42 | 0.04 | 0.835 | 0.113 | 0.520 |
| IGF1, ng/ml | 9.23 | 3.16 | 5.51 | 6.32 | 1.46 | 0.870 | 0.150 | 0.065 |
| NEFA, µEq/L | 460 | 287 | 334 | 299 | 65 | 0.463 | 0.188 | 0.379 |
| Glucose, mg/dl | 53.8 | 56.0 | 54.1 | 57.9 | 3.0 | 0.753 | 0.389 | 0.818 |

Discussion

The lower level of insulin, IGF-1 and glucose, and the higher level of NEFA agree with a relatively higher EB in prepartum cows as compared with postpartum cows. Prepartum lipogenesis rate in adipose tissue was increased in bST treated cows. Treatment with bST did not affect the mRNA abundance of the lipogenic enzymes ACC and FAS in adipose tissue of these cows (Donkin et al., 2000). Lanna et al. (1995) have found that lipogenesis was reduced by 97% in bST treated cows, concomitantly with a marked decrease in the activities of ACC and FAS. In these cows bST treatment resulted with a marked negative EB. Thus the reduced lipid synthesis in adipocytes was related to altered nutrient partitioning between the mammary gland and the adipose tissue. In contrast, in our precalving cows, the DM intake was 1.8% of BW, indicating that animals were in a positive EB according to NRC (2001). Also the relative high blood glucose and low blood NEFA concentrations are compatible with a positive EB during this time. Our data are in line with the lack of effect of bST on basal and stimulated lipolysis in dry cows maintained at constant EB (Ferlay et al., 1996).

In late pregnancy and early lactation, adipose issue becomes resistant to insulin, and the reduction in lipogenesis is associated with a reduction in insulin receptors in adipocytes (McNamara, 1995). While postpartum lipogenesis was definitely abolished in all treatments, our data indicate that three weeks before calving, cows were still capable of active lipogenesis. The reduction in lipogenesis occurs apparently later on, when DM intake is markedly reduced. We don't have a clear explanation for the positive effect of bST on lipogenesis. One possibility is that bST in transition cows maintained at positive EB may stimulate IGF-I in adipose tissue to locally maintain lipogenesis in a paracrine manner.

Treatment with M did not affect lipogenesis rate, or mRNA abundance of the lipogenic enzymes ACC and FAS in adipose tissue prepartum (Donkin et al., 2000). Likewise in sheep, M administration did not affect the activity of ACC and FAS (Wahle and Livesey, 1985). However, the expression of lipogenic enzymes was increased 2h after intravenous propionate infusion in

sheep (Lee and Hosner, 2002). In prepartum cows dietary M supply increased the glucose pool and distribution space (Arieli et al., 2001), in line with an increased propionate supply. The variation between our two studies might be related to the sampling timetable: lipogenesis rate was measured 3 weeks prepartum, whereas glucose kinetics was monitored a week later. A period longer than one week of M administration might be needed to reveal its effect on lipogenesis, or that this effect is manifested under a more marked negative EB state.

In conclusion, during positive EB in transition cows the prepartum adipose tissue lipogenesis rate is increased one week after treatment with bST. During this time daily administration of M had no effect on lipogenesis rate. In these cows two weeks postpartum, lipogenesis rate was markedly reduced and not affected by prepartum bST or M treatment.

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Studies on milk production of the Etawah crossbreed goat fed with Tempe waste

D.A. Astuti¹, E.B. Laconi² & D. Sastradipradja³

¹ Department of Physiology and Pharmacology, Faculty of Veterinary Medicine IPB, Bogor, Indonesia

² Department of Animal Nutrition, Faculty of Animal Science IPB, Bogor

³ Department of Biology, Faculty of Medicine, Krida Wacana Christian University, Jakarta

Summary

The present paper reports on results of a study aiming at enhancing milk production of indigenous ruminants, i.e. the Etawah crossbreed goat, by feeding local agroindustrial waste products. The study used 16 first lactation does (BW 50 ± 5.0 kg) assigned into four dietary level of feeding treatment groups of 4 animals each that received 50% king grass plus respectively 50% concentrate (R1), 25% concentrate and 25% fresh tempe (fermented soybean cake) waste (R2), 25% concentrate and 25 % dry fermented tempe waste (FTW) (R3), and 25 % concentrate and 25% dry gelatinized liquid tempe waste (LTW). The rations contained 14% CP. FTW was made by fermentation of solid tempe waste using *Aspergillus niger*, while LTW was gelatinized with maize flour. Daily milk production was monitored twice daily while glucose kinetics was assessed by radioisotope tracer technique. Mammary blood flow (MBF) and metabolite arteriovenous differences (Δ AVs) were measured to get uptake and mammary performance data. Results show that glucose kinetics at peak lactation were affected by ration type. Glucose pool size, space of distribution and flux in R3 were higher in comparison to the other treatments. Milk yield of R1, R2 and R4 were lower than R3, i.e. 30%, 54 %, and 63%, respectively. Milk protein, lactose and fat were also highest with R3. MBF values ranged from 18 to 20.82 l/h. Mammary glucose, triglyceride, acetate, phenylalanine, tyrosine and oxygen uptakes in R3 were highest in comparison to the other treatments. Glucose uptake was higher than the amount secreted as lactose. The excess glucose taken-up was used for other metabolic processes.

Keywords: Etawah crossbreed goat, fermented tempe waste, dry gelatinized liquid tempe waste

Introduction

Tempe is an Indonesian food product which is made by fermentation of soybean using *Rhizopus sp.* From tempe processing there are several waste products, such as liquid waste and solid waste. Solid waste (21,9 %) can be used as a fiber source for ruminants, while liquid waste which is still high in amino acid content can be made into a dry gelatinized product using maize flour. From a preliminary study it was found that the best dry gelatinized liquid tempe waste is made from maize flour rather than wheat flour or cassava flour. Tempe waste still contains high protein (approx. 16%) and essensial amino acids.

Research about the utilization of tempe waste should affix technologies for most advantageous use of this product. Fermentation processes of solid tempe waste using *aspergillus niger* and gelatinization of liquid tempe waste are the options to improve their utilization and include them as substitute concentrate feed and a source of microbial protein for lactating goats. Performance parameters of the animal cover glucose kinetics, mammary function and milk production characteristics.

Material and methods

Sixteen, first lactating Etawah crossbreed does (ave. BW 50 ± 5.0 kg) were used during a three month experiment. The animals were randomly allocated into four dietary treatment groups of 4 animals each, R1 to R4, receiving basal (50%) king grass ration plus respectively 50% concentrate (R1), 25% concentrate and 25% fresh tempe (fermented soybean cake) waste (R2), 25% concentrate and 25 % dry fermented tempe waste (FTW) (R3), and 25 % concentrate and 25% dry gelatinized liquid tempe waste (LTW) (R4). The complete rations contained around 14% CP. FTW was made by fermentation of solid tempe waste using 0.5 % (DM) *Aspergillus niger*, while LTW was gelatinized with 10 % maize flour. A one month adaptation period was allowed before glucose kinetics were measured in a one week trial. Milk yield were measured twice daily.

Tabel 1. Ration formulation and chemical composition of lactating Etawah crosbreed goat fed with tempe waste.

| Treatments | R1 | R2 | R3 | R4 |
|----------------------------------|-------|-------|-------|-------|
| King grass (% DM) | 50 | 50 | 50 | 50 |
| Concentrate (% DM) | 50 | 25 | 25 | 25 |
| Fresh tempe waste (% DM) | - | 25 | - | - |
| Dry ferment tempe w. (%DM) | - | - | 25 | - |
| Dry gelatin. liq. tempe w. (%DM) | - | - | - | 25 |
| Composition: | | | | |
| Dry matter | 91,22 | 93,74 | 93,74 | 94,02 |
| Crude protein (%DM) | 14,00 | 14,25 | 14,10 | 14,01 |
| Fat (%DM) | 4,83 | 3,99 | 3,80 | 4,70 |
| Crude fiber (%DM) | 20,61 | 29,94 | 29,27 | 20,0 |
| Energy (MJ.kg ⁻¹) | 16.94 | 1456 | 16.55 | 15.44 |

Glucose kinetics (glucose flux) was measured by single pulse labelling with glucose-2-³H through the jugular vein for the duration of 1 minute as described by Sastradipradja *et al.* (1976). At intervals of 20 minutes post injection, blood was sampled 6 times, immediately chilled on ice and subsequently the plasma collected and kept frozen at -20°C until analysis for radioactivity. Mammary blood flow (MBF) was measured according to the method of Cant *et al.* (1993) and metabolite uptake was calculated from arterovenous differences times MBF. Glucose and triglycerides were measured using commercial kits and read spectrophotometrically. Tyr and phe were measured using the HPLC technique. Oxygen concentration in blood was calculated as the sum of dissolved oxygen plus oxygen carried by hemoglobin. The equations used were:

$$\text{MBF (ml.min}^{-1}\text{)} = \{(\text{FYb} \times 0.965) + \text{FYf}\} / \text{FYa-v} \quad (\text{Cant et al., 1993}),$$

where

Fyb = Phe + Tyr output bound in milk protein (moles per hour);

FYf = free milk Phe and Tyr (moles per hour);

FYa-v = (Phe + Tyr) ΔAV (moles per liter);

Oxygen concentration = {0.0235 × pO₂ × 22.4}/{(44 × 760)};

HbO₂ = % Hb × 1.34 × % HbO₂

Results and discussion

The results on the metabolic and production performance of lactating Etawah crossbreed does are presented in Table 2. Glucose kinetics, i.e. pool size, space of distribution and flux in R3 are the highest among all the treatments groups. Dry FTW is more palatable than other rations for lactating does. Parameters of mammary gland function in R3 are also the best. The high glucose flux of R3 resulted in high glucose uptake. Uptake of other measured nutrients in R3 were also highest. Oxygen is used to support metabolic activity and its high uptake in R3 is in line with what would be expected in supporting mammary metabolism. R1 with 50 % concentrate was not as good as with ration R3. Fermentation using *Aspergillus niger* resulted in high palatability, digestibility and utilization of nutrients (Laconi, 1998, unpublished data). Data on milk yield and composition are also the best with ration R3. Milk yield in R3 is the highest, followed by R1, R2 and R4. Treatment with dry gelatinized LTW could not improve the metabolic and mammary performance of Etawah crossbreed does because of low palatability of dry gelatinized LTW. Milk protein with ration R3 is three times the amount produced with ration R4. Milk lactose and fat in R3 however are only twice the amount produced with the other rations. It was also evident that the lower milk yield in R1 compared to R3 is attributed to the lower glucose kinetics values.

Data on glucose uptake in the present study range from 2.7 (R4) to 4.99 (R3) g.h⁻¹. Expressed per kg milk produced would yield values of 74, 101.6, 77.5 and 114 g.kg⁻¹ for R1, R2, R3 and R4, respectively. The range 74 to 114 is comparable to the range of values, 70 to 80 g/kg reported by Kronfeld (1982) for dairy goats, but is lower than that reported for the Bali cow, a truly indigenous tropical ruminant, i.e. 120 to 140 g/kg milk (Sukarini *et al.* (2001)). There was strong indication that mass-wise glucose uptake in the indigenous Bali cow is 2 to 4 times the amount secreted as lactose, while little lactose is synthesized. Thus, the excess glucose is used for other metabolic processes taken place in the mammary gland. Whether such situation applies also for the Etawah crossbreed goat needs further investigation, considering the present results and findings of a previous study by Astuti *et al.* (1995) that MBF of lactating Etawah crossbred goats fed different levels of feeding showed a range of 300 to 1200 ml.min⁻¹ with milk yields around 500 to 900 ml.d⁻¹.

Table 2. Metabolic performance parameters of lactating Etawah crossbreed does.

| Metabolic parameters | R1 | R2 | R3 | R4 | SEM |
|--|---------------------|---------------------|----------------------|--------------------|-------|
| Glucose kinetics : | | | | | |
| - Pool size (uM.BW ^{-0.807}) | 2.19 ^q | 2.15 ^q | 3.07 ^p | 1.89 ^q | 0,20 |
| - Space of distribution (%BW) | 20 ^q | 19 ^q | 25 ^p | 18 ^q | 2.11 |
| - Flux (uM.min ⁻¹ .BW ^{-0.807}) | 5.22 ^q | 5.84 ^q | 7.14 ^p | 5 ^q | 0,90 |
| Milk : | | | | | |
| - Yield (g.d ⁻¹) | 1072 ^q | 700 ^r | 1544 ^p | 567 ^r | 14.00 |
| - Protein (g.d ⁻¹) | 45.82 ^q | 28.27 ^r | 67.51 ^p | 19.28 ^r | 4.60 |
| - Lactose (g.d ⁻¹) | 41.81 ^q | 20.81 ^r | 57.76 ^p | 23.24 ^r | 3.12 |
| - Fat (g.d ⁻¹) | 44.48 ^q | 21.98 ^r | 58.50 ^p | 23.23 ^r | 2.32 |
| Mammary function : | | | | | |
| - MBF (l.h ⁻¹) | 19.50 ^p | 18.78 ^q | 20.82 ^p | 18 ^q | 1.41 |
| - Glucose uptake (g.h ⁻¹) | 3.31 ^q | 3.38 ^q | 4.99 ^p | 2.70 ^r | 0.13 |
| - Triglyceride uptake (g.h ⁻¹) | 1.95 ^q | 2.82 ^q | 3.74 ^p | 1.80 ^q | 0.09 |
| - Acetate uptake (mM.h ⁻¹) | 195.00 ^q | 131.46 ^r | 312.30 ^p | 108 ^r | 11.10 |
| - Phe uptake (mg.h ⁻¹) | 5.80 ^p | 3.75 ^r | 6.24 ^p | 3.60 ^r | 0.56 |
| - Tyr uptake (mg.h ⁻¹) | 2.95 ^q | 3.75 ^q | 6.32 ^p | 1.80 ^r | 0.08 |
| - Oxygen uptake (ml.h ⁻¹) | 813.15 ^q | 751.20 ^q | 1361.63 ^p | 486.0 ^r | 17.15 |

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The effects of protein : energy ratio of the diet and feed intake on protein and energy metabolism of Iberian pigs growing from 50 to 100 kg

R. Barea, R. Nieto & J.F. Aguilera

Unidad de Nutrición Animal, Estación Experimental del Zaidín (CSIC), Camino del Jueves s/n, 18100 Armilla, Granada, Spain.

Summary

The effects of dietary protein content and feeding level on the utilization of metabolizable energy and on the rates of gain, protein and fat deposition have been studied in Iberian pigs growing from 50 to 100 kg body mass by means of comparative slaughter experiments. Growth rate improved with each decrease in dietary CP from 145 up to 95 g/kg dry matter (DM) and then levelled off. The level of intake had a positive significant effect on the rates of body gain, protein and fat deposition ($P<0.001$). Protein deposition reached a maximum value (69.2 g) when the diet providing 95 g CP/kg DM (5.12 g digestible ideal protein/MJ ME) was offered at the highest feeding level. With this diet the marginal efficiency of body protein accretion was 1.43 g/MJ ME and the rate of growth attained 19.85 g/MJ increase in ME intake, equivalent to an energy cost of 50.4 kJ ME/g gain. Between 50 and 100 kg the mean chemical composition of 1 kg gain was found to be 78.0, 588.2, 27.3 and 286.0 g for crude protein, fat, ash and water, respectively. Net efficiency of utilization of ME for energy gain (k_g) attained a mean value of 0.54 (± 0.020).

Keywords: Iberian pig, energy retention, protein deposition

Introduction

The nutritional requirements of the Iberian pig (*Sus mediterraneus*) are not well-defined and seem to differ from those of conventional pigs. Previous work (Serra *et al.*, 1998) has showed slower growth rates and enhanced fat deposition in the Iberian breed in comparison with conventional breeds of pigs. The response of the growing Iberian pig (15 to 50 kg body mass (BM)) to changes in energy and protein supply has been recently assessed in our laboratory (Nieto *et al.*, 2002) in terms of maximum daily rate of protein accretion (P_{max}) and marginal efficiency of body protein deposition (PD) at restricted ME intakes ($\Delta PD/\Delta ME$; g/MJ). These results clearly show great differences in metabolic activities between genotypes and, consequently, the need to accurately define the optimal protein:energy ratios for traditional unimproved breeds. The gathered information might help in providing an interesting contrast of value in the development of models for pig growth. In this work are reported preliminary results concerning the effects of dietary protein content and level of feeding on growth performance, protein deposition and energy utilization of Iberian pigs from 50 to 100 kg BM.

Materials and methods

The experiment was performed with 72 castrated males purebred Iberian pigs of Silvela strain which were randomly assigned at 50 BM to twelve experimental dietary treatments following a 4 (dietary protein content; DPC) \times 3 (feeding level; FL) factorial design with six replicates per treatment. Eighteen additional pigs were allocated to an initial slaughter group. Four diets of similar ME content were formulated to supply ideal crude protein at levels varying from 145 to 70 g/kg DM (Table 1). These diets were prepared by incorporation of a protein-free mixture to a diet

Table 1. Nutrient composition of the experimental diets obtained by dilution of a high protein content diet (HPC) with a mixture based on maize starch.

| | A | B | C | D |
|-----------------------|-------|-------|-------|-------|
| HPC diet ¹ | 1000 | 828 | 656 | 483 |
| Diluting mixture | 0 | 172 | 344 | 517 |
| CP, g/kg DM | 145 | 120 | 95 | 70 |
| DE, MJ/kg DM | 14.28 | 14.49 | 14.79 | 15.00 |
| ME, MJ/kg DM | 13.94 | 14.22 | 14.58 | 14.85 |

¹ Contains (g/kg): Lysine, 7.78; Methionine + cystine, 5.31; Threonine, 5.12; Tryptophan, 1.34; Isoleucine, 4.69; Leucine, 8.49; Histidine, 3.15; Phenylalanine + Tyrosine, 10.42; Valine, 6.57.

with a high protein content (HPC). Each diet was given at three daily rates: 0.95, 0.80 and 0.60 of *ad libitum* intake in two equal meals. Experimental procedures were as described in a previous paper (Nieto *et al.*, 2002). The comparative slaughter procedure was used together with classical digestibility and balance trials. The animals were slaughtered at 50 kg or 100 kg BM. After slaughter they were divided into four components (carcass, head and feet, viscera and blood) and kept at -20°C until analysis. The right half of the carcass and rest of body components were minced, freeze-dried and analysed separately for crude protein, gross energy and ash. Body fat was calculated assuming an energy content of 23.8 and 39.3 kJ/g for protein and fat, respectively.

Results and discussion

The effects of DPC and FL on performance and energy metabolism of the experimental animals are shown in Tables 2 and 3. No significant interactions between the main factors were observed. Mean daily gain was significantly affected by DPC and FL ($P<0.001$). It improved when decreasing the concentration of CP of the diet down to 95 g/kg DM and was maintained thereafter. Growth rate /MJ ME was also affected by both factors ($P<0.001$ and $P<0.01$ for DPC and FL, respectively). It decreased significantly on decreasing the dietary CP content down to 95 g/kg DM and with this diet achieved a value of 19.85 g /MJ increase in ME intake, equivalent to an energy cost of 50.4 kJ ME/g gain.

Decreasing the CP content of the diet resulted in significant increases in d(E) and m(E) in part as an effect of the dilution of the high protein diet with a mixture based on maize starch. The effect was higher for the latter, so that ME:DE ratio increased significantly ($P<0.001$) on decreasing dietary CP content from 145 to 95 g/kg DM. The average value obtained was 0.983. Protein deposition tended to be higher for diets of lower protein content. However, the effect was not significant. Our results seem to indicate that diet C provided the optimal amino acid-N supply for animal performance.. Fat gain was highly significantly affected by dietary protein supply ($P<0.001$) increasing as dietary CP content declined.

The level of intake had a positive significant effect on the rates of body gain, protein and fat deposition ($P<0.001$). Protein deposition reached a maximum value (69.2 g) when the diet providing 95 g CP/kg DM (5.12 g digestible ideal protein/MJ ME) was offered at the highest feeding level. With this diet a marginal efficiency of body protein accretion of 1.43 g/MJ ME was estimated. Between 50 and 100 kg the mean chemical composition of 1 kg gain was found to be 78.0, 588.2, 27.3 and 286.0 g for crude protein, fat, ash and water, respectively. Whole-body chemical composition at approximately 100 kg empty BM was on average (g/kg) 108.9, 463.1, 30.6 and 392.6 for crude protein, fat, ash and water, respectively.

Energy retained (kJ/kg BM $^{0.75}$.d) was related to ME intake (kJ/kg BM $^{0.75}$.d) by means of individual linear regression equations for each dietary protein content. Diets B, C and D showed

Table 2. Effect of dietary protein content on performance and energy partition of Iberian pigs growing from 50 to 100 kg BM and fed diets of similar energy content.

| | Dietary protein concentration (g/kg DM) | | | | SEM ¹ | LS ² |
|------------------------------|---|--------------------|---------------------|--------------------|------------------|-----------------|
| | 145 | 120 | 95 | 70 | | |
| d(E) | 0.791 ^a | 0.814 ^b | 0.838 ^c | 0.862 ^d | 0.0028 | *** |
| m(E) | 0.773 ^a | 0.798 ^b | 0.826 ^c | 0.853 ^d | 0.0028 | *** |
| ME/DE | 0.977 ^a | 0.981 ^b | 0.986 ^c | 0.989 ^c | 0.0012 | *** |
| MEI (MJ/d) | 35.79 ^a | 36.54 ^b | 37.10 ^b | 37.97 ^c | 0.233 | *** |
| DN intake ³ | 45.9 ^a | 37.8 ^b | 30.3 ^c | 22.4 ^d | 0.36 | *** |
| BM gain ³ | 594 ^a | 643 ^b | 674 ^c | 657 ^{bc} | 9.8 | *** |
| Protein gain ³ | 48 | 50 | 54 | 47 | 2.4 | ns |
| Fat gain ³ | 342 ^a | 368 ^b | 398 ^c | 411 ^c | 6.5 | *** |
| RE ⁴ | 596 ^a | 634 ^b | 688 ^c | 699 ^c | 10.0 | *** |
| RE _p ⁴ | 46 | 48 | 52 | 45 | 2.3 | ns |
| RE _f ⁴ | 549 ^a | 586 ^b | 637 ^c | 654 ^c | 10.5 | *** |
| RE _p /RE | 0.080 ^a | 0.078 ^a | 0.075 ^{ab} | 0.065 ^b | 0.0039 | * |
| RE _f /RE | 0.920 ^a | 0.922 ^a | 0.925 ^{ab} | 0.935 ^b | 0.0039 | * |

¹ Standard error of the mean

² Level of significance *** P<0,001; ** P<0,01; * P<0,05; ns, not significant

³ expressed in g/d; ⁴ expressed in kJ/kg BM^{0,75}.d

a, b, c, d, Within the same row, values bearing different superscripts differ significantly (P< 0.05)

Table 3. Effect of feeding level on performance and energy partition of Iberian pigs growing from 50 to 100 kg BM and fed diets of similar energy content.

| | Level of feeding (x ad libitum) | | | SEM ¹ | LS ² |
|---|---------------------------------|--------------------|--------------------|------------------|-----------------|
| | 0.95 | 0.80 | 0.60 | | |
| d(E) | 0.823 ^a | 0.820 ^a | 0.835 ^b | 0.0025 | *** |
| m(E) | 0.814 ^a | 0.806 ^a | 0.820 ^b | 0.0024 | *** |
| ME/DE | 0.985 | 0.982 | 0.982 | 0.0010 | ns |
| ME intake (MJ/d) | 44.24 ^a | 37.60 ^b | 28.71 ^c | 0.201 | *** |
| DN intake (g/d) | 40.0 ^a | 35.0 ^b | 27.2 ^c | 0.32 | *** |
| BM gain (g/d) | 780 ^a | 662 ^b | 484 ^c | 8.7 | *** |
| Protein gain (g/d) | 58 ^a | 49 ^b | 41 ^c | 2.1 | *** |
| Fat gain (g/d) | 471 ^a | 391 ^b | 277 ^c | 5.7 | *** |
| RE (kJ/kg BM ^{0,75} .d) | 810 ^a | 671 ^b | 481 ^c | 8.7 | *** |
| RE _p (kJ/kg BM ^{0,75} .d) | 56 ^a | 47 ^b | 40 ^c | 2.0 | *** |
| RE _f (kJ/kg BM ^{0,75} .d) | 754 ^a | 624 ^b | 441 ^c | 8.7 | *** |
| RE _p /RE | 0.069 ^a | 0.071 ^a | 0.084 ^b | 0.003 | ** |
| RE _f /RE | 0.931 ^a | 0.929 ^a | 0.916 ^b | 0.003 | ** |

¹ Standard error of the mean

² Level of significance *** P<0,001; ** P<0,01; * P<0,05; ns, not significant

a, b, c Within the same row, values bearing different superscripts differ significantly (P< 0.05)

homogeneous regressions coefficients and a pooled equation was calculated accordingly. This equation estimates the net efficiency of use of ME for growth (k_g) as 0.54 (± 0.020), a value unexpectedly low taking into account the relative proportion in which fat and protein are deposited in these animals. This pooled equation also underestimate ME requirements for maintenance ($ME_m = 230 \text{ kJ/kg BM}^{0.75} \cdot \text{d}$, a value far lower than that reported by Nieto *et al.*(2002) for the Iberian pig growing from 15 to 50 kg BM). Estimates of the partial efficiencies of ME utilization for protein deposition (k_p) and fat deposition (k_f) was calculated by means of a multiple regression equation (Kielanowski, 1965) using ME intake (kJ/kg BM $^{0.75} \cdot \text{d}$) as dependent variable and energy retained as protein (RE_p , kJ/kg BM $^{0.75} \cdot \text{d}$) and energy retained as fat (RE_f , kJ/kg BM $^{0.75} \cdot \text{d}$) as independent variables. Estimates of the energy cost of protein and fat deposition are 4.01 ($k_p = 0.25$) and 1.64 ($k_f = 0.61$), respectively, which differ considerably from the values reported by ARC (1981) and also are lower than previous estimates for lighter Iberian pigs (Nieto *et al.*, 2002). This equation predicts ME_m as 270 kJ/kg BM $^{0.75} \cdot \text{d}$. Further investigation is needed to deepen our knowledge on the energy metabolism of this lipogenic breed.

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Effect of a jugular infusion of essential AA on splanchnic metabolism of glucose and hormones in dairy cows fed a protein deficient diet

R. Berthiaume¹, M.C. Thivierge², G.E. Lobley³, P. Dubreuil⁴, M. Babkine⁴ & H. Lapierre¹

¹ *Dairy & Swine R&D Centre, Lennoxville, Québec, Canada*

² *Université Laval, Québec, Canada*

³ *Rowett Research Institute, Aberdeen, UK*

⁴ *Université de Montréal, St-Hyacinthe, Québec, Canada*

Summary

Six lactating Holstein cows were used to measure the effect of a jugular infusion of essential amino acids (AA) on splanchnic metabolism of glucose and hormones in dairy cows fed a protein deficient diet, according to a crossover design. A 15% CP total mixed ration (27% grass silage; 26% corn silage; 47% concentrate) was fed in twelve equal meals per d (mean DMI: 17.0 kg d⁻¹). Indwelling catheters had been surgically implanted in the mesenteric artery, the portal and hepatic veins for blood collection, and in two distal branches of the mesenteric vein to allow infusion of *p*-aminohippurate to determine plasma flow. After 5 days of continuous infusion of saline or AA (mixture of Arg; 36.7 g d⁻¹, His; 16.6 g d⁻¹, Ile; 44.3 g d⁻¹, Leu; 60.7 g d⁻¹, Lys; 59.3 g d⁻¹, Met; 17.4 g d⁻¹, Phe; 36.2 g d⁻¹, Thr; 44.9 g d⁻¹, Trp; 10.0 g d⁻¹, Val; 43.4 g d⁻¹), six hourly blood samples were collected to determine plasma concentrations of glucose, insulin, glucagon, somatotropin (GH) and insulin-like growth factor-1 (IGF-1). Yields of milk (29.2 vs 31.3 ± 0.46 kg/d and protein (912 vs 1047 ± 21.7 g/d) increased ($P < 0.05$) with AA infusion. Infusion of AA had no effect ($P > 0.05$) on arterial concentrations of glucose (3.20 vs 3.21 ± 0.02 mM), insulin (0.89 vs 0.79 ± 0.07 ng ml⁻¹), glucagon (0.11 vs 0.14 ± 0.02 ng ml⁻¹), GH (3.19 vs 3.35 ± 0.52 ng ml⁻¹) and IGF-1 (133.4 vs 140.6 ± 13.2 ng ml⁻¹). Moreover, neither net fluxes across the portal-drained viscera, nor net hepatic fluxes of glucose and glucagon were affected by infusion of AA. However, both the insulin:glucagon ratio and hepatic extraction of insulin were significantly decreased by the vascular infusion of AA.

Keywords: *amino acids, hormones, dairy cows*

Introduction

Nutrient-hormone interactions determine the efficiency with which dietary intake is converted into milk constituents in the lactating cow. Because absorbed nutrients are presented to tissues in sequence (gut, liver, mammary glands) the amounts of nutrients (AA) leaving the liver match or surpass the needs for milk production and are probably controlled by interactions between hormones (insulin, glucagon, IGF-1 and GH) at both hepatic and mammary glands sites. The importance of either individual hormones or combinations can be tested by increasing supply beyond the liver, for example by intra-jugular infusion of essential AA. This has been shown (MacRae *et al.*, 2000) to enhance milk yield, milk protein content and milk protein output but lead to reduced or even negative AA supply across the liver (Berthiaume *et al.*, 2002). The observed increases in milk yield suggest that vascular infusions of essential AA do increase glucose availability, the primary factor governing the volume of milk produced. The current study examines the impact of increasing mammary glands metabolism at the same time that marked changes occur in catabolism across the liver on endocrine balance and on glucose economy in order to further understand the site and nature of metabolic regulation.

Materials and Methods

Six multicatheterized lactating (159 ± 2 DIM) Holstein cows (610 ± 67 kg) were used to measure the effect of a jugular infusion of essential AA on splanchnic metabolism of hormones and glucose in dairy cows fed a protein deficient diet, according to a crossover design. A 15% CP total mixed ration (27% grass silage; 26% corn silage; 47% concentrate) was fed in twelve equal meals per d (mean DMI: 17.0 kg d^{-1}). This diet covered 90% of the protein requirements and 100% of the NE_I requirements of lactating cows (NRC, 1989). Indwelling catheters had been surgically implanted in the mesenteric artery, the portal and hepatic veins for blood collection, and in two distal branches of the mesenteric vein to allow infusion of *p*-aminohippurate (PAH) to determine plasma flow. The Fick principle was used to determine mammary plasma flow using Phe and Tyr as marker AA. After 5 days of continuous infusion of saline or AA (mixture of Arg; 36.7 g d^{-1} , His; 16.6 g d^{-1} , Ile; 44.3 g d^{-1} , Leu; 60.7 g d^{-1} , Lys; 59.3 g d^{-1} , Met; 17.4 g d^{-1} , Phe; 36.2 g d^{-1} , Thr; 44.9 g d^{-1} , Trp; 0.0 g d^{-1} , Val; 43.4 g d^{-1}), six hourly blood samples were collected to determine plasma concentrations of PAH, glucose, insulin, glucagon, GH and IGF-1. PAH concentrations were determined by a colorimetric method (Reynolds *et al.*, 1989). An enzymatic method (Kit #166391, Boehringer Mannheim, Dorval, Qc, Canada) was used to determine plasma glucose. For glucagon analysis, samples with aprotinin were centrifuged at 3000 RPM for 12 min and plasma samples were kept frozen until analysed. Plasma hormone concentrations were determined using double antibody RIA as described by Lapierre *et al.* (2000). Inter and intra-assay coefficients of variation for insulin, glucagon, GH and IGF-1 were 6.6, 13.2, 0.2, 8.4 and 9.1, 6.4, 0.7, 0.8%, respectively. GH and IGF-1 concentrations were determined on arterial plasma only while glucagon, insulin and glucose concentrations were determined on arterial, portal and hepatic plasma samples.

Results

Part of the data from this project was reported earlier (Berthiaume *et al.*, 2002). Briefly, yields of milk (29.2 vs $31.3 \pm 0.5 \text{ kg d}^{-1}$) and protein (912 vs $1047 \pm 21.7 \text{ g d}^{-1}$) increased ($P < 0.05$) with AA infusion.

Infusion of AA had no effect ($P > 0.05$) on arterial concentrations (*Table 1*) of glucose (3.20 vs $3.21 \pm 0.02 \text{ mM}$), insulin (0.89 vs $0.79 \pm 0.07 \text{ ng ml}^{-1}$), glucagon (0.11 vs $0.14 \pm 0.02 \text{ ng ml}^{-1}$), GH (3.19 vs $3.35 \pm 0.52 \text{ ng ml}^{-1}$) and IGF-1 (133.4 vs $140.6 \pm 13.2 \text{ ng ml}^{-1}$). However, the insulin:glucagon ratio in arterial plasma decreased ($P = 0.08$) due to AA infusion. Essential AA infusion had no effect on insulin:glucagon ratio in portal and hepatic plasma (data not shown).

*Table 1. Effect of essential AA infusion on arterial plasma concentrations of glucose and hormones in lactating Holstein cows*¹.

| Item | Treatment | | SEM | <i>P</i> |
|---------------------------------|-----------|--------|-------|----------|
| | Control | EAA | | |
| Glucose (mM) | 3.20 | 3.21 | 0.02 | 0.69 |
| Glucagon (ng ml ⁻¹) | 0.11 | 0.14 | 0.02 | 0.20 |
| Insulin (ng ml ⁻¹) | 0.89 | 0.79 | 0.07 | 0.23 |
| Insulin : Glucagon | 8.84 | 6.44 | 0.92 | 0.08 |
| GH (ng ml ⁻¹) | 3.19 | 3.35 | 0.52 | 0.84 |
| IGF-1 (ng ml ⁻¹) | 133.39 | 140.58 | 13.25 | 0.40 |

¹ Least squares means with SEM, n = 6.

Essential AA infusion had no effect on net TSP fluxes of glucose, glucagon and insulin (*Table 2*). Also, treatment had no significant effect on mammary uptake of glucose.

However, essential AA infusion reduced the proportion of insulin extracted by the liver (*Table 3*) but had no effect on hepatic extraction of glucagon and mammary extraction of glucose.

Discussion

In this study, the increment in milk production observed with AA infusion was not related with changes in circulating concentrations or net splanchnic fluxes of the measured hormones nor was it associated with an increase in the net TSP flux or mammary glands uptake of glucose which is surprising considering that glucose extraction has been assumed to determine the rate of milk secretion (Clark, 1975). Circulating levels and net splanchnic fluxes of glucose, insulin, glucagon and GH in the control group were similar to those reported by Reynolds *et al.* (1989). Surprisingly,

Table 2. Effect of essential AA infusion on splanchnic net fluxes of glucose and hormones and on mammary uptake of glucose and insulin in lactating Holstein cows¹.

| Item | Tissue ² | Treatment | | SEM | P |
|---------------------------------|---------------------|-----------|--------|-------|------|
| | | Control | EAA | | |
| Glucose (mmol h ⁻¹) | PDV | 51.9 | -20.0 | 75.2 | 0.51 |
| | Liver | 519.5 | 593.2 | 115.8 | 0.22 |
| | TSP | 568.1 | 546.4 | 95.7 | 0.85 |
| | MG | -373.0 | -409.3 | 39.3 | 0.36 |
| Glucagon (μg h ⁻¹) | PDV | 39.5 | 55.5 | 7.3 | 0.20 |
| | Liver | -29.0 | -30.2 | 3.5 | 0.82 |
| | TSP | 8.8 | 22.2 | 6.4 | 0.22 |
| Insulin (μg h ⁻¹) | PDV | 316.8 | 440.6 | 56.5 | 0.16 |
| | Liver | -171.1 | -143.0 | 32.4 | 0.52 |
| | TSP | 97.8 | 215.5 | 58.0 | 0.16 |

¹ Least squares means with SEM given for n = 6

² Tissue: PDV = portal-drained viscera; TSP = total splanchnic tissue; MG = mammary glands;

Table 3. Effect of essential AA infusion on hepatic extraction of insulin and glucagon and mammary extraction of glucose in lactating Holstein cows¹.

| Item | Treatment | | SEM | P |
|----------------------|-----------|-------|------|------|
| | Control | EAA | | |
| Hepatic ² | | | | |
| Insulin | 71.3 | 36.5 | 9.8 | 0.07 |
| Mammary ³ | Glucagon | 74.0 | 54.8 | 11.6 |
| Glucose | -19.3 | -21.3 | 1.6 | 0.41 |

¹ Least squares means with SEM, n = 6.

² calculated as: (net liver flux / net PDV flux) x 100.

³ calculated as: ((M - A) / A) x 100.

although hepatic extraction of AA increased with the infusion of AA, this effect did not appear to be mediated by glucagon. However, the significant decrease in the insulin:glucagon ratio due to treatment might have reflected an increased demand for endogenous glucose. Glucose precursors (e.g.: alanine) have been shown to elicit a fall in the insulin:glucagon ratio when infused in the jugular vein of humans (Unger & Dallas, 1971). Circulating levels of alanine, glycine and serine were all decreased (data not shown) by the essential AA infusion in the present trial. Also, short term jugular infusions of AA have been associated with increases in the circulating levels of insulin, but this was not the case in this study. The observed increase in milk protein output without an increase in mammary blood flow (data not shown) may have resulted from the higher delivery (blood flow x arterial AA) to the glands, with this impacting on transport into mammary cells.

Conclusion

When cows are fed a protein deficient diet, the increment in milk protein secretion induced by infusion of EAA probably results from the stimulation of cellular protein synthesis by one (or more) of the supplemental AA, rather than changes in either the number and/or sensitivity of endocrine receptors.

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A new concept for feed evaluation based on standardised digestible amino acids and potential physiological energy of nutrient fractions in pig feeds

S. Boisen

Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark.

Summary

A new concept for feed evaluation has been developed. The purpose was to obtain a scientifically more correct basis for pig feed optimisation according to the contribution of biologically available amino acids and energy from the ingredients into the diet and the actual requirements of the pig, respectively. The energy value of individual nutrient fractions is based on the potential physiologically available energy (PhE) which is conserved in adenosine triphosphate (ATP) when they are oxidised at cellular level, whereas the protein value is based on standardised digestible amino acids (SDAA). The contributions of PhE and SDAA from ingredients are generally additive in the complete diets that should be optimised according to the actual requirements of the pig for energy and ideal protein. On the other hand, only starch can be considered to be a pure energy source, whereas all other nutrient fractions have additional physiological properties depending on their contribution in the diet and the actual use of the diet. In future, feed evaluation should be based on a common international system based on all specific properties of the individual nutrients.

Keywords: feed evaluation, physiological energy, standardised digestible amino acids

Introduction

Feed evaluation has been under development during more than a century. Traditionally, the energy value of the feed has been considered to be the principal property. The basis for energy evaluation has moved from digestible energy (DE) and metabolisable energy (ME) to net energy (NE). Different feed evaluation systems based on NE have been developed in the former East Germany, Denmark, The Netherlands and France (Boisen & Verstegen, 1998). The general philosophy behind NE has been to more accurately describe the feed's production value for the animal (Chiba, 2000). However, the more the system attempts to describe the productive processes the more the value depends upon the animal itself, and since the animal factors increase the variability of the response, the less precise the measure becomes (Fuller, 1997). Therefore, estimation of NE is difficult and imprecise and influenced by many factors and the use of NE is too sensitive to be of practical use (NRC, 1988). Consequently, NE is unlikely to provide any greater precision in formulating diets or predicting responses compared with the ME or DE systems (Wittemore, 1993). Moreover, present feed evaluation systems are generally based on regression analyses of animal production results for estimating general energy coefficients for the different nutrient fractions. Therefore, the obtained energy values are significantly influenced by a large number of variable factors relating to the specific experiments for their determination, e.g. the feed, feeding strategy, environment etc. (Boisen & Verstegen, 2000).

The purpose for the present paper is to describe the outline for a new concept for feed evaluation based on the properties of the feed itself, i.e. the potential value of the feed according to its composition of biologically available nutrients which are defined according to their physiological utilisation.

New concept for feed evaluation

Physiological energy value

The physiological energy is a direct measure for the energy from the digested nutrients that is available for the animal and corresponds to the potential production of ATP at cellular level after complete oxidation of the nutrients. According to Figure 1 the metabolite, AcCoA, which contribute to a dominating portion of the ATP production, plays a central role in the oxidative degradation of the different nutrients. It follows, that the specific properties of the individual nutrients are quickly lost during oxidation and the remaining property is solely related to their energy value and, therefore, their relative energy value can be precisely determined.

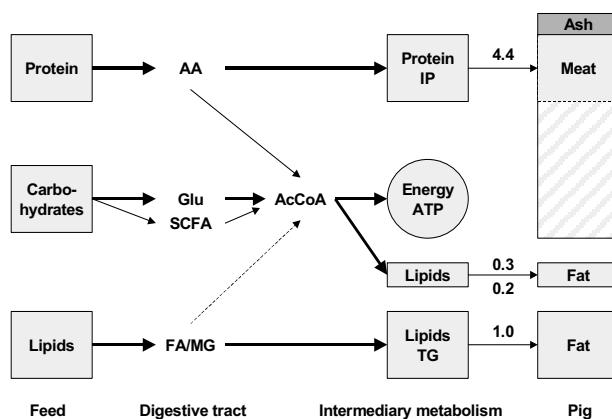


Figure 1. Metabolism of digestible nutrient fractions to energy or deposited nutrients in the pig. Abbreviation: AA: amino acids; IP: ideal protein; Glu: glucose; SCFA: short chained fatty acids; AcCoA: Acetyl Coenzyme A; FA: Fatty acids; MG: monoacyl-glycerols; TG: triacyl-glycerols (Boisen & Verstegen, 2000).

Generally, glucose from digested starch is the dominating energy source, in particular for growing pigs. Therefore, the potential energy value of the other nutrient fractions should be considered in relation to the energy value of starch, i.e. the relative energy value at cellular level when the nutrient replaces starch in the diet. Thus, for the growing pig dietary lipids save the animal for energy costs for fat synthesis from glucose because the pig deposits more fat during growth than the general dietary supply of lipids. The saved energy costs, when substituting starch with lipids, should be credited the energy value of the lipid fraction.

Figure 1 also illustrates that the dietary supply of protein has a major impact on growth and feed utilisation because 1 kg deposited protein is accompanied by 3.4 kg extra gain due to the associated retention of water and ash (minerals). On the other hand, surplus dietary protein increases the energy metabolism and has also negative environmental effects. Therefore, a precise protein supply is generally very important for animal production.

Standardised digestible amino acids

The digestibility of protein and amino acids is significantly influenced by various endogenous losses during digestion. These losses can be divided into 1) a basal loss which correspond to a minimum loss related to the feed intake and 2) extra losses which is caused by the specific properties of the feed and can be predicted from the undigested dry matter at ileal level (Boisen,

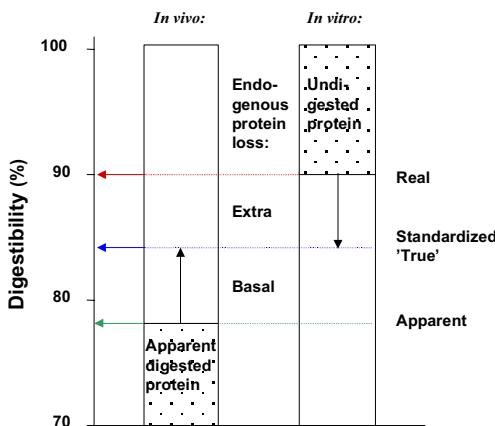


Figure 2. Calculation of standardised digestibility of protein from *in vivo* or *in vitro* analyses, respectively (Boisen, 2000).

1998). Standardised digestible amino acids can be calculated from the apparent digestibility, obtained by *in vivo* studies, or from the real digestibility obtained by *in vitro* analysis that is considered as a direct measure for the potential protein digestibility (Figure 2).

Feed optimisation

The dominant properties of the feed are generally accepted to be the energy value and the protein value, respectively. The protein value is defined by the amount of available ideal protein for the particular pig category. Available ideal protein corresponds to the contribution of standardised digestible essential and semi-essential amino acids relative to the specified requirements of the pig for the particular production. The energy value is defined by a feed unit for pigs (FUp) corresponding to the physiological energy value in a simple standard diet for slaughter pigs. Requirements are expressed in g standardised digestible amino acids per FUp.

Nutrient based feed evaluation

The described feed evaluation system is implemented in Denmark and is based on well-documented measurements of the potential physiologically available energy from the different nutrients. However, only starch can be considered as a pure energy source while all other nutrients have additional physiological effects that can influence the digestibility, metabolisability and utilisation of the nutrient itself as well as of the other nutrients.

Thus, lipids may improve the palatability of the diet, which increase voluntary feed intake. Lipids also have a negative effect on gastric emptying rate, which may improve the efficiency in the digestion of protein and carbohydrates. Furthermore, the digestibility of the lipids themselves is influenced by the composition of dietary fatty acids and can be reduced by a high proportion of animal fat that has a high proportion of saturated fatty acids.

On the other hand, dietary fibres have a general negative effect on the energy intake and digestibility of the other nutrient. Dietary fibres also increase the endogenous losses of protein and lipids during the digestion processes. Furthermore, they have significant effects on the development of the intestinal tissues and reduce the contribution of the carcass of the slaughter weight of the pigs.

The negative effects of surplus protein in the diet and the specific effects of antinutritional factors (ANF's) in many vegetable feedstuffs need also to be included in the feed evaluation.

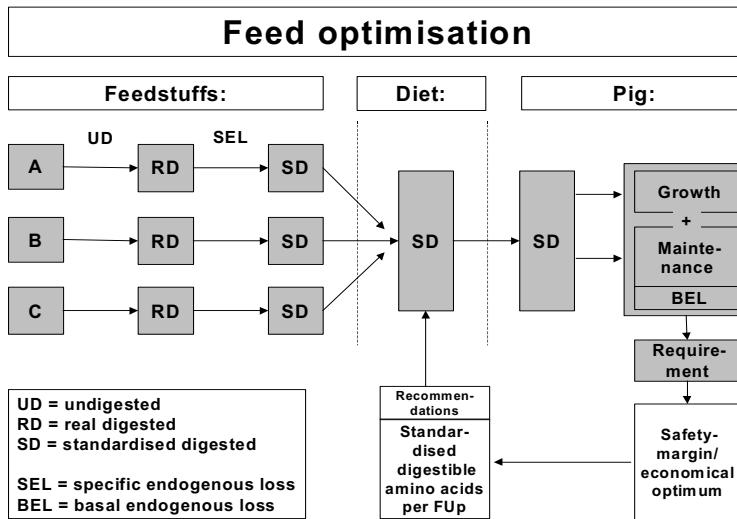


Figure 3. Feed optimisation based on standardised digestible nutrients in the feedstuffs and recommendations, respectively. Recommendations are based on requirements for growth and maintenance, which include basal endogenous losses of protein and lipids.

Conclusion

Feed evaluation for farm animals is still premature and need to be further developed. Future feed evaluation should be based on a common international system. The system should include all scientifically documented knowledge about the effect on the energy value, as well as the additional specific physiological properties, of the individual nutrient fractions for the different animal categories. A common international system would offer the optimal conditions for further scientific research within nutrition science as well as general trade with animal feeds.

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Numerical solution of an optimal control problem in mathematical biology

R. Burlacu

University of Agricultural Sciences and Veterinary Medicine-Bucharest, Dep. of Mathematics
Bvd. Marasesti 59, sector 1, cod:Ro-71331 Bucuresti, Romania

Summary

We apply the Dynamic Programming Method to an optimal control problem modeling some metabolic processes and prove that numerical integration of a 4 dimensional Hamiltonian system provides not only the numerical but also the complete theoretical solution in the form of an optimal feedback control.

Keywords: mathematical model, metabolic process, optimal control

Introduction

The aim of this paper is to report the numerical and also the theoretical solution of an optimal control problem modeling some metabolic processes.

Previous work of the authors in Burlacu, R. & S. Mirica (1997) in consisting in selection and organization of a coherent set of quantitative relations in the physiology of nutrition (e.g. Burlacu, Gh. *et al.* (1996), Vermorel, M. *et al.* (1987), Whittemore, C. (1993) etc.) resulted in a control system that describe the evolution of the total proteins and the total weight of the body (the “state variables”) corresponding to the feeding policies represented by the administrated digestible crude proteins and digestible energy (the “control variables”) in the food.

Adding reasonable “terminating rules” and optimality criteria we have obtained in Burlacu, R. & S. Mirica (1997) several non-trivial optimal control problems that are characterized by strong nonlinearities of the involved functions and by the fairly complex state and control constraints that classify these problems among the non-standard ones, defined by differential inclusions rather than by parameterized differential equations.

In order to solve one of these problems, aiming at the maximization of an aggregate of proteins and total weight on a fixed time-interval, we apply the Dynamic Programming Method as described in Mirica, S. (1990) and show that the numerical integration of a 4-dimentional Hamiltonian system provides not only the numerical solution but also its complete theoretical solution in the form of an optimal feedback control.

Mathematical model for some metabolic processes

We recall shortly the mathematical model in Burlacu, R. & S. Mirica (1997) of some metabolic processes that, in principle, are common to a large class of organisms; however, as a concrete example we consider the case of commercial suines of ages between 75-180 days.

We choose the following *state variables*:

$x_1(t)$ - proteins in the body (at time $t \geq 0$)

$x_2(t)$ - total weight of the body

and the following control variables:

$u_1(t)$ - digestible crude protein in the food (at time $t \geq 0$)

$u_2(t)$ - digestible energy in the food

For the considered class of organisms we have the *state constraints*:

$$x_1 \in (x_1^m, x_1^M) = (3; 20), x_2 \in (x_2^m, x_2^M) = (25; 120) \quad (2.1)$$

and the *control constraints*:

$$a_1(x_2) \leq u_1 \leq b_1(x_2), a_2(x_1, x_2, u_1) \leq u_2 \leq b_2(x_2) \quad (2.2)$$

defined by the following functions:

$$\begin{aligned} a_1(x_2) &:= 0.002077 \cdot x_2^{0.75}, b_1(x_2) := 0.002077 \cdot x_2^{0.75} + 0.1944 \\ a_2(x_1, x_2, u_1) &:= 59.332 \cdot u_1 + 0.3016 \cdot x_2^{0.75} + 3.6 \cdot x_1 \cdot x_2^{0.75} + 3.6 \cdot \\ x_1 \cdot \frac{(0.72 \cdot u_1 - 0.0015 \cdot x_2^{0.75})}{(9.66 + 0.23 \cdot x_1)}, b_2(x_2) := 55 \cdot (1 - e^{-0.0172}) \end{aligned} \quad (2.3)$$

The dynamics of the system is described by the control system:

$$\begin{cases} \dot{x}_1 = f_1(x_2, u_1(t)), x_1(0) = x_1^0 \in (x_1^m, x_1^M) \\ \dot{x}_2 = f_2(x_1, x_2, u_1(t), u_2(t)), x_2(0) = x_2^0 \in (x_2^m, x_2^M) \end{cases} \quad (2.4)$$

defined by the following functions:

$$\begin{aligned} f_1(x_2, u_1) &:= 0.72 \cdot u_1 - 0.0015 \cdot x_2^{0.75} \\ f_2(x_1, x_2, u_1, u_2) &:= x_2^{0.75} \cdot \left(\frac{0.001 \cdot x_1}{9.66 - 0.23 \cdot x_1} - 0.094 - \frac{0.006}{x^{0.145}} \right) + \\ u_1 \cdot \left(0.506 + \frac{3.16}{x^{0.145}} - \frac{0.0509 \cdot x_1}{9.66 - 0.23 \cdot x_1} \right) + 0.0196 \cdot u_2 \end{aligned} \quad (2.5)$$

The mathematical coherence of the model amounts to the following:

Proposition 2.1 (Burlacu, R. & S. Mirica, 1997) *The multifunction $U(\cdot)$ of admissible control parameters in (2.2) has nonempty values i.e.:*

$$\begin{aligned} U(x) &:= (u_1, u_2); a_1(x_2) \leq u_1 \leq b_1(x_2), a_2(x_1, x_2, u_1) \leq u_2 \\ &\leq b_2(x_2) \neq \emptyset, \forall x \in Y := (x_1^m, x_1^M) \times (x_2^m, x_2^M) \end{aligned}$$

Terminating rules and optimality criteria

As it is well known (e.g. Cesari, L., 1983), Mirica, S. (1990) etc.), from the point of view of “terminating rules” of a control system one may distinguish the following two classes of problems:

I. *Fixed time - interval problems* in which the time - interval $[0, T]$ is fixed, a subset $Y_1 \subset Y$ of “terminal states” is given and the *admissible trajectories*, $x(\cdot)$ (corresponding via (2.4) to *admissible controls*, $u(\cdot)$) are required to satisfy the *terminal constraints*:

$$x(T) \in Y_1 \quad (3.1)$$

II. *Variable time - interval problems* in which a set of terminal states $Y_1 \subset Y$ is given and the admissible trajectories are required to satisfy the condition:

$$\exists t_1 > 0 : x(t_1) \in Y_1 : x(t) \in Y_0 : Y_1 \cap Y_0 \neq \emptyset \quad (3.2)$$

For each of these classes of problems one may consider different types of optimality criteria that have practical significance; in our case one may aim at the maximization of protein and/or the total weight of the body, the maximization of the profit, minimization of the cost, etc.

As a concrete example we choose to fix time-interval problem defined by:

$$[0, T] = [0; 90], x(T) \in Y_1 := (13; 20) \times (95; 120) \quad (3.3)$$

and the following *cost-functional*:

$$C(u(\cdot)) := -\lambda \cdot x_1(T) + (1-\lambda) \cdot x_2(T), \lambda = 0.8 \quad (3.4)$$

whose minimization amounts to the maximization of a certain aggregate of proteins and total weight on the specified time-interval.

Therefore, in what follows we shall try to solve the following:

Problem 3.1: For any $(s, y) \in E_0 := [0, T] \times Y$, minimize the functional $C(u(\cdot))$ in (3.4) subject to conditions (2.1), (2.2), (2.4), (3.1) defined by the data in (2.3), (2.5), (3.3).

Outline of Dynamic Programming Method in the general case

For convenience of the reader we present shortly the main steps of Dynamic Programming Method as described in Mirica, S. (1990) for general Mayer-type optimal control problems of the form:

Minimize : $C(u(.)) := g(x(T))$

$$\text{Subject to : } \begin{cases} \dot{x} = f(x, u(t)), x(s) = y \\ u(t) \in U(x(t)) \text{ a.e. } [s, T] \\ x(t) \in Y \subset R^n, \forall t \in [s, T] \\ x(T) \in Y_1 \subset Cl(Y) \end{cases}$$

STEP I (*The Hamiltonian system*) consists in computing (characterization, description, etc.) of the *Hamiltonian and of the associated multifunction of minimizing control parameters* defined by:

$$H(x, p) := \min_{u \in U(x)} \langle p, f(x, u) \rangle, x \in Y \subseteq R^n, \hat{U}(x, p) := \{u \in U(x); \langle p, f(x, u) \rangle = H(x, u)\},$$

$$(x, p) \in Z \subseteq Y \times R^n$$

and the choice of a *Hamiltonian inclusion* of the form:

$$(x', p') \in d^{\#} H(x, p) \quad (4.2)$$

that generalize the *classical Hamiltonian system*:

$$p' = \left(\frac{\partial H}{\partial p}(x, p), -\frac{\partial H}{\partial x}(x, p) \right) \quad (4.3)$$

at the points $(x, p) \in Int(A)$ at which $H(., .)$ is differentiable.

If $H(., .)$ is a stratified function ([4]) then one may choose:

$$d^{\#} H(x, p) = d^{\#}_S H(x, p) := \{(x', p') \in T_{(x, p)} A; x' \in f(x, \hat{U}(x, p))\},$$

$$\langle x', v \rangle - \langle p', u \rangle = DH(x, p) \cdot (u, v) \quad \forall (u, v) \in T_{(x, p)} A \quad (4.4)$$

and otherwise one may choose Hamiltonian orientor fields either by its contingent semi-differentials or by Clarke's generalized gradient (e.g. Mirica, S. (1990)):

STEP II (*End-point conditions*) consists in adding to the differential system in (4.3) end-point condition which, in the case $Y_1 \subset R^n$ is open and $g(.)$ is differentiable are of the form:

$$X(T) = \xi \in Y_1, p(T) = Dg(\xi) \quad (4.5)$$

STEP III (*Construction of a Hamiltonian flow*) is the first main computational operation which consists in the “backwards integration” of the differential system (4.3) with the terminal condition in (4.6) and the choice of a certain *Hamiltonian flow*, $X^*(., \xi) = (X(., \xi), P(., \xi)) : (t^-(\xi), T] \rightarrow Z, \zeta \in Y_1$ (parameterised family of solutions of (4.3), (4.6)) that satisfy conditions of the form:
 $X(t, \xi) \in Y, \forall t \in (t^-(\xi), T].$ (4.6)

STEP IV (*value function and selection of admissible trajectories*) consists in computing (characterizing, etc.) the (possible) *value function* defined by:

$$(4.7) \quad W(s, y) := \begin{cases} g(y) & \text{if } (s, y) \in E_1 := \{T\} \times Y_1 \\ \inf_{x_{(s, \zeta)=y}} g(\zeta) & \text{if } (s, y) \in E_0 := [0, T] \times Y \end{cases}$$

the corresponding *marginal multifunction* :

$$\hat{\xi}(s, y) := \{\xi \in Y_1; X(s, \xi) = y, W(s, y) = g(\xi)\}, (s, y) \in E_0 \quad (4.8)$$

and the multi-selection of (possibly optimal) *admissible trajectories*:

$$\hat{E}_0 \ni (s, y) \rightarrow A(s, y) := \left\{ X(., \xi) / [s, T]; \xi \in \hat{\xi}(s, y) \right\} \quad (4.9)$$

STEP V (*theoretical arguments*) consists in using a suitable verification theorem for $W(\cdot, \cdot)$ (e.g. Cesari, L. (1983), Mirica, S. (1990)) to conclude the optimality of the trajectories in $A(\cdot, \cdot)$.

Dynamic Programming Method applied to Problem 3.1

Applying the algorithm above to Problem 3.1 we obtain the following results:

The Hamiltonian system: in view of the structure of the multifunction $U(\cdot)$ in (2.6) of admissible control parameters and the fact that the vector field in (2.4) - (2.5) is of the form.

$$f(x, u) = \hat{f}(x) + u_1 \hat{f}_1(x) + u_2 \hat{f}_2(x), \quad (5.1)$$

the Hamiltonian in (4.2) turns out to be a stratified function i.e., its domain, $Z := Y \times R^2$ admits a partition $\{Z_i; i = 1, \dots, 9\}$, into differentiable manifolds such that $H_i(\cdot, \cdot) : H(\cdot, \cdot)|Z_i, i = 1, \dots, 9$ are smooth functions; therefore we may choose the Hamiltonian inclusion in (4.3) defined by the stratified orientor field $\# H(\cdot, \cdot)$ in (4.5).

End-point (transversally) conditions: since the “terminal cost function” $g(\cdot)$ in (4.1) is given by:

$$g(x) = -\lambda x_1 - (1-\lambda)x_2, \quad \lambda = 0.8$$

the terminal conditions in (4.6) are the following:

$$x(T) = \xi \in Y_1 := (13; 20) \times (95; 120), p(T) = (-\lambda, \lambda-1) \quad (5.2)$$

Numerical computations: we verify first that for every $\xi \in Y_1$ the terminal value, $(x(T), p(T))$ in (5.1) belong to the open stratum Z_i defined by:

$$Z_1 := \{(x, p) \in Z; < p, \hat{f}_2(x) > < 0, < p, \hat{f}_1(x) + b_2(x_2) \hat{f}_2(x) > < 0\} \quad (5.3)$$

on which the marginal multifunction $\hat{U}(\cdot, \cdot)$ in (4.2) is given by:

$$\hat{U}(x, p) = \{\hat{u}(x, p)\}, \hat{u}(x, p) = (b_1(x_2), b_2(x_2)) \quad (5.4)$$

and the corresponding Hamiltonian is given by:

$$H_1(x, p) = < p, \hat{f}_0(x) + b_1(x_2) \hat{f}_1(x) + , b_2(x_2) \hat{f}_2(x) > \quad (5.5)$$

Further on, since $Z_1 \subset R^2 \times R^2$ is open, for any $\xi \in Y_1$ there exists $t_1(\xi) \in [0, T]$ such that the Hamiltonian inclusion in (4.3), (4.5), (4.6) has a unique solution $X^*(\cdot, \xi)$ which coincide on $[t_1(\xi), T]$ with the unique solution, $X_{-1}^*(\cdot, \xi) = (X_1(\cdot, \xi), P(\cdot, \xi))$ of the smooth Hamiltonian system:

$$(x', p') = \left(\frac{\partial H_1}{\partial p}(x, p), -\frac{\partial H_1}{\partial x}(x, p) \right), x(T) = \xi, \quad (5.6)$$

defined by the function $H_1(\cdot, \cdot)$ in (5.4); therefore, in what follows, an essential role is played by the function $t_1(\cdot)$ which, formally, is defined by:

$$t_1(\xi) := \inf \{t \in [0, T]; X_{-1}^*(s, \xi) \in Z_1 \forall s \in (t, T]\}, \xi \in Y_1 \quad (5.7)$$

We note that if $t_1(\xi) > 0$ for some $\xi_0 \in Y_1$ then we should study the possibility of continuing the solution $X^*(\cdot, \xi)$ for the $t \in [0, t_1(\xi_0))$.

On the other hand, due to the complexity of the functions in (2.3), (2.5), the only way of characterizing the function $t_1(\cdot)$ in (5.7) is the numerical integration of the smooth Hamiltonian system in (5.6), for which we have used the well known computer MATHCAD 7.0 and QUATTRO 8.0.

Choosing the following grid:

$$h_1 = 1, \xi_1^0 = 13, \xi_1^{i+1} = \xi_1^i + h_1, i \in \overline{0, 6}, h_2 = 3, \xi_2^{i_0} = 95$$

$$\xi_2^{i, j+1} = \xi_2^{i, j} + h_2, i \in \overline{0, 6}, j \in \overline{0, 8}, \tau = 1, t_k = T - k\tau, k \in \overline{0, 90}$$

and several experiments with more refined grids, we have integrated, numerically the differential system in (5.6) checking at each step the conditions:

$$x_1(t_k) \in (4; 20), x_2(t_k) \in (25; 120), h_2(t_k) := < p(t_k), \hat{f}_2(x(t_k)) > < 0$$

$$h_3(t_k) := < p(t_k), \hat{f}_1(x(t_k)) + b_2(x(t_k)) \hat{f}_2(x(t_k)) > < 0$$

that defines the function $t_1(\cdot)$ in (5.7) since these conditions are satisfied with margins largely greater than the possible numerical errors, we may consider that these numerical computations prove the following:

Proposition 5.1 *The function $t_1(\cdot)$ in (5.7) is given by: $t_1(\xi) = 0 \forall \xi \in Y_1$.*

Therefore, for any $\xi \in Y_1$, the Hamiltonian system in (4.3),(4.5),(4.6) for Problem 3.1 has a unique solution, $X^*(\cdot, \xi) : [0, T] \rightarrow Z$, that coincide with the smooth solution, $X_1^*(\cdot, \xi)$ of the smooth Hamiltonian system in (5.6).

Theoretical solution of problem 3.1: the statement in Prop. 5.1 and the existing theoretical results in Cesari, L. (1983), Mirica, S. (1990) etc., allow us to prove the following results that gives the complete theoretical solution of problem 3.1

Theorem 5.2. *Let $(X_1(\cdot, \cdot), P_1(\cdot, \cdot))$ be the maximal flow of the smooth Hamiltonian system in (5.6) defined by the data in (2.3), (2.5),(5.5) and let \check{E}_0 , $v(\cdot)$ be defined by:*

$$\check{E}_0 := \{(t, X_1(t, \xi)); \xi \in Y_1, t \in [0, T], v(x) := (b_1(x_2), b_2(x_2))\} \quad (5.8)$$

Then $v(\cdot)$ is the optimal feedback control of problem 3.1 restricted to the subset $\check{E} := \check{E}_0 \cup E_1$ in the following sense: for any $(s, y) \in \check{E}_0$, the unique solution $\tilde{x}(\cdot; s, y) : [s, T] \rightarrow Y$ of the differential equation:

$$\frac{dx}{dt} = f(x, v(x)), x(s) = y \quad (5.9)$$

is the optimal trajectory with respect to (s, y) and the mapping $\tilde{u}(\cdot; s, y) := v(x(\cdot; s, y))$ is the corresponding optimal control.

Proof. From (5.5), (5.6) and (5.9) it follows that the first component, $X_1(\cdot, \xi)$ of the smooth flow, $X_1^*(\cdot, \xi)$ coincide with the solution $x(\cdot; T, \xi)$ of the problem (5.9); on the other hand, from the general theory of ODE (e.g. Mirica, S. (1990)), it follows that there exists a smooth mapping $\hat{\zeta}(\cdot, \cdot)$ such that: $x(s; T, \hat{\zeta}(s, y)) = y \forall (s, y) \in E = [0, T] \times Y$ and therefore the value function in (4.8) is given by: $W(s, y) = g(\hat{\zeta}(s, y)) \forall (s, y) \forall \check{E}$ and, obviously, is a smooth mapping and also the value function of the above selection of admissible control; moreover, from the basic results in the theory of smooth Hamiltonian system (e.g. Mirica, S. (1990)) applied to the one in (5.6) it follows that $W(\cdot, \cdot)$ satisfies the relation:

$$DW(t, x) \cdot (I, f(x, u)) \geq DW(t, x) \cdot (I, f(x, v(x))) \equiv 0 \forall u \in U(x)$$

and therefore, according to the so called “elementary verification theorem” (e.g. Cesari, L. (1983), Mirica, S. (1990)), $W(\cdot, \cdot)$ coincides with the value function of problem 3.1 and $\tilde{u}(\cdot; s, y)$, $(s, y) \in \check{E}_0$ are optimal controls.

Remark 5.3 The numerical solution of the problem 3.1 may be described as follows: for any $(s, y) \in \check{E}_0$ one computes the numerical solution $x(\cdot; s, y)$ of the problem (5.9) which is taken as the optimal trajectory while $\tilde{u}(\cdot; s, y) := v(x(\cdot; s, y))$ is taken as optimal control and $W(s, y) := g(x(T; s, y))$ as the optimal value of the cost-functional in (3.4).

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Mathematical models of applied biology of some metabolic processes

R. Burlacu

*University of Agricultural Sciences and Veterinary Medicine-Bucharest, Dep. of Mathematics
Bd. Marasesti 59, sector 1, cod:Ro-71331 Bucuresti, Romania*

Summary

The mathematical model of metabolic processes in growing pigs developed by C.T. Whitmore (1983) is proved to represent an input-output discreet control system with fairly complicated control and state space constraints. We suggest some whose solutions may provide useful information on the feeding policies aiming at prescribed targets.

Keywords: mathematical model, metabolic process, optimal control

Notations

To facilitate the understanding of the exposition that follows, we give first of all a complete list of the notations we are going to use. In order to make the mathematical expressions more clear, we have simplified the notations used by most of the non-mathematician authors who, probably due to “mnemonic” reasons, use very complicated graphic expressions that make it difficult to “read” the mathematical formulas. Thus, the part of modulations and the part of “interpretation” of the mathematical results will use the following notations:

Body weight

- G = weight at an unspecified moment;
 G' = rate of weight retention at an unspecified moment;
 G_n = weight at the discrete moment (day) $n \geq 0$;
 $G(t)$ = weight at the moment $t \geq 0$;

Body water

- A = body water at an unspecified moment;
 A' = rate of water retention at an unspecified moment;
 \hat{A} = highest body water amount at an “ideal moment”, $n \geq 0$;
 $A(t)$ = body water at moment $t \geq 0$.

Body ash

- C = body ash at an unspecified moment;
 C' = rate of body ash retention at an unspecified moment;
 \hat{C} = highest amount of body ash at an “ideal moment” $t_c^* > 0$;
 C_n = body ash at moment $n \geq 0$;
 $C(t)$ = body ash at moment $t \geq 0$.

Body protein

- P = body protein at an unspecified moment;
 \hat{P} = highest amount of protein at an “ideal moment” $t_p^* > 0$;
 P_m = maintenance protein at an “unspecified moment”;
 PA = available protein;
 P' = rate of protein retention;
 \hat{P}' = highest rate of protein retention;
 P_n = body protein at the discrete moment (day) $n \geq 0$;
 $P(t)$ = body protein at moment $t \geq 0$.

Body lipids

- L = body lipids at an unspecified moment;
 L^* = highest amount of lipids at an “ideal moment” $t^* L > 0$;
 L' = rate of lipid retention;
 L_n = body lipids at a discrete moment (day) $n \geq 0$;
 $L(t)$ = body lipids at moment $t \geq 0$.

Digestible protein (administered in food)

- P^* = digestible protein administered at an unspecified moment;
 \hat{P}^* = highest amount of digestible protein that can be assimilated;
 P_n^* = digestible protein administered at the discrete moment $n \geq 0$;
 $P^*(t)$ = digestible protein administered at moment $t \geq 0$.

Digestible energy (administered in food)

- E = digestible energy administered at an unspecified moment;
 E^* = highest amount of digestible energy that can be assimilated;
 E_n = digestible energy administered at the discrete moment $n \geq 0$;
 $E(t)$ = digestible energy administered at moment $t \geq 0$.

Other symbols

- EM = metabolisable energy;
 EM_m = metabolisable energy for maintenance;
 EP = energy required for protein retention;
 EL = energy required for lipid retention;
 EU = urine energy;
 EPD = energy for protein desamination;
 ef = coefficient for the efficiency of amino acid utilization;
 vb = diet biological value.

Observation. As mentioned before, probably due to “mnemonic” reasons, the papers of researchers in biology and nutrition use more complicated notations that contain the initials of the used terms; for example: $P^* \rightarrow PBD$, $E \rightarrow ED$

Also, the notations $G_t \rightarrow G$, $A_t \rightarrow A$, $C_t \rightarrow C$, etc. (for the total amount) can be mistaken for the values $G(t)$, $A(t)$, etc. of the respective variables at the time moment $t \geq 0$.

Dynamic models commands. Discrete systems

The existence of estimates of the daily rate of protein, lipid, water and ash retention, leads naturally to a dynamic discrete model that determines the evolution over time of the variables of state P_n , L_n , C_n , A_n , G_n , $n \geq 0$, as soon as the initial states P_0 , L_0 , C_0 , A_0 , G_0 , and the feeding strategy (“command”) represented by the string $u(\cdot) = \{(P_n^*, E_n), n = 0, 1, 2, \dots\}$ of the daily values of P_n^* , E_n of the digestible protein and energy are known.

We shall now choose a certain set of relations among the most widely accepted and verified experimentally, referring to metabolism processes of commercial pigs aged 90 to 180 days and with a total body weight between 30 and 110kg. Other options are possible, leading in principle to mathematical problems of the same nature.

In order to avoid mathematical complications generated by restrictions such as equality, we shall modify the variation limits of the variables so that the restrictions in view are characterized by strict inequalities.

We shall write these relations in the dynamic context mentioned earlier, that is in the terms of G_n , P_n , L_n , A_n , C_n , P_n^* , E_n at discrete moments $n = 0, 1, 2, \dots$

We accept, thus, as first restriction, the inequality:

$$25 < G_n < 120 \quad n = 0, 1, 2, \dots \quad (1)$$

Subsequently, from among the experimental determinations for the considered organisms accepted by most of the authors, we adopt the following limits for protein variation:

$$P = 3,5 < P_n < \hat{P} = 18 \quad n = 0, 1, 2, \dots \quad (2)$$

Literature offers us enough arguments to accept formula (1.1.2.1) as being valid for each moment of time; we suppose thus that:

$$G_n = \bar{G}(P_n, L_n, A_n, C_n) := 1,05 (P_n + L_n + A_n + C_n) \quad n = 0, 1, 2, \dots \quad (3)$$

as well as the inequalities from (1.1.2.4) rewritten as

$$0,04 \cdot G_n < L_n < 0,19 \cdot G_n \quad (4)$$

which, together with the previous ones define "phase restrictions" of the general form:

$$y_n = (P_n, L_n, A_n, C_n) \in Y_0 \subset (R_+)^4 \quad (5)$$

As the rate of protein retention is concerned, we observe first that for the type of organism we have chosen, most of the authors (for ex. Whittemore, 1976 and 1983, Burlacu et al. 1987, 1996) consider that the highest rate \hat{P}' is given by:

$$\hat{P}' = 0,140 \quad (6)$$

To make a choice, from the many formulas we choose formula:

$$P'_n = \min \left\{ q_1 P_n^* - q_2 G_n^{q_3}, \hat{P}' \right\} \quad q_1 = 0,72, q_2 = 1,5, q_3 = 0,75 \quad (7)$$

This option leads to the conclusion that above a certain value, the digestible protein P_n^* does not increase any more the retention rate, P'_n , of protein and it is therefore reasonable to impose the condition:

$$q_1 P_n^* - q_2 G_n^{q_3} \leq \hat{P}'$$

which comes back to the restriction:

$$P_n^* \leq \frac{1}{q_1} \hat{P}' + \frac{q_2}{q_1} G_n^{q_3} = \alpha + \beta \cdot G_n^{q_3} \quad (8)$$

where $\alpha = 194,44$ and $\beta = 2,077$.

On the other hand, the logic of the physiological processes allows us to believe that there must be a lower limit $\hat{P}' \leq 0$ for the daily rate of protein retention, so that we must also put the condition: $q_1 P_n^* - q_2 G_n^{q_3} \geq \hat{P}'$

where from we infer:

$$P_n^* \geq \frac{1}{q_1} \hat{P}' + \frac{q_2}{q_1} G_n^{q_3} = \alpha + \beta \cdot G_n^{q_3} \quad (9)$$

Considering the particular case shown here (commercial pigs weighing between 25 and 120 kg), in the absence of experimental studies on the effects and limits of the negative rate of body protein and due to reasons belonging to the concrete nature of the studied problems, we shall assume $P' = 0$, therefore the fact that a feeding strategy is adopted which provides a retention rate $P'_n \geq 0$.

Based on these hypotheses of the evolution of body protein, we obtain the following equations:

$$\begin{cases} P_{n+1} - P_n = f_1(G_n, P_n^*) := q_1 P_n^* - q_2 (G_n)^{q_3} \\ a_1(G_n) := \beta G_n^{q_3} \leq P_n^* \leq b_1(G_n) := \alpha + \beta \cdot G_n^{q_3} \\ q_1 = 0,72, q_2 = 1,5, q_3 = 0,75, \alpha = 194,44, \beta = 2,077 \end{cases} \quad (10)$$

Subsequently, due to considerations of the same nature, for the rate of lipid retention, we use formula, which, in its dynamic variant is written as:

$$\left\{ \begin{array}{l} L_{n+1} - L_n = f_2(P_n, G_n, P_n^*, E_n) := \gamma_1 E_n - \gamma_2 P_n - \gamma_3 (G_n)^{\gamma_4} \\ \quad - \left(\gamma_5 + \frac{\gamma_6 \hat{P}}{\gamma_7 \hat{P} - P_n} \right) f_n(G_n, P_n^*) \\ \gamma_1 = 18,691, \gamma_2 = 0,213, \gamma_3 = 13,439, \gamma_4 = 0,63, \\ \gamma_5 = 0,217, \gamma_6 = 0,0878, \gamma_7 = 0,3 \end{array} \right. \quad (11)$$

As the second command parameter, digestible energy, (En) is concerned, in agreement with most of the authors we accept the higher limitation:

$$E_n \leq b_2(G_n) := 55 (1 - e^{-0,0204 G_n}) \quad (12)$$

Among the other biological restrictions we accept condition

(7) for $u_i = 1$, we suppose thus:

$$L_n' \geq P_n' \forall n = 0, 1, 2, \dots \quad (13)$$

condition which, together with the definition from (11) of the size $L_n' = L_{n+i} - L_n$ and (12) leads to the restriction of command:

$$\left\{ \begin{array}{l} a_2(P_n, G_n, P_n^*) \leq E_n \leq b_2(G_n), n = 0, 1, 2, \dots \\ a_2(P, G, P^*) := \delta_1 P^* + \delta_2 G^{q_3} + \left(\delta_4 + \frac{\delta_5 \hat{P}'}{\delta_6 \hat{P} - P_n} \right) f(G, P^*) \\ \delta_1 = 0,0114, \delta_2 = 0,719, \delta_3 = 0,63, \delta_4 = 0,0116 \\ \delta_5 = 0,005, \delta_6 = 0,3 \end{array} \right. \quad (14)$$

We observe here that, if we accept a condition as that from (8) than, condition $L_n' \leq u_2 P_n'$ leads to a restriction of the form $E_n \leq c_2(P_n, G_n, P^*)$, which must be compared to that in (12) and the model complicates furthermore, beyond the insufficient justified for the coefficient $u_2 > u_1 = 1$.

Finally, for the rate of water and ash retention we accept formulas:

$$\left\{ \begin{array}{l} A_{n+1} - A_n = A_n' = r_1 (f_1(G_n, P_n^*))^{r_2}, r_1 = 4,189, r_2 = 0,855 \\ C_{n+1} - C_n = 0,21 f_1(G_n, P_n^*) \end{array} \right. \quad (15)$$

As a conclusion, with the choices mentioned earlier and using the functions $G(\cdot)$, $f_1(\cdot, \cdot)$, $f_2(\cdot, \cdot, \cdot)$, $a_1(\cdot)$, $b_1(\cdot)$, $a_2(\cdot)$, $b_2(\cdot)$, defined in (3), (10)-(12), (14), we obtain the following mathematical description of the evolution of the state variables P_n , L_n , A_n , C_n and of the output variable G_n , function of the command parameters P_n^* , E_n :

$$\left\{ \begin{array}{l} P_{n+1} = P_n + f_1(G_n, P_n^*) \\ L_{n+1} = L_n + f_2(P_n, G_n, P_n^*, E_n) \\ A_{n+1} = A_n + r_1 (f_1(G_n, P_n^*))^{r_2} \\ C_{n+1} = 0,21 f_1(E_n, P_n^*) \\ G_n = (P_n, L_n, A_n, C_n) \end{array} \right. \quad (16)$$

that must verify the *phase restrictions*

$$\left\{ \begin{array}{l} 25 < G_n < 120 \\ P = 3,5 < P_n < \bar{P} = 18 \\ 0,04 G_n < L_n < 0,19 G_n \end{array} \right. \quad (17)$$

as well as the *command restrictions*

$$\begin{cases} a_1(G_n) \leq P_n^* \leq b_1(G_n) \\ a_2(P_n, G_n, P_n^*) \leq E_n \leq b_2(G_n) \end{cases} \quad (18)$$

In the current incipient stage of both the effort of acquiring mathematical models and the use of mathematical methods to solve concrete problems, it is normal to try to simplify as much as possible the model without changing, however, the nature of the principally, general accepted relations.

From the practical viewpoint, the existence of such a mathematical model suggests the problem of choosing a command ("strategy") $u(\cdot) = \{u_n = (P_n^*, E_n); n \geq 0\}$ which to determine a certain type of evolution of the system in accordance with the predetermined objectives.

A first stage, rather "rudimentary" from the scientific viewpoint, is that of the method of "simulation" which consists in the empirical generation, step by step, of a number (fatally finite) of commands (and trajectories) admitted and the selection of the most convenient from the viewpoint of the predetermined objectives. Such procedures are no more justified, however, under the conditions of existence of a mathematical theory of the command systems which allows, on one hand, to determine much more precise objectives, including some optimization criteria, and further more, to determine precisely the optimal commands and trajectories related to the proposed objectives.

From this viewpoint, the mathematical theory of the continuous command systems, much more elaborate than the theory of the discrete systems, seems to offer much more efficient methods to solve concrete problems.

Continuous command systems and concrete problems of optimal control

Beyond the difficulties practically impossible to overcome, to effectively solve the discrete problems of optimal control previous formulated, we must also take into consideration that in fact, the metabolic processes modeled above are continuous processes and it is therefore naturally to consider continuous mathematical models too.

First of all, instead of the "discrete" time interval $\{0, 1, 2, \dots, N\}$ we consider the "continuous" interval $[0, T]$, $T = N$, and instead of the discrete variables $y_n, G_n, u_n; n = 0, 1, \dots, N$, we consider the functions:

$$[0, T] \ni t \rightarrow y(t), G(t), u(t) \quad (19)$$

which associate to each moment of time $t \in [0, T]$, the values $y(t), G(t), u(t)$ of the variables y, G, u at the moment t .

Subsequently, the discrete moments $t = 0, 1, \dots, N$ can be replaced with analogue relations at smaller intervals, of length $\theta > 0$ which allow to pass to limit for $\theta \rightarrow 0_+$.

Without going further in details (which can be found for example in Boltianski, 1979), we admit that everything from § 2 remains valid on any interval of the form $[t, t + \theta]$, $t \in [0, T]$, and we obtain therefore:

$$\begin{cases} y(t + \Theta) = y(t) + \Theta \cdot g(y(t), G(t), u(t)), \quad t \in [0, T], \Theta > 0 \\ G(t) = \bar{G}(y(t)) \\ y(t) \in Y_0 \subset (R_+)^4, G(t) \in G_0 \in R_+ \\ u(t) \in U(y(t), G(t)) \quad \forall t \in [0, T] \end{cases} \quad (20)$$

Even in the general case in which the "average speed" of variation $g(y(t), G(t), u(t))$ also depends on the length $\theta > 0$ of the time interval, in reasonable hypothesis of continuity of the biological process, it results that the application $y(\cdot)$ can be derived and that its derivative is given by the relation:

$$\dot{y}(t) = \lim_{\Theta \rightarrow 0_+} \frac{y(t + \Theta) - y(t)}{\Theta} = g(y(t), G(t), u(t))$$

It results that in the case in which the modeled process has reasonable properties of continuity, the discrete system can be replaced with the following continuous command system:

$$\begin{cases} \dot{y}(t) = g(y(t), G(t), u(t)) \\ G(t) = \bar{G}(y(t)) \\ y(t) \in Y_0 \subset (R_+)^4 \\ u(t) \in U(y(t), G(t)) \end{cases} \quad (21)$$

Regarding the nature of applications $y(\cdot)$, $G(\cdot)$, $u(\cdot)$, we must observe first of all that in the situation of the models studied above, as well as in many other concrete problems, the commands $u(\cdot) : [0, T] \rightarrow (R_+)^2$ can be constant on several parts, continuous on other parts, described or even measurable; on the other hand, both from the mathematical viewpoint and from the viewpoint of the “physical” interpretation, the corresponding trajectories, $y(\cdot)$ as well as the output functions $G(\cdot)$ must be at least absolutely continuous, in particular regulated, or of class C^1 on certain parts. Regarding the **criteria of termination** of the continuous processes, just like in the case of the discrete systems, we have two major classes of problems:

I.A. Problems with fixed terminal time, in which $T \in R$ (in our case, $T > 0$) and two “terminal multitudes”, $Y_F \subseteq (R_+)^4$, $G_F \subseteq R_+$ are set, and an admitted trajectory, $y(\cdot) : [t_0, T] \rightarrow Y_0 \cup Y_F$ relative to the initial point $(t_0, y_0) \in [0, T] \times Y_0$ which corresponds to the admitted command $u(\cdot) : [t_0, T] \rightarrow U$ must verify the initial conditions:

$$y(t_0) = y_0 \quad (22)$$

as well as the “terminal conditions”:

$$y(T) \in Y_F, G(T) \in G_F \quad (23)$$

I.B. Problems with free terminal time in which only the terminal multitudes $Y_F \subseteq (R_+)^4$, $G_F \subseteq R_+$ are set, and for each $Y \in Y_F$, an admitted trajectory $y(\cdot) : [0, t_1] \rightarrow Y$ must verify the phase restrictions too:

$$y(0) = y_0, y(t) \in Y_0, G(t) \in G_0 \quad \forall t \in [0, \tau] \quad (24)$$

as well as the terminal restrictions too:

$$y(t_1) \in Y_F, G(t_1) \in G_F \quad (25)$$

We mention that in this formulation, the terminal moment $t_1 = t_1(y_0, y(\cdot)) > 0$ is defined as the smallest $\tau > 0$ for:

$$t_1 = \inf \{ \tau > 0; y(t) \in Y_0, G(t) \in G_0 \quad \forall t \in [0, \tau] \} \quad (26)$$

$$y(\tau) \in Y_F, G(\tau) \in G_F$$

As the criteria of optimization are concerned, the most general form of criteria of optimization for the continuous systems is of the form:

$$C(u(\cdot), y(\cdot)) = g(y(T)) + \int_{t_0}^T f_0(t, y(t), G(t), u(t)) dt \quad (27)$$

for the processes with fixed terminal time, and of the form:

$$C(u(\cdot), y(\cdot)) = g(t_1, y(t_1)) + \int_{t_0}^{t_1} f_0(t, y(t), u(t)) dt \quad (28)$$

Thus, according to the above equations, the discrete command system is replaced by the continuous command system:

$$\begin{cases} P'(t) = f_1(G(t), P^*(t)) \\ L'(t) = \bar{f}_2(P(t), G(t), P^*(t), E(t)) \\ G(t) = W(P(t), L(t)) := 1,05 [1,189 \cdot P(t) + L(t) + 4,889 (P(t))^{0,855}] \end{cases} \quad (29)$$

defined by the functions $f_1(\cdot, \cdot)$ and $\bar{f}_2(\cdot, \cdot, \cdot, \cdot)$ from (10), (11), respectively; the “input-output” type system is accompanied by the restrictions of state:

$$25 < G(t) < 120$$

$$\underline{P} := 3,5 < P(t) < \bar{P} := 20 \quad (30)$$

$$0,04 \cdot G(t) < L(t) < 0,19 \cdot G(t)$$

and by the command restrictions

$$a_1(G(t)) \leq P^*(t) \leq b_1(G(t)) \quad (31)$$

$$a_2(P(t), G(t), P^*(t)) \leq E(t) \leq b_2(G(t))$$

where the function $a_1(\cdot), b_1(\cdot), a_2(\cdot, \cdot, \cdot), b_2(\cdot, \cdot, \cdot)$, are defined in (10), (12), (14).

Before we going on we observe that the “input-output” type system from is equivalent to an usual command system, obtained by removing the output $G(t)$.

Thus, if we introduce the functions:

$$\hat{f}_1(P, L, P^*) := f_1(W(P, L), P^*), \hat{f}_2(P, L, P^*, E) := \bar{f}_2(P, W(P, L), P^*, E)$$

then, the system from is equivalent with the usual command system:

$$\begin{cases} P' = \hat{f}_1(P, L, P^*(t)) \\ L' = \hat{f}_2(P, L, P^*(t), E(t)) \end{cases}$$

accompanied by the restriction from (30) - (31) in which, variable G is replaced by the expression $W(P, L)$.

As concerns the criteria of optimization, from the multitude of possible variants we will approach first the issue of “maximizing the end product”, represented

by the mathematical expression $\lambda P(T) + (1 - \lambda) G(T)$ where $\lambda \in [0,1]$ is thus chosen as to express a certain proportion of the terminal protein, $P(T)$ out of the terminal weight $G(T)$.

We shall consider thus the problem of minimizing the functional:

$$C_1(u(\cdot)) := -\lambda \cdot P(T) - (1 - \lambda) \cdot G(T), \lambda \in [0,1] \quad (32)$$

Another reasonable criteria of optimization for the problem with the terminal rules (1.3.22) - (1.3.23) is that of the “least cost”, represented by functional

$$C_2(u(\cdot)) = \int_{t_0}^T (c_1 P^*(t) + c_2 E(t)) dt, \quad (33)$$

where $c_1, c_2 > 0$ represent the current cost per unit necessary to obtain the digestible protein P^* , respectively the digestible energy E , that were administered.

On the other hand, although apparently less natural from the point of view of the practical interpretation, problems can be formulated in which the terminal time is apparently free (not fixed) but which is in fact limited by the terminal conditions.

To make a choice, for problems with free terminal time, $t_1 > 0$ we shall impose terminal conditions of the form:

$$P(t_1) \in (\underline{P}, \bar{P}) = (13, 20), G(t_1) = 110, \gamma_1(P(t_1)) < \gamma_2(P(t_1)) \quad (34)$$

and we will try to minimize the functional:

$$C_3(u(\cdot)) = \int_0^{t_1} dt = t_1(u(\cdot)) \quad (35)$$

that is, we will study the “problem of the minimum time”, $t_1 > 0$ in which an initial point $(P_0, G_0) \in Y_0$ can be “transported” into a terminal point from the multitude Y_1 defined by:

$$Y_1 = \{(P, 110); P \in (\bar{P}_1, \bar{P}) = (13; 20), \gamma_1(P) < 110 < \gamma_2(P)\} \quad (36)$$

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Specific dietary selection for lysine, tryptophan and threonine by the piglet

T. Ettele & F.X. Roth

*Fachgebiet für Tierernährung und Leistungsphysiologie, Technische Universität München,
Hochfeldweg 6, 85350 Freising- Weihenstephan, Germany*

Summary

Dietary selection for lysine, tryptophan and threonine was studied in three separate experiments using 48 crossbred piglets each. In each trial two reference groups and two amino acid choice groups were built. The reference groups were fed a diet containing either 0.70 or 1.25 % lysine in experiment I, 0.11 or 0.20 % tryptophan in experiment II and 0.57 or 0.67 % threonine in experiment III. The two other groups had the possibility to choose from pairs of diets containing 0.70 / 1.15 % or 0.70 / 1.25 % lysine in experiment I, 0.11 / 0.16 % or 0.11 / 0.20 % trp in experiment II and 0.57 / 0.62 % or 0.57 / 0.67 % threonine in experiment III. When offered pairs of diets with concentrations of 0.70 / 1.15 % or 0.70 / 1.25 % lysine, 0.11 / 0.16 % or 0.11 / 0.20 % tryptophan and 0.57 / 0.62 % or 0.57 / 0.67 % threonine, the preference for the diet higher in the concentration of lysine, tryptophan or threonine was 61 or 71 %, 87 or 93 % and 57 or 47 %, respectively. Results of growth performance and the preference for the different amino acids suggest, that dietary selection for amino acids in piglets is highly related to the piglet's amino acid requirement.

Keywords: dietary selection, amino acids, piglets

Introduction

Especially observations on dietary selection behaviour of rats and chicks suggest, that an innate ability to regulate the intake of macro- and micronutrients still exists in domesticated animals. As a specific dietary selection behaviour was even observed in pigs, some authors pointed out the possible economic advantage of a choice-feeding for commercial pig and poultry production systems (Rose and Kyriazakis, 1991) and self-selection of dietary protein has been furthermore considered to become an attractive and alternative feeding strategy that would also allow to minimize nitrogen output (Henry, 1993). Nevertheless, data regarding to preferences for single amino acids in pigs are less available. Studies of Henry (1987, 1993) have shown that preference for lysine in growing pigs is influenced by the magnitude of the difference in lysine concentration of the diets offered for choice. Therefore, in three feeding trials selection behaviour of piglets offered diets with varying lysine, tryptophan or threonine concentrations was investigated. Except conception of diets, methods used in individual trials were held similar in order to be able to compare the results obtained for each single amino acid.

Material and methods

Each experiment (exp) was carried out over a period of 6 weeks and used 48 crossbred piglets (German Landrace x Piétrain) with initial body weights of 7.37 ± 0.69 kg (exp I, lysine), 8.20 ± 0.90 kg (exp II, tryptophan) and 9.14 ± 1.19 kg (exp III, threonine). Female as well as male piglets were randomly subdivided into four groups of twelve animals each. Animals of two of the four treatments (treat) were offered a pair of diets either with a low (diet 1) or an intermediate (diet 2) concentration of the respective amino acid (treat 2, amino acid (aa)- choice group 1) or a pair of

diets with a low (diet 1) or a high (diet 3) concentration of the respective amino acid (treat 3, amino acid- choice group 2). The amino acid concentrations from pairs of diets offered were 0.70 / 1.15 % (treat 2) or 0.70 / 1.25 % (treat 3) lysine in exp I, 0.11 / 0.16 % (treat 2) or 0.11 / 0.20 % (treat 3) trp in exp II and 0.57 / 0.62 % (treat 2) or 0.57 / 0.67 % (treat 3) threonine in exp III. The two remaining groups were used as reference groups with no dietary choice. Dietary amino acid concentrations of reference groups were 0.70 (treat 1, negative control group) and 1.25 (treat 4, positive control group) % lysine in exp I, 0.11 (treat 1) and 0.20 (treat 4) % tryptophan in exp II and 0.57 (treat 1) and 0.67 (treat 4) % threonine in exp III. The diets were based mainly on wheat, corn, barley, field bean or peas and varying amino acid concentrations where obtained by adding lysine as L-lysine · HCl in exp I and crystalline L-tryptophan or L-threonine in exp II and III. Measurement criteria were feed intake, proportions of ingested diets in amino acid choice groups, growth performance criteria and plasma amino acid pattern (exp II and III). Furthermore, in exp II and III at two dates a week animals of the aa- choice groups where kept without food for about 45 min and in this time period the position of the two diets in the feeders was changed. After the animals were given access to the feeders again, spontaneous food selection behaviour was observed for about 5 min.

The effect of the intake of the respective amino acid on food intake, growth performance and plasma amino acid pattern was tested for statistical significance ($p < 0.05$) by Student-Newman-Keuls test, time course of food selection by the Student's t-test and the spontaneous food selection behaviour by the Chi-square test (χ^2 - test).

Results

In exp I (lysine) daily feed intake of piglets fed the diet deficient in lysine (treat 1) was significantly lower than in self selection group 1 (treat 2), self selection group 2 (treat 3) and positive control group (treat 4), respectively (Tab. 1). As a mean of the experiment, animals of treat 2 and 3 chose a diet containing 61 and 71 % of the higher lys feeds, resulting in lys concentrations of the total diets of 0.97 and 1.10 %, respectively. At the start of the experiment both self-selection groups chose a diet with the same proportion of the lys deficient feed (about 42 %). In the following period animals of treat 3 lowered this proportion until the 5th experimental week (Fig 1), but self-selection behaviour of animals of treat 2 was inconsistent. Average daily gain in treat 2 and 3 was 496 and 459 g, respectively. Daily gain was significantly lower (239 g) in treat 1 and significantly higher (552 g) in animals of treat 4.

In exp II a significantly lower feed intake in treat 1 and 2 compared to treat 3 and 4 was observed. From the total diet treat 2 and 3 preferred on average 87 and 93 % of the feed with 0.16 and 0.20% trp. Resulting trp contents of total diets were 0.15 and 0.19 % in treat 2 and 3, respectively. In week 1 animals of treat 2 chose higher proportions of the trp deficient feed (44 % of total diet) than animals of treat 2 (31 %), but at the end of the experiment animals of both groups chose nearly exclusively the feed with the higher trp content. In tryptophan- choice groups 1 and 2 in 94 and 100 % of observations of spontaneous feeding behaviour animals chose the diets higher in tryptophan content within the observation period of 5 minutes. Animals of treat 2 had an average daily gain of 218 g, which was only slightly above daily gain of treat 1 with 198 g. Animals of treat 3 and 4 showed significantly higher average daily gain of 404 and 458 g, respectively. Plasma trp concentrations in groups 1 and 2 (5.88 and 4.96 $\mu\text{mol}/\text{ml}$) were significantly lower than in groups 3 and 4 (9.21 and 9.01 $\mu\text{mol}/\text{ml}$). The plasma concentration of urea in treat 2 (5.09 mmol/l) was significantly higher than in treat 3 and 4 (3.11 and 2.99 mmol/ml) whereas animals of treat 1 had intermediate plasma urea concentrations of 4.24 mmol/ml.

In exp III average daily feed intake was 856, 849, 936 and 917 g for treatments 1, 2, 3 and 4, respectively. Animals of treat 2 chose a diet containing on average 57 % of the diet high (0.62 % thr) in thr content, whereas animals of treat 3 showed no preference for the diet with the low or high thr (on average choice of 47 % of the 0.67 % thr diet) content. The resulting thr

Table 1. Daily feed intake (g) and weight gain (g) in exp I, II and III.

| | treat 1 | treat 2 | treat 3 | treat 4 |
|------------------------------|------------------------|------------------------|------------------------|------------------------|
| Lysine experiment | | | | |
| Feed intake (g/d) | 529 ± 65 ^b | 831 ± 109 ^a | 742 ± 140 ^a | 831 ± 104 ^a |
| Weight gain (g/d) | 239 ± 39 ^c | 496 ± 70 ^b | 459 ± 85 ^b | 552 ± 67 ^a |
| Tryptophan experiment | | | | |
| Feed intake (g/d) | 335 ± 102 ^b | 366 ± 103 ^b | 589 ± 281 ^a | 645 ± 131 ^a |
| Weight gain (g/d) | 198 ± 82 ^b | 218 ± 90 ^b | 404 ± 102 ^a | 458 ± 94 ^a |
| Threonine experiment | | | | |
| Feed intake (g/d) | 856 ± 138 | 849 ± 101 | 936 ± 117 | 917 ± 125 |
| Weight gain (g/d) | 469 ± 91 ^b | 498 ± 62 ^b | 559 ± 68 ^a | 549 ± 64 ^a |

^{a,b} Means within a row not sharing the same letter are significantly different at p<0.05

concentrations of the chosen total diet were 0.60 and 0.62 % for treat 2 and 3, respectively. In both treatments there was some variation in the preference for the different diets in consecutive weeks. Animals of treat 2 showed a slight increase of about 4 % in preference for the diet higher in threonine from week 1 to 6, whereas in treat 3 the opposite was the case. Evaluation of the spontaneous feeding behaviour in both amino acid- choice groups revealed no significant spontaneous preference for diets high or low in threonine concentration. Average daily gain in treat 1 was 469 g and was significantly lower than in treat 3 and 4 with 559 and 549 g, respectively. Animals of treatment 2 showed an intermediate live weight gain of 498 g/d. Plasma urea concentrations in treat 1 (4.32 mmol/l) was significantly higher than in treat 3 and 4 (3.47 and 3.56 mmol/l). Animals of treatment 2 had plasma urea concentrations of 4.20 mmol/ml. Plasma threonine concentration in treat 4 (143.5 µmol/ml) was significantly higher than in treat 1, 2 and 3 with 52.5, 59.7 and 87.4 µmol/ml.

Table 2. Cumulative intake (kg/animal and 6 weeks) of the diets on offer in the AA- choice groups.

| | treat 2 | | treat 3 | |
|-----------------------|------------|-------------|------------|-------------|
| | diet 1 | diet 2 | diet 1 | diet 3 |
| Feed intake (kg) | | | | |
| Lysine experiment | 14.0 ± 5.6 | 20.9 ± 4.5* | 8.7 ± 5.1 | 22.5 ± 6.3* |
| Tryptophan experiment | 1.8 ± 1.9 | 13.6 ± 5.1* | 1.9 ± 1.4 | 22.8 ± 4.5* |
| Threonine experiment | 15.4 ± 4.8 | 20.3 ± 3.7* | 20.8 ± 1.7 | 18.5 ± 4.2 |

* Marked value pairs are significantly different at p<0.05

Discussion

The presented results clearly demonstrate, that piglets are able to distinguish between diets differing in either lysine, tryptophan or threonine concentration and to show a preference for a diet with an amino acid concentration assumed to be more adequate to the piglets requirement. In agreement with data of Henry (1987), results of exp 1 and 2 indicate a more pronounced preference for a diet with a higher concentration of the tested amino acid, when the difference in the amino acid concentration of the two diets offered for choice is higher, but in the threonine experiment this observation was not made. As a mechanism of food selection the ability of animals to detect metabolic changes, e.g. a decline of a deficient amino acid in the brain (Gietzen, 1993),

caused by nutrient deficiency and the ability to partly redress them by modifying their food pattern is discussed (Kirchgessner et al., 1999). Because animals of threonine choice group 2 had nearly the same growth rate as animals in positive control group, it is assumed that the threonine supply was not the limiting factor for growth when the two diets were chosen at random and therefore there was no need to select for threonine in animals of this group. In all the other amino acid choice groups the performance was at least numerically lower than in the respective positive control group, resulting in the preference for diets higher in the concentration of the respective amino acid.

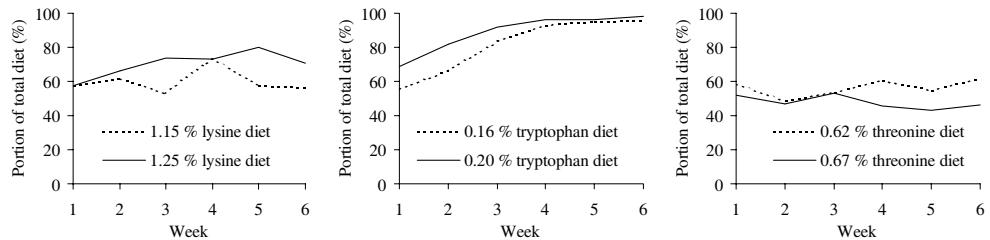


Figure 1. Ingested portions (% of total feed intake) of the amino acid supplemented diets by the dietary self-selection groups in exp I, II and III.

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The effect of reducing protein and energy intake of pregnant ewes on the subsequent muscle development of the offspring

A.J. Fahey, J.M. Brameld, T. Parr & P.J. Butterly

Division of Nutritional Biochemistry, School of Biosciences, Sutton Bonington Campus, The University of Nottingham, Leicestershire, LE12 5RD, U.K.

Summary

The aim of this study was to test the general hypothesis that carcass quality can be influenced by poor nutrition at strategic time points during an animal's development. Reduction in the total food intake of ewes bearing twin lambs to 50% of their maintenance requirement during days 30-70 of pregnancy was shown to result in a reduction in the number of fast muscle fibres with an increase in the number of slow fibres in the *Longissimus Dorsi* and *Vastus Lateralis* muscles, of the lambs 14 days after parturition. Reduction in food intake at days 55-95 and 85-115 had no significant effect compared with control animals.

Keywords: muscle development, muscle fibres, maternal nutrition

Introduction

As agricultural practices continue to become more extensive, variation in the nutrient supply to the animal is becoming more common. One period during an animal's life that is sensitive to changes in diet is early foetal life, when muscle fibres are being formed from myogenic cells. It is also during this period that the total numbers of muscle fibres for life are essentially set. Our hypothesis is that achieving maximal skeletal muscle fibre number is dependent upon adequate/optimal maternal nutrition during this period (Brameld *et al.*, 1998). Interestingly, muscle fibre number positively correlates with post-natal growth rate in pigs and rodents (Butterly *et al.*, 2000). Therefore, these effects of early maternal diet on foetal muscle development may also have long term effects upon postnatal growth rate, responses to later dietary manipulations and subsequent carcass quality. Reduced numbers of muscle fibres coupled with a relatively high plane of nutrition in later life may lead to inefficient growth and increased adiposity. We suggest that there could be a time during pregnancy when adequate feeding is essential to ensure maximum muscle development of the offspring, and therefore manipulation of diet during this time period could change muscle characteristics for meat production.

Materials and methods

32 pregnant ewes (North Country mules) carrying twins were used in this study. The ewes were mated naturally and checked every 2 days. Day zero of gestation was taken as the first day at which the ewes had an obvious raddle mark. At day 20 the ewes were individually housed and fed a pelleted diet consisting of straw nuts and soya. The amount given was calculated on an individual ewe basis to provide 100% of their daily maintenance (M) requirement (AFRC 1993). The diet was fed in two equal rations and supplied 8.6MJ/day at the start. The ewes were randomly allocated into one of four groups (n=8). Group d30-d70 ewes were fed M diet until d30, the diet was then dropped to 50% M until d70, they then returned to 100% M until term. Group d55-95 ewes were similarly restricted from d55-d95, group d85-115 ewes were restricted from d85-d115. Group 4 ewes were the control group and were fed 100% M throughout gestation. The time periods of restriction were allocated based on previous studies identifying when muscle

differentiation occurs (Fahey *et al* 2003). After parturition, the ewes were fed a normal commercial diet for the lactating ewe, calculated on an individual ewe basis, according to their live weight. On day 14 (after parturition) the lambs were euthanised by an overdose of pentobarbitone sodium Ph.Eur (1.33 ml/1kg body wt). Samples (10-20g) of the *Longissimus dorsi* (LD), and the *Vastus lateralis* (VL), were dissected and snap frozen in liquid nitrogen chilled isopentane to be used for histological techniques. Muscle fibre type was classified using myosin ATPase technique, the muscle fibre number was counted and diameter measured. The data was analysed using ANOVA using the tukey test as a Post Hoc analysis.

Results

The lambs born from the ewes restricted between d30-d70 showed an increase in the number of slow fibres in both LD and VL compared with the other treatment groups (* = $p < 0.001$ for both, see figure 1). The same group (d30-d70) showed a decrease in the number of fast fibres in both LD and VL compared with the other treatment groups (* = $p < 0.001$ for both, see figure 2). Group d30-d70 also showed an increase in fast fibre diameter (figure 3) compared to the other treatment groups in both LD (* = $p < 0.001$) and VL (* = $p < 0.005$). No significant change was observed in the slow fibre diameter.

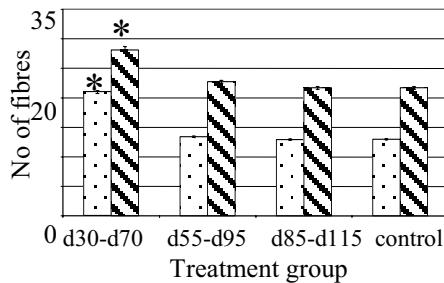


Figure 1. Number of slow fibres.

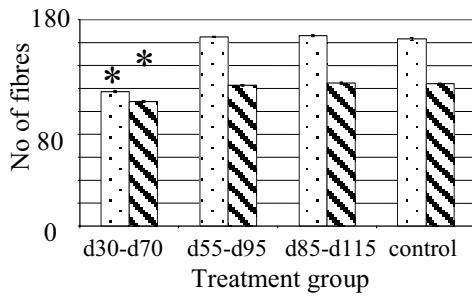


Figure 2. Number of fast fibres.

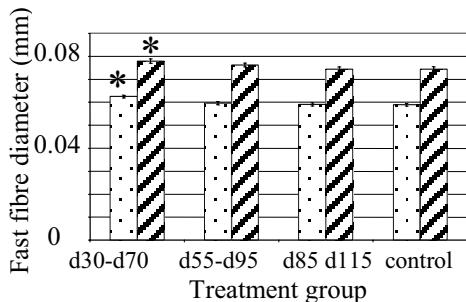


Figure 3. Fast fibre diameter.

Conclusions

This study indicates a reduction in the number of fast muscle fibres but an increase in the number of slow fibres in the lambs born from the ewes restricted between d30-d70. It is thought that only secondary fibres (fast fibres) can be affected by environmental factors, while primary fibres (slow fibres) appear resistant. We suggest that the changes seen in muscle fibre number are due to changes in the proportion of fibres, i.e. there are not more slow fibres but just proportionally more in the sample due to the reduction in fast fibres. The results indicate that during d30-d70 of gestation ovine muscle development can be manipulated by nutrition. It is yet to be seen whether these changes effect meat quality. However, studies have shown that the toughness of meat as judged by shear force, increases with overall fibre diameter (Seidmen *et al* 1987). In this study the lambs born from the ewes restricted between d30-d70 have larger fast fibre diameters in both the LD and VL compared to the other groups suggesting their may be an effect on meat quality.

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Comparative study of the gestational energy and protein balance of rabbit does

S. Fekete¹, I. Hullár¹, R. Romvári², Emese Andrásoszky¹ & Zs. Szendrő³

¹ Szent István University, Faculty of Veterinary Science Budapest, Institute of animal Breeding, Nutrition and Laboratory Animal Science, H-1400 Budapest, P.O. Box 2, Hungary

² Kaposvár University, Faculty of Animal Science, Diagnostic Centre, H-7401 Kaposvár, P.O. Box 16, Hungary

³ Kaposvár University, Faculty of Animal Science, Department of Small Animal Husbandry, H-7401 Kaposvár, P.O. Box 16, Hungary

Summary

Authors determined the total body composition of 4x10 female, approximately 5-month-old New Zealand White rabbits, using direct chemical analysis and computerized tomography (CT). Ten non-pregnant rabbits and three times ten pregnant rabbits on days 14, 21, and 28 of gestation were investigated.

With the advancement of pregnancy, the does' body weight increased significantly, while the dry matter content of the does' body slightly decreased (43.02 vs. 42.93, 40.77, and 40.94%). On the 28th day of pregnancy, the crude protein content of the pregnant does' body was significantly lower (20.15 vs. 18.13%, P(0.05), than that of the non-pregnant does. The same decreasing tendency could be observed during the pregnancy in case of ether extract (20.15 vs. 18.84, 19.04. and 18.13%) and ash content (3.06 vs. 2.88, 3.08, and 2.88%). With the advancement of pregnancy, the total energy content of the uterus and foetuses (1.75, 2.70, and 4.71% respectively) gave higher and higher proportion of the total energy content of does' body, but 5 percent of the energy level of total does body was not reached by that of the foetuses and uteri even at 28 days of pregnancy. According to the equations created from the CT pictures, body fat ($R^2=0.871$) and energy ($R^2=0.926$) content can be predicted with high accuracy. The accuracy of the prediction of body protein content is much lower ($R^2=0.356$), which can be improved by introducing the body weight as a new dependent variable ($R^2=0.797$).

The foetal growth proved to be allometric. In contrast to other animal species, conceptus building in rabbits is not only considerable in the last trimester but also from the second half of the pregnancy.

Keywords: rabbit, body composition

Introduction

The success of feeding experimental living (intact) animals is largely dependent on the thorough knowledge of the main chemical components in the diet. To measure the relative proportion of the main chemical components, during and at the end of the experiment, indirect *in vivo* methods are needed. The understanding of the composition of the body is immensely important not only for feed sciences (net energy determination) but also for veterinary studies. Thus, dosing anaesthetics depends on fat/whole body ratio of the animal. Body composition is influenced by both genetic and environmental factors. The concept of environment involves nutrient supply, as-well.

In this experiment the chemical composition of the entire does' body and that of the uteri and foetuses was investigated by both direct chemical analysis and computerised tomography (CT). Correlations were calculated between the CT examination data and the real chemical composition, in order to create equations for predicting the chemical compounds of the body according to the

CT investigations. By these equations we sought to take steps on behalf of animal protection. We also hope, that these results can add data for working out a net energy system for the energetic evaluation of rabbit feeds.

Materials and methods

This experiment involving 4x10, approximately five-month-old New Zealand White rabbit does was conducted to determine the body composition by direct chemical analysis and computerised tomography (CT). Ten empty (non-pregnant), and 3x10 pregnant rabbits at 14, 21, and 28 days of gestation were examined at the RCT Siemens Biological Centre by means of a third generation tomography, type: SOMATOM DRG (Horn, 1991). After receiving anaesthetics, rabbits were positioned on their abdominal side and a cross-sectional scan was taken at each of the following positions. "B": between the last dorsal and first lumbar vertebra; "C": between the 2nd and 3rd; "D": between the 4th and 5th; "E": between the 6th and 7th lumbar vertebra; "H": at the head of the femur. This was followed by Wendee analysis (AOAC., 1975) of rabbits, measuring their gastrointestinal content and pregnant uteri (empty uterus and foetuses separately). For estimating the regression between CT-data and chemical composition the interval between -150 and +120 HU (Hounsfield Unit) was used (Hounsfield, 1980). Similar legends were used: "B", "C", "D", "E", "H" for cross-sectional points; H6-H32 for group of ten sequential figures of HU-units, intervals between -150, -140, etc. and +120; "W" for body weight in grams.. Five scans for each of the animals or 27 HU-values for each of the scans were displayed in a 162 column-chart where column indicated the dependent variables, plus body weight for the 163rd column. Independent variables were: protein, fat and energy. Functions were generated by MGLH stepwise methods using the function generating software programme SYSTAT, Version 5.01. Figures were made by using STATGRAF programme.

Results and discussion

With advancement of pregnancy (*Table 1.*) does' body weight increased significantly (3255 vs. 3760 g, P(0.05; 3255 vs. 3815 g, P(0.05; and 3255 vs. 3938 g, P(0.01), while the dry matter content of does' body slightly decreased (43.02 vs. 42.93, 40.77, and 40.94%). Accordingly, it can be supposed, that the increment of the body water content also plays a role in the does' body weight gain, consequently the body compositions also change during the pregnancy (Milisits et al., 1999). On day 28 of pregnancy the crude protein content of the pregnant does' body was significantly lower (20.15 vs. 18.13%, P(0.05), than that of the non-pregnant does. But the decrement of the protein level does not mean a negative protein balance of does (Xiccato et al., 1992). The same decreasing tendency can be observed during the pregnancy in case of ether extract (20.15 vs. 18.84, 19.04. and 18.13%) and ash content (3.06 vs. 2.88, 3.08, and 2.88%) but the differences are statistically not significant.

On the 14th day of pregnancy, the energy content of does' body exceeded the energy content of non-pregnant does by 16.26 absolute percent (40.40 vs. 46.97 MJ, P(0.05), and the values were higher during the whole pregnancy. Although the energy content of pregnant does' body, as an absolute value, is higher than that of the non-pregnant does (40.40 vs. 46.97 MJ, P(0.05, 40.40 vs. 46.51 MJ, P(0.05), but when calculating the data related to 1 kg of body weight, values do not differ significantly, moreover they slightly decrease with advancement of pregnancy (12.41 vs. 12.49, 11.67, and 11.81 MJ). This tendency also shows the changes of the body composition, and the increment of the water content of the pregnant does' body (Parigi-Bini et al., 1992).

Due to the enlargement of uteri and to the foetal growth, the retained energy, in form of uterus with foetuses (GEUF), significantly increased (0.824 vs. 1.202 MJ, P(0.01; 0.824 vs. 2.189 MJ, P(0.001; 1.202 vs. 2.189 MJ, P(0.001). By all means the same tendency can be observed when evaluating the foetal energy content, although in this case, only the day 21 and 28 can be compared

Table 1. Chemical composition of the whole does' body (carcass + hide + foetuses + viscera)

| Parameters | Control (non-pregnant) | Pregnant does | | |
|-------------|------------------------|---------------------|--------------------|--------------------|
| | | d 14 | d 21 | d 28 |
| W, g | 3255 ^a | 3760 ^b | 3815 ^b | 3938 ^c |
| ± | 506 | 245 | 256 | 242 |
| DM, % | 43.02 | 42.93 | 40.77 | 40.94 |
| ± | 3.44 | 2.55 | 5.87 | 4.78 |
| CP, % | 20.15 ^a | 18.84 | 19.04 | 18.13 ^b |
| ± | 2.06 | 0.67 | 0.91 | 0.74 |
| EE, % | 19.13 | 20.48 | 18.49 | 18.89 |
| ± | 5.29 | 2.88 | 1.68 | 2.20 |
| Ash, % | 3.06 | 2.88 | 3.08 | 2.88 |
| ± | 0.41 | 0.27 | 0.31 | 0.44 |
| CP/Ash | 6.58 | 6.54 | 6.18 | 6.30 |
| EE/Ash | 6.25 | 7.11 | 6.00 | 6.56 |
| GE, MJ | 40.40 ^a | 46.97 ^b | 44.52 | 46.51 ^b |
| ± | 4.74 | 4.78 | 4.36 | 4.07 |
| % | 100 | 100 | 100 | 100 |
| GE, MJ/kg W | 12.56 | 12.52 | 11.71 | 11.82 |
| ± | 1.52 | 1.38 | 1.27 | 0.89 |
| GEUF, MJ | - | 0.824 ^{ac} | 1.202 ^c | 2.189 ^d |
| ± | - | 0.243 | 0.193 | 0.348 |
| %* | - | 1.75 | 2.70 | 4.71 |
| GEF, MJ | - | 0.018 | 0.256 ^c | 1.090 ^d |
| ± | - | - | 0.032 | 0.265 |
| %* | - | 0.04 | 0.58 | 2.34 |

n, number of animals per treatments; W, live weight of does'; DM, dry matter; CP, crude protein (N*6.25); EE, ether extract; GE, gross energy content of the whole does' body; d = day; GEUF, gross energy content of uterus with foetuses; GEF: foetal gross energy; *, proportion of the GEUF or GEF related to the GE as a 100%, ab: significant ($P<0.05$) difference between the values in the same row, ac: significant ($P<0.01$) difference between the values in the same row, cd: significant ($P<0.001$) difference between the values in the same row.

statistically (0.256 vs. 1.090 MJ, $P<0.001$). Taking the total body energy content of pregnant does as 100%, with advancement of pregnancy, both the total energy content of uterus and foetuses (1.75, 2.70, and 4.71% respectively), and the energy content of foetuses alone (0.04, 0.58, and 2.34% respectively) gave higher and higher proportion of the total energy content of does' body. These data confirm the results according to those 5 per cent of the energy level of total does body is not reached by that of the foetuses and uteri even at 28 d of pregnancy (Kamphues, 1985, Partridge et al., 1986).

According to the equations created from the CT pictures, body fat ($R^2=0.871$) and energy ($R^2=0.926$) content can be predicted with high accuracy. The accuracy of the prediction of body protein content is much lower ($R^2=0.356$), which can be improved by introducing the body weight as a new dependent variable ($R^2=0.797$).

With the advancement of pregnancy, does' body weight increased, while the dry matter, crude protein and ether extract content of does' body decreased. According to that it can be concluded, that the increment of the body water content also plays a role in the does' body weight gain,

consequently the body compositions also change during the pregnancy. The slight decline of the relative energy content of does' body (MJ/kg of body weight) also shows the changes of body composition, and increment of water content of pregnant does' body. The foetal growth proved to be allometric. Data show that 5 per cent of the energy level of total does body was not reached by that of the foetuses and uteri even at 28 d of pregnancy. In contrast to other animal species, conceptus building in rabbits is not only considerable in the last trimester but also from the second half of the pregnancy.

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Comparative serum metabolites and hormonal profile of Iberian and Landrace growing pigs fed equilibrated or lysine-deficient diets

I. Fernández-Figares¹, M. Lachica¹, M.G. Rivera-Ferre¹, R. Nieto¹, C. García del Río² & J.F. Aguilera¹

¹ Unidad de Nutrición Animal, Estación Experimental del Zaidín (CSIC), Camino del Jueves s/n, 18100 Armilla, Granada, Spain

² Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Avenida de Madrid s/n, 18014 Granada, Spain

Summary

Fifteen Iberian and fifteen Landrace gilts, 20 kg body mass, allocated in metabolic cages, were fed four times daily at 90% *ad libitum* on either adequate amino acid profile diets, based on wheat and maize gluten, containing 120 (A12) or 160 (A16) g CP/kg as fed, or diets deficient in lysine (DLys) given at 120 g CP/kg for Iberian and 160 g CP/kg for Landrace pigs, respectively. Diets were approximately isoenergetic (13.5 kJ ME/g DM). Gilts were fed four times daily at 90% *ad libitum* for 10 d. On day 11th blood samples were taken 4 h postprandial, serum obtained and frozen at -20°C until analysis. Glucose, urea, cholesterol, triglycerides, growth hormone (GH), insulin like growth factor I (IGF-I), insulin, and leptin were determined. Landrace gilts had significantly higher serum glucose ($P<0.05$) and creatinine ($P<0.001$) than Iberian gilts. Iberian and Landrace gilts showed distinct metabolic hormones profiles. Significantly higher serum level of insulin, IGF-I and leptin was encountered (65, $P<0.05$; 53, $P<0.05$ and 204%, $P<0.001$, respectively) in Iberian gilts compared to Landrace and a trend ($P=0.18$) towards lower (48%) serum growth hormone. Serum leptin concentration increased (42%, $P<0.05$) as dietary crude protein increased while the other hormones studied were not affected ($P>0.10$). Lysine deficient diets decreased serum IGF-I concentration (43%, $P<0.05$) and there was a trend towards lower serum leptin and insulin concentrations (36%, $P=0.051$; 39%, $P=0.052$), while GH remained unaffected ($P>0.10$).

Keywords: Iberian pig, hormonal profile, lysine deficiency

Introduction

Protein deposition is determined by genotype and endocrine status. The Iberian breed has lower potential for protein deposition compared to modern high-performing pigs such as Landrace (Nieto et al., 2002). Previous work from our laboratory has shown that during the 25-35 kg period, the Iberian pig growth rate is 24% lower than that observed in Landrace pigs (Rivera-Ferre, 2003). Furthermore, whole body protein turnover is 25-27% lower in Iberian compared to Landrace pigs (Rivera-Ferre et al., 2003) of approximately 28 kg body mass. The existing metabolic differences between Iberian and modern breeds could be at least partially explained by a different hormonal status. Iberian pigs have traditionally been raised in extensive production systems where amino acid imbalance, especially lysine deficiency, is not rare. No information is available concerning hormonal levels in Iberian pigs. The aim of the present study was to investigate the influence of genotype and a severe lysine deficiency on serum hormones and metabolites of Iberian and Landrace pigs.

Materials and methods

Fifteen Iberian gilts of Silvela strain and fifteen Landrace gilts were weighed and randomly allotted to experimental treatments. Pigs were fed for ten days, 90% *ad libitum*, four times daily, with isoenergetic (13.3-13.9 kJ ME/g dry matter) and semi-synthetic diets based on wheat and gluten feed, designed to cover nutritional requirements for growing pigs. They were fed on either adequate amino acid profile diets containing 120 (A12) or 160 (A16) g CP/kg diet as fed, or diets deficient in lysine. Amino acid deficient diets were given at 120 and 160 g CP/kg for the Iberian and Landrace breed, respectively, and provided approximately 35% of recommended lysine, according to the ideal protein concept. Gilts were allocated in individual pens in a controlled environment room. Water was freely available. Average body mass during the experiment was 21.7 ± 0.3 and 24.9 ± 0.5 kg for Iberian and Landrace pigs, respectively. The eleventh day of treatment blood samples were taken at 4 hours postprandial, let to clot in ice for 3 hours and centrifuged at 1000g for 15 min. Then serum was stored in different aliquots at -20°C until analysis. The experimental data were analysed by two factorials, the first was: 2 (protein level (PL): 120 (A12) or 160 (A16) g CP/kg) \times 2 (breed: Iberian vs Landrace) and the second: 2 (amino acid profile (AAP): adequate (A) or lysine deficient) \times 2 (breed: Iberian vs Landrace). Hormones were determined using available commercial kits following instructions of the manufacturer. Assays were validated for porcine serum in our laboratory. All samples were assayed in duplicate. GH was analyzed using a Linco porcine/canine RIA kit (cat. # PGH-46HK; Linco, St. Louis, MO). The sensitivity of the assay was 1.4 ng/ml, average recovery was 104.2% and an intra and inter-assay CV were 11 and 19%, respectively. Leptin was analyzed using a two-site immunoradiometric assay (IRMA) manufactured by Diagnostic Systems Laboratories, Inc. (Webster, TX, cat. # DSL-82100). The sensitivity of the assay was 1.0 ng/ml, mean recovery was 112% and intra and inter-assay coefficients of variation were 1.9 and 8.1%, respectively. Insulin was analyzed using a Linco porcine insulin RIA kit (cat # PI-12K; Linco, Saint. Louis, MO). The sensitivity of the assay was 3 μ units/ml, average recovery was 95% and intra and inter-assay CV were 2.6 and 8.4%, respectively. IGF-I was analyzed using an IRMA manufactured by Diagnostic Systems Laboratories, Inc. (Webster, TX, cat. # DSL-5600). The sensitivity of the assay was 4.8 ng/ml, mean recovery was 98.7% and intra and inter-assay CV were 5.2 and 7.2%, respectively. Serum glucose, creatinine, urea, cholesterol (Chol) (total-, HDL- and LDL-) and triglycerides were determined colorimetrically using an automated Advia 1650 (Dublin, Ireland).

Results and discussion

Serum glucose and creatinine were 8 and 46% greater, respectively, in Landrace compared to Iberian gilts with $P < 0.05$ and 0.001 , respectively (Table 1). No other serum biochemical parameters showed significant differences attributable to breed. Serum urea increased ($P < 0.001$) and cholesterol (total-, $P < 0.01$; HDL- and LDL-, $P < 0.05$) decreased when gilts consumed the diets containing the highest protein level (Table 1). Serum triglycerides significantly ($P < 0.05$) increased in Iberian gilts fed on diet A16, while this parameter was not affected ($P < 0.10$) in Landrace gilts (Table 1).

Severe lysine deficiency (Table 2) elicited a marked increase in serum urea (55%, $P < 0.001$) and a slight decrease in serum glucose (8%, $P < 0.05$). Serum creatinine, cholesterol and triglycerides were not affected ($P > 0.10$).

Iberian and Landrace gilts showed a distinct metabolic hormones profile (Table 3). Compared to Landrace, Iberian gilts had higher serum insulin, IGF-I and leptin concentrations, (65 ($P < 0.05$), 53 ($P < 0.05$) and 204% ($P < 0.001$), respectively). A 48% decrease in serum GH concentration was not statistically significant ($P > 0.10$) when Iberian were compared to Landrace gilts. The different growth rate between both breeds could not be explained by a divergent serum GH concentration. The greater serum insulin and IGF-I in Iberian compared to Landrace gilts contrasts with the slow

Table 1. Biochemical serum parameters (mg/dl) in pigs of Iberian and Landrace breeds fed with diets adequate in their AA profile but differing on their protein level (PL, 12 or 16% CP).

| | PL 12 | | PL 16 | | Pooled SEM | PL | Breed | PL×Breed |
|---------------|---------|----------|---------|----------|------------|-----|-------|----------|
| | Iberian | Landrace | Iberian | Landrace | | | | |
| Glucose | 85.6 | 94.2 | 85.6 | 91.3 | 3.41 | ns | * | ns |
| Urea | 15.8 | 13.2 | 29.8 | 28.8 | 2.26 | *** | ns | ns |
| Creatinine | 0.54 | 0.79 | 0.53 | 0.77 | 0.038 | ns | *** | ns |
| Total Chol | 100.8 | 93.2 | 84.6 | 78.7 | 5.29 | ** | ns | ns |
| HDL-Chol | 35.6 | 27.4 | 24.6 | 24.8 | 2.98 | * | ns | ns |
| LDL-Chol | 60.7 | 60.4 | 52.4 | 48.5 | 4.14 | * | ns | ns |
| Triglycerides | 22.4 | 26.6 | 38.2 | 27.3 | 3.74 | * | ns | 0.055 |

Table 2. Biochemical serum parameters (mg/dl) of pigs of Iberian and Landrace breeds fed with diets containing adequate protein level for each breed with either adequate or lysine deficient amino acid profile (AAP).

| | Lysine deficient | | Adequate | | Pooled SEM | AAP | Breed | AAP×Breed |
|---------------|------------------|----------|----------|----------|------------|-----|-------|-----------|
| | Iberian | Landrace | Iberian | Landrace | | | | |
| Glucose | 78.4 | 83.8 | 85.6 | 91.3 | 3.3 | * | ns | ns |
| Urea | 24.6 | 44.5 | 15.8 | 28.8 | 3.0 | *** | *** | ns |
| Creatinine | 0.494 | 0.723 | 0.540 | 0.768 | 0.051 | ns | *** | ns |
| Total Chol | 102 | 102 | 101 | 79 | 8.9 | ns | ns | ns |
| HDL-Chol | 29 | 33 | 36 | 25 | 3.6 | ns | ns | ns |
| LDL-Chol | 69 | 65 | 61 | 48 | 6.8 | ns | ns | ns |
| Triglycerides | 21 | 23 | 22 | 27 | 3.2 | ns | ns | ns |

Table 3. Serum insulin, IGF-I, GH and leptin concentration of Iberian and Landrace pigs fed with diets adequate in their AA profile but differing on their protein level (PL, 12 and 16% CP).

| | PL 12 | | PL 16 | | Pooled SEM | PL | Breed | PL×Breed |
|-----------------|---------|----------|---------|----------|------------|----|-------|----------|
| | Iberian | Landrace | Iberian | Landrace | | | | |
| Insulin ((U/ml) | 24.5 | 15.6 | 30.3 | 17.6 | 4.16 | ns | * | ns |
| IGF-I (ng/ml) | 374 | 191 | 305 | 251 | 44.0 | ns | * | ns |
| GH (ng/ml) | 0.9 | 1.6 | 1.5 | 2.8 | 0.78 | ns | ns | ns |
| Leptin (ng/ml) | 7.2 | 2.0 | 9.6 | 3.6 | 0.88 | * | *** | ns |

growing nature of the former. Other elements such as receptors or binding proteins must be regulating IGF-I activity. The greater serum leptin concentration in Iberian is probably due to their higher adiposity compared to Landrace gilts (Serra et al., 1998).

Serum leptin concentration increased (42%, $P<0.05$) when animals were fed with the highest protein level diet. No changes ($P>0.10$) in serum insulin, IGF-I and growth hormone were observed when dietary protein level was considered.

Gilts fed on lysine deficient diets (Table 4) had significantly lower levels of serum IGF-I (43%, $P<0.05$) while serum GH concentration was not affected ($P>0.10$). There was a trend towards lower serum leptin and insulin concentrations (36%, $P=0.051$ and 39%, $P=0.052$, respectively) when gilts were fed with lysine deficient diets.

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Table 4. Serum insulin, IGF-I, GH and leptin concentration of Iberian and Landrace pigs fed on diets containing the adequate protein level for each breed, with either adequate or lysine deficient amino acid profile (AAP).

| Breed | Lysine deficient | | Adequate | | SEM | Pooled | AAP | | | |
|-----------------|------------------|----------|----------|----------|------|--------|-----|--|--|--|
| | AAP×Breed | | | | | | | | | |
| | Iberian | Landrace | Iberian | Landrace | | | | | | |
| Insulin ((U/ml) | 13.5 | 12.1 | 24.5 | 17.6 | 4.6 | 0.052 | ns | | | |
| IGF-I (ng/ml) | 210 | 145 | 374 | 251 | 51.6 | * | ns | | | |
| GH (ng/ml) | 3.1 | 3.1 | 0.9 | 2.8 | 1.02 | ns | ns | | | |
| Leptin (ng/ml) | 4.8 | 2.1 | 7.2 | 3.6 | 0.93 | 0.051 | ** | | | |

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Effects of low protein supply on energy metabolism and milk production of the mink (*Mustela vison*)

R. Fink¹, A-H. Tauson¹, A. Chwalibog¹, N.B. Kristensen² & S. Wamberg³

¹ Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark

² Department of Animal Nutrition and Physiology, P.O. Box 50, DK-8830 Tjele, Denmark

³ Department of Physiology and Pharmacology, Institute of Medical Biology, University of Southern Denmark, Winsløwparken 21, DK-5000 Odense C, Denmark

Summary

Thirty mink dams were allocated into 3 groups and fed diets with decreasing ratio of metabolisable energy (ME) derived from protein during the four first weeks of lactation. In 12 dams energy metabolism was studied by means of balance and respiration experiments, and milk production was estimated by measurements of milk intake of the kits by the deuterium dilution technique. The remaining 18 dams were kept under normal farming conditions and used as reference for the animals kept in the laboratory. Metabolisable energy intake by feed was not significantly affected by dietary treatment. However, dams fed the HP diet had a higher ($P<0.05$) total heat production (HE), higher ($P<0.05$) protein oxidation (OXPHE) and a higher ($P<0.05$) nitrogen (N) excretion than dams fed the LP diet. Dams fed the LP diet, however, had a higher ($P<0.05$) milk production in weeks three and four of lactation, resulting in a higher live weight of the kits four weeks postpartum than dams fed the HP diet.

Keywords: nutrient oxidation, nitrogen output, preweaning kit growth

Introduction

The mink is a strict carnivore and hence its diet, compared to that of omnivores, is rich in animal protein and fat whereas it is low in carbohydrates. However, the actual protein and amino acid requirement of mink during different physiological stages, including lactation, is still incompletely known. Presently, in the Scandinavian countries, recommendations for supply of protein, fat and carbohydrates during the lactation period are a minimum of 40% of metabolisable energy (ME) from protein, 40-50% of ME from fat and a maximum of 20% of ME from carbohydrates (Hansen *et al.*, 1991). However, dietary protein supply is usually considerably higher than the minimum recommended, and a commercial Danish lactation diet typically consists of 55:33:12 % of ME derived from protein:fat:carbohydrate, respectively.

Milk from the dam is the only source of nutrient for mink kits during their first 24-26 days of life, and due to selective breeding programmes, litter size, and thereby the energetic demands of the dams, has increased profoundly in the farmed mink. Wamberg & Tauson (1998) and Fink *et al.* (2001) found that a female mink with a live weight of about 1 kg nursing an average litter of 6 kits produces about 4 kg milk during the four first weeks of lactation. Usually, voluntary feed intake of the dams is insufficient to meet nutrient and energy demands for maintenance and milk production, and extreme mobilisation of body reserves can in severe cases result in emaciation and death (Wamberg *et al.*, 1992). However, dietary nutrients requirement for milk production has been offered only limited attention in mink. Thus, since protein metabolism is an energetically expensive process (Tauson *et al.*, 1997) there is an urgent need for research in this area.

Materials and methods

A total of 30 mink dams nursing litters of 6 kits were fed ad libitum with diets containing different ratios of ME derived from protein:fat:carbohydrates (high protein (HP): 65:32:3, medium protein (MP): 48:37:15 and low protein (LP): 34:33:33(from parturition until the fourth week of lactation. Twelve dams were transferred to the laboratory, where the effects on energy metabolism were studied by means of balance and respiration experiments by indirect calorimetry in an open-air circulation system. Milk production was estimated by means of measurements of milk intake of the kits by the deuterium dilution technique as described in detail by Fink *et al.* (2001). The remaining 18 dams were kept under normal farming conditions, where feed intake and dams' and kits' live weights were measured and used as reference material for the animals kept in the laboratory.

Deuterium in plasma was analysed by means of isotope ratio mass spectroscopy. Response variables were tested for the fixed effects of dietary treatment by the GLM-procedure in SAS (SAS Institute Inc., 1990).

Results

The feed intake increased ($P<0.001$) as lactation progressed in all dams, dams fed the HP diet consumed approximately 21% more ($P<0.01$) per day in week four postpartum than dams fed the LP diet. However, the ME intake was not significantly affected by dietary treatment (Table 1). The main water supply was provided by feed intake in all dams, however, four weeks postpartum dams fed the HP diet consumed about 50% more drinking water per day than dams fed the LP diet. Thus, dams fed the HP diet had a higher ($P<0.001$) water intake than in dams fed the MP and LP diets (Table 1).

The N intake increased ($P<0.05$) with increased protein content of the diets and consequently, the N excretion by faeces and urine were higher ($P<0.001$) in dams fed the HP diet than in dams fed the MP and LP diets (Table 1).

Calculated in relation to metabolic live weight of dam with litter the HE (kJ/kg^{0.75}) was relatively constant during the lactation period, but approximately 20% higher ($P<0.05$) in dams fed the HP diet than in dams fed the LP diet (Table 1). The oxidation of protein (OXP) in relation to total HE was about double as high ($P<0.001$) in dams fed the HP diet than in dams fed the LP diet (Table 1).

Absolute live weight of the dams in week 1, 2, 3 and 4 postpartum were due to large individual variation not significantly different between dietary treatments. However, the individual weight loss, calculated in % of individual body weight from week one to week four postpartum, was significantly ($P<0.001$) higher in dams fed the HP diet than in dams fed the MP and LP diets, respectively (Table 1).

Daily milk intake of the kits and thereby the daily milk production was higher ($P<0.05$) in dams fed the LP diet than in dams fed the HP and MP diets during weeks three and four of lactation (Figure 1). This resulted in individual body weights of kits nursed by dams fed the LP diet being 10% and 6% higher ($P<0.001$) than those of kits nursed by dams fed the HP and MP diets, respectively, four weeks postpartum (Figure 1).

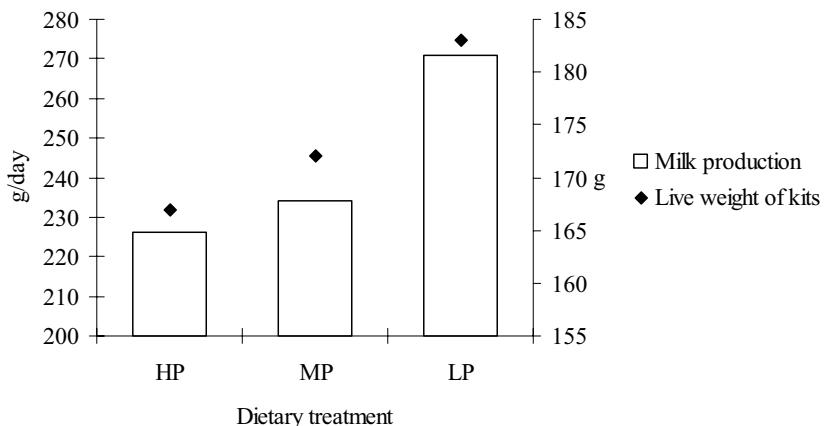


Figure 1. Milk production and live weight of kits 4 weeks postpartum.

Table 1. Effect of dietary treatment (high protein (HP), medium protein (MP) and low protein (LP)) on feed and drinking water consumption, nitrogen (N) excretion by faeces and urine, total heat production (HE), protein oxidation (OXP) and weight loss of the dams.

| | Dietary treatment | | | RMSE ¹ | <i>P</i> , group |
|------------------------------|-------------------|-------|-------|-------------------|------------------|
| | HP | MP | LP | | |
| Feed intake, MJ ME/day | 1.5 | 1.4 | 1.5 | 0.2 | 0.14 |
| Drinking water, (g/day) | 88.0 | 65.0 | 49.9 | 8.7 | <0.001 |
| N excretion, g/day | 6.8 | 4.7 | 3.2 | 0.5 | <0.001 |
| HE, (kJ) | 668.1 | 640.8 | 548.4 | 114.2 | 0.04 |
| OXP, (% of HE) | 43.4 | 30.9 | 21.2 | 4.5 | <0.001 |
| Weight loss ² , % | 9.2 | 3.6 | 1.4 | | 0.001 |

¹RMSE: root mean square error.

²Calculated in % of individual body weight from week one to week four postpartum.

Discussion

Knowledge of dietary interactions with milk production is necessary to secure animal health and welfare of the high producing female mink and her offspring. In this study the ME consumption was not affected by dietary protein content, indicating that mink, as previously found by Tauson (1988), similar to pigs (Forbes, 1995) within certain limits are able to compensate for decreased dietary energy concentration by an increased feed intake. Dams fed the HP diet, however, had a significantly higher HE and OXP contributed more to HE than dams fed the LP diet, confirming that when the protein supply is above the requirement, the excess protein is deaminated and used as an energy source. The N intake increased by increased protein content of the diets and consequently the N excretion by urine and the drinking water consumption were higher in dams fed the HP diet than in dams fed the LP diet. This corresponds to the fact that excess N is excreted in the urine, which is an energetically costly, and water requiring process. The energetic costs were also reflected in the significantly higher weight loss, in % of body weight from week one to week 4 of lactation, of dams fed the HP diet compared to dams fed the LP diet. Furthermore, was the milk production about 20% lower in the fourth week of lactation in dams fed the HP diet

compared to dams fed the LP diet. This, indicates that the LP diet covered the dams' protein requirement for maintenance and milk production, however, the dietary amino acid composition and the actual amino acid requirement of the mink needs to be further elucidated. The lower milk production of dams fed the HP diet was reflected in the live weights of the kits being significantly lower than in dams fed the MP and LP diets four weeks postpartum. Protein constitutes the most expensive part of the mink feed and a shortage of protein feedstuffs is expected in the near future. Furthermore, has the demand to reduce nitrogen emission from farming enterprises gained increasing importance. Thus, though this area needs to be further elucidated, these findings suggests that there is a considerable potential to reduce the protein supply to lactating mink with positive effects on animal performance and with beneficial effects for the environment.

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Growth performance, energy and nitrogen balance of weanling pigs fed cereal based diets supplemented with Chinese rhubarb

S. Gebert¹, R. Straub², M. Wanner² & C. Wenk¹

¹ Institute of Animal Sciences, Nutrition Biology, Swiss Federal Institute of Technology Zurich, 8092 Zurich, Switzerland

² Institute of Animal Nutrition, University of Zurich, 8057 Zurich, Switzerland

Summary

The present experiment was designed not only as performance but also as balance and respiration trial with weanling pigs. The cereal based diet, supplemented with four different dosages of Chinese rhubarb (0.0 % 0.25 %, 0.5 %, 1.0 %), was offered ad libitum to forty four week old Large White piglets. Gaseous exchange was measured and urine, faeces and blood samples were taken. The addition of 0.25 % dietary rhubarb tended to increase feed intake (+32 %) and daily weight gain (+17 %) in relation to the control diet. Energy and nitrogen losses by urine and faeces were reduced two weeks after weaning. Consequently piglets consuming 0.25 % dietary rhubarb were able to convert the greater amount of energy- and nitrogen into body protein. This effect was also reflected in slightly reduced serum urea concentrations of animals fed diets supplemented with 0.25 % rhubarb. Nonsignificant depressed feed intake (-15 %) and reduced daily weight gain (-35 %) were observed in piglets fed 1.0 % dietary rhubarb compared to control piglets. They converted slightly less energy and nitrogen into body mass. Serum IGF-1 concentration was lowest in animals fed 1.0 % dietary rhubarb and dry matter of faecal samples was reduced as a result of the laxative activity of Chinese rhubarb. The addition of 0.5 % dietary rhubarb had no relevant effect neither on growth performance nor energy- and nitrogen metabolism of animals.

Keywords: weanling pig, respiration study, feed additive

Introduction

For over fifty years animals have been fed antibiotics such as chlortetracycline, oxytetracycline and carbadox in sub-therapeutic doses in order to improve growth performance and to prevent diseases in livestock environments. However, increased occurrences of resistant pathogens (Swann, 1969) as well as growing demand for natural and healthy food by end consumers finally lead to the prohibition of antibacterial growth promoters in Switzerland in 1999. In order to prevent a negative impact on the industry the research for more reliable and effective alternatives such as enzymes, organic acids, herbs and spices was intensified.

Dried roots of two species of rhubarb (*Rheum palmatum* and *Rheum officinale*) have been widely used in human medicine by the Chinese for more than two thousand years. As the main active agents of Chinese rhubarb, anthraquinone derivatives are assumed to stimulate peristaltic contractions of the large intestine as well as mucus and active chloride secretion (Garcia-Villar *et al.*, 1980). They are also known to have antibacterial, antifungal and antiviral properties (Cyong *et al.*, 1987). The astringent effect was due to the tannins found in rhubarb roots. They are thought to supersede the anthraquinone activity resulting in a lower water content of the stool. Astringent and antibacterial properties may be used in order to prevent diarrhoea in weanling pigs and to improve growth performance of the animals.

Materials and methods

Forty Large White piglets were weaned at four weeks of age and randomly assigned to four different dietary dosages of Chinese rhubarb. Groups of two animals were penned in a environmentally controlled facility under normal husbandry conditions. During a 13-day adaptation period piglets were accustomed to the new housing and feeding regimens. The piglets were gradually adjusted to the experimental feed formulated to meet energy- and nutrient requirements of growing pigs as of day 6. The cereal based pelleted diets (maize 25.5 %, barley 25 %, wheat 19.5 %, oat flakes 5 %, soybean meal 7 % and fish meal 2.5 %, amino acids, minerals and vitamins) were supplemented with 0.25 %, 0.5 % and 1.0 % Chinese rhubarb, whereas no rhubarb (0.0 %) was added to the control diet.

Individual animals were weighed at weekly intervals and daily feed consumption was monitored. After two weeks the piglets were placed in a stainless steel metabolic cage ($84 \times 95 \times 140$ cm) to get them used to the open indirect respiratory calorimetry system. The temperature in the respiration chamber was maintained at 22°C with a concomitant relative humidity of 34 to 41 %. At the pig level the air flow rate was set at $5.0 \text{ m}^3/\text{h}$. In a 221/2 hours pre-run on day 17 a spontaneous urine sample was collected. In the following two weeks gaseous exchange (O_2 , CO_2 and CH_4) was monitored. During two subsequent 4-day balance periods (only the first is shown) faeces and urine were separately collected. On day 17 after 12 hour fasting, serum samples were taken by vena cava cranialis punctuation.

Results and discussion

Pigs entered the trial with an average body mass of 8.4 ± 0.4 kg and at the end of the 13-day adaptation period all animals weighed 10.3 ± 0.2 kg (Figure 1). Final body mass of the piglets was increased ($P = 0.014$) if diets contained 0.25 % rhubarb compared to animals treated with 1% dietary rhubarb.

The addition of 1 % rhubarb to the diet resulted in decreased daily weight gain ($P = 0.004$) and feed intake ($P = 0.032$) of the piglets compared to animals receiving 0.25 % dietary rhubarb. Diets supplemented with 0.5 % rhubarb had no particular effect on growth performance as results were very close to the control group. Gebert *et al.* (1999) reported a nonsignificant reduction in the growth performance of animals consuming 0.25 % and 0.5 % dietary rhubarb. Whereas diets supplemented with 0.1 % rhubarb increased daily feed intake (+9.4 %) and daily weight gain (+11 %). The authors detected a higher content of anthraquinones in the dried rhubarb roots (1.53 %).

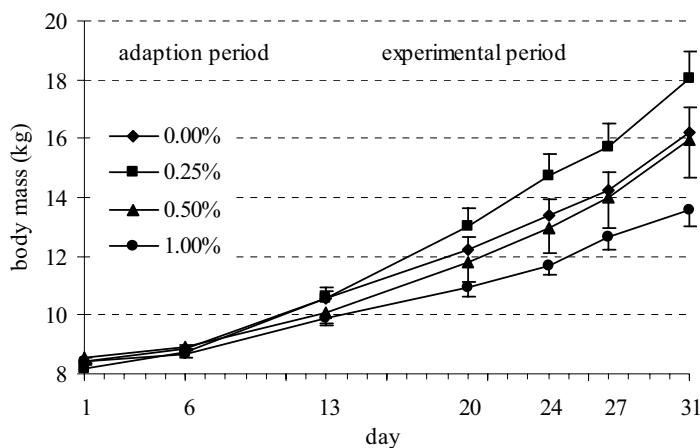


Figure 1. Treatment effects on body mass of weanling pigs.

than in the herb used in the present experiment (1.02 %). Thus variable concentration of active ingredients may have caused different effects on growth performance observed in animals fed the same doses of rhubarb (0.25 %).

Reduced feed intake may correspond to the laxative effect of medicinal rhubarb, as anthraquinones were proved to have purgative action and predominantly act on large intestine motility after their degradation by colonic microorganisms (Garcia-Villar *et al.*, 1980). They also stimulate chloride and water secretion into the stool. The laxative action of the feed additive was also reflected by slightly decreased dry matter of faeces excreted by all animals consuming 1 % rhubarb (Table 1). Still, increased serum protein indicated a slight dehydration of animals fed 1 % dietary rhubarb. Although animals lost more water by faeces their water intake was not affected (data not shown). Regarding the first balance period (Table 1), feed intake was slightly increased in animals consuming 0.25 % rhubarb compared to the control diet, although differences were statistically not significant. As a consequence they ingested more energy and nitrogen. Energy and nitrogen losses via faeces and urine tended to be decreased of animals in this treatment. Slightly reduced serum urea concentrations of animals fed 0.25 % dietary rhubarb confirmed the positive effect on the nitrogen balance. Heat expenditure tended to be increased in pigs fed 0.25 % dietary rhubarb indicating enhanced body protein synthesis, as heat production was related to whole body protein synthesis. Maintenance energy requirements corresponded to the rate of energy expenditure estimated for ME for maintenance (ME_m) at low physical activity. Piglets fed 0.25 % tended to need less energy for maintenance, whereas highest energy requirements were noted in animals fed 0.5 % dietary rhubarb. ME_m and partial efficiency of utilisation of ME for growth corresponded well to other findings in literature. Contrary to the beneficial effects of 0.25 % dietary rhubarb 1 % impaired nitrogen and energy metabolism in the present period. This effect may be due to the purgative action of rhubarb, when it is fed in higher doses (Wagner, 1988). Consequently transit in the lower digestive tract is accelerated and contact time between intestine

Table 1. Treatment effects on nitrogen and energy metabolism in weanling pigs (first balance period).

| Rhubarb (%) | Treatment | | | | SEM | P-value |
|---|--------------------|--------------------|--------------------|-------------------|-------|---------|
| | 0.00 | 0.25 | 0.50 | 1.00 | | |
| Body mass (kg) | 12.8 ^{ab} | 13.9 ^a | 12.4 ^{ab} | 11.3 ^b | 0.576 | 0.018 |
| Feed intake (g/d) | 532 ^{ab} | 658 ^a | 537 ^{ab} | 404 ^b | 40.2 | 0.001 |
| N intake (g/BM ^{3/4}) | 2.19 | 2.54 | 2.28 | 1.81 | 0.188 | 0.089 |
| DN (g/BM ^{3/4}) | 1.94 | 2.31 | 2.05 | 1.66 | 0.169 | 0.091 |
| NB (g/BM ^{3/4}) | 1.64 ^{ab} | 2.12 ^a | 1.65 ^{ab} | 1.44 ^b | 0.159 | 0.049 |
| RN _p (g/BM ^{3/4}) | 10.2 ^{ab} | 13.2 ^a | 10.4 ^{ab} | 9.01 ^b | 0.992 | 0.049 |
| GE intake (kJ/BM ^{3/4}) | 1368 | 1563 | 1381 | 1109 | 116 | 0.090 |
| DE (kJ/BM ^{3/4}) | 1271 | 1471 | 1294 | 1047 | 110 | 0.095 |
| Q (kJ/BM ^{3/4}) | 664 | 699 | 679 | 586 | 30.7 | 0.086 |
| EB (kJ/BM ^{3/4}) | 578 | 746 | 583 | 443 | 81.8 | 0.117 |
| RE _p (kJ/BM ^{3/4}) | 243 ^{ab} | 315 ^a | 246 ^{ab} | 214 ^b | 23.6 | 0.048 |
| ME _m (kJ/BM ^{3/4}) | 434 | 374 | 458 | 441 | | |
| k _g | 0.725 | 0.681 | 0.757 | 0.762 | | |
| Dry matter of faeces (%) | 41.8 | 37.3 | 37.7 | 30.3 | | |
| Serum Protein (mmol/l) | 45.8 ^a | 46.0 ^{ab} | 46.3 ^{ab} | 51.7 ^b | 1.6 | 0.033 |
| Urea (mmol/l) | 3.06 | 2.47 | 2.55 | 2.84 | 0.344 | 0.495 |
| IGF-1 (ng/l) | 38.1 | 34.6 | 35.9 | 34.4 | 7.71 | 0.976 |

content and intestinal wall shortened. As a result fluid and nutrient absorption is reduced. The addition of 0.5 % had relevant effect neither on growth performance nor on energy and nutrient metabolism. Maybe beneficial antimicrobial effects were reduced by a slightly laxative action, confirmed in lower dry matter of faeces.

In conclusion, the addition of 0.25 % dietary rhubarb tended to increase growth performance as well as slightly improved energy and nitrogen metabolism of weanling pigs. This positive effect was restricted to the very early days after weaning. Whereas higher doses of rhubarb (1 %) depressed growth performance as well as energy and nitrogen utilisation. The dose responding action of rhubarb was observed in all animals fed 1 % showing clinical signs of diarrhoea. However, standardisation of rhubarb may be difficult as the concentration of active ingredients depends on many factors such as place of origin and time of harvesting. Still, the exact mechanism of rhubarb on feed stimulation or increased energy and nitrogen utilisation have to be found out yet.

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Effect of dietary energy sources on energy metabolism of growing and fattening pigs: a model simulation

V. Halas¹, J. Dijkstra², L. Babinszky¹, M.W.A. Verstegen² & W.J.J. Gerrits²

¹ University of Kaposvár, Faculty of Animal Science, Department of Animal Nutrition, P.O. Box 16, H-7400 Kaposvár, Hungary

² Wageningen Institute of Animal Sciences, Animal Nutrition Group, PO Box 338, 6700 AH Wageningen, the Netherlands

Summary

The aim of the study is to present the preliminary results of a dynamic, mechanistic model of nutrient metabolism in pigs. The model was developed to predict the partitioning of ingested nutrients to protein and fat deposition in various organs and tissues in growing pigs. The state variables of the model are lysine, acetyl-CoA equivalents, glucose, VFA, and fatty acids as metabolite pools, and protein and fat in muscle, hide, bone and viscera and body ash as body constituent pools.

The model was run with increasing fat/carbohydrate ratio (F/CH) from 0.05 to 0.52 kJ/kJ. The daily feed intake was iso-nitrogenous and iso-caloric (17.19 g ileal digestible protein/kg^{0.75} and 1.491 MJ DE/kg^{0.75}). At the weight range of 20–75 kg, the simulated protein deposition rate and the average daily gain decreased and the fat deposition rate increased with increasing F/CH. With increasing F/CH the simulated glucose oxidation obviously decreased, while amino acid, fatty acid and VFA oxidation slightly increased. The *de novo* fatty acid synthesis decreased with increasing F/CH. The released energy from oxidation of metabolites gives the heat production. At the extreme levels of F/CH (0.05 and 0.52), the simulated daily total heat production was 800 and 700 kJ/kg^{0.75} and the energy efficiency (NE/ME) was 0.755 and 0.816, respectively. In conclusion, the model predictions are in agreement with the literature data regarding to some relevant rates of energy utilisation and metabolism.

Keywords: modelling, energy utilisation, pig

Introduction

Nutrition provides a means to manipulate the body composition of pigs. A mechanistic, dynamic growth model was developed to predict the partitioning of nutrients to fat and protein deposition in various parts of the body of growing and fattening pigs. A detailed description of the model is in preparation (Halas et al., submitted). The model includes a description of all energy costs and gains of nutrient fluxes. Therefore, it may be used as an aid in understanding the effects of changes in nutrient supply upon energy metabolism. This is of particular importance since the *in vivo* measurement of energy metabolism is expensive and there are only a few facilities for calorimetry trials.

The aim of the present study is to evaluate the nutrient partitioning predicted by this model to variations in the dietary fat to carbohydrate ratio (F/CH) and compare the simulated results with independent data on energy metabolism.

Material and methods

The model is aimed at the integration of protein and energy metabolism in pigs of 20–105 kg live weight. The model is driven by nutrient inputs. The state variables of the model are lysine, acetyl-

CoA equivalents, glucose, VFA, and fatty acids as metabolite pools, and protein and fat in muscle, hide, bone and viscera and body ash as body constituent pools. For distribution of nutrients between pools, standard saturation kinetics are assumed. The differential equations for the state variables were solved numerically for a given set of initial conditions and parameter values, using a fourth order fixed-step-length Runge-Kutta method (Halas et al., submitted). In this nutrient-based model, the energy metabolism can be simulated as affected by changing nutrient supplies. In the simulations to evaluate the effects of F/CH, the initial live weight of the pig was 20 kg and the simulations were performed for 58 days with a cumulative feed intake close to 100 kg. The diets contained 11.2 g/kg and 169 g/kg ileal digestible lysine and ileal digestible protein and 14.85 MJ DE/kg, respectively. The pigs were fed at the level of 3.2 times maintenance energy requirement. The daily fat intake increased from 1.4 to 9.4 g/kg^{0.75} in steps of 1 g/kg^{0.75} per day, whilst the daily starch intake decreased from 50.1 to 32.1 g/kg^{0.75} to obtain a constant DE intake level. This resulted in a F/CH increase from 0.05 to 0.52 kJ/kJ.

The average daily gain, protein and fat deposition rates were predicted at the 58-day period. The oxidation of glucose, fatty acids, amino acids and VFA and the *de novo* fatty acid synthesis from acetyl-CoA in response to changes in F/CH was compared at the 58th day. The ME intake was calculated from digested nutrients corrected for the energy in simulated urinary N (334 [kJ/d] + N-urine [g/d]*30.6 [kJ/g])) and methane production (-269.3 [kJ/d] + 0.0947*fermentable cell wall components [g/d]*17.6 [kJ/g]) as suggested by Bakker et al. (1996). The heat production (HP) was calculated by subtracting simulated energy retention from simulated ME intake.

Results and discussion

The simulated effects of F/CH on average daily gain, protein and fat deposition rates are presented in Figure 1a. The final simulated body weight was some 75 kg (within a range of 73.6 to 76.7 kg). Increasing F/CH from 0.05 to 0.52 kJ/kJ decreases the simulated daily protein deposition and the weight gain by 14 and 6 % respectively, whereas the fat deposition rate is increased by 26 %. The large increase in body fat gain does not give rise to an increased daily gain, since a considerable amount of water is deposited along with protein. The oxidation of the metabolites and the utilisation of acetyl-CoA to *de novo* fatty acid synthesis and the concentration of acetyl-CoA can be seen in Figure 1b. The increasing F/CH obviously decreased the glycolysis since the daily starch intake decreased. Amino acid, fatty acid and VFA oxidation slightly increased with increasing F/CH. The rate of glucose oxidation depends amongst others on the diet composition,

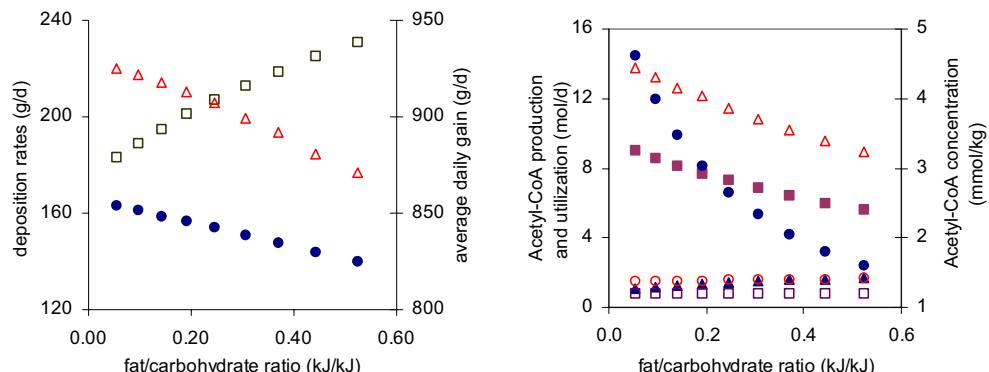


Figure 1. The simulated effect of increasing fat to carbohydrate ratio (F/CH) on (a) average daily gain (Δ), protein (\bullet) and fat deposition rates (\square) and on (b) metabolite oxidation (acetyl -CoA production from glucose - Δ , fatty acid - \blacktriangle , amino acid - \circ , VFA - \square), acetyl-CoA utilisation to fatty acid synthesis (\blacksquare) and acetyl-CoA concentration (\bullet).

however even at the 0.52 F/CH ratio the main source of acetyl-CoA is the glycolysis. A decreased glycolysis reduced acetyl-CoA concentration and thus *de novo* fatty acid synthesis decreases with increasing fat/CH. In the model, the protein synthesis depends on the concentration of both lysine and acetyl-CoA. At the digestible lysine and energy contents of the diet used in the present exercise, the concentration of acetyl-CoA is more limiting to protein deposition than that of lysine. Since the production of acetyl-CoA, and consequently its concentration, decreases upon an increase in F/CH, protein deposition is reduced. Fatty acid oxidation hardly increased with increasing fat intake. This result is in agreement with observations of Chwalibog et al. (1992) who found negligible fatty acid oxidation when the energy from carbohydrates was enough to supply the energy requirement for maintenance and growth.

The released energy from oxidation of metabolites gives the heat production. The plot on oxidation of metabolites (Figure 1b) suggests that the heat production decreases with increasing fat/CH. The results for two extreme diets in the simulation are presented in Table 1. The ME intake hardly differed from each other since there was only a slight change in amino acid oxidation. The ME/DE ratio was 0.947 in both diets. In general, the energy losses via urine and methane production for pigs is about 5 % (Boisen and Verstegen, 2000), which is in agreement with our simulations. Feeding different types of diets, van Milgen et al. (2001) found that the ME was between 94.7 and 97.1 % of DE. The ME/DE ratio was highest with high fat diets. In the present simulation, the dietary NE intake (defined as the energy intake required for maintenance and energy deposited in the body) at 0.05 kJ/kJ F/CH was lower than that at 0.52 F/CH, viz. 1067 vs. 1153 kJ/kg^{0.75/d}. Our results agree with values of Noblet et al. (1994) who reported NE/ME of 82 % for digestible starch and 90 % for digestible ether extract. Van Milgen et al. (2001) found significantly higher NE/ME ($P<0.01$) when the experimental diet was supplemented with lipid. The additional lipid provided 95.3 g/kg fat compared to 27.3 g/kg fat of the basal diet and resulted in 78.1 % vs. 73.9% NE/ME. The increase in NE/ME was not significant if the ME efficiency of lipid supplemented diet was compared to a starch supplemented diet. Bakker et al. (1996) however reported a decrease in energy and fat retention with increasing fat replacement of maize starch. It has to be noted that Bakker et al. (1996) did a comparative slaughter trial, while the others measured the NE by indirect calorimetry.

A 15 % lower heat production was obtained at the high F/CH level (Table 1). This is caused by the vast majority of extra ingested fat, which is directed towards fat deposition. The reduced intake of

Table 1. Simulated effect of fat to carbohydrate ratio on energy utilisation, heat production and net energy cost of protein deposition at 58th day.

| | fat/carbohydrate ratio (kJ/kJ) | |
|--|--------------------------------|-------|
| | 0.05 | 0.52 |
| Daily protein deposition at 58 th day (g) ¹ | 211 | 170 |
| Daily fat deposition at 58 th day (g) ¹ | 274 | 349 |
| Daily energy requirement for maintenance (kJ/kg ^{0.75}) ¹ | 454 | 441 |
| Daily DE intake (kJ/kg ^{0.75}) ¹ | 1491 | 1491 |
| Daily ME intake (kJ/kg ^{0.75}) | 1413 | 1412 |
| Daily NE intake (kJ/kg ^{0.75}) ² | 1067 | 1153 |
| ME/DE | 0.947 | 0.947 |
| NE/ME | 0.755 | 0.816 |
| Daily energy retention (kJ/kg ^{0.75}) ² | 612 | 711 |
| Daily HP (kJ/kg ^{0.75}) | 800 | 700 |

¹ predicted; ² NE = energy retention + energy requirement for maintenance

carbohydrate, therefore directly causes a reduction in heat production. Hillcoat and Annison (1974) noted that heat production of growing pigs tended to decrease with increasing dietary fat level. The total heat production was 53 and 49 % of the ME intake when pigs were fed either a semipurified diet without oil or a with 90 g soya oil/kg feed (Chwalibog et al., 1992). It should be noted, however, that the results of the present simulations depend on the protein intake level. Under protein limiting conditions, an increased F/CH ratio would probably not result in very pronounced differences, since in that case energy is not the major limiting factor towards protein deposition anymore.

In conclusion, the model predictions are generally in line with literature data with respect to energy metabolism of growing and fattening pigs. With increasing fat to carbohydrate ratio, the fat deposition increases whilst the heat production decreases. The ME/DE ratio is not affected by the replacement of carbohydrate to fat. The NE content of the diet increases with increasing F/CH.

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Energy and nitrogen balance in growing mink kits fed bacterial protein

Anne Louise Frydendahl Hellwing, Anne-Helene Tauson & André Chwalibog

The Royal Veterinary and Agricultural University, Department of Animal Science and Animal Health, Grønnegaardsvej 3, DK-1870 Frederiksberg C, Copenhagen, Denmark

Summary

A new protein source based on bacterial protein, Bioprotein meal (BPM) was evaluated in a dose-response experiment with growing male mink kits. The dietary supply of BPM was 0 % (D I), 20 % (D II), 40 % (D III) or 60 % (D IV) of the digestible nitrogen (DN). Animals given the highest content of BPM in their diet had a significantly ($P<0.05$) lower live weight gain and a lower retention of energy than animals on the other diets. The apparent digestibility of nitrogen and energy decreased with increasing content of BPM in the diet. The retention of nitrogen was highest on D II but it was only significantly different ($P<0.05$) from D IV. Heat production was almost identical on all diets. It was concluded that BPM could provide up to 40% of DN without altering quantitative N and energy metabolism traits in growing male mink kits.

Keywords: bioprotein, nitrogen retention, substrate oxidation

Introduction

Bioprotein is a new protein source produced from spray-dried bacterial cells (mainly *Methylooccus capsulatus* (Bath)). The chemical composition of bioprotein meal (BPM) is very similar to that of fish meal regarding crude protein content and amino acid composition, but with a high DNA and RNA content which makes up about 10 % of the dry matter in BPM (Skrede et al., 1998). Bioprotein is mainly intended as protein source for monogastric animals. The aim of the present study was to investigate the impact of increasing dietary contents of BPM on energy and protein metabolism in growing male mink.

Material and methods

Sixteen growing mink kits of the standard brown colour type were allocated to 4 diets and 2 blocks according to day of birth and live weight. Diet I was a control diet without BPM. In diets II, III and IV fish meal was substituted with BPM (Norferm DA, Norway) on a digestible nitrogen (DN) basis, BPM making up 0 % (D I), 20 % (D II), 40 % (D III) and 60 % (D IV) of DN. The dietary composition is given in Table 1. Five balance periods comprising a one-week adaptation period and a 4-days collection period were conducted from a kit age of 10 to 26 weeks of age. During each period all animals were measured in a 22-hour respiration experiment by means of indirect calorimetry in an open-air circulation system. Between balance periods the animals were housed in the mink farm and fed a conventional farm diet. All results reported are means of the five balance periods. The data were analysed by means of the procedure MIXED in SAS with block, diet and balance period as fixed effects and mink as random effect. The two-way interaction between diet and balance period was also included the model. The results are reported as LSmeans and differences were considered significant if $P<0.05$.

Table 1. Dietary composition in %.

| | D I | D II | D III | D IV |
|----------------------------|-----|------|-------|------|
| Fish meal (NorSeaMink) | 12 | 8 | 4 | 0 |
| Bioprotein | 0 | 4.5 | 9 | 13.5 |
| Cod fillet | 15 | 15 | 15 | 15 |
| Whole chicken | 12 | 12 | 12 | 12 |
| Carbohydrates ¹ | 16 | 16 | 16 | 16 |
| Soya or rapeseed oil | 4 | 4 | 4 | 4 |
| Sugar beet pulp | 2 | 2 | 2 | 2 |
| Water | 39 | 38.5 | 38 | 37.5 |

¹ Potato mash powder, heat-treated wheat and barley and rolled oats

Results

The main results are reported in Table 2. The feed intake was highest on D III with $122 \text{ g/kg}^{0.75}$ and lowest on diet D IV with $104 \text{ g/kg}^{0.75}$, the difference between diets being non-significant. During the balance periods only animals on D III had a positive live weight gain with a daily gain of 8.2 g/day, which was significantly higher than on D I and D II. The lowest and negative daily live weight gain of -12.1 g/day was recorded for animals on D IV.

The diets had the same content of nitrogen (N) and therefore the ingested nitrogen (IN) in $\text{g/kg}^{0.75}$ followed the same pattern as the feed intake. The apparent digestibility of nitrogen (ADN) decreased significantly ($P<0.01$) with increasing dietary content of BPM. The reduced ADN did not result in any significant difference in DN but there was a tendency for a lower DN for D IV, which indicates that animals on this diet had less N available for metabolism. Animals on D I excreted $0.4 \text{ g/kg}^{0.75}$ N more in urine (UN) than animals fed D IV, this difference being of the same magnitude as the difference in DN between these two diets. The retention of N (RN) was highest on D II and D III with $0.54 \text{ g/kg}^{0.75}$ and $0.52 \text{ g/kg}^{0.75}$, values that were significantly ($P<0.05$) higher than $0.40 \text{ g/kg}^{0.75}$ which was recorded for D IV. The utilisation of DN for RN was not significantly different between diets although the numerical difference between DIII and DIV was almost 5 percent units.

The apparent digestibility of energy (ADE) was similar to ADN, decreasing with increasing content of BPM in the diet. The reduction of ADE was caused by decreasing ADN and a concomitant decline in fat digestibility (data not shown) with increasing dietary content of BPM. This resulted in a significantly lower intake of metabolisable energy (ME) for animals on D IV compared to the other diets.

The respiratory quotient was about 0.8 for all diets, and the heat production (HE) was almost the same for animals on all diets. Therefore, the retention of energy (RE) showed the same pattern as the ME intake with the lowest RE for D IV and nearly the same RE for D I, D II and D III. Animals on D IV only retained $8 \text{ kJ/kg}^{0.75}$ and because RN was positive this resulted in a negative fat retention (data not shown), a result in accordance with the negative body gain. For the three other diets both RN and fat retention were positive.

The total HE given in Table 2 is the sum of the HE from oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF). The contribution of the respective nutrients to HE is shown in Figure 1 in percent of total HE. Only OXP was significantly affected by diet with animals on D IV oxidising less protein than animals on the other diets. The lower OXP was caused by the lower DN and the utilisation of DN for RN being similar to that on other diets making less protein available for oxidation.

Table 2. Nitrogen and energy metabolism data for mink kits fed diets with increasing levels of Bioprotein meal. Feed intake (g/kg^{0.75}), weight gain (g/day), ingested N (IN; g/kg^{0.75}), digested N (DN; g/kg^{0.75}), apparent digestibility of N (ADN; %), urinary N (UN; g/kg^{0.75}), retained N (RN; g/kg^{0.75}), DN/RN (%), apparent digestibility of energy (ADE; %), respiratory quotient (RQ), metabolisable energy (ME; kJ/kg^{0.75}), heat production (HE, kJ/kg^{0.75}), retained energy (RE; kJ/kg^{0.75}) and, P-values and square root of residuals (RR).

| | Diet | | | | RR | P-values | | | |
|-------------|--------------------|--------------------|--------------------|--------------------|------|----------|--------|-------|-------|
| | D I | D II | D III | D IV | | Diet | Period | D*P | Block |
| Feed intake | 117 | 117 | 122 | 104 | 19.6 | 0.14 | <.001 | 0.65 | 0.10 |
| Weight gain | -2.7 ^b | -0.3 ^b | 8.2 ^a | -12.1 ^c | 13.8 | 0.002 | <.001 | 0.55 | 0.10 |
| IN | 2.8 | 2.8 | 2.9 | 2.5 | 0.5 | 0.19 | <.001 | 0.86 | 0.08 |
| DN | 2.3 | 2.3 | 2.3 | 1.9 | 0.4 | 0.07 | <.001 | 0.81 | 0.07 |
| ADN | 83.3 ^a | 81.6 ^{ab} | 79.3 ^{bc} | 77.2 ^c | 2.6 | 0.002 | 0.001 | 0.23 | 0.29 |
| UN | 1.9 | 1.7 | 1.7 | 1.5 | 0.3 | 0.10 | <.001 | 1.00 | 0.06 |
| RN | 0.46 ^{ab} | 0.54 ^a | 0.52 ^a | 0.40 ^b | 0.2 | 0.05 | <.001 | 0.03 | 0.43 |
| RN/DN | 17.7 | 21.5 | 21.7 | 17.1 | 7.8 | 0.19 | <.0001 | 0.09 | 0.96 |
| ADE | 84.1 ^a | 81.4 ^b | 79.3 ^{bc} | 74.5 ^c | 2.8 | <.001 | 0.02 | 0.21 | 0.06 |
| RQ | 0.79 | 0.80 | 0.81 | 0.77 | 0.03 | 0.09 | <.001 | 0.70 | 0.01 |
| ME | 816 ^a | 792 ^a | 814 ^a | 652 ^b | 141 | 0.03 | <.001 | 0.55 | 0.03 |
| HE | 640 | 620 | 621 | 644 | 65 | 0.87 | <.001 | <.001 | 0.62 |
| RE | 176 ^a | 174 ^a | 193 ^a | 8 ^b | 134 | 0.01 | <.001 | 0.26 | 0.05 |

^{a,b,c} values that share no common superscript differ significantly (P<0.05)

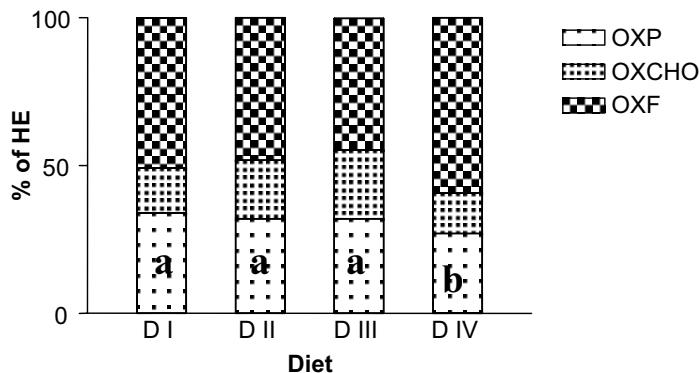


Figure 1. The oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF) in percent of heat production (HE), (^{a,b} columns that share no common superscript differ significantly (P<0.05)).

Discussion

On the highest inclusion level BPM provided 60% of DN in this experiment, and animals on D IV generally performed worse on this very high level of BPM than animals fed diets providing up to 40% of DN from BPM. Indeed, there was a trend for animals on D III to have an improved performance. This finding deviates somewhat from results of a production experiment in which

Ahlstrøm et al. (2002) reported a significantly reduced feed intake and live weight gain of male mink kits receiving a diet containing 8 % BPM.

The feed transit time through the mink digestive tract is very short (Charlet-Lery et al. 1981). The decreasing nutrient digestibility with increasing dietary supply of BPM may be caused by BPM containing intact bacterial cell walls which must be decomposed by digestive enzymes before matrix proteins can be digested and absorbed (Kihlberg, 1972), and it is possible that the feed transit time in mink is too rapid for this process to be completed. Moreover, the RNA and DNA fractions in BPM contribute with a significant amount of non-amino N, mainly in the form of purine and pyrimidine bases. On a molar basis the non-amino N content of BPM can be estimated to be in the order of 10-20%. It is not known if this fraction is digestible for mink, but for rats a high digestibility of between 90 % and 100 % has been reported for yeast-RNA (Greife and Molnar, 1980). Based on the present results it cannot be concluded if the decreased ADN was caused by low amino-N digestibility or that purine and pyrimidine bases were not absorbed. The end-product of purine base metabolism in nonprimate mammals is allantoin, which is excreted in urine, and an increased urinary allantoin excretion has been observed in pigs fed a bacterial protein source (Braude et al. 1977). Although the dietary amino-N decreased with increasing supply of BPM, RN was only impaired on D IV, which most likely was a combination of a lower DN intake and a slightly lower utilisation of DN for retention. Taken together the N balance data suggest that the dietary levels of BPM used in this experiment supported the protein requirement of growing male mink kits. The metabolism of purine and pyrimidine bases in BPM had no influence on heat production, and energy retention was similar on all diets except D IV. These results and the N balance data suggest that BPM could provide up to 40% of DN in a diet to growing male mink kits without inflicting measurable effects on the quantitative N and energy metabolism.

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Energy and nitrogen balance in slaughter chickens fed bacterial protein

Anne Louise Frydendahl Hellwing, Anne-Helene Tauson & André Chwalibog

The Royal Veterinary and Agricultural University, Department of Animal Science and Animal Health, Grønnegaardsvej 3, DK-1870 Frederiksberg C, Copenhagen, Denmark

Summary

Bioprotein meal (BPM) is a microbial protein with an amino acid pattern similar to that of fish meal. This new protein source was evaluated in a respiration and balance experiment with slaughter chickens fed different levels of BPM. The experiment comprised five balance periods carried out from a chicken age of 3 to 35 days. The inclusion levels of BPM were respectively 0 % (D 0), 2 % (D 2), 4 % (D 4) and 6 % (D 6) in the four diets. The growth performance, feed utilization and energy metabolism traits were nearly the same on all four diets. There was a tendency for ($P=0.08$) higher retention of nitrogen (RN) among animals fed the control diet D 0. It was concluded that the applied levels of BPM supported a normal animal performance, and that N balance and energy metabolism traits were not affected.

Keywords: poultry, bioprotein, nitrogen retention

Introduction

BioProtein meal (BPM) is a bacterial protein produced from spray-dried cells of mainly *Methylococcus capsulatus* (Bath). The protein content and the amino acid composition are very similar to that of fish meal, but the content of RNA and DNA is nearly 10 % of the dry matter (Skrede et al., 1998). The aim of the present study was to investigate the influence of increasing dietary level of BPM on protein and energy metabolism in slaughter chickens.

Material and methods

Seventy-two day old Ross chickens were allocated to four diets, each in three replicates, according to live weight. They were housed in twelve metabolic cages. From the age of 3 to 35 days five balance periods, each comprising a 4-days collection of droppings, were carried out. The original number of animals per cage (six in the first balance period) was subsequently reduced with one chicken per balance period until two in the fifth period. During each period two replicates per diet were measured in a 22-hour respiration experiment by means of indirect calorimetry in an open-air circulation system. In the first two periods all chickens were used but from then on measurements were made on individual chickens. In the diets fish meal was replaced with BPM (Norferm DA, Norway) on a weight basis. The contents of BPM were 0% (D 0), 2 % (D 2), 4 % (D 4) and 6 % (D 6). For dietary composition see Table 1. The data were analysed by means of the procedure MIXED in SAS with diet and balance period as fixed effects, cage as random effect, and two-way interaction between diet and balance period. Data are presented as LSmeans for the five balance periods and differences between observations were considered significantly different when $P<0.05$.

Table 1. Dietary composition in %.

| | D 0 | D 2 | D 4 | D 6 |
|-----------------|------|------|------|------|
| Fish meal | 6 | 4 | 2 | 0 |
| Bioprotein | 0 | 2 | 4 | 6 |
| Soybean meal | 20 | 20 | 20 | 20 |
| Oats | 19.2 | 19.1 | 19.0 | 18.6 |
| Wheat | 26 | 25.9 | 25.9 | 26 |
| Maize | 22 | 22 | 21.9 | 22 |
| Soya Oil | 3 | 3.2 | 3.4 | 3.6 |
| Minerals | 2.4 | 2.4 | 2.4 | 2.4 |
| Vitamins | 0.2 | 0.2 | 0.2 | 0.2 |
| Other additives | 1.2 | 1.2 | 1.2 | 1.2 |

Results

The main results are reported in Table 2.

Table 2. Live weight (LW) gain (g/day), feed intake (g/day), feed:gain (kg feed/kg live weight gain), ingested N (IN; g/day), Ndroppings (g/day), retained N (RN; g/day), respiratory quotient (RQ), metabolisable energy intake (ME; kJ/kg^{0.75}), heat production (HE; kJ/kg^{0.75}), and retained energy (RE; kJ/kg^{0.75}) in chickens fed different levels of Bioprotein meal. P-values and square root of residuals (RR). n=12 for all observations except ME, HE and RE where n=8.

| | Diet | | | | RR | P-value | | |
|-------------|------|------|------|------|-------|---------|--------|-------|
| | D 0 | D 2 | D 4 | D 6 | | Diet | Period | D*P |
| LW gain | 69 | 69 | 66 | 67 | 0.55 | 0.75 | <.001 | 0.98 |
| Feed intake | 104 | 102 | 101 | 102 | 0.76 | 0.90 | <.001 | 0.90 |
| Feed:gain | 1.4 | 1.4 | 1.5 | 1.5 | 0.21 | 0.38 | <.001 | 0.47 |
| IN | 3.8 | 3.4 | 3.5 | 3.5 | 0.81 | 0.21 | <.001 | 0.10 |
| Ndroppings | 1.5 | 1.3 | 1.3 | 1.3 | 0.86 | 0.32 | <.001 | 0.21 |
| RN | 2.33 | 2.15 | 2.20 | 2.15 | 0.13 | 0.08 | <.001 | 0.002 |
| RQ | 0.92 | 0.94 | 0.94 | 0.93 | 0.73 | 0.88 | <.001 | 0.60 |
| ME | 1040 | 1031 | 1062 | 1088 | 57.5 | 0.16 | <.001 | 0.08 |
| HE | 790 | 860 | 858 | 792 | 184.7 | 0.95 | <.001 | 0.74 |
| RE | 256 | 170 | 206 | 290 | 187.1 | 0.88 | <.001 | 0.18 |

The live weight gain, feed intake and feed utilisation were not significantly affected by diet. The live weight gain was around 70 g/day and the intake of feed was about 100 g/day, giving a feed utilization of 1.4 kg feed/kg live weight gain. The intake of nitrogen (IN) was somewhat higher on D 0 than the other diets but the difference was not significant. Also the excretion of N in droppings was slightly higher on D 0 than on the other diets. Although the differences in IN and excretion of N between diets were small there was a tendency (P=0.08) for a higher retention of nitrogen (RN) of 2.33 g/day on D 0 to be compared with 2.15 g/day and 2.20 g/day on the BPM containing diets. The differences between diets in intake of metabolisable energy (ME) were, similar to many other traits in this study, small and non-significant. The respiratory quotient (RQ)

values varied between 0.92 and 0.94 for the 4 diets. The heat production (HE) calculated without correction for urinary N, was not significantly different between treatment groups. The largest difference in energy retention (RE) between groups was 120 kJ/kg^{0.75}, which was non-significant.

Discussion

The present data suggest that at the applied inclusion levels BPM could substitute fish meal in slaughter chicken diets without effect on animal performance, N balance and quantitative energy metabolism traits. This was in agreement with Skrede et al. (2003), who found that up to 6% dietary BPM supported a normal weight gain in chickens although a higher BPM content resulted in a reduced weight gain. A chicken study with a flash-dried bacterial protein source showed a slight increase in performance when the bacterial protein supply was 96 g/kg, but performance was reduced when the inclusion level was 290 g/kg (D'Mello & Acamovic, 1976). Another type of bacterial protein gave a reduced growth at levels of 90 and 150 g/kg feed but 60 g/kg feed supported a normal weight gain (Plavnik et al., 1981).

The combined picture of studies with BPM and early types of bacterial protein hence suggest that a dietary supply of more than 6 to 10 % of the bacterial protein source may impair chicken performance. Both Plavnik et al. (1981) and Skrede et al. (2003) observed a reduced feed intake, when the content of bacterial protein increased, which may explain the reduction in body gain. Bacterial protein sources have a high content of RNA and DNA which possibly might contribute to the reduced feed intake when high dietary levels of bacterial protein are fed. The contents of RNA and DNA in feedstuffs like fish meal and maize are respectively 0.6 and 0.2 g/kg dry matter (Greife, 1984), but in BPM it is 100 g/kg dry matter. On the other hand, RNA and DNA can provide an absorbable non-protein nitrogen source. When fed to rats RNA was almost 100% digestible (Greife & Molnar, 1980) and in chickens the purine and pyrimidine bases were absorbed and a substantial part of them were metabolised by the intestine (Karasawa et al., 1991). Furthermore, purine and pyrimidine bases can be utilised as N source for chickens fed diets with a limiting content of non-essential amino acids (D'Mello, 1979). Therefore, it is likely that RNA and DNA from BPM were absorbed by the chickens but our experimental data could not confirm this.

However, this possible absorption of metabolites from the RNA and DNA degradation did not change the energy metabolism traits, which concurs with the results of an experiment with BPM as protein source for mink kits (Hellwing et al., 2003).

In conclusion, the present experiment has shown that a dietary BPM content up to 6 % had no measurable influence on the animal performance and quantitative nitrogen and energy metabolism traits of slaughter chickens.

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Nutritional memory on protein metabolism in Wistar rat

K. Kanskan & J.S. Keller

Warsaw Agricultural University, Faculty of Human Nutrition and Consumer Sciences,
Department of Dietetics and Functional Foods, Nowoursynowska 159C, 02-787 Warsaw

Summary

The experiment was carried out in 13 male *Wistar* rats weighing initially 250 g on the average. Six-week-experimental period was divided into three equal parts of 2 weeks. The animal consumed a high (20%) protein diet in the first and the third periods and a low protein diet in the second one. The casein was a sole source of protein in both diets. 24-hours urine collection was performed every 7 days and total N, urea, uric acid and creatinine was determined. The results indicated significant ($p<0.05$) the upward and downward change of the urea excretion level as compared to the sudden changes in protein intake. Creatinine excretion increased slightly during reduced protein intake period as compared to the preceding phase of experiment and remained at the same level during the period of protein realimentation. Uric acid excretion increased considerably ($p<0.05$) during the second period but decreased in the period of protein recovery. These results indicate the existence of so-called "nutritional memory" phenomenon in protein metabolism in rats, which persist more than 2 weeks after the change of protein intake level.

Keywords: nutritional memory, protein metabolism, rat

Introduction

In recent decades, there has been a tremendous interest in considering how different nutritional states such as undernutrition or overnutrition can exert short or long-term effects on metabolic processes in the body of various species.

Long-term consequences of the altering nutrition level during the period of growth and development upon the body composition, metabolic processes, health status and longevity of adult organisms are rather well known and the literature was reviewed by Mc Cance (1962) and Lucas (1998). These relationships are called "metabolic hysteresis" (Mobbs, 1994; Junghans, 1998), "metabolic programming" (Lucas, 1991; Lucas, 1998) or "nutritional imprinting" (Levin, 2000). The sudden changes in the level of protein intake are often observed in animal and human nutrition; however, there is little information on the dynamics of metabolic adaptation to that kind of changes (Tauson et al., 2000).

The purpose of this study was to examine the extent and the time scope of some adaptive processes in protein metabolism to the sudden decrease and increase in the level of protein intake in rats.

Material and methods

Thirteen male eight-week-old *Wistar* rats with an initial mean body weight of 250 ± 15.5 g were used for this investigation. The animals were housed individually in wire cages in a temperature - controlled room (23°C) with relative humidity approximately 65%, under a 12h light-dark cycle with lights on from 8.00 h to 20.00 h.

Three-day-long preliminary adaptation was applied, prior to the study followed by the six-week-experimental period divided into three equal periods of fortnight. All rats were offered approximately 20g of diet daily throughout the investigation. Two experimental diets were composed according to National Research Council (NRC, 1995) recommendations for rodents

with some slight modifications. During the first and the third periods animals were assigned HP diet (20% of crude protein), whereas in the second one LP diet (10% of crude protein) was applied. Body weight, feed consumption were recorded every week as well as 24-hours urine collection was performed for each animal individually. The urine volume was measured and stored immediately after collection in labeled flasks at -20°C. 1 ml sample of urine was used for nitrogen determination using Kjeldahl method on 1026 Kjeltec analyzer (Foss Tecator, Sweden). The urea, uric acid and creatinine content in centrifuged urine (10000 g for 3min.) was assayed using Roche Diagnostics kits on Hitachi 704 (Boehringer Mannheim, Germany) automated analyzer. Data were analyzed by one-way ANOVA and Fisher's least significant difference (LSD) procedure by means of STATGRAPHICS Plus version 4.1. Differences were considered significant at $p<0.05$.

Results

The influences of sudden decrease and increase in protein intake on protein metabolism are presented in Table 1.

Table 1. Adaptation of protein metabolism to decreased (Period II) and increased (Period III) protein intake.

| | Period II as compared to Period I (%) | | Period III as compared to Period II (%) | |
|------------------------|--|----------------------|--|----------------------|
| | 7 th day | 14 th day | 7 th day | 14 th day |
| Protein intake | 42.4 | 40.0 | 152.3 | 160.0 |
| Urinary total N | 42.6 | 44.0 | 270.0 | 240.0 |
| Urinary urea excretion | 91.0 | 77.0 | 130.9 | 129.7 |

As shown in Table 1 relative urea excretion level diminished statistically significant ($p<0.05$) as compared to period 1 after sudden decrease in protein intake but not as much as one could expect, and this delayed reaction existed more than 14 days.

As indicated by Table 1 relative nitrogen and urea excretion did not increase after sudden increase in protein intake as much as one could expect but markedly significant ($p<0.05$) and this delayed reaction persisted more than 14 days.

Both delays in adaptation of protein metabolism to decreasing and increasing levels of protein intake evidence the phenomenon of so-called "nutritional (metabolic) memory" in protein metabolism in rat and it lingered on more than 14 days.

The influences of sudden decrease and increases in protein intake on creatinine and uric acid excretion are presented in Table 2.

Based on the results highlighted in Table 2, it can be concluded that decreasing protein intake influenced the statistically significant increase of creatinine excretion ($p<0.05$) in second week of Period II, what could be related to the increase of the metabolic rate as a consequence of higher physical activity of hungry animals looking for feed. Considerably higher uric acid excretion ($p<0.05$) during the period of reduced protein intake might be related to the lower rate of cells rebuilding after normal degradation.

Thorough analysis of Table 2 shows that increasing protein intake did not influence the level of creatinine excretion ($p>0.05$), but showed statistically significant decrease in the urinary excretion of uric acid.

Table 2. Adaptation of creatinine and uric acid excretion to decreased (Period II) and increased (Period III) protein intake.

| | Period II as compared to Period I (%) | | Period III as compared to Period II (%) | |
|------------------------------|--|----------------------|--|----------------------|
| | 7 th day | 14 th day | 7 th day | 14 th day |
| Protein intake | 42.4 | 40.0 | 152.3 | 160.0 |
| Urinary creatinine excretion | 113.1 | 122.2 | 103.7 | 104.5 |
| Urinary uric acid excretion | 214.2 | 186.8 | 48.8 | 68.3 |

Discussion

Classical point of view presented in that experiment is that the level of daily urinary nitrogen excretion is a linear function of daily protein intake. In our study daily excretion of urea showed the delay of more than 14 days as compared to the time of a sudden change of protein intake, what is interpreted as the result of the so-called "nutritional (metabolic) memory".

Lower or higher level of protein intake has not affected dietary purines consumption. It correspond to Krebs (1978) indicating no influence of dietary protein on uric acid level where purine free diet such as a casein diet is consumed. Thus, changes of uric acid excretion observed in this experiment might be related to the variable degradation rate of endogenous nucleic acids, what might be connected to the well known phenomenon of body cells apoptosis.

Creatinine excretion level is related to the metabolic rate of an organism. Therefore, lower rate of protein synthesis was mirrored by the decreased rate of metabolism during the second period of the experiment.

It has been concluded that described results indicate the existence of the so-called nutritional memory phenomenon in protein metabolism in rats, which persists more than 2 weeks after the change of protein intake level. The present experimental design seems to require some modifications, which will be beneficial for further research.

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Effects of a low lysine diet on glucose metabolism in skeletal muscle of growing pigs

M. Katsumata, M. Matsumoto & Y. Kaji

National Agricultural Research Center for Kyushu Okinawa Region, Kumamoto, 861-1192, Japan

Summary

Six-week-old barrows were pair-fed a control or low lysine (LL) diet for three weeks. The control diet was designed to contain optimal amounts of all essential amino acids including 1.18% lysine. The LL diet was similar but contained only 0.70% lysine. D-glucose (3g/kg BW^{0.75}) was orally given to the pigs 1 h before the samplings. *Longissimus dorsi* (*l. dorsi*) and *rhomboideus* muscles were sampled for measurements of activities of hexokinase (EC 2.7.1.1) and citrate synthase (EC 4.1.3.7), and for contents of glucose 6-phosphate and glycogen. Plasma free lysine concentration was significantly lower in pigs fed on the LL diet ($P<0.01$). GLUT4 mRNA expression in *rhomboideus* muscle was significantly higher in pigs fed on the LL diet ($P<0.05$). Activity of hexokinase was unaffected by dietary lysine level while that of citrate synthase was higher both in *l. dorsi* and *rhomboideus* muscles of pigs fed on the LL diet ($P<0.05$). In *l. dorsi* muscle, glucose 6-phosphate content was higher in pigs fed on the LL diet ($P=0.0666$). Glycogen content was higher both in *l. dorsi* and *rhomboideus* muscles of pigs fed on the LL diet ($P=0.0745$ & $P<0.01$, respectively). From these observations, we conclude that dietary lysine level plays a role in regulating glucose metabolism in porcine skeletal muscle.

Keywords: dietary lysine, glucose metabolism, porcine skeletal muscle

Introduction

We have reported that a low lysine diet selectively up-regulates muscle GLUT4 gene and protein expression in growing pigs (Katsumata et al. 2001). However, the role of dietary lysine level in regulating post-GLUT4 glucose metabolism is unknown. We decided, therefore, to conduct a study to elucidate effects of a low lysine diet on glucose metabolism in skeletal muscle of growing pigs.

Materials and methods

Seven litters each of two barrows aged 6 weeks were used. Each littermate was assigned to one of two diets, control or low lysine (LL), which were isoenergetic and isoprotein; providing 14.2 MJ/kg DE and 16.0% crude protein. The control diet was designed to contain all essential amino acids in the recommended amounts (NRC 1998), including 1.18% lysine. The LL diet was similar but contained only 0.70% lysine. A pair-feeding design was used. The amount of food was increased as the pigs grew and the final daily intake was 950-1050 g. At 9 weeks old, *l. dorsi* and *rhomboideus* muscles were sampled 16-17 hours after the last meal. D-glucose (3g/kg BW^{0.75}) was orally given to the pigs 1h before the samplings in order to make the most of predicted up-regulation of GLUT4 expression. Plasma free amino acid concentration was determined by chromatographic separation method by using an automatic amino acids analyzer (L-8800, Hitachi Ltd, Tokyo, Japan). GLUT4 mRNA abundance in *rhomboideus* muscle was determined by RNase protection assay. Activities of hexokinase and citrate synthase were determined spectrophotometrically. The enzymes were chosen to be analyzed because the coordinated up-regulation of GLUT4 and these two enzymes had been observed (Hjeltnes et al. 1998, Henriksen

& Halseth 1995). Contents of glucose 6-phosphate and glycogen were measured by enzymatic methods. Further, in order to have insights into effects of dietary lysine level on mitochondrial oxidative phosphorylation, we measured state 3 and state 4 oxygen consumption rates of subsarcolemmal mitochondria isolated from *rhomboideus* muscle. The data were subjected to analysis of variance for randomized block design, where litter was block and dietary lysine level was the main effect.

Results

Growth rate was significantly lower in pigs fed on the LL diet compared with the control diet (350 g/d vs. 454 g/d; $P<0.01$). GLUT4 mRNA abundance in *rhomboideus* muscle was 1.9-fold higher in pigs fed on the LL diet ($n=5$, $P<0.05$), while plasma glucose concentration was unaffected. Plasma free lysine concentration was significantly lower in pigs fed on the LL diet compared with the control diet ($P<0.01$, Figure 1). Interestingly, concentrations of plasma free threonine, isoleucine, phenylalanine, and histidine were higher, and that of leucine was lower in pigs fed on the LL diet ($P<0.01$, Figure 1).

Although hexokinase activity was not affected by dietary lysine level, that of citrate synthase was significantly higher in both *l. dorsi* and *rhomboideus* muscles of pigs fed on the LL diet ($P<0.05$, Figure 2). Glucose 6-phosphate content in *l. dorsi* muscle tended to be higher in pigs fed on the LL diet compared with the control diet ($P=0.0666$, Figure 2). Further, glycogen content in *l. dorsi* tended to be higher in the LL diet group ($P=0.0745$, Figure 2), while that in *rhomboideus* was significantly higher in the LL diet group ($P<0.01$, Figure 2). State 3 and 4 oxygen consumption rates of subsarcolemmal mitochondria isolated from *rhomboideus* muscle were not affected by dietary lysine level ($n=4$).

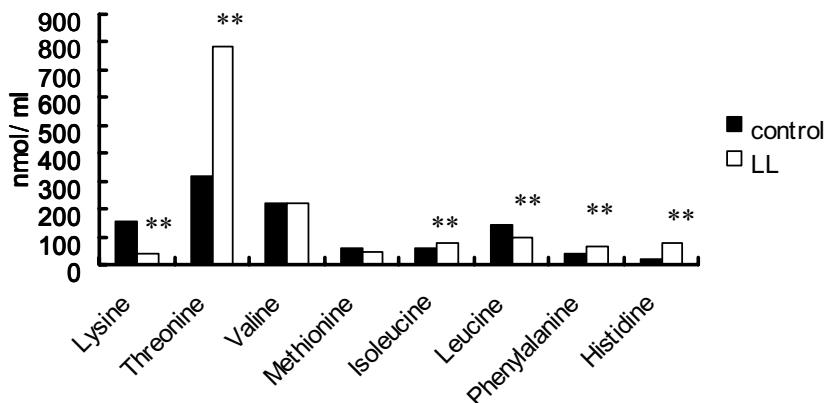


Figure 1. Concentrations of plasma free amino acids. Values are the least squares means. ***; significantly different from the control diet group ($P<0.01$).

Discussion

We predicted that plasma glucose level would be lower in pigs fed on the LL diet because of the up-regulation of muscle GLUT4 expression. Orally given glucose to the pigs 1 h before the samplings strengthened this prediction. Indeed, plasma glucose concentration after 3 h removal of diet tended to decrease with decreasing dietary lysine level in *ad lib* fed finishing pigs (Goodband et al. 1990). However, plasma glucose concentration of pigs fed on the LL diet did not differ from that of pigs fed on the control diet. It was shown by Lörhrke et al. (2001) that quality of dietary protein affected insulin-stimulated glucose uptake to muscle cells *in vitro*; pigs given soy protein

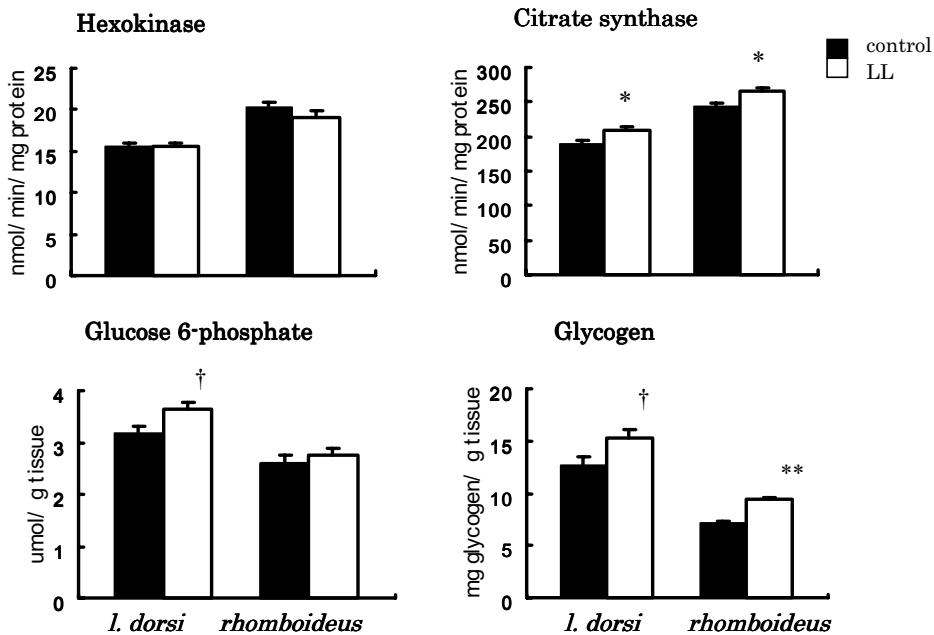


Figure 2. Activities of hexokinase and citrate synthase, and contents of glycogen and glucose 6-phosphate in *l. dorsi* and *rhomboideus* muscles ($n=5$). Values are the least squares means \pm standard errors. †, * and **; significantly different from the control diet group ($P<0.1$, $P<0.05$, and $P<0.01$, respectively).

isolate as protein source had higher basal glucose uptake to muscle cells but they were less sensitive to insulin compared with pigs given a diet based on casein. The LL diet in this study is comparable to their diet based on soy protein isolate. Thus, sensitivity of muscle to insulin might be affected by dietary lysine level. It is necessary to have further studies to elucidate the effect of dietary lysine level on glycemia in relation to peripheral sensitivity to insulin.

One of the interesting results obtained in this study is concentration of plasma free amino acid. As predicted, pigs fed on the LL diet had lower plasma free lysine concentration. However, other free amino acid responded differently. As shown in Figure 1, concentrations of free threonine, isoleucine, phenylalanine, and histidine were higher, whereas that of leucine was lower in pigs fed on the LL diet compared with the control diet. Higher threonine, isoleucine, phenylalanine, and histidine concentrations of pigs fed on the LL diet agree with results obtained by Goodband et al. (1990) where all these amino acid concentrations linearly increased with decreased dietary lysine levels. Overall protein synthesis rate in pigs fed on the LL diet might be lower due to the shortage of lysine in the diet. Thus, excess amino acids that were not utilized for body protein synthesis kept circulating in the blood stream, and as a consequence, concentrations of these four essential amino acids might be maintained higher. However, the reason that explains lower concentration of leucine in pigs fed on the LL diet is unknown.

We hypothesized that activities of both hexokinase and citrate synthase in skeletal muscle would be higher in pigs fed on the LL diet because the coordinated up-regulation of GLUT4 and these enzymes had been observed (Hjeltnes et al. 1998, Henriksen & Halseth 1995). However, in this study, dietary lysine level did not affect the activity of hexokinase although that of citrate synthase was higher in pigs fed on the LL diet. The coordinated up-regulation of GLUT4 expression and these enzymes were induced by exercise (Hjeltnes et al. 1998, Henriksen & Halseth 1995), while GLUT4 expression in this study was up-regulated by a low level of dietary lysine. Thus,

underlying mechanisms up-regulating GLUT4 expression by exercise and dietary lysine level may be different. Although activity of hexokinase was unaffected by dietary lysine level, content of glucose 6-phosphate tended to be higher in the muscle sample of the pigs fed on the LL diet. Further, content of glycogen in the muscle sample was higher in pigs fed on the LL diet. These results indicate that the amount of glucose entering the glycolysis and/or glycogen synthesis in skeletal muscle is higher in pigs fed on the LL diet. The higher activity of citrate synthase in the muscle sample of pigs fed on the LL diet may be partly explained by higher amount of glucose entering glycolysis. In addition, amino acids may also play a role. The amino group of excess amino acid circulating in blood stream is removed and eventually excreted as urea. The remaining carbon skeleton can be completely oxidized by the combined action of the TCA cycle and oxidative phosphorylation. The carbon skeletons of amino acids are firstly converted to pyruvate, acetyl-CoA, or some other intermediates of the TCA cycle. As shown in Figure 1, concentrations of four amino acids in the plasma were higher in pigs fed on the LL diet. Thus, the TCA cycle in skeletal muscle might be activated to oxidize the carbon skeletons of these excess amino acids. Indeed, three out of four pigs examined had higher state 4 oxygen consumption rate of subsarcolemmal mitochondria isolated from *rhomboideus* muscle although the effect of the LL diet was not statistically significant. As citrate synthase catalyzes synthesis of citrate, an intermediate of the TCA cycle, from acetyl-CoA and oxaloacetate, the activity of this enzyme might be affected by the plasma free amino acid concentration.

In summary, we conclude that dietary lysine level plays a role in regulating glucose metabolism in porcine skeletal muscle as well as regulation of GLUT4 expression.

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Influence of metabolic memory on the dietary protein utilization in young adult men

D. Kluszczyńska & J.S. Keller

Department of Dietetics and Functional Foods, Warsaw Agricultural University,
Nowoursynowska 166, 02-787 Poland

Summary

In the experiment lasting 12 weeks, took part ten young adult persons, all were volunteers. They consumed for the first 4 weeks diet containing protein at the level of 1g /d/ kg body mass, then during the next 4 weeks diet containing protein at the level of 0,5 g / kg body mass and for the last 4 weeks - diet containing protein on the level as during the first period. It was found, that during the first 3 weeks of the reduced protein intake the total nitrogen, ^{15}N from glycine and urea excretion were relatively higher, than it was expected and they were decreasing gradually, reaching a level adequate to the protein consumption after 4 weeks of the treatment. During the realimentation period total nitrogen, ^{15}N and urea excretion was increasing gradually, reaching the level almost the same as during the initial period just after 4 weeks. The uric acid excretion was constant during all twelve weeks of the experiment. The creatinine excretion showed the insignificant tendency to decrease in the time of protein malnutrition, and to rise in the realimentation time. The dynamics of ^{13}C -leucine oxidation in postabsorptive state, determined during 3 hours, did not show any changes depending on the level of protein consumption on the day preceding the measurement, what indicated the mechanism of the muscle proteins protection against the increase of oxidation. The results show that protein metabolism in human organism adapts to the changes of dietary protein intake for the period of at least 3 weeks.

Keywords: nutritional memory, protein metabolism

Introduction

Periods of inadequate consumption of some nutrients, including protein are often observed in human nutrition. This phenomena occurs in clinical nutrition, preoperative and postoperative states, as well as in healthy people nutrition, for example during the application of weight reducing diets. So far, there hasn't been sufficiently investigated the range and the rate of metabolic adaptation to the changing level of nutrition. The study of these adaptive processes is essential for establishing rational nutritional/dietetics recommendations. The changing nutritional level in young organisms may influence the long-term changes in functioning of the adult organism (Lucas, 1991, Waterland, 1999, Levin, 2000), and in adult organism can cause reversible adaptive processes in the metabolism (Junghans *et al.*, 1998). The aim of this research was to investigate the dynamics of adaptive, metabolic processes occurring in protein metabolism as consequence of sudden changes in the level of protein intake.

Material and methods

There were made 10 twelve-weeks measurements on young, adult person, all were volunteers. They were fed according to their individual energetical needs, determined on the base RMR measurements with the use of indirect calorimetry in metabolic chamber taking into account the count coefficient 1,4. The volunteers consumed for the first 4 weeks diet containing protein at the level of 1g / kg body mass, then during next 4 weeks diet containing protein at the level of 0,5 g

/ kg body mass and for the last 4 weeks - diet containing protein on the level, as during first period (1g/kg body mass). Every seven days each person was examined; all volunteers were then given the ^{13}C -leucine in postabsorptive state and ^{15}N -glycine with the breakfast meal. The dynamic of ^{13}C -leucine oxidation in postabsorptive state, during 3 hours from the application of the marker, was evaluated, and then 24-hour urine was collected from each person, and the total nitrogen, ^{15}N , urea, uric acid and creatinine were determined. The statistic analysis was made by the use of *Statistica 5.0* programme, using one-way analysis of variance, $p < 0,05$.

Results and discussion

Excretion of total nitrogen, ^{15}N , urea and ^{13}C -leucine oxidation

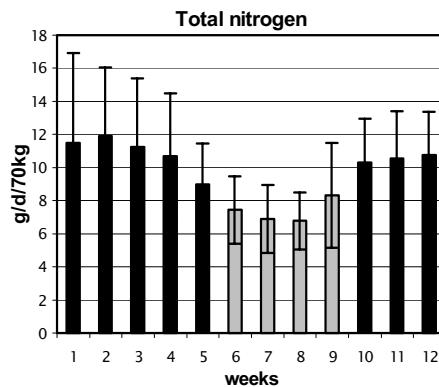


Figure 1. 24-hour excretion of total nitrogen.

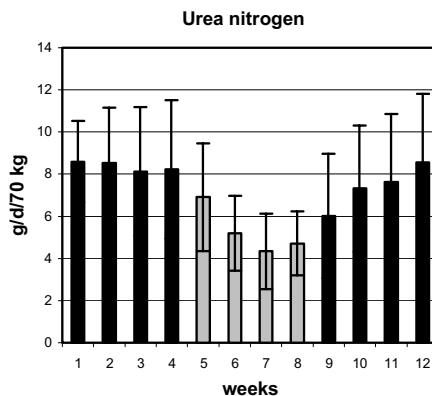


Figure 2. 24-hour excretion of urea nitrogen.

The reduced dietary protein level of 0,5 kg/d/kg body mass resulted in the decreasing of protein intake by about 40 g per day. Such decrease should diminish the nitrogen excretion by about 6,4 g, nevertheless that level has not been obtained before 3 weeks of the experimental period. The excretion of ^{15}N from glycine as well as of urea were decreasing gradually during 3 weeks after sudden decrease of protein intake. When the daily protein intake increased by 40 g, the excretion of all fractions of nitrogen was not so high as expected, but was increasing gradually during 3 weeks after the sudden increase of protein intake level. These results show, that the rate of body

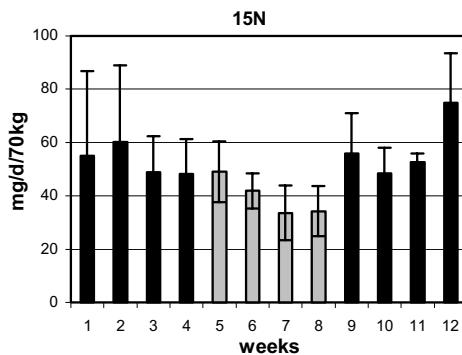


Figure 3. 24-hour excretion of ^{15}N .

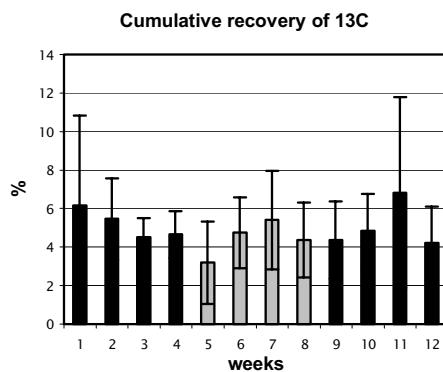


Figure 4. Cumulative recovery of ^{13}C - leucine after 180 min of breath test.

proteins degradation showed the tendency to remain on the level characteristic for the preceding period and was changing gradually during 3 weeks at least.

^{13}C - leucine utilization

The observed constant rate of ^{13}C - leucine oxidation should be related to the constant level of muscle protein degradation during the all three periods of experiment. It confirms the opinion of the protection of muscle proteins during decrease of protein intake level.

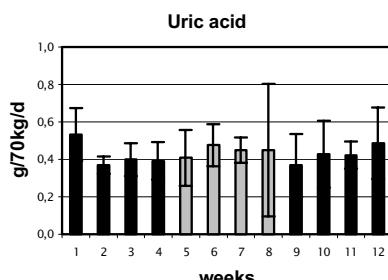


Figure 5. 24-hour uric acid excretion.

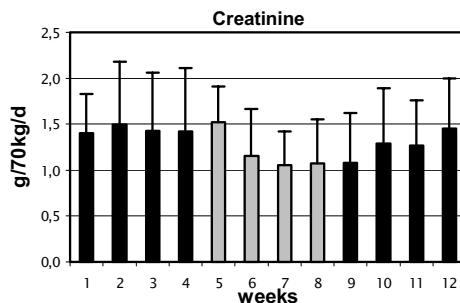


Figure 6. 24-hour creatinine excretion.

Decreased level of protein intake should be partly related to the lower level of purine intake, nevertheless the uric acid excretion has not been decreased. It can result from the lower rate of rebuilding of body cells broken up during this period of reduced protein intake.

In present research the creatinine excretion was decreasing by about 16% just after 3 weeks of reduced protein intake and returned to the initial level after 4 weeks of realimentation. It seems to be characteristic, that dynamics of the creatinine excretion indicated one-week delay in relation to the changes occurring on the level of protein intake, what was visible as well in the period of reduced protein intake as during the realimentation. 16% decrease of muscle mass during 4-weeks of reduced protein intake, and then adequate increase of muscle mass, seems to be absolutely incredible, that is why we should suppose, that observed changes in creatinine excretion were connected with the changes of the metabolic rate. The reduced protein intake to the half had to decrease the rate of protein synthesis in the muscles, while the energyl cost of protein synthesis plays significant role in energy metabolism (Keller, 2000).

Conclusions

1. Protein metabolism in human organism adapted to the sudden changes in the protein intake level but this adaptation persisted at least 3 weeks. During the period of reduced protein intake the metabolic adaptation consisted on slow, but not sudden decreasing of the oxidation rate of amino acids releasing from the labile protein pool of the organisms, what appeared in slow decreasing rate of total nitrogen, ^{15}N excretion (coming from labelled glycine), as well as excretion of the urea in 24-hour urine. During the realimentation period total nitrogen, ^{15}N and urea excretion increase gradually, reaching the level almost the same as during the initial period just after 4 weeks. This gradual, not immediate metabolic adaptation was the result of short-time metabolic (nutritional) memory.
2. The constant rate of ^{13}C -leucine oxidation rate showed, that muscle protein degradation does not change significantly during the period of reduced dietary protein intake, what indicated the mechanism of protection of muscle protein degradation during the period of low protein intake.
3. Negative nitrogen balance rising temporary in organism after sudden reducing of protein intake level probably decreased the rate of the rebuilding of body cells breakdown, what was manifested in maintaining the constant level of uric acid excretion in 24-hour urine in spite of decreasing purines intake in diet.
4. The tendency to decrease the creatinine excretion, observed during the period of reduced protein intake, seems to be related to the decrease of the metabolic rate what was probably connected with the decreasing protein synthesis rate in organism.

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Insulin-stimulated net utilisation of plasma glucose and amino acids in growing pigs

S.J. Koopmans¹, Z. Mroz¹, R. Dekker¹, H. Corbijn¹, J. Wijdenes¹, S. v.d. Crabben², M. Ackermans² & H.P. Sauerwein²

¹ ID-Lelystad, Department of Animal Nutrition, Edelhertweg 15, P.O. Box 65, 8200 AB Lelystad, The Netherlands

² AMC, Academic Medical Center Amsterdam, Department of Endocrinology and Metabolism, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands

Summary

The aim of the present study was to quantify the effect of elevated plasma insulin concentrations on the net utilisation and turnover of plasma glucose and amino acids in conscious growing pigs of approximately 40 kg BW. Therefore, basal and insulin-stimulated glucose and amino acid clamp experiments in combination with $6,6\text{-}^2\text{H}$ -glucose and $1\text{-}^{13}\text{C}$ -leucine infusions were performed. Insulin, glucose and amino acids were infused into the portal vein, and arterial blood was clamped at the desired concentrations. Under insulin-stimulation (3-fold increase over baseline insulin concentration), the net utilisation of glucose and amino acids from blood into body tissues appeared to be 17.3 ± 1.7 and 1.72 ± 0.19 mg/kg.min ($\sim 10:1$), respectively. As plasma urea concentrations declined during the clamp experiments, it can be regarded that the value of 1.72 mg/kg.min for amino acids reflects the net body protein synthesis. We found that glucose turnover decreases (hepatic glucose release into blood was completely inhibited) and that leucine turnover increases during the clamp experiments. The latter implies that insulin stimulates net protein synthesis under conditions of increased protein turnover in growing pigs.

Keywords: pig, insulin, nutrient turnover

Introduction

After a meal, carbohydrates and proteins are enzymatically digested in the gastro-intestinal tract and, subsequently, the fluxes of glucose and amino acids increase in the portal vein. The postprandial rise in blood glucose and amino acid concentrations triggers insulin secretion from the pancreatic beta-cells into the portal vein. The purpose of postprandial insulin secretion is to facilitate and stimulate the postprandial utilisation of blood glucose and amino acids by the general anabolic action of insulin. The portal mixture of glucose, amino acids and insulin enters firstly metabolic pathways in the liver and afterwards via the hepatic vein it is delivered for further cellular processes in the whole body. The stimulation of glucose and amino acid utilisation by insulin mainly reflects anabolic processes, except for the stimulation of glucose oxidation, like the net metabolic syntheses of glycogen, lipids and/or protein (Newsholm & Leach, 1989 and DeFronzo & Ferrannini, 1992).

Material and methods

Animals and experimental design

The experimental protocol describing the experimental design, management, surgical procedures, and animal care was approved by the Animal Care and Use Committee (Lelystad, The Netherlands). Eight crossbred male pigs (Yorkshire x [Dutch Landrace x Finnish Landrace]) of

approximately 30 kg were used in this study. Each pig was an experimental unit. Data are expressed as mean \pm SEM. Statistical comparisons between "Baseline" and "Clamp" were performed with the paired Student's t-test at p<0.01.

Surgery

Pigs were housed individually in metabolic cages and after a 10 days habituation period, they were anesthetized by intramuscular injection of 2 mg azaperone/kg (Stressnil; Janssen, Tilburg, The Netherlands) followed by an intravenous injection of 15 mg Nesdonal/kg (Rhone Merieux, Lyon, France). Pigs were intubated and general anesthesia was maintained by inhalation anesthesia with O₂, N₂O, and isoflurane. Polyethylene catheters (Tygon, i.d. 1.02 mm, o.d. 1.78 mm, length 1 m; Norton, Akron, Ohio, USA) were placed into the right carotid artery and the right external jugular vein according to a modified procedure (Koopmans et al., 1991) and into the portal vein via the splenic vein. The catheters were inserted and advanced until the tip of the catheter reached the aorta (carotid artery catheter) or the antrum (jugular vein catheter) or the portal vein (just one cm distal of the liver). The catheters were fixed firmly at the place of insertion and were tunneled subcutaneously to the back of the pig and exteriorized between the shoulder blades. The catheters were filled and sealed with physiological saline containing 50 IU heparin and 150.000 IU penicillin (Procpen; AUV, Cuijk, The Netherlands) per mL and kept in and protected by a back pack which was glued to the skin of the pig's back. During surgery the pig was given an intramuscular injection of antibiotic (300.000 IU procaïne penicilline G, Depocilline, Mycofarm Nederland B.V., De Bilt, The Netherlands) and anodyne (50 mg flunixin, Finadyne, Schering-Plough N.V./S.A., Brussel, Belgium). After 10 days of recovery, the pigs were used for the study.

Measurements of plasma glucose and amino acid turnover and net utilisation

To calculate glucose- and leucine turnover (Rate of appearance, Ra) at baseline (preprandial) plasma insulin concentrations, a bolus-constant intraportal infusion of 6,6-²H-glucose (72 mg-1.2 mg/min) and 1-¹³C-leucine (24 mg-0.4 mg/min) started after overnight fasting. Subsequently, hyperinsulinaemic euglycaemic euaminoacidaemic clamp experiments were induced by a constant intraportal infusion of insulin (1 mU/kg.min) to the infuse of 6,6-²H-glucose and 1-¹³C-leucine. Simultaneously, intraportal infuses of D-glucose (330 g/L) and a tailor-made mixture of 20 amino acids (Table 1) have been provided in order to maintain euglycaemia and euaminoacidaemia.

Frequent arterial blood sampling and the rapid on-line measurement of plasma glucose, phenylalanine and tryptophan concentrations enabled to adjust the infusion rates of the D-glucose and amino acid infuses and to maintain euglycaemic and euaminoacidaemic status. With the intraportal infusion rates of D-glucose and all these amino acids, the whole body insulin-stimulated net utilisation of glucose and these amino acids could be calculated.

Table 1. Tailor-made mixture of 20 amino acids (g/L).

| | | | |
|-----------------|--------------------|--------------------|-----------------|
| Alanine, 4.6 | Glutamine, 6.9 | Leucine, 5.5 | Serine, 3.3 |
| Arginine, 3.5 | Glutamate, 4.8 | Lysine-HCl, 3.6 | Threonine, 3.0 |
| Asparagine, 1.9 | Glycine, 7.7 | Methionine, 1.6 | Tryptophan, 1.0 |
| Aspartate, 0.2 | Histidine-HCl, 2.0 | Phenylalanine, 2.3 | Tyrosine, 2.2 |
| Cysteine, 0.7 | Isoleucine, 3.2 | Proline, 4.9 | Valine, 4.1 |

Results

The effects of insulin stimulation on glucose and amino acid metabolism in growing pigs are presented in Tables 2, 3 and 4.

During the clamp experiments, steady state plasma insulin concentrations were increased 3-fold (to 21 ± 3 mU/L), plasma glucose and amino acid concentrations remained constant (5.1 ± 0.1 mmol/L and 3.9 ± 0.1 mmol/L, respectively) and plasma urea concentrations declined by 17% (to 2.0 ± 0.1 mmol/L, $p<0.01$). Under these hyperinsulinaemic euglycaemic and euaminoacidaemic conditions, hepatic glucose release into blood was completely inhibited whereas whole body leucine release into blood was elevated 2-fold (to 1.09 ± 0.04 mg/kg.min, $p<0.01$). At the same time, the uptake of plasma glucose into body tissues (R_d) was increased 4-fold (to 17.1 ± 1.8 mg/kg.min, $p<0.01$) and of leucine 2.4-fold (to 1.25 ± 0.05 mg/kg.min, $p<0.01$). Insulin-stimulated net utilisation of the intraportal infused glucose and amino acids was 17.3 ± 1.7 mg/kg.min and 1.72 ± 0.19 mg/kg.min, respectively.

Table 2. Plasma insulin, glucose, amino acid and urea concentrations before (baseline), and during the steady state period of the hyperinsulinaemic euglycaemic euaminoacidaemic clamp experiments in pigs.

| | Baseline | CV(%) baseline | Clamp | CV(%) |
|----------------------|-------------|----------------|---------------|-------|
| clamp | | | | |
| Insulin (mU/L) | 7 ± 2 | 31 | $21\pm3^*$ | 21 |
| Glucose (mmol/L) | 4.8 ± 0.2 | 6 | 5.1 ± 0.1 | 8 |
| Amino acids (mmol/L) | 3.9 ± 0.1 | 5 | 3.9 ± 0.1 | 7 |
| Urea (mmol/L) | 2.4 ± 0.1 | 5 | $2.0\pm0.1^*$ | 12 |

CV=coefficient of variation within pigs. * $p<0.01$ compared to baseline.

Table 3. Hepatic glucose release, whole body glucose uptake, whole body leucine release and whole body leucine uptake before (baseline), and during the steady state period of the hyperinsulinaemic euglycaemic euaminoacidaemic clamp experiments in pigs.

| | Baseline | Clamp |
|--|---------------|------------------|
| Hepatic glucose release (mg/kg.min) | 4.6 ± 0.4 | $-0.2\pm0.4^*\#$ |
| Whole body glucose uptake (mg/kg.min) | 4.6 ± 0.4 | $17.1\pm1.8^*$ |
| Whole body leucine release (mg/kg.min) | 0.53 ± 0.03 | $1.09\pm0.04^*$ |
| Whole body leucine uptake (mg/kg.min) | 0.53 ± 0.03 | $1.25\pm0.05^*$ |

* $p<0.01$ compared to baseline. # not different from 0.

Note: glucose and leucine turnover were calculated by the isotope ($6,6^{-2}\text{H}$ -glucose and 1^{-13}C -leucine) dilution technique.

Table 4. Whole body insulin-stimulated net utilisation of glucose, leucine and total amino acids (as calculated by the intraportal infusion rates of D-glucose and the tailor-made mixture of 20 amino acids).

| | |
|--|---------------|
| Net utilisation of glucose (mg/kg.min) | 17.3 ± 1.7 |
| Net utilisation of leucine (mg/kg.min) | 0.16 ± 0.02 |
| Net utilisation of total amino acids (mg/kg.min) | 1.72 ± 0.19 |

Discussion and conclusion

After a meal, portal fluxes of glucose, amino acids and insulin increase. The portal mixture of glucose, amino acids and insulin enters firstly hepatic metabolism and subsequently a peripheral metabolic pool is reached. Postprandial whole body protein turnover is increased: meal-stimulated protein accretion was shown to be associated with higher rates of both protein synthesis and protein breakdown (Reeds et al., 1981). In addition, diet-induced hyperinsulinaemia was shown to be accompanied by both increased muscle growth and muscle protein turnover (Jepson et al., 1988).

To properly mimic the hepatic and peripheral effects of insulin on whole body glucose and amino acid metabolism, we chose to infuse insulin, glucose and amino acids via the portal vein during the clamp studies.

The present study demonstrates that portal infusion of insulin at a rate of 1 mU/kg.min resulted in a 3-fold rise in arterial plasma insulin concentrations. The rates of portally infused D-glucose and amino acids to maintain arterial euglycaemia and euaminoacidaemia were used to assess the net utilisation of glucose and amino acids. The net utilisation of glucose (17.3 ± 1.7 mg/kg.min, i.e. ~997 g/day) was 10 times greater than the net utilisation of amino acids (1.72 ± 0.19 mg/kg.min, i.e. ~99 g/day). The net utilisation of amino acids most likely reflects net protein synthesis (i.e. protein accretion) since plasma urea concentrations decreased during the clamp experiments. Decreasing plasma urea concentrations during hyperinsulinaemic-euglycaemic clamps (without and with amino acid infusion via the jugular vein) in neonatal piglets were also observed by Wray-Cahen et al. (1997). Finally, our study shows that insulin-stimulated protein accretion occurs under conditions of increased protein turnover (as extrapolated from leucine turnover) in growing pigs.

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Predictions of energy digestibility in pig feeds

Jan Erik Lindberg & Carsten Pedersen

Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management,
P.O. Box 7024, SE-75007, Uppsala, Sweden

Summary

Feed samples were collected from feedstuffs and some diets that had been evaluated *in vivo* for digestibility in balance trials with growing pigs in Finland (n=33), Denmark (n=67) and Sweden (n=37). The samples covered a wide range of feedstuffs used in pig feeds and were analysed for neutral detergent fibre (NDF) and their *in vitro* digestibility of dry matter or organic matter (IVDOM) according to a three-stage method (total tract). The effect of NDF content and IVDOM on the dE in the pig feeds were subjected to linear regression analysis.

The content of NDF (g kg^{-1} DM) was closely related ($P<0.001$) to the *in vivo* variations in dE (digestibility of energy) (%) in samples for each country, as well as for all samples including all countries ($dE = 92.9 - 0.09 \times \text{NDF}$; $n=137$, $R^2=0.82$, RSD=4.62). Also, the *in vitro* digestibility of OM (IVDOM, %) was closely related ($P<0.001$) to the *in vivo* variations in dE (%) in samples for each country, as well as for all samples including all countries ($dE = -6.61 + 1.02 \times \text{IVDOM}$; $n=144$, $R^2=0.87$, RSD=3.99).

It was concluded that NDF and IVDOM could be used to predict the dE in pig feeds with similar precision. This provides an alternative approach for estimating the energy value of pig feeds to that currently used, and could form the basis for a future common energy evaluation system in the Nordic countries.

Keywords: digestibility, *in vitro*, NDF

Introduction

Currently, important differences exists in the adopted digestibility coefficients of nutrients for typical feedstuffs in the Nordic countries (Lindberg, 1997). As a result, the same feedstuff or diet with identical ingredient composition will be given different energy values depending on the data base used. An important first step when aiming at harmonizing the energy evaluation of diets for pigs would be to use the same principles to estimate the content of digestible energy, as this could form a common basis for predictions of either metabolizable or net energy content (Noblet & Henry, 1993). Furthermore, the use of a similar methodology when predicting the digestibility of energy would make comparisons of energy values in pig feeds between countries possible.

It has earlier been shown that the dietary fiber content can be used to predict the *in vivo* total tract digestibility of energy (dE) in pig feeds (Just, 1982; Noblet & Perez, 1993). More recently, Boisen & Fernandez (1997) showed that the *in vitro* digestibility of organic matter could be used to predict dE in pig feeds. To our knowledge, there are no comparisons available using the same feed samples to show the precision in estimating dE in pig feeds from either the content of fibre or from the *in vitro* digestibility of organic matter.

The aim of this work was to test the hypothesis that the total tract *in vivo* digestibility of energy could be predicted with similar precision from the dietary fibre content and from the *in vitro* digestibility.

Materials and methods

Feed samples

The feed samples used in this study were collected from feedstuffs and some diets that had been evaluated *in vivo* for digestibility in balance trials with growing pigs in Finland (n=33), Denmark (n=67) and Sweden (n=37). The samples covered a wide range of feedstuffs (cereals, cereal co-products, oilseed meals, legume seeds, forage meals), and also included some barley-based pig feeds. They were analysed for neutral detergent fibre (NDF) and their *in vitro* digestibility of dry matter or organic matter (IVDOM) according to a three-stage method. The range in *in vivo* total tract energy digestibility (dE) was 31-91%, and the range in analysed content of NDF was 17-586 g kg⁻¹ DM and in IVDOM 43-96%.

The effect of NDF content and IVDOM on the dE in the pig feeds were subjected to linear regression analysis.

In vivo digestibility

The dE in feedstuffs and diets used for the present study has been determined in different projects over a period of approximately 10 years.

In Denmark, the dE has been determined using growing pigs weighing 40 to 60 kg. The mean values for each sample were based on five replicates, obtained from total collections for 7 days that was preceded by a 5 day adaptation period.

In Finland, the dE has been determined using growing pigs weighing 40 to 80 kg. The mean values for each sample were based on five replicates, obtained from total collections for 5 days that was preceded by a 5 day adaptation period.

In Sweden, the dE has been determined using growing pigs weighing 40 to 70 kg. The mean values for each sample were based on four or five replicates, obtained from total collections for 5 days that was preceded by a 7 or 10 day adaptation period.

In vitro digestibility

All *in vitro* analyses were performed according to the method described by Boisen and Fernández (1997). In Finland and Denmark the *in vitro* digestibility (IVD) of OM (IVDOM) was determined, while in Sweden the IVD of DM (IVDDM) was determined.

To verify the use of DM the relationship between IVDOM and IVDDM was evaluated in one data set of 114 samples analysed for both parameters in Denmark. The relationship found was: IVDOM = 4.83 + IVDDM * 0.95 ($r^2 = 0.99$). Thus, both measures of *in vitro* digestibility will rank the feeds similarly.

NDF content

In the samples from Sweden and Finland, and most of the samples from Denmark, the content of NDF was determinated according to Robertson and van Soest (1977, 1981). For some of the Danish samples where *in vivo* data was available there were no feed samples left for renewed chemical analysis. In this case, for feedstuffs low in starch, values obtained by using the method of Goering & van Soest (1970) were adopted. For feedstuffs rich in starch, the NDF content was estimated from the proximate analysis as; (crude fibre + nitrogen-free extracts) - soluble carbohydrates.

Results

The content of NDF (g kg^{-1} DM) in feedstuffs and diets was strongly ($P<0.001$) related to the variations in dE in growing pigs in samples from Denmark ($n=67$), Finland ($n=33$) and Sweden ($n=37$) (Table 1). The residual standard deviation (RSD) was lowest for the Swedish dataset, followed by the Danish and the Finnish. The coefficients of variation (CV) was lowest for the Danish dataset, followed by the Swedish and Finnish. When data from all samples ($n=137$) were merged into one dataset the relationship remained strong ($P<0.001$), although the precision in the estimate was reduced compared with the country-based estimates. The country had a significant ($P<0.001$) influence on the regression for all samples, and increased the precision in the estimate when included in the model (Table 1).

Table 1. Relationship between content of NDF (g kg^{-1} DM) and the in vivo total tract digestibility(%) of energy in growing pigs.

| Samples | N | Intercept | Slope | R ² | RSD ¹ | CV ² |
|------------------|-----|-----------|--------|----------------|------------------|-----------------|
| Denmark | 67 | 97.1 | - 0.10 | 0.88 | 3.26 | 4.02 |
| Finland | 33 | 96.7 | - 0.08 | 0.65 | 4.53 | 5.57 |
| Sweden | 37 | 95.1 | - 0.10 | 0.92 | 3.18 | 4.30 |
| All | 137 | 95.5 | - 0.09 | 0.78 | 5.03 | 6.39 |
| All [#] | 137 | 92.9 | - 0.09 | 0.82 | 4.62 | 5.87 |

¹ RSD = residual standard deviation; ² CV = coefficient of variation; [#] The effect of country was significant ($P<0.001$) and was included in the model.

The *in vitro* digestibility of OM (%) in feedstuffs and diets was strongly ($P<0.001$) related to the variations in *in vivo* total tract digestibility of energy in growing pigs in samples from Denmark ($n=74$), Finland ($n=33$) and Sweden ($n=37$) (Table 2). The RSD and CV were lowest for the Swedish dataset, followed by the Finnish and Danish (Table 2). The relationship remained strong ($P<0.001$) when data from all samples ($n=144$) had been merged into one dataset. However, the precision in the estimate was reduced compared with the country-based estimates. In contrast to the NDF content, the country had no influence ($P>0.05$) on the regression for all samples (Table 2).

Table 2. Relationship between the in vitro digestibility of OM (%) and the in vivo total tract digestibility (%) of energy in growing pigs.

| Sample | N | Intercept | Slope | R ² | RSD ¹ | CV ² |
|------------------|-----|-----------|-------|----------------|------------------|-----------------|
| Denmark | 74 | - 12.67 | 1.09 | 0.84 | 4.60 | 5.75 |
| Finland | 33 | 0.07 | 0.95 | 0.80 | 3.39 | 4.18 |
| Sweden | 37 | - 1.92 | 0.98 | 0.93 | 2.93 | 3.96 |
| All [#] | 144 | - 6.20 | 1.02 | 0.87 | 3.99 | 5.01 |

^{1,2} see Table 1; [#] The effect of country was not significant ($P>0.05$) and was excluded from the model.

Discussion

It is well known that there is a variation in digestibility and energy content between and within feedstuffs used to pigs (Just, 1982; Henry et al., 1988). This is in particular true for cereal grains

(Jørgensen, 1997), which are the primary feedstuffs used for pigs in Scandinavia and in most parts of Europe. However, due to the current basis used for estimating the energy value of feedstuffs for pigs (i.e. proximate analysis), in combination with a lack of simple and reliable methods to predict the energy value, variation between batches is not systematically used in feed formulation.

In agreement with earlier reports, the present study has shown that the variation in dE in pig feeds can be described by using the dietary content of NDF (Noblet & Perez, 1993) and the *in vitro* digestibility of OM (Boisen & Fernandez, 1997). Furthermore, when accounting for the between country variation using NDF the precision in the estimates of dE using either NDF or IVDOM was of a similar order of magnitude. Although the precision in the estimates differed between countries, a coherent picture was found for the separate *in vivo* data sets evaluated with regard to the parameters tested. This provides support to introduce alternative approaches for estimating the energy value of pig feeds to that currently used, and could form the basis for a future common energy evaluation system in the Nordic countries.

Conclusion

It was concluded that NDF and IVDOM could be used to predict the dE in pig feeds with similar precision.

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Effect of body weight and dietary crude protein on energy utilisation in growing pigs and broilers

J. Noblet¹, J. van Milgen¹, B. Carré², P. Dimon¹, S. Dubois¹, M. Rademacher³ & S. van Cauwenbergh⁴

¹ INRA, UMRVP, Domaine de la Prise, 35590 Saint Gilles, France

² INRA, SRA, 37380 Nouzilly, France

³ Degussa AG, Rodenbacher Chaussee 4, 63403 Hanau, Germany

⁴ Ajinomoto Eurolysine, 153, rue de Courcelles, 75817 Paris, France

Summary

Feed intake, BW gain, energy, protein and fat balance and components of heat production (indirect calorimetry) were measured in individually-housed barrows (6 per treatment) and group-housed male broilers (4 groups per treatment). Measurements were conducted at 25, 55 and 85 kg in pigs and at 3 and 6 weeks of age in broilers. Animals were fed a conventional or a low protein diet where protein was partly replaced by starch. The difference in dietary crude protein level was about 4.5 points but both diets provided equivalent levels of essential amino acids. The absolute levels of dietary crude protein and essential amino acids were adapted to each stage of growth and each species. In both species, heat production due to physical activity represented more than 10% of ME intake. In growing pigs, the lower crude protein level significantly reduced total heat production, which was essentially due to a reduction in the thermic effect of feeding. In broilers, heat production was not affected by dietary crude protein content. Consequences for estimating energy value of feeds are discussed.

Keywords: pig, broiler, crude protein

Introduction

Advances in determination of amino acids requirements for pigs and broilers and the increased availability of industrial amino acids allow a reduction of the dietary crude protein (CP) level without a marked effect on performance (at least in pigs), whereas nitrogen excretion is markedly reduced. This reduction of the dietary CP level, generally by a partial replacement of dietary crude protein by starch, results in a lower thermic effect of feeding and an improved efficiency of utilisation of ME in pigs (Noblet *et al.*, 1994; Le Bellego *et al.*, 2001). This improved efficiency reflects differences in ME utilisation of CP (~60%) and starch (~80%) (Noblet *et al.*, 1994; van Milgen *et al.*, 2001), and reduced turn-over of body proteins at lower dietary CP levels (Roth *et al.*, 1999). It can be hypothesised that the difference in ME utilisation between starch and CP is affected by body weight (BW) and by species. The objectives of the present trials were to evaluate the effect of dietary CP level and BW on the efficiency of ME utilisation in both broilers and growing pigs.

Material and methods

A conventional diet (NP) and a low protein diet (LP) were fed to 25, 55 and 85 kg barrows (stages 1, 2 and 3, respectively; trial 1) and to 3- and 6-wk male broilers (stages 1 and 2, respectively; trial 2). Diets were based on corn, wheat, soybean meal and rapeseed oil. The difference in dietary CP level between NP and LP was about 4.5 points but both diets provided equivalent levels of essential amino acids, expressed as digestible amino acids per unit of energy (ME for broilers and

NE for pigs). This was achieved by replacement of soybean proteins in NP by corn starch and free amino acids (HCl-lysine, methionine, threonine, tryptophan, valine and isoleucine in pigs and broilers; arginine in broilers) in LP. The difference in dietary crude protein between both diets corresponded therefore to an equivalent difference in starch. The absolute levels of dietary crude protein and essential amino acids were adapted to each stage of growth and for each species and the ratios between essential amino acids and lysine were kept constant and above recommended level. The CP levels for broilers were 24.1 and 22.4% for NP and 19.5 and 17.8% for LP, at stages 1 and 2, respectively. For pigs, the values were 21.9, 19.4 and 17.4% for NP and 17.2, 14.5 and 12.7% for LP, at stages 1, 2 and 3, respectively.

Feed intake, BW gain, energy, protein and fat balances and components of heat production (HP) were measured in respiration chambers for 6 consecutive days in individually-housed pigs (6 couples of 2 littermates at each stage and one pig per couple on each diet) and in group-housed broilers (4 groups per treatment; 14 birds and 7 birds per group at stages 1 and 2, respectively). Pigs were used only once while the 7 birds used in stage 2 originated from the 14 birds used in stage 1 and were fed the same type of diet (LP or NP). Pigs were fed four meals per day with artificial lighting from 8h30 to 21h30 using identical feed intake levels for the two diets (200, 200 and 185 g/kg $BW^{0.60}/d$ for stages 1, 2 and 3, respectively). Pigs in stage 1 could move freely within the metabolism cage whereas those in stages 2 and 3 could not. Broilers were offered feed ad libitum and a 2-hr darkness period was imposed starting at 0h00. Animals were kept for an additional day in the respiration chamber and fasted to estimate the activity-free fasting heat production (FHP). All measurements were conducted at 24°C. Excreta were cumulated over the 6-d period while gas exchanges (O_2 , CO_2 and CH_4) were continuously measured over 24-h periods. Physical activity was estimated according to the response of force sensors on which the cage was mounted. Modelling techniques were used to partition daily HP between activity-related heat production (AHP), thermic effect of feeding (TEF) and FHP (van Milgen *et al.*, 1997). Energy, protein and fat balance were calculated according to standard procedures (Noblet *et al.*, 2001) and expressed per unit of metabolic body weight (kg $BW^{0.60}$; Noblet *et al.*, 1999).

Data of each trial were subjected to analysis of variance with diet (n=2), stage of growth (n=3 and 2 in trials 1 and 2, respectively) and the interaction between diet and stage (DxS) as main effects. For trial 1, litter (within stage) was also included in the model.

Results and discussion

Apart from BW gain in trial 2, the interaction between diet and stage of growth was not significant ($P > 0.05$) for the criteria presented in tables 1 and 2. Consequently, only mean effects of growth stage (table 1) and of diet composition (table 2) are given. With regard to BW gain in trial 2, results were similar for both diets at stage 1 but lower for LP at stage 2 (94 vs 114 g/d/bird). Nitrogen excretion was significantly reduced for LP at all stages and in both species (-6 to -10% per point of dietary CP). As anticipated, protein gain was affected by growth stage, especially in broilers (table 1) and was lower for LP (table 2). The latter result is inconsistent with the absence of effect of dietary CP level on BW gain. This discrepancy is partly due to the overestimation of protein gain according to the balance technique, the error becoming more pronounced for diets with high CP level (Quiniou *et al.*, 1995). Changes in BW gain with stage of growth were anticipated. Composition of BW gain indicates that protein to fat ratios are rather similar in broilers and young pigs (stage 1); in agreement with the higher RQ value in heavier pigs, this ratio decreases with increasing BW in pigs.

Energy balance data are often expressed per kg $BW^{0.60}$ resulting in constant maintenance energy requirements or FHP (Noblet *et al.*, 1999). This is confirmed in pigs, at least for stages 2 and 3, but not in broilers for which the most appropriate exponent appeared to be 0.68. For similar housing systems (free movement) and expressed as a percentage of ME intake, AHP represented

Table 1. Effect of stage of growth on performance and energy utilisation in growing pigs and broilers¹.

| Species | Pigs (trial 1) | | | Broilers (trial 2) | |
|---|-------------------|-------------------|-------------------|--------------------|-------------------|
| | 1 | 2 | 3 | 1 | 2 |
| Stage of growth | | | | | |
| Body weight, kg | 26.7 ^a | 56.8 ^b | 88.6 ^c | 0.58 ^A | 2.35 ^B |
| Body weight gain, g/d | 861 ^a | 923 ^{ab} | 950 ^b | 57 ^A | 104 ^B |
| Feed intake, g/d | 1306 ^a | 2067 ^b | 2580 ^c | 94 ^A | 197 ^B |
| Energy balance, kJ/kg BW ^{0.60} | | | | | |
| ME intake | 2620 ^a | 2624 ^a | 2451 ^b | 1670 ^A | 1598 ^B |
| Heat production | 1398 ^a | 1395 ^a | 1327 ^b | 856 | 867 |
| Protein gain | 504 ^a | 365 ^b | 259 ^c | 379 ^A | 327 ^B |
| Respiratory quotient | 1.11 ^a | 1.14 ^b | 1.15 ^b | 1.04 | 1.03 |
| Components of heat production, kJ/kg BW ^{0.60} | | | | | |
| Fasting heat production | 661 ^a | 765 ^b | 774 ^b | 423 ^A | 479 ^B |
| Thermic effect of feed | 438 ^a | 469 ^a | 378 ^b | 249 ^A | 172 ^B |
| Activity heat production | 294 ^a | 155 ^b | 167 ^b | 185 ^A | 215 ^B |
| Heat increment ² | 738 ^a | 631 ^b | 553 ^c | 434 ^A | 388 ^B |

¹ Values with different superscripts (within trial) are significantly different ($P<0.05$)

² Sum of activity heat production, thermic effect of feed and “ghost” effect

Table 2. Effect of dietary crude protein on energy utilisation in growing pigs and broilers¹.

| Species | Pigs (trial 1) | | Broilers (trial 2) | |
|---|--------------------|--------------------|--------------------|-------------------|
| | Normal | Low | Normal | Low |
| Dietary protein level | | | | |
| Body weight, kg | 57.6 | 57.2 | 1.47 | 1.46 |
| Body weight gain, g/d | 925 | 897 | 85 ^A | 76 ^B |
| Feed intake, g/d | 2002 | 2013 | 145 | 145 |
| Energy balance, kJ/kg BW ^{0.60} | | | | |
| ME intake | 2564 | 2566 | 1626 | 1642 |
| Heat production | 1402 ^a | 1346 ^b | 862 | 861 |
| Protein gain | 393 ^a | 358 ^b | 375 ^A | 331 ^B |
| Respiratory quotient | 1.11 ^a | 1.15 ^b | 1.01 ^A | 1.06 ^B |
| Components of heat production, kJ/kg BW ^{0.60} | | | | |
| Fasting heat production | 735 | 731 | 446 | 456 |
| Thermic effect of feed | 453 ^a | 404 ^b | 201 | 220 |
| Activity heat production | 207 | 204 | 216 ^A | 185 ^B |
| Heat increment ¹ | 667 ^a | 614 ^b | 417 | 404 |
| Diet energy values, kJ/g | | | | |
| ME | 14.04 | 13.98 | 13.36 | 13.34 |
| NE ² | 10.36 ^a | 10.61 ^b | 10.00 | 10.01 |
| NE/ME (x100) | 73.9 ^a | 75.9 ^b | 74.8 | 75.0 |

¹ See table 1 ² Adjusted for similar levels of physical activity (within species)

approximately 12% of ME intake in broilers and pigs at stage 1; this value was lower (6% of ME intake) in stages 2 and 3 when pigs were not able to move freely.

The hypothesis that the effect of diet on HP in pigs depends on growth stage was not confirmed. In agreement with previous results (Noblet *et al.*, 1994; Le Bellego *et al.*, 2001; van Milgen *et al.*, 2001), total HP and the TEF component were significantly lower for LP, resulting in a higher NE to ME ratio (table 2). In broilers, HP and its components were not affected by the dietary CP level and the NE to ME ratio was independent on the dietary CP level. Consequently, the relative energy values of diets differing in starch or CP level are similar when estimated on a ME or a NE basis in broilers. In contrast, in pigs the hierarchy depends on the energy system and a NE system is preferred. Generalisation of these conclusions to other nutrients (fat and dietary fibre) requires further studies in broilers. The increased RQ value observed with LP is consistent with the higher contribution of starch to lipogenesis.

In conclusion, our results indicate both similarities and differences in energy utilisation between pigs and broilers. Among similarities, the high energy cost of physical activity (>10% of ME intake) and the similar contributions of FHP, AHP and TEF to total HP are remarkable. Differences between growing pigs and broilers concern the partitioning of energy gain between protein and fat and the subsequent differences in RQ values. More importantly, pigs and broilers differ in the response to changes in diet composition, especially the effect of dietary CP on heat production. Further studies on the metabolic utilisation of other nutrients (fat or dietary fibre) are required, especially in broilers.

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Ruminant growth and composition model

J.W. Oltjen¹, A.B. Pleasants², T.K. Soboleva² & V.H. Oddy³

¹ Department of Animal Science, University of California, Davis, California 95616, USA

² AgResearch, Ruakura Research Centre, Hamilton, New Zealand

³ Meat and Livestock Australia, 165 Walker Street, North Sydney NSW 2060, Australia

Summary

We have refined a prediction system for ruminant animal growth and composition developed previously. The model represents body protein in two pools, viscera and non-viscera (muscle). Using sheep datasets, we have simplified the adjustments in the model for protein gain and loss of body fat at near maintenance feeding, and more precisely estimated variable maintenance parameters. In the model muscle and viscera each have an upper bound (m^* and v^* , respectively). For muscle m^* is genetically fixed; however, v^* is affected by energy intake and muscle (protein) mass. Net energy intake above maintenance is used for muscle and viscera gain before its use for fat accretion. Our new work has allowed simplification of previous equations for the adjustment allowing gain of muscle or viscera at zero retained energy and for visceral growth. Maintenance energy includes a variable coefficient on body weight which results in a lag in change of maintenance requirements after intake changes. Sheep growth and composition is more accurately predicted with the revised model, and the model predicts sheep empty body weight and fat content (± 2.1 kg and 2.3%-units, respectively) more accurately than the current Australian feeding system. New additions refine predictions at levels of energy intake at or below maintenance.

Keywords: growth, composition, modelling

Introduction

We have refined the prediction system for ruminant animal growth and composition developed previously in a collaborative international effort (Oltjen et al., 2000). The model represents body protein in two pools, viscera (v) and one closely associated with carcass muscle (m). Using sheep datasets from Nebraska (Ferrell et al., 1986) and New South Wales (unpublished), we have simplified the adjustments in the model for gain of muscle protein and loss of body fat (f) at near maintenance feeding, and more precisely estimated variable maintenance parameters.

Model description

In our model muscle and viscera each have an upper bound (m^* and v^* , respectively). For muscle m^* is genetically fixed; however, v^* is affected by energy intake and muscle (protein) mass. Net energy intake above maintenance (net energy for gain or retained energy, NEG) is used for visceral and muscle tissue gain before its use for fat accretion. The model is expressed in terms of energy (kJoules), with parameters k_m , k_v , c_m and c_v . Our new work has allowed simplification of previous equations:

$$dm/dt = k_m (NEG + c_m f_a) (1 - m/m^*) \quad (1)$$

$$dv/dt = k_v (v^* - v) \quad (2)$$

$$df/dt = NEG - dm/dt - dv/dt \quad (3)$$

The adjustment allowing gain of muscle or viscera at zero retained energy (Figure 1) is now:

$$f_a = (1-m/m^*)^{e2} \quad (4)$$

where the estimated value of $e2$ is 3.4, and

$$v^* = cs_1 \text{ MEI} + cs_2 m \quad (5)$$

where k_m (0.353), c_m (1340 kJ d⁻¹), k_v (0.050 d⁻¹), cs_1 (0.314 d) and cs_2 (0.0416) are estimated parameters.

Previously maintenance energy (HP_{maint}) was similar to the Australian feeding system (SCA, 1990) based on Corbett et al. (1987), but with a variable coefficient on body weight:

$$HP_{\text{maint}} = \alpha_t EBW^{0.75} + 0.09 \text{ MEI} \quad (6)$$

$$\alpha_t = \alpha_0 (1 + b (\text{MEI}_t/\text{MEI}_0 - 1)(1-e^{-t/\tau})) \quad (7)$$

which results in a lag in change of maintenance requirements after intake changes from MEI_0 to MEI_t . Here EBW is empty body weight, t is time (days), b and τ are constants; MEI_0 and α_0 are original values of intake and the maintenance coefficient, respectively. Fit of the Nebraska data (Ferrell et al., 1986) shows that the double correction for variable maintenance is not necessary; the previously used coefficient on MEI, 0.09, is not different than zero. Thus (Figure 2)

$$HP_{\text{maint}} = \alpha_t EBW^{0.75} \quad (8)$$

with improved estimates for b (0.116) and τ (20.0 d). Alternatively, with the new equation for viscera, the multiple regression prediction of heat production using m , v and their accretion (Oltjen and Sainz, 2001) is also improved (data not shown).

Overall, these changes significantly improve the prediction of body fatness as a function of body weight and gain (Figure 3).

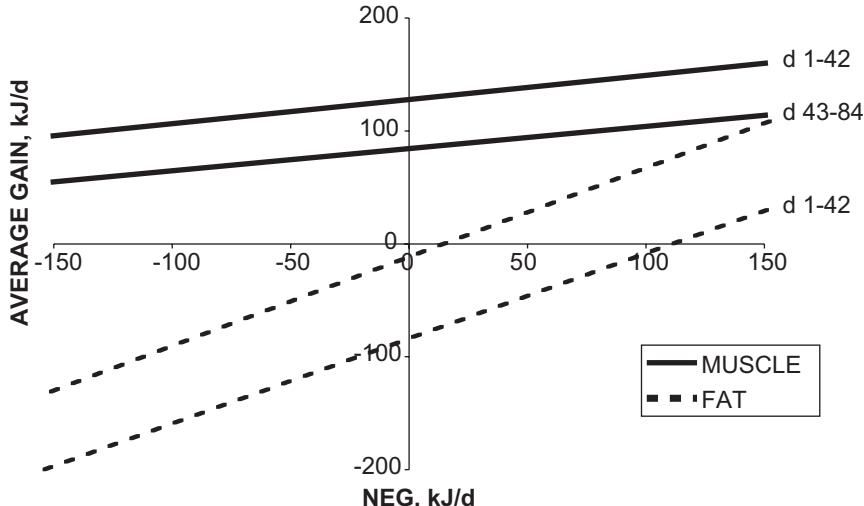


Figure 1. Relationship between retained energy (NEG) and gain of muscle or fat during initial and final 42 day treatment periods (Ferrell et al., 1986).

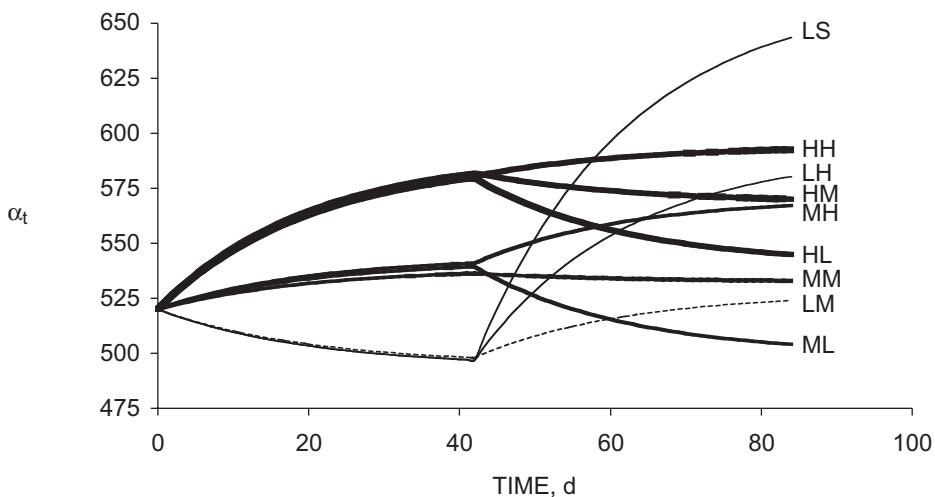


Figure 2. Model predicted maintenance coefficient (α) as a function of time (t) for nine treatment groups of Ferrell et al. (1986).

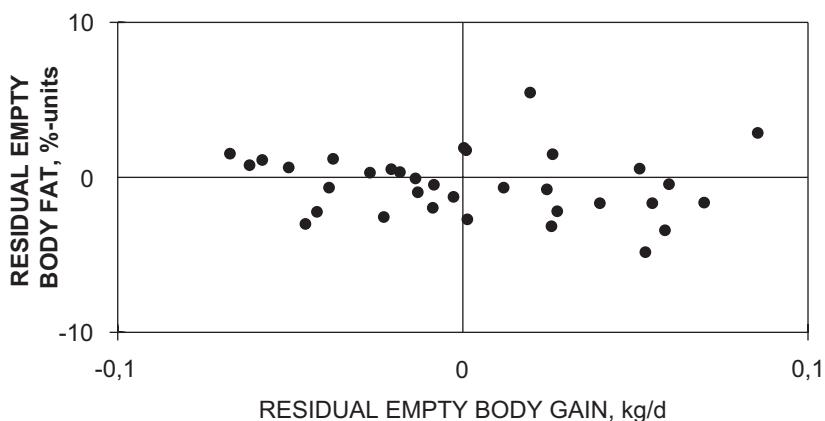


Figure 3. Residual (predicted minus observed) empty body fat and gain for rams fed 84 d (Ferrell et al., 1986).

Implications

Sheep growth and composition is more accurately predicted with the revised model, and the model predicts empty body weight and fat content more accurately (± 2.1 kg and 2.3%-units, respectively, Ferrell et al., 1986) than the current feeding system (SCA, 1990). New additions refine predictions at levels of energy intake at or below maintenance. The model provides the structure for predicting composition of growing cattle as well, but has yet to be completely parameterized and tested.

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Ruminal acetate/propionate pattern and energy-yielding nutrient fluxes across splanchnic and hindlimb tissues in growing lambs

I. Ortigues-Marty & L. Majdoub

Equipe Nutriments et Métabolismes, Unité de Recherches sur les Herbivores, INRA, Theix, 63122 Saint Genès Champelane, France

Summary

In growing lambs, the ruminal fermentation pattern was significantly correlated to the balance of nutrients appearing in the portal vein ($r=+0.83$), being released by the splanchnic tissues ($r= +0.58$) or being taken up by the hindlimb ($r=+0.52$). As for cetogenic nutrients, their net portal or splanchnic release was directly related to their utilisation by peripheral tissues, even though partition of utilisation among peripheral tissues could differ. As for glucogenic nutrients, their net splanchnic release and hindlimb utilisation appeared to be regulated by the supply of cetogenic nutrients which might also interact with the insulin regulations.

Keywords: nutrients, splanchnic tissues, hindlimb

Introduction

In growing ruminants, the quantity and the balance among energy-yielding nutrients supplied to the peripheral tissues may influence their utilisation therein and affect the orientation of energy metabolism in muscle and hence its composition (Hocquette et al., 1998). Prediction of those would be useful to improve feeding standards. However, because of substantial nutrient transformations in splanchnic tissues, there is no direct relationship between the ruminal fermentation pattern and the balance between glucogenic and ketogenic nutrients appearing in the portal vein and being delivered by the splanchnic tissues to peripheral tissues.

By combining individual results from two experiments carried out in lambs and characterised by changes in the molar ratio of (acetic + butyric) acids to propionic acid concentrations (C_2+C_4/C_3) in the rumen from 1.0 to 3.1, we addressed the following questions: 1) to which extent does the ruminal fermentation pattern determine the balance among glucogenic and ketogenic nutrients appearing in the portal vein ?, 2) to which extent does the pattern in the net portal appearance of nutrients determine their net splanchnic release ?, and 3) is the net uptake of nutrients by the hindlimb directly related to their net splanchnic release? A companion abstract is focused on nitrogenous nutrients (Savary-Auzeloux et al., 2003).

Materials and methods

Two experiments (Majdoub et al., 2003a, b) were conducted using growing lambs (32 to 40 kg LW) surgically equipped for measurements of nutrient fluxes across the portal-drained viscera (PDV), the liver and the hindlimb. In both experiments, the basal diet was constituted of frozen rye-grass (1.5 fold the maintenance requirements in metabolisable energy, MEm), and the ruminal fermentation profile was modified by supplementation either with barley (0.5 x MEm) or with an intraruminal infusion of propionate (0.15 or 0.25 x MEm). Animals were fed at 2 hourly intervals. Net fluxes were measured for volatile fatty acids (VFA), glucose, L-lactate, β -hydroxybutyrate, total free amino acids and insulin across all three tissue beds. Results from each experiment are being published separately (Majdoub et al., 2003a, b). The present work aims at combining all observations ($n = 28$ for the splanchnic tissues and $n=18$ for the hindlimb) and analysing them

either using a simple or multiple regression approach or using the Principal Component (PC) Analysis procedures (SAS) to identify the relationships which may exist among nutrients and among the different anatomical sites.

Results and discussion

Animals used in this study presented a wide range of ruminal fermentation profiles. Total volatile fatty acid (VFA) concentrations varied from 54 to 111 mM, and the range in the molar proportions of C2, C3 and C4 was 47-64, 20-40 and 5-15 % respectively.

Relationships between the ruminal fermentation pattern (C2/C3) and the balance among glucogenic and ketogenic nutrients appearing in the portal vein

Results indicated that the ketogenic/glucogenic $[(C_2 + C_4 + \beta\text{-hydroxybutyrate})/(C_3 + \text{lactate})]$ ratio of nutrients appearing in the portal vein was highly determined by the ruminal $[(C_2+C_4)/C_3]$ molar ratio ($r=+0.83$, $P<0.01$, Figure 1a), reflecting the strong correlations which exist between VFA patterns in the rumen and in the portal vein (Nozière et al., 2002).

Relationships between the pattern in the net portal appearance of nutrients and their net splanchnic release

In the liver, no significant relationship could be established between the balance of ketogenic/glucogenic nutrients appearing in the portal vein and that being released by the liver. When considering each nutrient individually, net hepatic uptake of propionate and butyrate increased linearly with influx at rates of 0.79 and 0.55 ($r=+0.98$ and $+0.95$, respectively, $P<0.01$). Such was not the case for the other nutrients, nor for insulin.

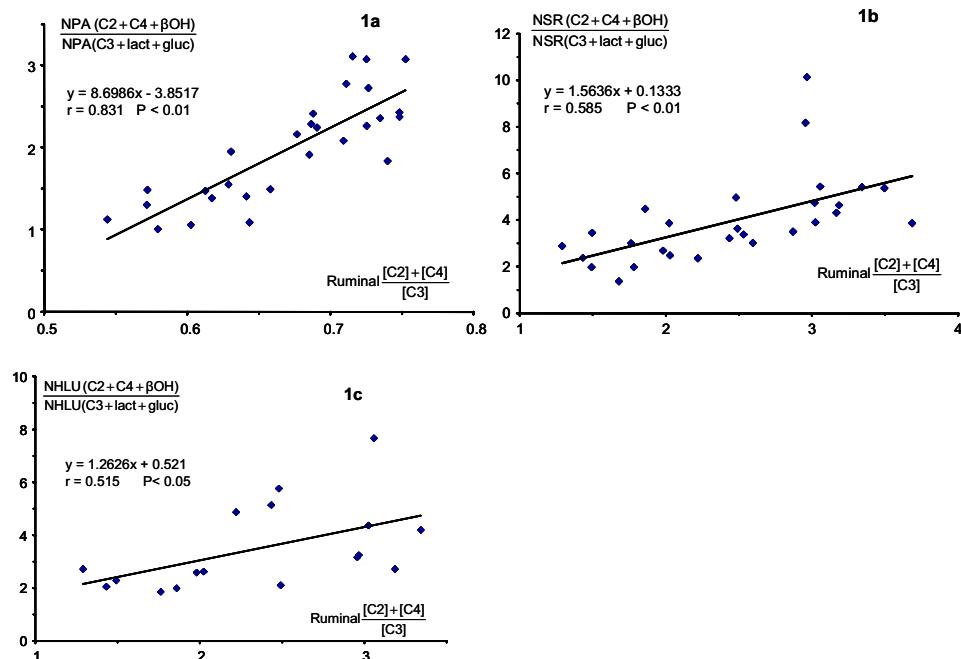
Interrelationships between net portal appearance and net hepatic fluxes of nutrients (C2, C3, C4 glucose, lactate, β -hydroxybutyrate, total amino acids, insulin) were further investigated by PC analysis. 47% of the total variance in the 16 measured variables was explained by two axes. The first axis (27% of the variance) was essentially defined by the net fluxes of cetogenic nutrients (C2, C4 and β -hydroxybutyrate), which were highly cross-correlated. This first axis corresponded to nutrients whose hepatic fluxes are directly responsive to inflows. The second axis (20% of the variance) corresponded to the net fluxes of glucogenic nutrients (C3, L-lactate, glucose), whose net hepatic release was less directly responsive to inflow because of fine metabolic regulations therein (Majdoub et al., 2003a). Insulin is known to play an important rôle in the utilisation of glucogenic substrates. Interestingly, the PC analysis showed that the pancreatic release of insulin (as measured by its net portal appearance) was positively correlated to the net portal appearance of lactate ($r=+0.49$, $P<0.01$) and C3 ($r=+0.37$, $P<0.05$) and was found close to the second axis. This is coherent with the insulinotropic effect of the glucogenic substrates (Harmon, 1992). Conversely, the net hepatic uptake of insulin was positively correlated to the net fluxes of β -hydroxybutyrate at the portal drained viscera ($r=+0.51$, $P<0.01$) and hepatic ($r=+0.58$, $P<0.01$) levels and was found close to the first axis. Little is known on the factors which regulate the net hepatic uptake of insulin. Present results suggest that the net splanchnic fluxes of cetogenic nutrients might be involved. Consequently, the correlation between the ketogenic/glucogenic $[(C_2 + C_4 + \beta\text{-hydroxybutyrate})/(C_3 + \text{lactate})]$ ratio of nutrients being released by the splanchnic tissues and the ruminal $(C_2+C_4)/C_3$ molar ratios (Figure 1b) was lower than that noted for the net portal release of nutrients, but still significant ($r=+0.58$, $P<0.01$).

Relationships between the net splanchnic release of nutrients and their net uptake by the hindlimb

In the hindlimb, net uptake of β -hydroxybutyrate, butyrate and acetate was highly correlated to their inflows ($r=+0.89$, $+0.77$ and $+0.62$, respectively, $P<0.01$); correlation was inexistent for glucose ($r=+0.16$, NS).

Subsequently, a PC analysis was conducted to determine to which extent the net hindlimb uptake of nutrients (C2, C3, C4 glucose, lactate, β -hydroxybutyrate, total amino acids, insulin) was determined by their net splanchnic release. Two axes explained 50% of the total variance among data. The first axis (29% of the variance) was defined by the net hindlimb glucose and amino acid uptake which were negatively correlated to the net splanchnic release of β -hydroxybutyrate ($r=-0.62$ and -0.31 , respectively) and C2 ($r=-0.52$ and -0.66 , respectively). The second axis (21% of the total variance) characterised the net hindlimb uptake of C2 and C4. Altogether, these results suggest that the higher the net splanchnic release of ketogenic nutrients, the lower the glucose and amino acid uptake by the hindlimb and the higher the acetate utilisation by other peripheral tissues such as the subcutaneous and perirenal adipose tissues.

Interestingly, the balance of nutrients taken up by the hindlimb was still significantly correlated to the rumen fermentation profile ($r=+0.52$, $P<0.05$) even though no significant correlation was found with the net portal appearance of nutrients or their net splanchnic release.



Figures 1a, 1b and 1c. Correlations between the ruminal fermentation profile ($C2+C4/C3$) and the net portal appearance (NPA, Figure 1a), the net splanchnic release (NSR, Figure 1b) or the net hindlimb uptake (NHLU, Figure 1c) of keto- to glucogenic nutrients [$(C2+C4+\beta$ -hydroxybutyrate)/(C3+lactate+glucose)].

Conclusions

In conclusion, significant correlations exist between the ruminal fermentation pattern and balance of nutrients being released by the portal drained viscera or by the splanchnic tissues, or being utilised by the hindlimb in growing ruminants. These correlations are however of decreasing magnitude probably because of the regulations involved in the metabolism of glucogenic nutrients in the liver. Net hindlimb uptake of glucose was directly related to the net portal appearance of glucogenic nutrients but not to the net splanchnic release of glucose. Insulin which is known to regulate hepatic glucose metabolism might itself be regulated for its secretion by glucogenic nutrients and for its hepatic degradation by cetogenic nutrients. The net hindlimb uptake of glucose was also negatively influenced by the net splanchnic release of cetogenic nutrients. Finally, the net hindlimb uptake of C4 and β -hydroxybutyrate was directly related to the net portal appearance of cetogenic nutrients, while the higher the net portal absorption of acetate, the higher its utilisation by peripheral tissues other than the hindlimb.

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Dietary treatment effects on growth, reproduction, and circulating leptin levels of Targhee ewe lambs

Wolfgang Pittroff^{1,3}, Duane Keisler² & Verne LaVoie¹

¹ U.S. Sheep Expt. Station, Dubois

² University of Missouri, Dept. of Animal Science, Columbia

³ present address: University of California, Davis, Dept. of Animal Science

Summary

An experiment was conducted to evaluate the effect of feeding regime on onset of puberty and initial reproductive performance in sheep. The hypothesis was tested that onset of puberty in ewe lambs coincides with the inflection point of the growth curve. Ewe lambs of two genetically different Targhee lines (Line 41: selected for total weight of lamb weaned; Line 42: unselected control line) were fed to achieve maximally different body composition and growth curve trajectories using diets designed to maximize growth (treatment LEAN), maximize fat deposition (treatment FAT), or control (conventional roughage-grain diet, treatment CONT). The results indicated that major differences in growth trajectory and body composition were achieved, that inflection point of the growth curve does not coincide with the onset of puberty as measured by the 0.5 ng progesterone threshold, and that the animals with the lightest weight, but highest proportion of fat, exhibited the highest pregnancy and parturition rates. Further, treatment and treatment - sampling day interaction effects were highly significant for circulating concentrations of leptin, and consistent with the findings in regards pregnancy and parturition data. However, age at threshold levels of progesterone as an indicator of puberty did not exhibit a consistent pattern in relation to circulating leptin levels, nor to age at the inflection point of the growth curve. Clearly, the relationship between age inflection point of the growth curve and initial reproductive performance was inverse. It is concluded that recent suggestions upholding the validity of the critical weight hypothesis for onset of puberty may not be appropriate.

Keywords: sheep, puberty, growth curve, leptin

Introduction

It is well known that onset of puberty in mammals is associated with age, body weight, and nutritional status of the animal. Brody (1945) concluded that the onset of puberty coincides with the inflection point of the growth curve, a point of view that is still widely accepted, and has been the basis for many mathematical models of growth, including bio-economic production system models (Sanders and Cartwright, 1979; Blackburn and Cartwright, 1987). It has become customary to accept a measured value of 0.5 ng/ml or 1.0 ng/ml circulating progesterone as a threshold indicating that a female has reached puberty.

The discovery of leptin was rapidly followed by reports suggesting a key role of this hormone in the regulation of reproductive processes. Ahima et al. (1997) showed that when starving mice where given exogenous leptin, typical changes in gonadal and thyroid functions which are sequelae of starvation are strongly attenuated. Indeed, the intricate relationships between leptin and the biology of reproduction are of great interest and possible economic relevance to livestock producers. Barash et al. (1996) found that ob/ob mice treated with leptin for 14 days had significantly higher serum levels of LH and higher uterus and ovary weight. In leptin treated males, higher weights of testicle and seminal vesicles were observed along with increased serum levels of FSH. Ahima et al. (1997) reported that exogenous leptin lowered the age of onset of puberty in

normal female mice fed ad libitum. However, it seems to be still unclear as to whether leptin is a primary trigger, or whether it acts in permissive fashion. Cheung et al. (2001) recently suggested that leptin may not work as a primary trigger because (a) leptin levels in terminally collected mice did not show a significant increase until adulthood was reached, (b) in situ hybridization biochemistry of hypothalamic expression of mRNA encoding the leptin receptor did not show any age related differences in various hypothalamic areas, and (c), although exogenous leptin advanced onset of puberty in nutritionally restricted mice, this response was not different from age at first estrus for ad libitum fed mice. The conclusion of this group was that leptin is permissive to, but not a trigger for onset of puberty. A recent review (Delemarre-van de Waal, 2002) concurred with this interpretation and found these results in agreement with the concept of critical weight for puberty (Frisch and Revelle, 1971). Thus, the examination of weight - age and weight - body composition relationships in the study of onset of puberty seems to benefit from monitoring of circulating leptin levels in order to arrive at a more coherent conclusions. A study was conducted to test the critical weight hypothesis for onset of puberty in sheep, evaluating the effects of differential feeding and resulting differences in growth trajectories on onset of puberty and initial reproductive performance.

Material and methods

Animals and feeding procedures

Targhee ewe lambs of 2 different genetic lines (*Line 41* - selected for total weight of lamb weaned vs. *Line 42* - random mating control) were assigned to 3 nutritional treatments: (1) LEAN - lambs fed a pelleted ration ad libitum so as to maximize growth and development (9.83 MJ/kg ME; 20.3% CP, w/w), (2) FAT - lambs fed a pelleted ration ad libitum so as to provide an energy surplus while protein-limiting lean growth (ME concentration 12 MJ/kg; 7.9% CP, w/w), and (3) CONT - lambs fed chopped alfalfa hay ad libitum and approx. 250 g barley/hd/d. It was hypothesized that feeding a diet with very high energy concentration, but minimal crude protein content would lead to maximal fat deposition in growing lambs, thereby creating the largest possible difference in body composition compared to a diet formulated to maximize lean growth. Group size per line - treatment combination was varied between 17 and 19 animals. Body composition was determined in terminal measurements on randomly selected animals in each group in a serial slaughter design. Overall, pregnancy was tested in 107 animals. Ewe lambs were bred for two cycles. Pregnancy was assessed via ultrasound.

Data analysis

Weight and gain data were analyzed with linear models with genetic line and nutrition treatments as main effects; reproductive performance was analyzed within genetic lines and treatments with categorical response models. Hormone data were analyzed with mixed model procedures for repeated measures, modeling the effects of treatment, line, sampling date and interactions. A series of alternative co-variance structures was fitted. A polynomial model fitting a quadratic regression on time was identified as the superior model, using Akaike's Information Criterion for the evaluation of fit. All statistical analyses were performed with SAS Version 8.1 (SAS, 1999). Growth curves were fitted to the logistic curve using non-linear algorithms. Attempting to fit a new growth model proposed by Lopez et al. (2000) allowing for flexible modeling of inflections did not lead to convergence

Results

Reproduction

Pregnancy was tested in 107 animals. Among line 41 lambs (selected for high fertility and growth) pregnancy percentages were 75% (CONT), 68.4% (FAT), and 77.8% (LEAN). Among line 42 (unselected) lambs, pregnancy percentages were 50% (CONT), 83.3% (FAT), and 72.2% (LEAN). Pregnancy percentages observed for line 42-FAT lambs were the highest percentage ever obtained for that line; those lambs also had the lowest weight of all groups studied. Differences in reproductive performance were statistically significant only for line 42 FAT vs. line 42 CONT lambs. This effect was due to the small subcell size varying from 16 to 19 animals and a clear line by treatment interaction.

Weight, growth and leptin responses

The effect of treatment on average daily gain (ADG) during the week prior to the initiation of mating was highly significant ($p=.0001$) with the lowest ADG observed for FAT lambs, and the highest ADG observed for LEAN lambs. Only line effects were significant for ADG computed for the period bracketing the mating period ($p=.015$) with line 41 lambs > line 42 lambs. Line and treatment effects were highly significant for weight at the beginning of mating ($p=.0001$); the contrast between LEAN and FAT was highly significant ($p=.0005$). There was an increasing trend for leptin levels for all treatments (data shown for Line 42, Figures 1-3); this trend was particularly clear for treatment FAT. Treatment and the treatment-sampling day interaction were

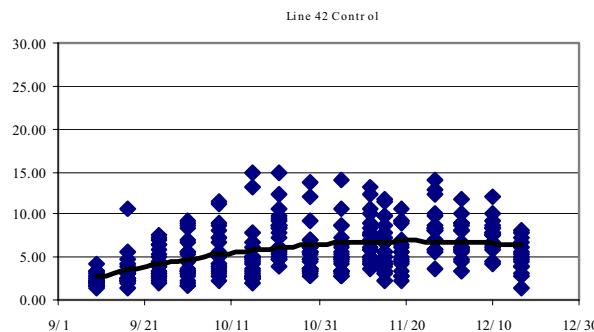


Figure 1. Circulating leptin levels, Line 42, 'CONT'.

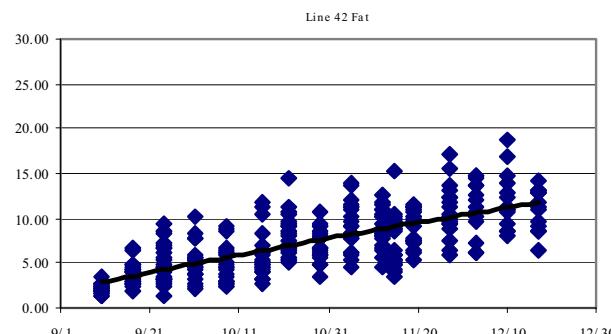


Figure 2. Circulating leptin levels, Line 42, 'FAT'.

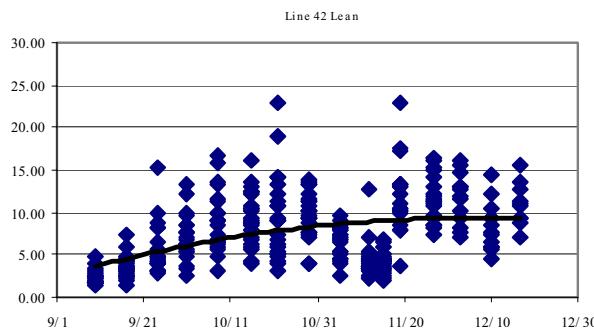


Figure 3. Circulating leptin levels, Line 42, 'LEAN'.

highly significant for leptin concentrations ($p<.0001$). Raw means for leptin (in ng/ml) ranged from 5.1 in CONT Line 41 to 7.72 in FAT Line 41. Body composition analysis indicated that the intended differentiation in body composition was achieved (data not shown).

The inflection point of the growth curve did not coincide with the onset of puberty, as assessed by age and progesterone threshold levels (Table 1).

Treatment and the treatment-sampling day interaction were highly significant for leptin concentrations ($p<.0001$). Raw means for leptin (in ng/ml) ranged from 5.1 in CONT Line 41 to 7.72 in FAT Line 41.

Table 1. Inflection point of average growth curves and age at two threshold levels of progesterone.

| Line | Treatment | Age progesterone was 0.5 ng/ml (days) | Age progesterone was 1.0 ng/ml (days) | Age Inflection Point (days) |
|------|-----------|--|--|--------------------------------|
| 41 | Control | 210.3 | 218.9 | 124.2 |
| 41 | Lean | 195 | 206.2 | 162.5 |
| 41 | Fat | 203.2 | 207.3 | 162 |
| 42 | Control | 207.4 | 216.4 | 113.2 |
| 42 | Lean | 209 | 220 | 154.2 |
| 42 | Fat | 209.8 | 217.6 | 174.4 |

Discussion

A relationship between inflection point of the growth curve and onset of puberty was not discernible. Likewise, there seemed to be no relationship between age at progesterone level thresholds and treatment. While high leptin levels were also observed for treatment LEAN, the treatment effect for leptin was significant, and highest average levels were observed for treatment FAT. The fact that the group with the lowest average weight had the highest initial reproductive success raises the possibility that attributes such as weight, degree of maturity or ADG prior to mating may not be the most accurate estimators of initial reproductive performance in sheep. Further, the inflection point of the growth curve and the onset of puberty were not congruent. Our data suggest that the question of the role of critical weight vs. body composition in onset of puberty does not have a definitive answer yet.

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Effect of different levels of metabolizable protein on splanchnic fluxes of amino acids in lactating cows

G. Raggio¹, G.E. Lobley², D. Pacheco³, D. Pellerin¹, G. Allard¹, R. Berthiaume³, P. Dubreuil⁴ & H. Lapierre³

¹ *Université Laval, Québec, Canada, G1K 7P4*

² *Rowett Research Institute, Aberdeen, UK, AB21 9SB*

³ *Agriculture and Agri-Food Canada, Lennoxville, Québec, Canada, J1M 1Z3*

⁴ *Université de Montréal, St-Hyacinthe, Québec, Canada, J2S 7C6*

Summary

The effect of different levels of metabolizable protein (MP) was measured in six catheterized multiparous Holstein cows. Three isoenergetic diets, balanced to provide increasing amounts of MP: 1922 (Low), 2264 (Medium) and 2517 (High) g of MP per day, were fed according to a replicated Latin Square with 21-d experimental periods. On d 18, 19 or 20, six hourly blood samples were collected to determine net fluxes of nutrients across the portal-drained viscera (PDV), the liver and the mammary glands (only 3 samples). Yields of milk and protein increased ($P < 0.01$), as did urinary N excretion ($P < 0.05$) with increasing MP. Portal absorption of essential amino acids (EAA) increased linearly ($P < 0.01$) with MP supply. Liver removal of His, Met, Phe increased linearly ($P < 0.10$), while liver removals of the branched-chain AA (BCAA) and Lys were not affected by the diets. The ratio of milk output to total splanchnic tissues flux for the BCAA, Thr ($P < 0.10$) and Lys ($P < 0.20$) decreased linearly with increasing MP, indicating additional oxidation of these AA in the peripheral tissues. These data demonstrate that increased efficiency of transfer of absorbed EAA into milk protein with low protein diet (equivalent to 13% CP) occurred through a decreased catabolism of EAA in the liver (His, Met, Phe and Thr) and/or in peripheral tissues (BCAA, Lys and Thr).

Keywords: *amino acids, splanchnic, dairy cows*

Introduction

Optimal conditions for milk production can be defined as either when output is maximal or when the efficiency of transfer of dietary protein into milk protein is high. Maximizing output involves increases in dietary protein supply resulting in higher portal absorption of amino acids (AA: Bach et al., 2000; Blouin et al., 2002). As supply approaches from recommendations, however, the efficiency of transfer of dietary protein into milk protein decreases (Doepel et al., 2002). Highest transfer efficiencies are, therefore, achieved at low dietary protein supply but with reduced yields. In practical terms, the sensible husbandry strategy lies between these extremes but to achieve this requires knowledge of how alterations in supply affect the partition of individual AA between catabolism and anabolism. The current study investigates the impact of changing MP supply, under conditions where the efficiency of transfer of N intake into milk-N will become increased, with alterations in both removal of individual AA by the liver and in the post-splanchnic supply to support metabolism at the mammary glands and other peripheral tissues.

Materials and methods

Six multiparous Holstein cows, averaging 656 ± 60 kg BW and 96 ± 8 DIM at the beginning of the study, were used in a double 3x3 Latin Square design with 21-d experimental periods.

Approximately one mo before the start of the experiment, all cows were surgically implanted with chronic catheters into the portal vein, one hepatic vein, and one mesenteric artery for blood sampling, and two distal mesenteric veins for p-aminohippurate (pAH) infusion (Huntington et al., 1989). Using NRC (2001), three diets were balanced to provide the same energy (6.27 MJ NEI kg DM⁻¹) but with increasing amounts of metabolizable protein (MP), averaging 1922 (Low), 2264 (Medium) or 2517 (High) g MP d⁻¹. The diets were fed as total mixed rations in equal quantities every 2 h from automated feeders, except long hay (1 kg d⁻¹) that was offered once a day. Fixed quantities of DM were offered throughout the study. Cows were housed in a tie stall barn, lit from 0600 to 2200. Cows were milked twice daily (0600 and 1800) and production was recorded at each milking.

On d 18, 19 or 20 (2 cows per day), plasma flows were measured by continuous infusion (14.4 g h⁻¹) of Na-pAH (100 g l⁻¹) into one mesenteric vein catheter, preceded by a priming dose (2 g). After at least 40 minutes of pAH infusion, blood was collected every hour for 5 h (n=6) simultaneously from the arterial, portal and hepatic catheters. Blood samples were collected from the mammary vein by venepuncture, every other hour (n=3). The Fick principle was used to determine mammary plasma flow using Phe and Tyr as marker AA. On d 13 to 18 of each period, total collection of faeces and urine was performed to determine N balance. The experimental protocol was approved by the Institutional Committee for Animal Care of the Lennoxville Research Centre and animals were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

Results and discussion

Data from one cow were excluded from analyses due to a non-patent hepatic vein catheter.

Table 1. Effect of the supply of metabolizable protein (MP) on the yield and composition of milk in Holstein cows¹

| Milk | Treatments | | | SEM | <i>p</i> ² | |
|-----------------------------------|------------|-----------|---------|------|-----------------------|-----------|
| | Low MP | Medium MP | High MP | | linear | quadratic |
| Yield, kg d ⁻¹ | 33.97 | 35.70 | 36.24 | 0.43 | 0.01 | 0.29 |
| Protein yield, kg d ⁻¹ | 0.81 | 0.87 | 0.92 | 0.12 | <0.01 | 0.79 |
| Protein concentration, % | 2.37 | 2.44 | 2.55 | 0.05 | 0.08 | 0.72 |
| Fat yield, g d ⁻¹ | 1.28 | 1.31 | 1.19 | 0.30 | 0.09 | 0.10 |
| Fat concentration, % | 3.76 | 3.65 | 3.29 | 0.08 | 0.01 | 0.30 |

¹Least squares means presented with pooled SEM, n=5

²Probability corresponding to the null hypothesis

Raising MP supply resulted in a linear increase in essential AA (EAA) absorption across the PDV. Overall, however, the efficiency of transfer of absorbed EAA into milk protein decreased (from 63 to 49%) as MP supply increased. This decreased efficiency was accompanied by increased hepatic removal of EAA, except for BCAA and lysine. As hepatic synthesis of plasma proteins was not affected by treatments (Raggio et al., 2002), the increase in hepatic removal of AA elevated ureagenesis which, combined with increased ammonia removal (data not shown), led to more urinary-N, averaging 79, 122 and 165 g N d⁻¹ for the Low, Medium and High MP, respectively.

Table 2. Effect of the supply of metabolizable protein (MP) on splanchnic net fluxes of amino acids (mmol h^{-1})¹.

| Amino Acid | Tissue ² | Treatments | | | SEM | <i>p</i> ³ | |
|---------------|---------------------|------------|-----------|---------|------|-----------------------|-----------|
| | | Low MP | Medium MP | High MP | | Linear | quadratic |
| Histidine | PDV | 7.83 | 12.00 | 14.15 | 0.92 | <0.01 | 0.39 |
| | LIVER | -2.98 | -4.38 | -7.23 | 0.80 | 0.01 | 0.48 |
| | TSP | 4.83 | 7.51 | 6.96 | 0.89 | 0.17 | 0.18 |
| | MG | -6.63 | -6.60 | -6.91 | 0.38 | 0.64 | 0.72 |
| | MILK | 5.79 | 6.42 | 6.55 | 0.04 | <0.01 | 0.79 |
| Isoleucine | PDV | 20.96 | 26.55 | 28.80 | 1.27 | <0.01 | 0.31 |
| | LIVER | 2.55 | 4.44 | 1.63 | 1.47 | 0.69 | 0.23 |
| | TSP | 23.48 | 30.77 | 30.53 | 0.92 | <0.01 | 0.02 |
| | MG | -17.02 | -19.89 | -22.43 | 0.96 | 0.01 | 0.89 |
| | MILK | 14.96 | 16.61 | 16.92 | 0.04 | <0.01 | 0.79 |
| Leucine | PDV | 35.55 | 43.21 | 49.61 | 1.62 | <0.01 | 0.75 |
| | LIVER | 1.04 | 6.02 | 2.38 | 2.68 | 0.75 | 0.23 |
| | TSP | 36.57 | 49.12 | 52.04 | 1.82 | <0.01 | 0.07 |
| | MG | -28.34 | -32.10 | -38.33 | 1.72 | 0.01 | 0.56 |
| | MILK | 24.85 | 27.58 | 28.11 | 0.04 | <0.01 | 0.57 |
| Lysine | PDV | 29.09 | 37.96 | 40.22 | 1.95 | <0.01 | 0.20 |
| | LIVER | -0.06 | 0.86 | -4.89 | 2.44 | 0.24 | 0.29 |
| | TSP | 28.97 | 38.50 | 35.46 | 1.26 | 0.02 | <0.01 |
| | MG | -24.88 | -26.48 | -30.05 | 0.09 | 0.01 | 0.41 |
| | MILK | 18.89 | 20.96 | 21.36 | 0.04 | <0.01 | 0.79 |
| Methionine | PDV | 10.00 | 13.49 | 14.27 | 0.38 | <0.01 | 0.03 |
| | LIVER | -2.96 | -4.19 | -6.58 | 0.59 | 0.01 | 0.44 |
| | TSP | 7.02 | 9.27 | 7.70 | 0.62 | 0.50 | 0.04 |
| | MG | -6.62 | -6.94 | -7.87 | 0.48 | 0.14 | 0.62 |
| | MILK | 6.24 | 6.93 | 7.06 | 0.04 | <0.01 | 0.79 |
| Phenylalanine | PDV | 24.41 | 29.84 | 35.41 | 1.35 | <0.01 | 0.96 |
| | LIVER | -11.78 | -15.09 | -20.70 | 1.25 | <0.01 | 0.47 |
| | TSP | 12.62 | 14.67 | 14.75 | 1.38 | 0.35 | 0.57 |
| | MG | -11.10 | -12.04 | -13.05 | 0.62 | 0.09 | 0.96 |
| | MILK | 9.87 | 10.95 | 11.16 | 0.04 | <0.01 | 0.79 |
| Threonine | PDV | 20.36 | 23.75 | 34.50 | 2.70 | <0.01 | 0.30 |
| | LIVER | -6.71 | -6.56 | -14.52 | 3.48 | 0.19 | 0.36 |
| | TSP | 13.69 | 17.45 | 19.87 | 1.87 | 0.07 | 0.77 |
| | MG | -11.81 | -13.82 | -13.23 | 0.84 | 0.33 | 0.26 |
| | MILK | 12.01 | 13.33 | 13.59 | 0.04 | <0.01 | 0.79 |
| Valine | PDV | 28.39 | 34.39 | 40.53 | 3.51 | 0.07 | 0.98 |
| | LIVER | 1.34 | 5.30 | 6.18 | 2.77 | 0.29 | 0.65 |
| | TSP | 29.70 | 39.49 | 46.80 | 4.51 | 0.05 | 0.82 |
| | MG | -19.98 | -26.99 | -27.96 | 2.40 | 0.07 | 0.33 |
| | MILK | 18.75 | 20.52 | 21.21 | 0.04 | <0.01 | 0.79 |

¹Least squares means presented with pooled SEM, n=5

²PDV: portal-drained viscera; TSP: total splanchnic tissues; MG: mammary glands

³Probability corresponding to the null hypothesis

The BCAA, threonine and lysine, were catabolised by peripheral tissues, because their post-liver supply exceeded output in milk protein. The contribution of the mammary gland to the post-splanchnic catabolism of lysine and leucine was greater (40-50%) than for isoleucine and valine (20-25%). In contrast, the mammary gland did not oxidize threonine and thus other peripheral tissues must have been responsible for catabolism of the 12-35% excess available beyond the liver. These data demonstrate the better transfer of AA into milk protein with Low MP diets, with associated reduction in urinary-N loss. However, with Low MP post-liver supply of histidine was insufficient to account for the milk output. Therefore, other sources of histidine must have been mobilized from body reserves (e.g. released from carnosine or ‘spared’ by reduced hemoglobin synthesis). Such a negative histidine balance could not have been maintained for a long period without adverse effects to the animal and so the apparent improved efficiency may hide adverse metabolic demand to the cow.

Conclusion

When cows are fed low levels of MP, the catabolism of EAA is dramatically reduced such that the transfer of absorbed AA to milk is greatly enhanced, allowing a very moderate decrease in milk protein output through an increased efficiency of utilization of the dietary supply. Caution should be taken when interpreting gross efficiency, as the supply to the mammary gland at low level of MP might also originate from endogenous sources. These would artificially increase the calculated efficiency for diets that could not be offered to dairy cows on a long term basis without detrimental effects.

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Dietary protein sources and lysine balance on the efficiency of energy utilisation in broiler chickens

S.P. Rose¹, V.R. Pirgozliev^{1,2}, J. Courtney¹ & S.D. Hare¹

¹ The National Institute of Poultry Husbandry, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK

² Queen's University, AESD, Newforge Lane, Belfast, BT9 5PX, UK

Summary

Two experiments were conducted to examine whether the efficiencies of utilisation of ME for retained energy in broiler chickens from two protein concentrates (fishmeal and maize gluten meal) were affected by the protein concentration of the basal feed. The objective of the second experiment was to examine whether dietary lysine concentration affected the determined energy retention of a broiler feed. In experiment 1, four dietary treatments were prepared by blending each of the two feedstuffs with a high protein and low protein basal feed. The efficiency of energy retention was determined in broiler chickens from 7 to 21d of age. TMEn of both feedstuffs was determined in adult cockerels. The second experiment compared the efficiency of energy retention between three diets that varied in their lysine concentrations (25, 80 and 111 g/kg of protein). The determined TMEn of the fishmeal and maize gluten meal were 13.27 and 15.52 MJ/kg DM respectively (SEM = 0.312). The efficiencies of utilisation of TMEn for the fishmeal were greater ($P<0.05$) in the low protein basal compared to the high protein basal feed, but the efficiency of utilisation tended ($P>0.05$) to be reduced for the maize gluten meal with the low protein basal feed. This interaction ($P<0.05$) may have been caused by a deficiency of one or more limiting amino acids, most probably lysine. Birds fed the 25g lysine/kg diet in the second experiment had a reduced ($P<0.001$) energy retention compared to the higher lysine intake treatment groups. Birds fed the excess lysine (111g/kg) diet had a reduced ($P<0.05$) growth rate, but these birds had a higher carcass fat content and so total carcass energy retention was not significantly reduced.

Keywords: chicken, energy efficiency, lysine

Introduction

There are a large number of protein concentrates that are available to poultry feedstuff manufacturers. Although the protein contents and amino acid balances of the protein concentrates are of major importance, quantification of the concentration of available energy in the individual feedstuffs is also required to make economic decisions on which protein concentrate to select. Energy availability is conventionally determined by empirical measurement of the metabolisable energy (ME) of feedstuffs, but the efficiency of utilisation of ME varies between diets and individual feedstuffs. It is important to understand these causes of variation in practical feedstuffs and to quantify the effect that the amino acid balance of the total protein supply has on energy utilisation.

This study comprised two experiments: The objective of the first experiment was to examine whether the efficiency of utilisation of ME for retained energy in broiler chickens of two protein concentrates was affected by the protein concentration of the basal feed. The objective of the second experiment was to examine whether three lysine concentrations of a feed (25, 80 and 111g/kg CP) affected the determined energy retention of broiler chickens.

Material and methods

Experiment 1

A sample of fishmeal and a sample of maize gluten meal were obtained. The two protein concentrates had approximately the same crude protein concentrations (647 and 620 g/kg respectively) but very different amino acid concentrations (for example, 80.3 and 17.4 g lysine/kg protein respectively). The TMEs of the samples were determined in adult cockerels using a rapid ME determination assay in which the previously un-fed cockerels were precision-fed 50g of the sample and their droppings collected for the following 48 h. Five birds were used for each feedstuff sample and the method was adapted from the procedure described by McNab and Blair (1988). The carcass energy retained per kg of additional protein concentrate fed was determined by substitution of the protein concentrate into two basal diets (40 parts protein concentrate : 50 parts basal) that were fed to broiler chickens from 7 to 21 d of age. Each diet was fed to six replicate cages of birds (floor area of 930 cm² with two birds per cage). The basal diets differed mainly in their protein concentrations (Table 1). The feed intakes of the birds were restricted to 90% of *ad libitum* in order to equalise nutrient intakes between replicate birds within treatments and to equalise basal feed intakes. Differences in determined TME were compared by a fully randomized analysis of variance and differences in broiler energy retention were compared by a randomized block analysis of variance with a 2 x 2 factorial treatment structure.

Table 1. Ingredient composition (kg/tonne) of the basal feeds used in experiments 1 and 2.

| Feedstuff | Experiment 1 | | Experiment 2 |
|---|-------------------|----------------------|------------------------|
| | Low protein basal | Higher protein basal | Lysine deficient basal |
| Ground wheat | 785.7 | 578.7 | 701.5 |
| Full fat soya | 65.0 | 300.0 | 30.0 |
| Fishmeal | 52.0 | 40.0 | |
| Maize gluten meal | | | 220.0 |
| Lysine hydrochloride | 1.3 | 1.0 | |
| Methionine | 2.7 | 2.0 | |
| Threonine | | | 1.0 |
| Tryptophan | | | 0.5 |
| Soya oil | 40.0 | 30.0 | |
| Dicalcium phosphate | 20.0 | 15.0 | 25.0 |
| Salt | | | 2.0 |
| Vitamin mineral premix | 33.3 | 33.3 | 20.0 |
| Nutrient content | | | |
| Crude protein (CP) (g/kg) | 148 | 201 | 226 |
| Lysine (g/kg CP) | 53 | 58 | 25 |
| Methionine plus cystine (g/kg CP) | 50 | 42 | 41 |
| Metabolisable energy (calculated) (MJ/kg) | 13.4 | 13.6 | 13.1 |

¹ The proprietary supplement was supplied by Ian Hollows Feed Supplements Ltd., Whitchurch, UK. It provided the following nutrients (mg/kg supplement) : 38.4 retinol acetate, 0.6 cholecalciferol, 2000 α-tocopherol acetate, 240 thiamin, 800 niacin, 1200 pantothenic acid, 240 pyridoxine, 1.2 cyanocobalamin, 20,000 choline chloride, 10 biotin, 120 folic acid, 1600 iron, 80 copper, 8000 manganese, 6400 zinc, 80 iodine and 16 selenium.

Experiment 2

A basal feed was formulated that was deficient in lysine (25g/kg CP) but that was otherwise nutritionally complete. Lysine hydrochloride was used to give two further lysine concentrations (80 and 111g/kg CP). The three diets were fed each to eight replicate pens of broiler chicks from 7 to 21d of age at 95% of predicted *ad libitum* intakes so that all birds had the same energy intakes. The retained energies in the carcasses of the birds at 21d of age were determined. Differences between treatment groups were examined by randomised block analyses of variance.

Results

Experiment 1

The determined TME_n of the fishmeal and maize gluten meal were 13.27 and 15.52 MJ/kg DM respectively (SEM = 0.312). There was a significant ($P<0.05$) interaction in the carcass energy retentions and the efficiency of utilisation for energy retention in the broiler chickens fed the two protein concentrates that depended upon the protein concentration of the basal feed. The efficiencies of utilisation of TME_n for the fishmeal were greater ($P<0.05$) with the low protein basal compared to the high protein basal feed, but the efficiency of utilisation tended ($P>0.05$) to be reduced for the maize gluten meal with the low protein basal feed.

Table 2. Efficiency of energy retention for two high protein feedstuffs when given with a high and low protein basal feed.

| Basal feed | Fishmeal (FM) | | Maize gluten meal (MGM) | |
|--------------|---------------------------------------|--|--|--|
| | Energy retained in carcass (MJ/kg FM) | Efficiency of utilisation of TME _n for energy retention | Energy retained in carcass (MJ/kg MGM) | Efficiency of utilisation of TME _n for energy retention |
| Low protein | 8.41 | 0.633 | 7.76 | 0.500 |
| High protein | 7.41 | 0.558 | 8.57 | 0.552 |

SEM for carcass energy retention = 0.467 and SEM for efficiency of utilisation of TME_n = 0.0223

Experiment 2

The birds fed the 80 g/kg CP lysine diet tended ($P>0.05$) to have a higher weight gain but only the birds fed the 25g/kg diet had a reduced ($P<0.001$) energy retention (Table 3).

Table 3. The effects of three dietary lysine concentrations on weight gains and energy retention of broiler chickens.

| Dietary lysine concentration (g/kg CP) | Weight gain (g/bird) | Carcass energy retention (MJ/kg of feed intake) |
|--|----------------------|---|
| 25 | 97.0 | 2.17 |
| 80 | 112.4 | 2.99 |
| 111 | 92.2 | 2.72 |
| SEM | 10.61 | 0.180 |

Discussion

Experiment 1 indicated that the efficiency of utilisation of TMEn for carcass energy retention in broilers fed different protein concentrates was affected by the total protein supply to broiler chickens. The low efficiency of utilisation of the maize gluten meal with the low protein basal feed suggests that the problem may have been caused by a deficiency of one or more limiting amino acids, most probably lysine. The mean daily lysine requirement of the broiler chickens was calculated to be approximately 1.1 g/day. The maize gluten diet with the low protein diet provided only 0.76 g lysine/day whereas the maize gluten with the high protein diet provided 0.94 g lysine/day (NRC 1994). Lysine was probably the first limiting amino acid in both these diets and the 24% increase in the daily supply of this amino acid would have enabled a much more efficient utilisation of the total dietary protein supply in the high protein basal diets. The fishmeal diets supplied approximately twice the daily requirement for lysine with both the high and low protein basal feeds. The lower efficiency of energy utilisation of the fish meal in the high protein, compared to the low protein, basal feed may have been due to the energy cost of dealing with the excess total protein intake that resulted from this dietary treatment.

Experiment 2 was conducted to quantify the effect of a deficiency of lysine on the energy retention of broiler chickens. The 25 g/kg lysine diet provided a mean daily lysine intake of 0.5 g/day compared to the expected requirement of 1.1 g/day. The 80 g/kg and 111 g/kg diets provided lysine intakes of 1.7 g/day and 2.3 g/day respectively. The birds fed the lysine deficient diet had a 27% reduction in carcass energy deposition compared to the birds given the 80g/kg diet. Although the excess lysine diet reduced growth rate, these birds had a higher carcass content of fat and so total carcass energy retention was not significantly reduced.

In summary, these experiments have demonstrated the need for amino acid deficiencies to be avoided when determinations of the efficiency of energy utilisation are being conducted on practical feedstuffs. The work has also quantified the large effect of lysine deficiency on the efficiency of energy retention in broiler chickens.

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Ruminal acetate/propionate pattern and nitrogenous nutrient fluxes across splanchnic and hind limb tissues in growing lambs

I. Savary-Auzeloux, L. Majdoub & I. Ortigues-Marty

Equipe Nutriments et Métabolismes, Unité de Recherches sur les Herbivores, INRA Clermont-Ferrand Theix, 63122 St Genès Champelan, France

Summary

The influence of modifications in the ruminal fermentation pattern on the quantity and the profile of nitrogenous nutrients (ammonia, urea and amino acids (AA)) appearing in the portal vein, being released by the splanchnic tissues and being used by the hindlimb, was tested by combining data from 2 experiments and using statistical multivariate analyses. At the liver level, the net branched chain AA uptake was correlated to the net hepatic insulin extraction rate as well as the net portal appearance of the ketogenic/glucogenic nutrient ratio. In the hindlimb, the net AA uptake was linked to a more glucogenic balance of nutrients, suggesting the nature of the energy-type nutrients influences muscle protein metabolism.

Keywords: nitrogen, splanchnic tissues, hindlimb

Introduction

The objective of this study was to test to which extent a modification in the ruminal fermentation pattern could determine the quantity and the profile of the nitrogenous nutrients (as ammonia, urea or amino acids) appearing in the portal vein and being delivered by the splanchnic tissues to the hind limb. Results from two experiments characterised by changes in the molar ratio between acetic+butyric versus propionic volatile fatty acid concentrations (C_2+C_4/C_3) in the rumen, the amount of crude protein (CP) ingested and the ratio of CP / ME (metabolisable energy) intake were combined.

The main questions addressed focussed on the relationships between : 1) the ruminal fermentation pattern, the CP/ME intake, and the amount and profile of the nitrogenous (N) nutrients appearing in the portal vein, 2) the net portal appearance (NPA) of N nutrients, their utilisation by the liver and their splanchnic release, 3) the net splanchnic release of N nutrients and their net uptake by the hind limb. A companion abstract is focused on energy-type nutrients (Ortigues-Marty *et al.*, 2003)

Materials and methods

Both experiments were conducted using growing lambs (32-40 kg BW) surgically equipped for measurements of nutrients fluxes across the portal drained viscera (PDV), the liver and the hind limb. The basal diet consisted of frozen ryegrass (1.5 x MEm), and the ruminal fermentation pattern and/or CP intake were modified by supplementation either with barley or with intraruminal infusions of propionate. Net fluxes were measured for total AA, essential AA, non essential AA, branched chain AA, urea, ammonia (NH_3), volatile fatty acids (VFA), glucose, lactate, β -hydroxybutyrate and insulin across all three tissue beds. Results from each experiment are being published separately (Majdoub *et al.*, 2003a, b; Savary-Auzeloux *et al.*, 2003a, b). The present work aims at combining all observations (n=28 for the splanchnic tissues and n=18 for the hind limb) and analysing them using multivariate Principal Component (PC) analysis procedures (SAS) to detect the relationships which may exist between the net nitrogenous nutrients fluxes and that of other nutrients at the different anatomical sites.

Results and discussion

In our data set, the dietary CP/ME intake ratio ranged from 1.11 to 1.84 g/kJ and ruminal NH₃ concentrations ranged from 4.3 to 15.8 mM. Those wide ranges reflected large differences in dietary CP contents. In addition, the ruminal fermentation profile evaluated by the molar C2+C4/C3 ratio varied from 1.0 to 3.1 (Ortigues-Marty *et al.*, 2003). Diets were insufficiently characterised in terms of ruminal organic matter digestibility and N degradability to allow precise predictive correlations between the NPA of AA and intake or ruminal fermentation characteristics. In other respects, the transfer of blood urea to the lumen of the digestive tract was clearly related to the ruminal volatile fatty acid profile. 39% of the variance in net urea transfer were attributed to the ruminal C2+C4/C3 molar ratio ($r=+0.69$, $P<0.001$), with transfer increasing in parallel with ruminal C3 concentrations.

Relationships between the net portal appearance of nitrogenous nutrients and their splanchnic release

At the hepatic level, the interactions between the energy and N type nutrients are complex due to the multiple fates of the N nutrients in the liver and because of regulations by insulin and the metabolic demand by the peripheral tissues.

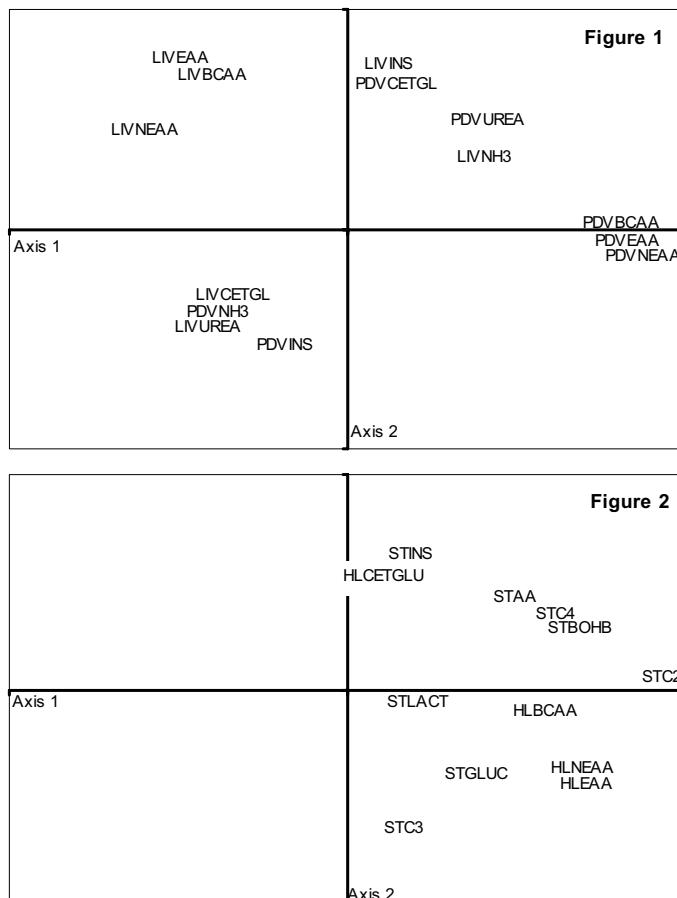
Interrelationships between net PDV and hepatic fluxes of N and ceto- to glucogenic compounds were studied by a PC analysis (Figure 1). 26% of the total variance among data was attributed to the NPA of amino acids (first axis). Another 24% of the variance (second axis) was attributed to the net hepatic uptake of branched chained amino acids which was positively correlated to the net hepatic extraction of insulin ($r=+0.45$, $P<0.05$) and to the net portal appearance of ceto- to glucogenic nutrient ratio ($r=+0.38$, $P<0.05$). The more cetogenic the nutrients released by the liver, and the lower the net hepatic extraction of insulin, the lower the net hepatic uptake of branched chained amino acids. This analysis confirmed, using a larger number of observations, the conclusions obtained by Savary-Auzeloux *et al.* (2003a) on the effects of propionate supplementation on hepatic AA metabolism.

Relationships between the net splanchnic release of nitrogenous nutrients and their net uptake by the hind limb

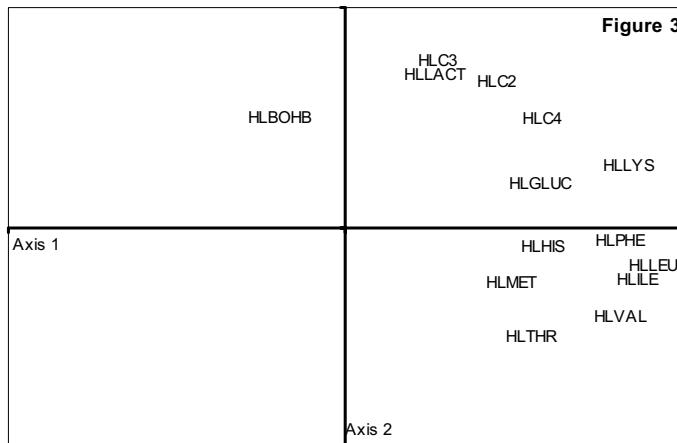
In the hind limb net AA uptake was not strongly related to the splanchnic release of AA, which suggests that the N supply to the animals was sufficient to sustain the requirements. A PC analysis was carried out to identify relationships between the net splanchnic release of nutrients (energy type and AA) with the net nutrient uptake by the hindlimb (Figure 2). The first axis (30% of the total variance) was determined by the net AA uptake by the hindlimb together with the net splanchnic release of C2 and β-hydroxybutyrate. These results suggest that the higher the net splanchnic release of cetogenic nutrients, the lower the net hindlimb uptake of AA. A subsequent multivariate analysis identified relationships between the net hindlimb uptake of essential AA and that of energy-type nutrients (Figure 3). First of all, 40% of the variance among data (first axis) was attributed to the uptake of the essential AA (6 of which carried the greater weight : Ileu, Leu, Val, Lys, Phe, Thr), while another 19% of the variance (second axis) was attributed to the uptake of C2, C3, C4, lactate and β-hydroxybutyrate. Interestingly, the uptake of glucose was positioned close to the first axis, indicating positive correlations between net essential AA uptake and net glucose uptake by the hindlimb. Therefore, a glucogenic balance of nutrients released by the splanchnic tissues and / or taken up by the hindlimb is associated with a greater AA uptake by the hindlimb.

Conclusions

The results presented here show the role of the ruminal fermentation pattern on the net splanchnic fluxes of essential AA, especially branched chain ones. The regulatory role of insulin at the splanchnic level is highly probable since insulin extraction rates by the liver are closely related to the extraction rates of the energy and N nutrients. The hind limb AA uptake was not strongly related to the splanchnic release of AA but seemed to be regulated by the glucogenic nutrient balance. Involvement of insulin is also probable.



Figures 1, 2 and 3. Principal component analyses showing relationships between 1) net PDV and hepatic fluxes of nitrogenous and ceto- to glucogenic compounds (Figure 1), 2) the net splanchnic release of nutrients (energy type and amino acids) with the net nutrient uptake by the hindlimb (Figure 2), and 3) the net hindlimb uptake of essential amino acids and that of energy-type nutrients (Figure 3). Abbreviations of the variables used were as follows : PDV, LIV, ST, HL (net fluxes across the portal-drained viscera, the liver, the splanchnic tissues and the hindlimb), for the following nutrients C2 (acetate), C3 (propionate), C4 (butyrate), GLUC (glucose), LACT (lactate), BOHB (β -hydroxybutyrate), INS (insulin), AA (amino acids), EAA (essential AA), BCAA (branched-chained AA), NEAA (non-essential AA), CETGLU [$(C2+C4+BOHB)/(C2+LACT+GLUC)$], HIS (Histidine), ILE (Isoleucine), LEU (Leucine), LYS (Lysine), MET (Methionine), PHE (Phenylalanine), THR (Threonine), VAL (Valine), NH3 (ammonia) and urea.



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The selection of a modified Gompertz-Function for description of postnatal growth of hogs

M. Schlegel

*University of Rostock, Faculty of Agricultural and Environmental Sciences, Department of Agroecology, Institute of Agroeconomy and Process Engineering, Justus-von-Liebig-Weg 6
18059 Rostock, Germany*

Summary

It have to be differed in general conclusions asserted in a comparison of groups (**I.**) and in conclusions which asserted in a comparison of individuals (**II.**).

I.

- Hogs with high finishing weight have a small growth intensity (small parameter value k).
- Hogs with small finishing weight show a high growth intensity (high parameter value k).
- Live weight curves with small finishing weight display concavities with markedness. By contrast pigs with high finishing weight and small growth intensity show a stretched shape of curve.

II.

- Is the finishing weight the same there are various growth intensities (k).
- At the same growth intensity various finishing weights appear.

At the end the application of growth function for breeding, for the verification and coordination of dates during the period of husbandry as well as feeding should be emphasized. The coherences mentioned top should be analyzed in further research in order to enhance the effectiveness of meat production with mathematical auxiliary means.

Keywords: growth function, pigs

Introduction

The pork production is of importance in Germany. Of less importance, following pork production, is beef-, chicken-, turkey- and other poultry production. Due to annual work programs and economic reasons partially there is nowadays a transition to larger production units. The use of large production units arises to problems concerning the effectiveness of meat production. It is known that there can be differences in the growth progress of individual pigs, even if they have about the same live weight and age at the beginning of the fattening process. With increasing fattening time there will also be increases in differences of individual live weight, resulting in different nutrient needs for the individual animal. But individual feeding would be too expensive for some farmers. Therefore it is important to use homogeneously growing animal groups for group feeding. The task is to investigate the growth progress of hogs under production conditions.

Material and methods

The study was conducted in a production unit with 70 000 pigs, including about 6000 sows and about 20 000 feeding pigs. 600 male feeding pigs (hogs) were registered for the study. Male pigs were used because they get a tattoo right after birth, making it possible to keep record of them even later on. The basis for the range of random sampling was the registration of the differences of the final fattening mass of all feeding pigs. Between the animals there had been a range of weight between 70 kg and 145 kg at the age of about 230 days. All the animals had about the same

genetic design and the same environmental surroundings during the breeding process. It was aspired to weigh the animals five times. Age and live weight had been recorded at the following stages:

- 1. day birth
- 31. - 33. day changeover pig rearing/ store pig rearing
- 109. - 111. day changeover store pig rearing/ fattening period I
- 169. - 171. day changeover fattening period I/ fattening period II
- 216. - 245. day barn leave

Due to different factors, such as death losses or the impossibility to recognise the tattoos and so on, in the end there had been 267 animals for further research. There had been a difference between the calculated sampling range of 600 animals and the 267 animals that had been finally used. First of all it would like to point out that the differences in the growth progress of the 267 animals was big enough to enable scientific statements. The different growth progresses had been assorted. The assortment was carried out due to increasing asymptotic end weight. The asymptotic end weight (mass) $x_E = a/k$ is the asymptotic value of live weight that will never be reached in reality. The 267 individuals had been divided into 11 groups counting about 25 animals each. The applied growth function, a modified Gompertz-Function from Lehmann

$$x = e^{\frac{a}{k} - \frac{1}{ke^{k(t-c)}}} \quad (1)$$

contains three parameters: a , k , and c which can be used to interpret growth. The graph of the growth function, the curve of live weight (*Figure 1.*) shows a typical s-shaped growth progress. The curve subdivides into a progressively and a degressively increasing curve. The turning-point (t_{Zmax}) lies in between. Growth processes are also called self accelerating and self stopping phase. Furthermore, the figure also shows daily increase in live weight, it arises from the first derivation of the growth function (see function 1). Besides that aspect, the figure also points out the turning-points and the point of time when maximum daily increase (point of inflection) in live weight is reached. First of all, the turning-points and the maximum daily increase in live weight are prominent signs of visually checking the curve. Second, they are of help since they are means that can be calculated to determine the exact time when fundamental biological changes occur. This prominent signs should be called growth relevant point of time.

Results and discussion

In Table 1 it can be shown that in group 1 are located animals with small asymptotic end weights and in group 10 animals with high asymptotic end weights. Also it can be seen that the parameter value of k at animals with small asymptotic end weights (group 1) is higher than at animals with high asymptotic end weights (group 10). The higher parameter value of k the higher is the growth intensity. Accordingly small asymptotic end weight associates with a high growth intensity and contrariwise. Perusing the values of growth relevant points of time in Table 1 it can be highlighted that the distances between t_{W1} and t_{W2} at group 10 are greater than at group 1. It can be traced back to the fact of lower growth intensity of group 10.

Table 1. Set of parameters (a , k , c), asymptotic end weight (X_E) as well as the growth relevant points of time (t_{W1} , t_{Zmax} , t_{W2}) of the groups 1 and 10.

| | Parameter | | | X_E ing kg | Growth relevant points of time | | |
|----------|-----------|--------|------|--------------|--------------------------------|--------------------|----------|
| | a | k | c | | t_{W1} | t_{Zmax} in days | t_{W2} |
| Group 1 | 0,062 | 0,0125 | -226 | 142 | 48 | 125 | 202 |
| Group 10 | 0,04 | 0,0069 | -503 | 322 | 81 | 221 | 361 |

This fact can be visualized in Figure 1. The curves are extrapolated over the end of fattening period (218 days) in order to show the different shapes. The curves and the growth relevant points of time of group 1 are gray colored. The smaller distances between the growth relevant points of time can be seen. The higher maximum of daily increase of live weight and the slow passing through the growth relevant points of time at group 10 causes in the end a higher asymptotic end weight.

In Figure 2 are illustrated two individuals: hog 59 and 117. Both individuals feature the same asymptotic end weight but show different parameter values of k . There are animals with the same asymptotic end weight but have different growth intensities. That means the hog 117 with the higher parameter value of k passes through the growth relevant points of time faster than the hog 59. This conclusion which should be considered for economic topics doesn't contradict the conclusion asserted in the comparison of groups. It is an enlargement. With high plausibility it can be assumed that the coherences between growth intensities and asymptotic end weights can be carried forward to finishing weight. It should be analyzed in further research in order to enhance the effectiveness of meat production with mathematical auxiliary means.

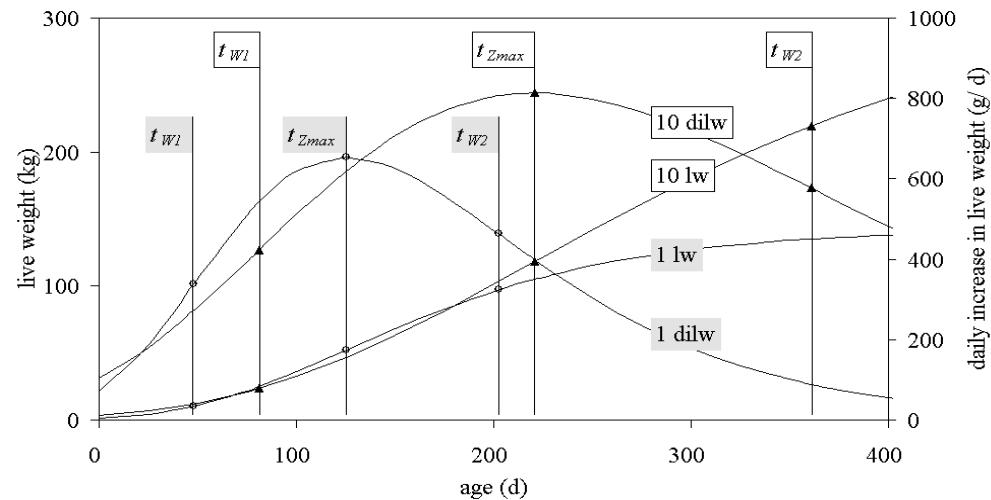


Figure 1. Curves of live weight (lw) and daily increase in live weight (dilw) as well as growth relevant points of time of group 1 and 11.

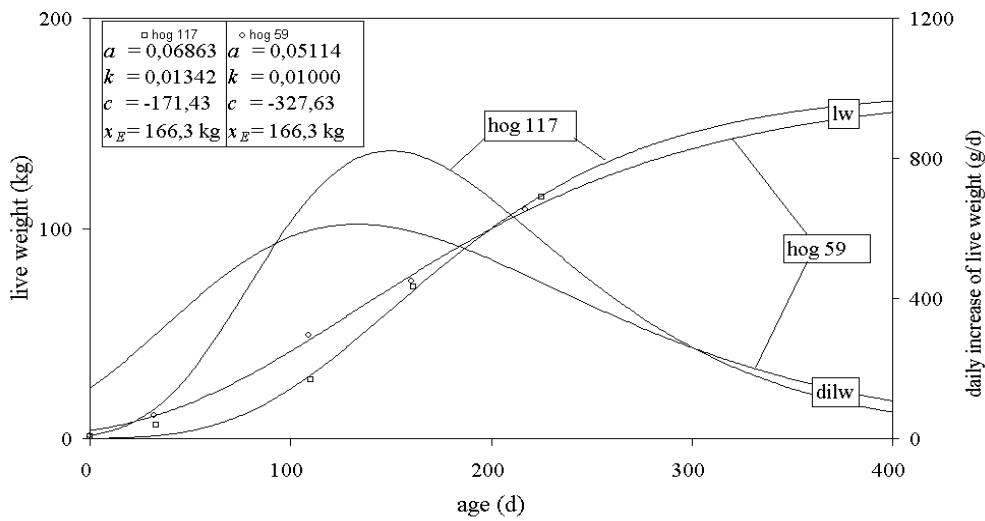


Figure 2. Curves of live weight (lw) and daily increase in live weight (dilw) as well as growth relevant points of time of hog 59 and 117.

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Mathematical analysis of [$^{13}\text{CO}_2$]-expiration curves from human breath tests using [$1\text{-}^{13}\text{C}$]-amino acids as oral substrate

V.V.A.M. Schreurs¹ & K. Krawielitzki²

¹ *Human and Animal Physiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen, The Netherlands*

² *Research Institute for the Biology of Farm Animals, Department of Nutritional Physiology "Oskar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany Institute, Dummersdorf-Rostock, Germany*

Summary

A [$^{13}\text{CO}_2$]-breath test examines the expiration of [$^{13}\text{CO}_2$] as function of time after oral intake of a [^{13}C]-labelled test substrate (single dose). In clinical settings, breath test studies are often used as a simple and non-invasive tool to diagnose the activity of metabolic functions. From a nutritional point of view, breath test studies can also be used to trace the catabolic fate of dietary nutrients. The focus of this paper was a mathematical analysis of [$^{13}\text{CO}_2$]-expiration curves. Human breath test results for [^{13}C]-labelled amino acids (leucine, methionine, lysine) under different dietary conditions were used to illustrate this approach. The mathematical parameters were meant to characterise changes in the catabolic fate of dietary amino acids as influenced by the ingestion of a meal.

Keywords: *amino acid, oxidation, breath test*

Introduction

Stable isotopes are more and more used as a tool in clinical diagnostics. A popular application is the 'Urea breath test' to diagnose a Helicobacter Pilori infection of the stomach. In this test, a known amount of [^{13}C]-urea is ingested and the amount of [$^{13}\text{CO}_2$] recovered in the breath is used as indicator of the severity of the infection. Based on the same principle a scope of other [^{13}C]-labelled substrates have been used to monitor various metabolic functions (Krumbiegel, 1991). [$^{13}\text{CO}_2$]-expiration curves (At%-excess vs. time) can be very informative about the kinetics of the labelled substrate through metabolic pathways. In general peak shaped responses can be expected for the expiration rate of [$^{13}\text{CO}_2$] in time. However, the characteristics of such a peak shaped response can vary in many ways, depending on the nature of the substrate and other breath test conditions. Similar responses can be presumed for related substrates and conditions. This paper describes a mathematical analysis of human [$^{13}\text{CO}_2$]-expiration curves for different [^{13}C]-labelled amino acids in relation to dietary conditions. The aim was to characterise the influence of a meal on the metabolic fate of orally ingested amino acids

Materials and methods

In the present study the expiration of [$^{13}\text{CO}_2$] as function of time has been studied for [$1\text{-}^{13}\text{C}$]-labelled amino acids (leucine, lysine and methionine) as oral substrate (single dose). In all cases the [^{13}C]-intake was 6 mg. The labelled amino acids were ingested either alone (as aqueous solution) or in combination with a test meal containing: 1.4 MJ ME, 19.5 g protein, 30.8 g carbohydrate and 14.6 g fat. More details about subjects, test substrates etc. will be presented in an extensive full paper (in preparation).

Breath sampling procedure

After ingestion of the test substrate (ca. 10.30 a.m.) the breath was spot sampled 15-times during a period of 6 hours. The subjects used a straw to expire smoothly at the bottom of a 10-ml exetainer tube (Labco, High Wycombe, UK) for at least 5 sec (to avoid air from dead respiratory volume). Tubes were analysed for [¹³C]-enrichment at the WIAS-IRMS facility with a Finnigan Delta C Isotope Ratio Mass Spectrometer (IRMS) equipped with breath device. The Atom%-excess values of the breath were plotted against time.

Calculations and statistics

The mathematical approach is based on the whole body model for protein and amino acid metabolism from Sprinson & Rittenberg (1949) as modified by Krawielitzki *et al.* (1997) and shown in figure 1. The mathematical parameters of the expiration curves were determined by an iterative mathematical principle (Cademix for Windows, Biorat GmbH) as originally described by Rash *et al.* (1987). This programme provides S_r -values (rest deviation) to establish statistical differences between the curves.

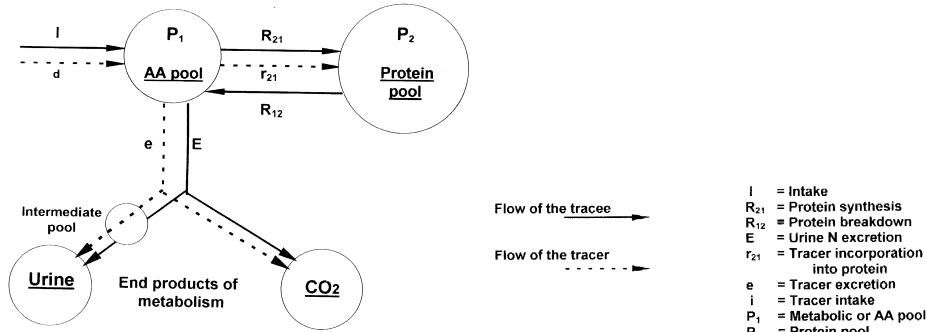


Figure 1. Whole body model for protein and amino acid metabolism. Flow of tracee and tracer are indicated separately (Krawielitzki *et al.*, 1997).

The metabolic pool of amino acids comprises all free amino acids of the body from different compartments. The model presumes a constant size of the metabolic pool in time and a rapid exchange between compartments. With respect to tracer kinetics, the metabolic pool is charged by intestinal absorption (linear process) and cleared by protein synthesis and amino acid degradation (exponential processes).

Results and discussion

Figure 2. shows breath test results for [¹³C]-labelled amino acids (leucine, lysine and methionine) ingested alone (left panel) or in combination with a test meal (right panel). All responses were peak shaped but clearly a-symmetric. This indicates that the metabolic pool is charged and cleared by processes with different kinetics. The ascending part (charging of the metabolic pool) was always steepest and took, depending on the treatment, 15 - 120 min. The descending part (clearance of metabolic pool) took at least 4 hours.

The descending part of the curves was described as an exponential function: $Y_t = D \cdot e^{-C \cdot t}$. The value D represents the (maximal) enrichment of the breath (At%-excess) in case the metabolic pool would have been charged by the entire tracer dose at $t = 0$. This D -value is obtained by extrapolation of the exponential part to $t = 0$. The value C represents the fractional decrease of

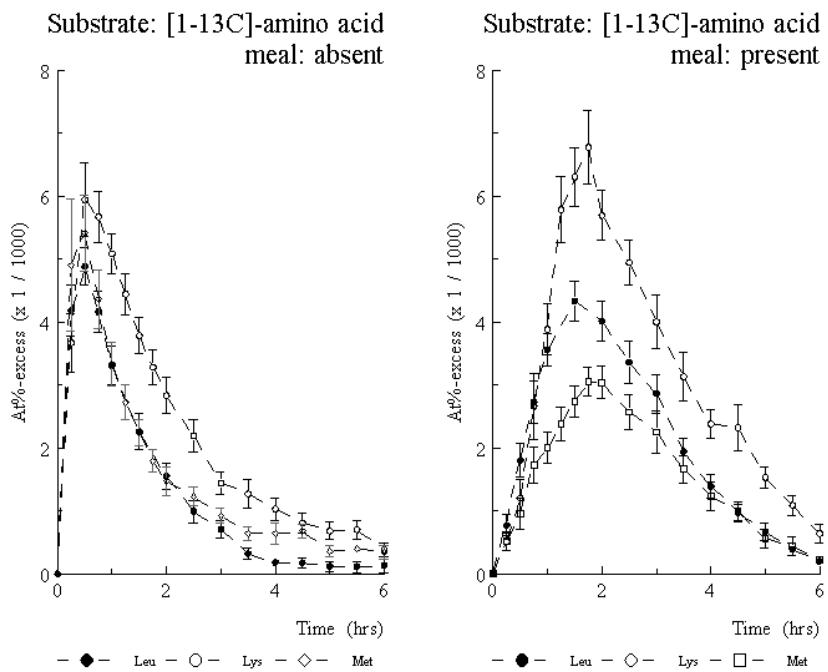


Figure 2. $[^{13}\text{CO}_2]$ -expiration curves from human breath tests using $[1-^{13}\text{C}]$ -amino acids (leucine, lysine, methionine) as oral substrate, either alone (left panel) or in combination with a meal (right panel).

the enrichment per hour and allows to calculate the half-life value: $T_{1/2} = \ln 2 / C$. The values D and C have been determined for the descending part of the measured curves.

The presence of an ascending part indicates that some time is required to complete (linear) absorption of tracer. To establish this lag time (T_{lag}) the differences between the extrapolated and measured values were described by a linear equation. This line with negative slope represents a correction for the enrichment due to incomplete absorption of tracer. The crossing point with the X-axis corresponds to the time required to reach complete absorption and maximal enrichment of the metabolic pool. Calculated parameters are shown in table 1.

The measured $[^{13}\text{CO}_2]$ -expiration curves as well as their mathematical parameters represent the overall result of the flow of label through all physiological and metabolic processes involved in the

Table 1. Mathematical parameters characterising exponential $[^{13}\text{CO}_2]$ -expiration curves after oral ingestion of a $[1-^{13}\text{C}]$ -labelled amino acid, either alone or in combination with a meal.

| | $[1-^{13}\text{C}]$ -Leucine | | $[1-^{13}\text{C}]$ -Lysine | | $[1-^{13}\text{C}]$ -Methionine | |
|---------------------------|------------------------------|--------|-----------------------------|--------|---------------------------------|--------|
| | - meal | + meal | - meal | + meal | - meal | + meal |
| D (At%-excess.) | 7.1 | 12.8 | 6.4 | 11.4 | 8.5 | 14.2 |
| $T_{1/2}$ (min) | 49 | 70 | 58 | 65 | 71 | 89 |
| T_{lag} (min) | 22 | 100 | 18 | 100 | 24 | 87 |
| S_R (\pm At%-excess) | 0.08 | 0.19 | 0.08 | 0.09 | 0.11 | 0.21 |

expiration of [$^{13}\text{CO}_2$] from orally ingested [1- ^{13}C]-labelled amino acids. These processes enclose gastric emptying, digestion, absorption and metabolism.

These preliminary results indicate that amino acids, ingested alone, are rapidly absorbed and metabolised. Complete absorption is reached after a lag time of 18 - 24 min. Ingested in combination with a meal the complete absorption of tracer takes more time. The lag time increased to 87 - 100 min. Also the half-life values increased indicating that a meal lowers the kinetics of the tracer. This can be caused by a reduced gastric passage rate or by an increased competition with non-labelled amino acids for absorption.

A longer half-life indicates that more time is required to clear the tracer from the metabolic pool. Such a situation increases the risk for a postprandial decarboxylation of ingested amino acids. However, the total oxidative loss of ingested amino acids not only depends on the time required to clear them from the metabolic pool. Also the partitioning of amino acids over the anabolic (protein synthesis) and catabolic (decarboxylation) pathways of clearance has a great influence. This partitioning is reflected by the calculated D-values. The D-values represent the enrichment of the breath (At%-excess) in case the metabolic pool would have been charged by the entire tracer dose at $t = 0$. Based on the same CO_2 -production at $t = 0$ in the different situations, the D-values are proportional to the fraction of ingested label responsible for the enrichment of expired air. An increase of the D-values after ingestion of a meal, indicates that a larger fraction of ingested label is available to enrich the expired air. In other words a larger fraction of the amino acids is cleared from the metabolic pool by decarboxylation.

The overall conclusion is that human [$^{13}\text{CO}_2$]-breath tests can be used to study the metabolic fate of orally ingested amino acids. Results can be described by mathematical parameters based on the whole body model for protein and amino acid metabolism. This study shows that a meal has the same general effects for individual amino acids, but mathematical parameters can be helpful to describe the differences.

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Whole body and hindlimb protein degradation is differentially altered by feeding in 10- and 28-d-old piglets

M.C. Thivierge^{1&2}, H.V. Nguyen¹, J.A. Bush¹, A. Suryawan¹, R. Orellana¹, C.W. Liu¹, D.G. Burrin¹, F. Jahoor¹ & T.A. Davis¹

¹ Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, 77030, Houston, Texas

² Department of Animal Science, FSAA, Université Laval, G1K 7P4, Québec, Canada

Summary

The neonatal period is characterized by a high rate of muscle protein accretion, which is partly due to an elevated rate of skeletal muscle protein synthesis in response to feeding. However, little is known about the regulation of muscle protein accretion by protein breakdown in response to feeding during neonatal life. To determine the feeding-induced response of protein breakdown at the whole body level and across the hindlimb in neonates, overnight-fasted 10- and 28-day-old piglets ($n = 6/\text{age group}$) were infused for 7 h with [$1\text{-}^{13}\text{C}$]phenylalanine and [ring- $^2\text{H}_4$]tyrosine during an initial 3-h fasting period, followed by a 4-h feeding period. Feeding was achieved by a continuous intra-duodenal infusion of an elemental diet. Plasma samples were obtained simultaneously from the carotid artery and the vena cava; blood flow of the caudal aorta was recorded using ultrasonic flow probes. Feeding resulted in increased whole body removal of phenylalanine from plasma for hydroxylation into tyrosine, its subsequent oxidation and its use for protein synthesis. Whole body endogenous flux of phenylalanine tended to be greater increased ($P = 0.12$) in 10- than 28-d-old piglets. Although young neonates are known to be very anabolic and efficient to use dietary protein for body protein deposition, older neonates tended ($P = 0.12$) to be more responsive to decrease phenylalanine appearance from proteolysis with feeding than young neonates. Fasting resulted in a net mobilization of phenylalanine from body proteins and was not further altered by age. Hindlimb protein metabolism was overall increased with feeding including increased phenylalanine net flux, its use for protein synthesis and its appearance from protein breakdown. Hindlimb proteolysis was increased by feeding but was not further altered by age. The reduction in proteolysis seen at the whole body level with feeding was not seen in the hindlimb, suggesting that other tissues were responsible for the whole body decrease in proteolysis in neonates. Net phenylalanine mobilization from hindlimb proteins was also not occurring during fasting for both age groups, which contrasts with the whole body mobilization.

Keywords: hindlimb, piglets, proteolysis

Introduction

Protein synthesis capacity of newborns is phenomenal and crucial to sustain their rapid rate of growth. Such regulation is transitory during the neonatal period and decreases with development. Investigations have highlighted some of the mechanisms that underlay such regulation. The novel technique of hyperinsulinemic-euglycemic-euaminoacidemic clamps revealed that young neonates are more sensitive and response to insulin than old neonates (Wray-Cahen et al., 1997). Further specific explorations of molecular aspects behind that regulation clarified that insulin receptor numbers and its substrates, their activation and downstream cascades of intermediates were more activated in young neonates (Suryawan et al., 2001). These findings put in light the consistent articulation of biological events that drive the neonatal anabolism.

Although protein synthesis is a primary mechanism sustaining protein accretion and muscle growth, proteolysis rate in muscle is not without consequence on muscle mass. Data on neonates mainly from human baby studies reveal that neonates are more resistant to reduce protein breakdown given free amino acids are constantly required in the free cellular pool to sustain the constant and rapid protein remodeling (Pointdexter et al., 1997). These few investigations on the regulatory influence of protein degradation on protein metabolism have always been realized at the whole body level and no data are available specifically on skeletal muscle regulation. Since changes in proteolysis can significantly alter endogenous protein stores, it is of both physiological and clinical interest to better define the regulation of skeletal muscle proteolysis in newborns.

A study was purposely designed to delineate the involvement of proteolysis on growth regulation in neonates where whole body and hindlimb protein metabolism were simultaneously investigated in fast and fed 10- and 28-d-old piglets.

Materials and methods

Repeated measurements comparing the effect of fasting and feeding on proteolysis were carried out in an incomplete bloc design comparing two age groups of six 28-d-old piglets and five 10-d-old piglets. The animals remained with the sow until 2 or 20 days after birth and the surgical procedures were carried out on the following day after an overnight fast. The catheter set up consisted in a feeding catheter in the duodenum, a catheter for tracer infusions in a jugular vein, sampling catheters in a carotid and the inferior vena cava. A perivascular flow probe was placed around the caudal aorta. Twenty-four hours after the surgery, the animals were infused i.v. an elemental diet to accelerate the recovery and were introduced to a commercial milk replacer. The measurements were carried out at 10 and 28 days of age following a recovery period of 7 d. The tracers used to quantify protein metabolism were [$1\text{-}^{13}\text{C}$]phenylalanine and [ring- $^2\text{H}_4$]tyrosine during an initial 3 h of fasting, followed by a 4-h feeding period. Feeding was achieved by a continuous intra-duodenal infusion of an elemental diet. Plasma samples were obtained simultaneously from the carotid artery and the vena cava every 30 min during the last 2 h of fasting and feeding periods; blood flow of the caudal aorta was recorded using ultrasonic flow probes.

Results

Feeding increased the overall rate of whole body phenylalanine metabolism with the exception of protein breakdown that decreased with feeding (Table 1). Whole body proteolysis decreased by 39% and 53% in 10 and 28-d-old piglets, respectively, this differential response between age groups reached a tendency. Whole body oxidative process measurements show that catabolic processes are likely not developmentally regulated.

Plasma flow to the hindlimb was increased by feeding more in older than in younger piglets (Table 2). Feeding enhanced glucose net uptake by the hindlimb. Similarly, net flux of phenylalanine as well as protein synthesis increased with feeding and was increased more at 28 days. In contrast to whole body proteolysis, hindlimb protein breakdown increased with feeding and this was not further altered by age.

Further examination of the data showed that accretion of phenylalanine in body proteins is related to the arterial concentration of phenylalanine and this regulation is differentially altered by age (Figure 1). In contrast, such differential effect of hindlimb arterial supply of phenylalanine on phenylalanine accretion into hindlimb proteins is not seen.

Discussion

Feeding increased whole body disposal of phenylalanine for protein synthesis and reached a numerical higher rate in 10-d-old piglets when compared to 28-d-old piglets. Simultaneously,

Table 1. Arterial insulin and glucose and whole body phenylalanine metabolism ((mol/(kg·h)) in response to feeding and development in neonatal piglets.¹

| | 10-d-old | | 28-d-old | | SEM | P | | |
|-------------------------|----------|------|----------|------|------|------|-------|------|
| | fast | fed | fast | fed | | Age | State | AxS |
| Insulin; (U/ml | 1.7 | 18.1 | 0.9 | 25.5 | 2.1 | 0.18 | <0.01 | 0.08 |
| Glucose; mM | 4.92 | 9.76 | 6.36 | 9.02 | 0.69 | 0.61 | <0.01 | 0.13 |
| Endogenous flux | 141 | 255 | 129 | 231 | 4 | 0.09 | <0.01 | 0.12 |
| Hydroxylation | 6.4 | 15.8 | 5.1 | 27.7 | 2.0 | 0.19 | <0.01 | 0.01 |
| Oxidation | 28.2 | 68.9 | 22.6 | 73.7 | 7.4 | 0.91 | <0.01 | 0.47 |
| Protein synthesis | 112 | 170 | 105 | 144 | 12 | 0.36 | 0.002 | 0.43 |
| Protein breakdown | 140.5 | 85.2 | 129.1 | 61.1 | 3.9 | 0.09 | <0.01 | 0.12 |
| Accretion into proteins | -28 | 85 | -24 | 96 | 10 | 0.23 | <0.01 | 0.73 |

¹Least square means are presented; 10-d-old piglets n = 5, 28-d-old piglets n = 6.

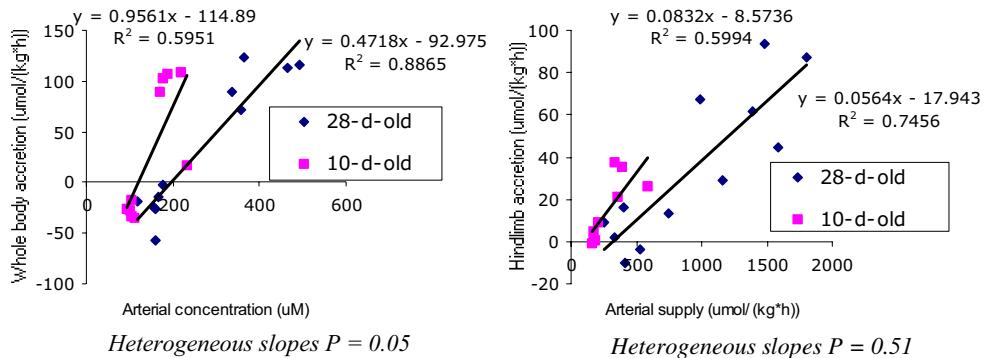
Table 2. Hindlimb plasma flow and net uptake of glucose (mmol/(kg·h)) and phenylalanine metabolism ((mol/(kg·h)) in response to feeding and development in neonatal piglets.

| | 10-d-old | | 28-d-old | | SEM | P | | |
|-------------------------|----------|-------|----------|-------|-------|------|-------|------|
| | fast | fed | fast | fed | | Age | State | AxS |
| Plasma flow, l/(kg·h) | 1.73 | 2.21 | 2.81 | 3.60 | 0.07 | 0.01 | <0.01 | 0.04 |
| Glucose net uptake | 0.442 | 1.320 | 0.525 | 1.783 | 0.132 | 0.21 | <0.01 | 0.12 |
| Net uptake | 2.86 | 29.66 | 4.41 | 63.9 | 7.0 | 0.06 | <0.01 | 0.02 |
| Protein synthesis | 22.8 | 56.2 | 33.9 | 100.9 | 8.2 | 0.01 | <0.01 | 0.04 |
| Protein breakdown | 19.9 | 26.5 | 29.4 | 37.1 | 3.6 | 0.18 | 0.04 | 0.87 |
| Accretion into proteins | 2.86 | 29.7 | 4.41 | 63.9 | 7.0 | 0.06 | <0.01 | 0.02 |
| FSR; %/d ¹ | - | 15.4 | - | 5.6 | | | | |

¹FSR means fractional rate of protein synthesis; Paired-t test showed that the FSR was different between 10- and 28-d-old piglets ($P = 0.004$). STDev = 1.4 and 3.1 for 10- and 28-d-old piglets.

whole body protein metabolism in a fed state was accompanied by a decreased proteolysis, as typically observed when insulin or amino acids were provided to adults (Gelfand et al., 1998; Garlick and Grant, 1988) and newborns (Goldspink and Kelly, 1984; Pointdexter 1997). Whole body proteolysis tended to decrease less with feeding in 10- than 28-d-old piglets. Such regulation would be consequent to a resistance to depression of protein breakdown in newborn babies given their rapid and continual remodeling of muscle protein requiring constant amino acid availability at cellular level (Pointdexter et al., 1997). Fasting resulted in net mobilization of body proteins in both groups of age. Phenylalanine accretion into body proteins is sensitive to arterial phenylalanine and phenylalanine accretion in body proteins decreases more drastically in young neonates with the reduction in plasma concentration of phenylalanine.

Feeding resulted in a global increase of hindlimb muscle protein turnover in neonatal piglets, including an increase in hindlimb proteolysis which contrasts with reduced whole body protein degradation. An increase in intake frequently results in improved nitrogen retention accompanied by higher rates of both protein synthesis and breakdown (Lobley, 1998; Reeds et al., 1980). In



*Figure 1. Heterogeneity of slopes between age groups for phenylalanine accretion into body or hindlimb proteins ((mol/(kg*h))) according to arterial concentration ((M) or arterial supply of phenylalanine ((mol/(kg*h))).
(n = 5 and n = 6 for 10- and 28-d-old piglets respectively)*

the current study, vascular tone adjustments of capillary beds in hindlimb muscles are involved likely in order to sustain tissue recruitment for nutrients to support muscle anabolism (Gaudreault et al., 2001). Feeding increased phenylalanine accretion into hindlimb proteins but it was greater enhanced in 28- than 10-d-old piglets. The regulatory influence of arterial phenylalanine supply is also seen on phenylalanine accretion into hindlimb proteins. Although age has positive influence on accretion of phenylalanine, it does not exert further control on phenylalanine accretion into hindlimb proteins through differential arterial phenylalanine supply, raising interesting contrasts between whole body and hindlimb. Whole body accretion of phenylalanine into proteins is more sensitive to phenylalanine arterial concentration in 10-d-old piglets, this differential sensitivity between ages is not further seen in the hindlimb. In consequence, other tissues than the hindlimb are responsible for this differential regulation.

Conclusion

The current experiment has shown that whole body protein accretion in response to feeding, accompanied by fed concentrations of insulin, is mainly driven by increased protein synthesis and reduced protein breakdown in neonates. However, young neonatal piglets tended to exhibit more resistance to reduce proteolysis as observed in human newborns. Although amino acids appear to be the primary regulator of protein breakdown during neonatal life, hindlimb kinetics of phenylalanine highlight the circumstance that skeletal muscle turnover in the hindlimb was increased in response to fed concentrations of insulin and to supplemental arterial supply of nutrients. We showed that overnight fasting induced net mobilization of phenylalanine from body proteins and such mobilization was not seen in hindlimb, which indicates that other body pools prevail. Furthermore, young neonates exhibit a sensitive capacity to reduce phenylalanine mobilization from body proteins according to the arterial concentration of phenylalanine in comparison to old neonates, although this was not seen for muscle hindlimb. We then conclude that in early post-natal life, high rates of growth are sustained by adaptive mechanisms which efficiently use amino acids for protein accretion in skeletal muscle. In consequence, muscle growth in neonates appears as a metabolic priority in healthy subjects.

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Modified mitochondrial functions by a natural uncoupler and a cell-synthesized uncoupling protein

M. Toyomizu & Y. Akiba

Animal Nutrition, Life Science, Graduate School of Agriculture Tohoku University, 1-1 Tsustsumidori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan.

Summary

Screening for both exogenous chemicals and cell-synthesized proteins to promote heat production can be an attainable goal. Reducing body fat is an attractive target for not only the development of antiobesity therapies, but also for the production of low-fat animal meat for humans to eat. In this study, we describe two works on: (i) a natural uncoupler, anacardic acid, which was isolated from cashew nutshell oil and (ii) a cell-synthesized uncoupling protein in chicken. Both have potential to promote heat production rather than ATP production. Lipid-soluble anacardic acid could have the potential to act as an uncoupler since it has alkyl side chains with various degrees of unsaturation, as well as carboxyl and hydroxy groups. Addition of anacardic acid to reaction mixtures containing mitochondria isolated from rat liver resulted in increased State 4 rates and RCR and decreased ADP/O ratio, which is strong evidence that anacardic acid has an uncoupling effect on oxidative phosphorylation. A 10-12 d period of acclimation of chickens to 5 °C exhibited larger palmitate-induced increments in respiration of subsarcolemmal skeletal muscle (*pectoralis superficialis*) mitochondria compared with controls. Further, the results of the Northern blots hybridized as probes using both *avUCP* (uncoupling protein) and *avANT* (adenine nucleotide translocator) cloned here revealed an increase in their mRNA levels in the skeletal muscle of cold-acclimated chickens. Such an increase is presumably accompanied by a decrease in $\Delta\psi$ in muscle mitochondria, thereby making it more sensitive to fatty acid-induced uncoupling. These findings are significant for understanding body composition and energy utilization in animals.

Keywords: uncoupler, uncoupling protein, mitochondrial function

Introduction

As controlling the uncoupling of oxidative phosphorylation in mitochondria could be the most effective way to alter heat production in animals, it may allow manipulation of body composition with body fat reduced. The uncoupling of oxidative phosphorylation can be induced by both chemical compounds and cell-synthesized proteins, that is, uncoupling protein (UCP) (Skulachev, 1998), increasing the membrane proton permeability and collapsing the transmembrane proton gradient, ΔpH , by shuttling protons across the membrane (Liberman & Skulachev, 1970). For the former, lipid-soluble 2,4-dinitrophenol (DNP) is representative of classical uncouplers (Hanstein, 1976). For the latter case, UCP1 is well-established to contribute to the heat production by mitochondrial uncoupling of oxidative phosphorylation in brown adipose tissue (BAT) in rodents during overfeeding (Trayhurn *et al.*, 1982) as well as during cold-acclimation (Nicholls & Locke, 1984). In this study, we describe two works on: (i) a natural uncoupler, anacardic acid, which was isolated from cashew nutshell oil and (ii) a cell-synthesized uncoupling protein in chicken.

Uncoupling effect of anacardic acid*

Anacardic acid

Anacardic acid (AA, 2-hydroxy-6-pentadecylbenzoic acid; Figure 1) is the product of hydrogenation of the naturally occurring unsaturated anacardic acids, which are the chief constituents (about 75%) of cashew nut shell liquid (Shobha & Ravindranath, 1991). These compounds exhibit antitumor (Kubo *et al.*, 1993) and antimicrobial (Muroi & Kubo, 1993) activities as well as having potent molluscicidal (Kubo *et al.*, 1986) effects. Thus, many of their significant biological actions may involve the fundamental processes of living cells and organisms.

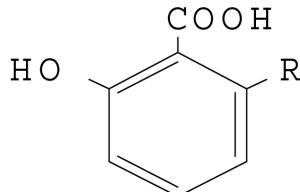


Figure 1. Structure of anacardic acid. R=C_{15:0}, 6-pentadecylsalicylic acid.

Uncoupling effect on oxidative phosphorylation

Anacardic acids are not only lipophilic or organic solvent-soluble, but are also salicylic acid derivatives with a linear long alkyl chain, suggesting that anacardic acids may act as protonophores, uncoupling electron transport from ATP synthesis in respiration-linked ATP production system. Therefore, we investigated the effects of AA on oxidative phosphorylation in rat liver mitochondria using succinate as a substrate. Figure 2 demonstrates results of the ADP/O ratio, RCR and state 4 following incubation with 2-16 µM of AA, in comparison with those doing incubation with 5-40 µM DNP. The concentrations required for a 50% decrease (IC50) of AA were 13 µM for ADP/O ratio and 5 µM for RCR. The decrease in RCR value was derived mainly from the increase in state 4; the half-maximal effective dose (ED50) of AA for the increase in state 4 was 13 µM when the rate in DNP-uncoupled state 4 was used as the maximum. The IC50 values of DNP for ADP/O ratio and RCR were 26 and 8 µM, respectively, while the ED50 value of DNP for state 4 was 16 µM. This study provides strong evidence that AA has an uncoupling effect on oxidative phosphorylation.

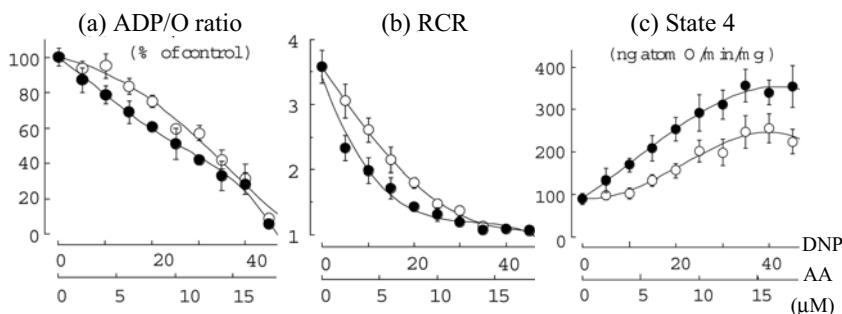


Figure 2. Effects of anacardic acid (○, AA) and DNP (●) on ADP/O ratio (a), RCR (b) and state 4 (c). All values are mean ± SD (n=6).

Reducing effect on body fat pads in rats

In order to clarify whether or not AA could be used advantageously as a special feed/food supplement to reduce fat deposition through the uncoupling action, the experiment was conducted to determine quantitatively the effect of dietary AA (0.1% w/w) supplementation on growth and fattening in rats fed normal and high carbohydrate (CHO) diets. As shown in Table 1, there were no significant differences in body weight gain, and feed consumption among the experimental groups.

For the total fat pad content, including inguinal and epididymal fat, significant interaction was shown between both treatments: dietary AA at 0.1 % w/w significantly decreased the total fat pad content only in rats fed the CHO diet, but not in rats fed the normal diet. This result reveals a unique function of AA in that, for dietary conditions enhancing body fat deposition, that is consumption of a diet high in carbohydrates, dietary AA has the potential to decrease body fat deposition. A possible mechanism for differences observed in AA-induced regulation of body fat pad content between rats fed the normal and CHO diets, will be based on uncoupling action of AA on the mitochondrial oxidative phosphorylation.

Table 1 Cumulative feed consumption, body weight gain and weight of various organs in rats fed normal diet or CHO diet (D) containing anacardic acid (AA)¹

| Anacardic acid in diet, % | Normal | | CHO | | Pooled SE | Statistical significance of: | | |
|------------------------------|--------------------|--------------------|-------------------|-------------------|--------------|---------------------------------|----|--------|
| | 0 | 0.1 | 0 | 0.1 | | D | AA | D x AA |
| Number of rats | 5 | 5 | 5 | 5 | | | | |
| Weight gain,g | 263.7 | 260.8 | 260.0 | 236.5 | 16.2 | NS | NS | NS |
| Intake,g | 514.5 | 514.8 | 515.5 | 487.6 | 15.2 | NS | NS | NS |
| Tissue weight,g | | | | | | | | |
| Muscle ² | 3.436 | 3.561 | 3.828 | 3.509 | 0.19 | NS | NS | NS |
| Heart | 1.184 | 1.185 | 1.204 | 1.031 | 0.06 | NS | NS | NS |
| Inguinal fat | 8.74 ^{ab} | 10.17 ^a | 9.09 ^a | 7.10 ^b | 0.61 | ** | NS | ** |
| Epid. Fat ³ | 3.94 ^{ab} | 3.95 ^{ab} | 4.82 ^a | 3.23 ^b | 0.76 | NS | * | * |

¹All groups were fed ad libitum. ²Pectoral muscle. ³Epididymal fat. NS, not significant.

P* < 0.10, *P* < 0.05. ^{a-d}Means with no common superscript differ significantly (*P* < 0.05).

Cell-synthesized uncoupling protein - avian UCP - **

Screening not only for exogenous chemicals but also for cell-synthesized proteins, such as UCP homologues expressed in the mitochondria of domestic animals, such as bovine (Stone *et al.*, 1999) and piglet (Damon *et al.*, 2000), to convert surplus energy to heat is a realistic research objective. In this section, we conducted two experiments (i) to examine the effects of cold acclimation (5 °C for 10-12 d) on the fatty acid-induced uncoupling of oxidative phosphorylation in skeletal muscle mitochondria and (ii) to clone the cDNA of UCP and ANT homologues from chicken skeletal muscle and study differences compared to controls in expression levels of their mRNAs in the skeletal muscle of cold-acclimated chickens.

Palmitate-induced uncoupling action of cold acclimation on subsarcolemmal mitochondria of chicken skeletal muscle

Palmitate-stimulated increments in respiration were significantly greater for subsarcolemmal mitochondria from the cold-acclimated group compared with the controls at the cumulative concentrations of palmitate above 11.25 μM , while no differences were observed between groups for intermyofibrillar mitochondria (not shown). Figure 3 shows that suppression of palmitate-induced uncoupling by carboxyatractylate was greater in the subsarcolemmal skeletal muscle mitochondria from cold-acclimated chickens than that for control birds, suggesting that ANT and other mitochondrial anion carriers in cold-acclimated chickens may dominate in mediating fatty acid-induced uncoupling of mitochondrial oxidative phosphorylation processes.

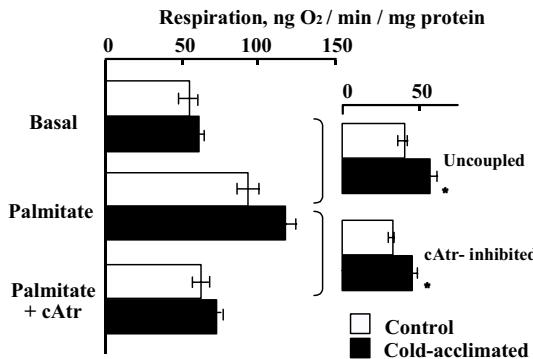


Figure 3. Effect of cold acclimation on palmitate-stimulated resting state respiration of the subsarcolemmal mitochondria of chicken skeletal muscle in the presence and absence of 5 μM carboxyatractylolide (cAtr; f.c.). Respiration was stimulated by the addition of palmitate to a final concentration of 3.75 μM . Values are means \pm SE ($n=4$).

* $P < 0.05$.

Expression of the mRNAs for avUCP and avANT in skeletal muscle of cold acclimated chicken

Figure 4a shows that both *avUCP* and *avANT* transcripts produced much stronger signals in skeletal muscle for the cold-acclimated group compared to the control group. The levels of *avUCP* and *avANT* mRNAs, which were quantified by scanning photodensitometry and normalized using corresponding GAPDH mRNA expression were increased 1.5 and 2.0-fold by cold acclimation, respectively (Figure 4b). The cold acclimation-induced increase in chicken UCP mRNA expression observed here is also in good agreement with data of Raimbault *et al.* (2001). These increases are presumably accompanied by a decrease in $\Delta\psi$ in muscle mitochondria, thereby making them more sensitive to fatty acid-induced uncoupling. Considering that body weight gain was lower in the cold-acclimated animals compared to controls (control chickens, 174 \pm 2 g; cold-acclimated chickens, 114 \pm 3 g; 34% decrease: $P < 0.001$) even though the cold-acclimated chickens ate more feed than the controls (540 \pm 20 g and 590 \pm 8 g; 9% increase: $P < 0.05$), it is obvious that cold acclimation induced more heat production. These results suggest that the simultaneous increments in levels of *avANT* and *avUCP* mRNA expression may be involved in the regulation of thermogenesis in skeletal muscle of cold-acclimated birds.

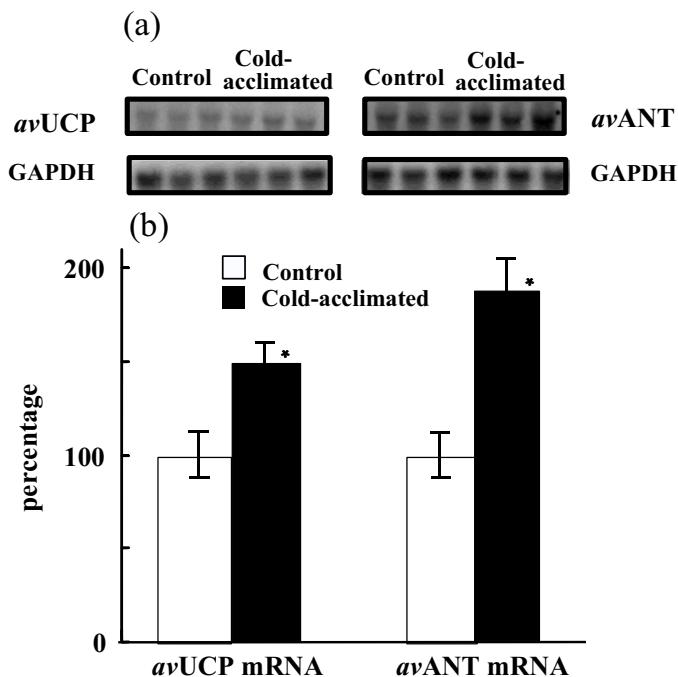


Figure 4. Effect of cold acclimation on the expression of the mRNAs for *avUCP* and *avANT* in chicken skeletal muscle. (a), Northern blot analyses of RNA (30 mg/lane) from pectoralis superficialis muscle were performed. (b), The results are shown as percentage of control group. Blots were hybridized with a GAPDH cDNA probe to correct for differences in the amounts of RNA. Values are means \pm SE ($n=5-6$). * $P<0.05$.

Conclusions

Anacardic acid can contribute to controlling body fat in animals through dissipation of $\Delta\psi$ and ΔpH . The cell synthesized uncoupling protein also shows decreasing feed efficiency probably through dissipation of $\Delta\psi$ and ΔpH . Modification of the oxidative phosphorylation state in mitochondria by using an uncoupler or by increasing mitochondrial anion carriers (MAC) expressions can regulate not only the thermogenesis capacity but also metabolism and body composition in animals.

Therefore, further screening for dietary exogenous chemicals from natural product to assist the uncoupling of oxidative phosphorylation and clarifying the mechanism of regulations of chicken MAC expressions and their activations may provide new insights into applications to control body composition and growth in animal, where we now know that controlling animal heat production via mitochondrial uncoupling could result in alterations to body composition.

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Nutrient availability and metabolic hormone relationship in dairy cows dry or in different stage of lactation

E. Trevisi, F. Piccioli-Cappelli, R. Lombardelli & G. Bertoni

Istituto di Zootecnica - Facoltà di Agraria, Via E. Parmense, 84, 29100 Piacenza, Italy

Summary

With the aim to evaluate the hormonal response and the metabolite changes to a short overload of glucose and/or amino acids, 6 average genetic merit dairy cows randomly received 4 intrajugular infusions for 6 hours of an overload of: glucose, amino acid mixture, both, pyrogen free water. The infusions were repeated in dry period and at 20-40, 70-90, 120-140 days in milk. Glucose was infused to maintain glycemia around 50% above starting values. The amount of amino acids (AA) infused was 3.5 times less. Blood metabolic-endocrine profile was checked hourly during infusions, beside milk yield and composition.

The load of glucose, alone or with AA, significantly increased blood levels of glucose and insulin, while GH, glucagon were reduced, as well as NEFA, β -OHB and AA. The load of AA alone increased only their and glucagon blood levels, while other metabolites and hormones were not affected in a significant extent. In a short period, only high blood glucose level seems to affect mammary synthesis: more lactose, equal protein and less fat.

Keywords: dairy cow, nutrients, hormones

Introduction

Nutrient availability (push hypothesis) is not *per sé* a guaranty of high milk yield, it has been therefore suggested a “pull hypothesis” (Knight *et al.*, 1994), according to which the mammary gland drives the extraction of nutrients (Bequette *et al.*, 1998). A clear response in favour to one or both the hypotheses is not available yet (Lescoat *et al.*, 1996). Still recently some studies have showed how the glucose availability can have positive or negative effect on milk synthesis (Rigout *et al.*, 2002), while Aikman *et al.* (2002) did suggest: “milk protein response to supplemental metabolizable protein depends on a number of factors, including stage of lactation, basal protein and energy status, and the amount and composition of the protein or amino acid supplied”. Thus, both hypothesis could be true; in fact, in a simulation analysis of substrate utilization in the mammary gland of cows, Cherepanov *et al.* (2000) did conclude that there is probably no one simple relationship linking milk yield, fat and protein contents, and the concentration of substrate in the blood; the active secretory tissue mass, the energy status of the secretory cell, and the rate of expression of regulatory proteins could be the factors of major importance. But many of these knowledge are lacking and “we have not been able to identify the right combination of factors that the mammary gland requires to take full advantage of its potential to synthesize and secrete milk” (Bequette *et al.*, 1998). However, some of these factors are known and described by Aikman *et al.* (2002); one of them is the stage of lactation that modifies the hormone levels (Bertoni *et al.*, 1995), the responsiveness of tissues to many hormones (Vernon, 1989), but it can also modify the feed intake and blood nutrient availability and then the hormone levels (Bertoni *et al.*, 1995).

In conclusion, it is extremely difficult to separate the effects of hormone and nutrient levels - as affected by lactation stage, feeding, mobilization of reserves, etc. - from those due to the different uptake by the mammary gland activity: i.e. nutrient changes and then hormone level variations. A first and partial attempt to overtake the difficulty could be a very short overload of glucose, or amino acids or their mixture, with the aim to evaluate mammary gland response and hormone

changes in relationship with metabolite levels, while body tissue intrinsic response could remain relatively unchanged.

Material and methods

Six average genetic merit dairy cows, housed in a tied stall barn with temperature kept between 19-26°C, humidity between 50-70% and 14h/d of light, were used. During the experimental period the cows received a diet based on: corn silage (6 kg in dry period and 16 kg in lactation), chopped alfalfa hay (2 kg in dry period and 3 kg in lactation), chopped grass hay (6-8 kg in dry period and 2-3 kg in lactation), 1.5-2 kg of concentrate during dry period and a different one in lactation distributed with the ratio of 1 kg every 3 kg of milk. About one month before calving and at 20-40, 70-90, 120-140 days in milk (DIM), the cows alternatively received for 6h (starting at 10:00 a.m.) an intrajugular infusion of: a) glucose (LG), infused to maintain blood glucose the 50% higher than basal value. In particular, according to some preliminary trials, after a bolus of 10g, glucose was infused at the rate of 0.075 and 0.15 g/kg b.w./h in dry period and lactation respectively, and the rate was increased of a 15% every 2 h; b) mix of amino acids (LAA) characterised by a profile similar to that of casein, infused at doses 3.5 times lower than glucose in LG (Riis et al., 1990), i.e.: a bolus of 5.0g and then an infusion rate of 0.021 and 0.043 g/kg b.w./h in dry period and lactation respectively, increased of a 15% every 2 h as in LG; c) glucose and mix of amino acids together (LGAA) as the previous; d) pyrogen-free water (CI).

Within treatments there was an interval of 3-4 days. Cows were milked at 6:00 a.m. and 4:00 p.m., the milk was weighted at each milking and a representative sample was collected for fat, total protein and lactose determination. During the experiment, blood samples were withdrawn from the opposite jugular vein at: -2, -1, 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 h from infusion start, for the determination of glucose, non-esterified fatty acids (NEFA), β -hydroxybutyrate (β -OHB), triglycerides (TG), urea, amino acidic nitrogen (AAN), creatinine, and the hormones: insulin, growth hormone (GH), glucagon, cortisol and the thyroid hormones T₄ and T₃. Statistical analysis was performed by using the PROC MIXED of SAS. Only data from sampling during infusions (from 0.5 to 6 hour) were analysed.

Results and discussion

During the 6 hours of infusion the total amount of glucose administered (bolus included), with LG and LGAA was in average 324, 559, 573, 599g as foreseen, while the total amount of AA administered with LAA and LGAA was slightly lower than the foreseen and namely 81, 140, 140, 145g in the 4 stages. Effects of treatments on milk yield and composition have been described by Piccioli-Cappelli *et al.* (2003). Briefly, average milk yield was 32.3, 33.4, 30.8 kg/d in the 3 lactation stages; the treatments affected milk yield and composition only in milking after infusions, and in particular the infusion of glucose (LG and LGAA) always increased milk yield (nearly 10%) and lactose output (nearly 12.3%). On the contrary protein output remained almost unchanged (with content reduced), while milk fat output and content have been markedly decreased. When AA were infused alone (LAA) only a slight increase in milk yield and protein output was observed. Nevertheless, when milk yield was markedly increased (glucose), both fat and protein contents have been reduced (dilution).

TG, urea and creatinine, as well as the levels of T₃, T₄ and cortisol have not been shown because remained almost unchanged (a not significant increase of urea has been observed in LAA, but only in late lactation). Main results on blood plasma metabolites and hormones are shown in Table 1. In all phases the plasma glucose was higher than the control level and relatively constant during glucose infusion (LG and LGAA); nevertheless, the 50% of increase was reached only in the 3rd stage of lactation and because glucose infused and milk yield were not so different, a different peripheral utilisation of glucose can be suggested in the 3 stages. So high level of plasma glucose

have significantly increased the level of insulin, but its amplitude resulted different depending on stage of lactation. In particular the increase was less pronounced and similar during dry period and first stage of lactation, and much more marked during last two periods of lactation (particularly in the second half of infusion), with the highest levels at 120-140 DIM, when glucose level was also maximum. Otherwise, the level of glucose did not affect GH level when administered in dry period, whereas GH was significantly (not always) decreased during lactation. Plasma glucagon levels were always and significantly reduced by glucose when infused alone; otherwise, when infused with AA, the levels of glucagon were almost unchanged. The metabolite changes after glucose infusion and partly consequence of the previous hormone variations, are particularly clear for NEFA (and similar for β -OHB, data not shown), significantly reduced but only in the case of quite high basal values (dry and fresh cows, $P<0.01$). The blood NAA changes were extremely small, but a significant reduction was sometime observed.

Table 1. Means of some plasma metabolites and hormones during 6 h of infusion of placebo (CI), a load of glucose (LG) of amino acids (LAA) or their mix (LGAA) in 6 average genetic merit dairy cows during dry and 3 different periods of lactation.

| DIM | Infusions | | | | Contrast ¹ | | | | | | |
|-----------------------|-----------|-------|-------|-------|-----------------------|----|----|----|----|----|----|
| | CI | LG | LAA | LGAA | C1 | C2 | C3 | C4 | C5 | C6 | |
| Glucose mmol/l | dry | 3.97 | 5.38 | 3.97 | 5.31 | ** | NS | ** | ** | NS | ** |
| | 20-40 | 3.81 | 5.50 | 3.97 | 5.24 | ** | NS | ** | ** | NS | ** |
| | 70-90 | 3.97 | 5.73 | 4.02 | 5.59 | ** | NS | ** | ** | NS | ** |
| | 120-140 | 3.95 | 5.99 | 4.04 | 5.71 | ** | NS | ** | ** | NS | ** |
| NEFA mmol/l | dry | 0.078 | 0.032 | 0.068 | 0.029 | ** | NS | ** | * | NS | * |
| | 20-40 | 0.144 | 0.055 | 0.132 | 0.036 | ** | NS | ** | ** | NS | ** |
| | 70-90 | 0.047 | 0.026 | 0.055 | 0.032 | NS | NS | NS | NS | NS | NS |
| | 120-140 | 0.027 | 0.029 | 0.022 | 0.024 | NS | NS | NS | NS | NS | NS |
| AAN mg/dl | dry | 29.9 | 26.2 | 35.2 | 30.4 | * | ** | NS | ** | ** | ** |
| | 20-40 | 35.4 | 32.7 | 42.2 | 38.5 | NS | ** | * | ** | ** | * |
| | 70-90 | 35.6 | 35.4 | 42.2 | 36.5 | NS | ** | NS | ** | NS | ** |
| | 120-140 | 37.6 | 31.1 | 40.6 | 37.8 | ** | NS | NS | ** | ** | NS |
| Insulin μ U/ml | dry | 8.2 | 18.9 | 8.5 | 20.2 | ** | NS | ** | ** | NS | ** |
| | 20-40 | 7.6 | 19.8 | 8.9 | 18.5 | ** | NS | ** | ** | NS | ** |
| | 70-90 | 9.4 | 27.6 | 10.0 | 27.0 | ** | NS | ** | ** | NS | ** |
| | 120-140 | 10.3 | 32.4 | 11.8 | 32.2 | ** | NS | ** | ** | NS | ** |
| Glucagon pg/ml | dry | 62.7 | 44.0 | 67.6 | 54.2 | * | NS | NS | ** | NS | NS |
| | 20-40 | 69.6 | 52.5 | 71.7 | 60 | * | NS | NS | * | NS | NS |
| | 70-90 | 84.1 | 62.8 | 97.3 | 70.4 | ** | NS | NS | ** | NS | ** |
| | 120-140 | 91.6 | 63.2 | 102.0 | 79.7 | ** | NS | NS | ** | * | ** |
| GH ng/ml | dry | 2.80 | 2.35 | 2.90 | 2.33 | NS | NS | NS | NS | NS | NS |
| | 20-40 | 3.84 | 2.96 | 3.15 | 2.66 | * | NS | ** | NS | NS | NS |
| | 70-90 | 2.98 | 1.90 | 2.61 | 1.95 | * | NS | * | NS | NS | NS |
| | 120-140 | 2.41 | 1.53 | 2.41 | 1.73 | * | NS | NS | * | NS | NS |

¹Contrast: C1 = CI vs LG; C2 = CI vs LAA; C3 = CI vs LGAA; C4 = LG vs LAA; C5 = LG vs LGAA; C6 = LAA vs LGAA. NS= not significant; * = $P<0.05$; ** = $P<0.01$.

The infusion of AA (LAA) was able to increase AAN level and in a significant way particularly vs LG, because it tended to decrease AAN level. However, LAA was not able to modify any hormone (except an increase of glucagon but never significantly) or energy metabolite (neither glucose or NEFA), maybe because the infusion rate was lower than expected and the blood AAN increase was too small.

Conclusions

Of the infused nutrients, only glucose was able to strongly increase its blood level; maybe for this reason, only glucose infusion was able to modify milk yield and composition, as well as some plasma metabolites and hormones. Nevertheless, milk synthesis changes (more lactose, unchanged proteins and less fat) seem mainly justified by glucose level *per sé*, i.e. any effect disappears in the following milking (16 h later), while the pattern of changes was similar in the 3 lactation stages despite insulin, glucagon, GH and NEFA levels were different.

Therefore the mammary gland tissue seems always sensible to stimulation effect of quite high glucose availability, but some enzyme could limit it (Rigout *et al.*, 2002), while the partition rate of nutrients can be modified by hormone changes, but only in the longer period.

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The use of nutritional models as a tool in basic research

J. van Milgen¹, F. Gondret¹ & D. Renaudeau²

¹ Institut National de la Recherche Agronomique, Unité Mixte de Recherches sur le Veau et le Porc, Domaine de la Prise, 35590 St-Gilles, France

² Institut National de la Recherche Agronomique, Unité de Recherches Zootechniques, Domaine Duclos, 97170 Petit-Bourg, Guadeloupe, France

Summary

The purpose of this study is to show how relatively simple models of nutrient metabolism can be used in basic research. A framework (in spreadsheet form) of a generic model of stoichiometry was used, which allowed quantification of nutritional balances. The model was used to test the consistency of an experimental data set, which related nutrient extraction from the blood to nutrient output in sow milk. The observed nutrient balance corresponded reasonably well with the calculated theoretical balance. A margin of error of 10% in estimated milk production or blood flow was sufficient to ensure correspondence between observed and stoichiometric balances. The framework was also represented as a generic compartmental system of cellular metabolism in order to facilitate numerical calculations and test biochemical scenarios. It is suggested that NADPH synthesis by isocitrate dehydrogenase may be functionally related to glutamine synthesis in adipocytes.

Keywords: modelling, nutrient metabolism, biochemistry

Introduction

Modeling has become an increasingly popular tool in nutritional research. Relatively advanced models have been developed based on the transformation of digested nutrients to end-products. These models typically include mechanisms of nutrient regulation, allowing simulation and prediction of different metabolic conditions. Full understanding of these models can be quite a challenge both for novice and experienced modelers due to the numerous and sometimes complex systems of equations that are involved. The objective of this paper is to illustrate that relatively simple models can also be used to analyze experimental data thereby providing a useful tool in basic, analytical research.

Checking the consistency of organ balance data

The basis of this paper is a model describing the stoichiometry of nutrient transformation in mammals (van Milgen, 2002). In short, this model describes the major catabolic and anabolic pathways as a function of nutrients and metabolic co-factors (called ‘pivots’). Six carbon-chain pivots (glucose, pyruvate, acetyl-CoA, oxaloacetate, α -ketoglutarate, and serine) and eight metabolic co-factors (ATP, mitochondrial NADH, cytoplasmic NADH, NADPH, FADH₂, and NH₃) were used, describing a total 54 reactions. The model can be represented as a spreadsheet with 14 columns (pivots) and 54 rows (reactions). The model can be easily expanded to include additional pivots or reaction equations. For example, in its initial form, glycolysis was represented as the reaction glucose → pyruvate. Consequently, glycogen metabolism may be difficult to accommodate in the model structure. This may be resolved by including glucose 6-phosphate (G6P) as a pivot in the model. For similar reasons, glyceraldehyde 3-phosphate (G3P) was included to simplify representing the anabolic origin (and catabolic fate) of glycerol. The

transformation of glucose to pyruvate (one row in the original model) will now be represented by three reactions (glucose → G6P, G6P → G3P, and G3P → pyruvate, each associated with appropriate stoichiometry). Also lactose synthesis (i.e., the equivalent of 1 glucose, 1 G6P and 1 ATP) and lactate metabolism (i.e., the equivalent of 1 pyruvate and 1 cytoplasmic NADH) were included in the model.

The purpose of this study is to use the above-mentioned model to check the consistency of observed experimental data. For this purpose, data concerning the milk production in sows were used (Renaudeau et al., 2003; only data for sows kept at 20°C were used). The original published data concern arterio-venous nutrient differences and blood flow to the mammary gland, (estimated) milk production, and milk composition. Fluxes are expressed as molar quantities on a per-day basis. With the exception of the individual non-essential amino acids, nutrient balance data can be obtained from the original publication. In short, 6.88 mol/d of glucose is extracted from the blood and 1.91 mol/d of lactose is exported in the milk. Although only 0.60 mol/d of free fatty acid is taken up by the mammary gland, 1.38 mol/d of fatty acid is required for milk fat synthesis. For most essential amino acids, the quantities found in the milk were of similar magnitude as those taken up from the blood. However, large differences existed for non-essential amino acids. For example, only 37% of serine extracted from the blood was excreted in the milk, whereas aspartate excretion in the milk was 14-fold greater than that taken up from the blood. The overall observed nutrient balance can be expressed as a model pivot balance (Table 1, input balance). Amino acids taken up from the blood were supposed to be in free form whereas those excreted in the milk were proteins (peptide synthesis was assumed to require 5 ATP). The negative values for oxaloacetate and α -ketoglutarate are due to the net synthesis by the mammary gland of aspartate (or asparagine) and glutamate (or glutamine), respectively. Negative values for acetylCoA and NADPH are due to fatty acid synthesis, whereas the negative value for G6P is due to lactose synthesis. Based on this pivot balance, a negative balance for certain nutrients (e.g., NADPH, G6P) may be supplied by excess of other nutrients. Table 1 (intermediate 1) shows the intermediate balance after glucose is used to cover the requirements for G6P, G3P, and NADPH via the pentose phosphate pathway; the remaining glucose is then converted to pyruvate. Excess serine is also converted to pyruvate. Intermediate 2 is based on the same principle, but after the negative balances for oxaloacetate and α -ketoglutarate are covered by pyruvate; all remaining pyruvate is converted to acetylCoA. The final balance is obtained by transporting the cytoplasmic NADH to the mitochondrion and subsequent oxidation of NADH and FADH₂. It shows that (the equivalent of) 2.1 mol/d acetylCoA and 39 mol/d ATP were not accounted for. The observed respiratory quotient of the mammary gland was 0.99 whereas the calculated RQ was 3.95. There are several reasons for these discrepancies. First, not all nutrients were analyzed in the plasma and thus accounted for in the model. More importantly, the balance data depend greatly on the estimated blood flow and milk production. Reducing the estimated milk production by 9.8% or increasing the measured blood flow by 10.7% would balance all pivots (Table 1). Applying one of these corrections would also virtually eliminate the observed numerical synthesis rates of some essential amino acids. In summary, with a margin of error of 10% for either the output (milk production) or input (blood flow), the observed nutrient balance data of Renaudeau *et al.* (2003) correspond to theoretical stoichiometric model calculations. Nevertheless, the balance data do not account for nutrient utilization by the mammary gland itself, which would further increase the apparent nutrient deficiency. Although the model provides an overall balance at the organ level, it does not identify the metabolic origin of synthesized nutrients.

Construction of a dynamic model

The model described above can be used to quantify nutrient balances at the cell, organ or animal level. In its present form, the model does not include concepts of regulation but rather evaluates, *a posteriori*, the overall nutrient flows that have to occur in order to obtain a given situation. The

Table 1. Nutrient balance of the sow mammary gland (mol/d; data from Renaudeau et al., 2003).

| | ATP | NADHc | NADHm | FADH ₂ | NADPH | CO ₂ | NH ₃ | OAA ¹ | α KG ¹ | PYR ¹ | ACA ¹ | GLC ¹ | G6P ¹ | G3P ¹ | SER ¹ | |
|--------------------------------|--------|-------|-------|-------------------|-------|-----------------|-----------------|------------------|--------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------|
| Input balance | -47.14 | -7.58 | -0.52 | 0.16 | -5.46 | 0.04 | -0.78 | -0.77 | -0.24 | -0.32 | 0.30 | -6.89 | 4.98 | -1.91 | -0.38 | 0.46 |
| Intermediate 1 | -45.02 | -2.73 | -0.52 | 0.16 | 0.00 | 2.77 | -0.78 | -0.30 | -0.24 | -0.32 | 5.62 | -6.89 | 0.00 | 0.00 | 0.00 | 0.00 |
| Intermediate 2 | -45.58 | -2.73 | 4.86 | 0.16 | 0.00 | 7.60 | -0.78 | -0.30 | 0.00 | 0.00 | 0.00 | -2.14 | 0.00 | 0.00 | 0.00 | 0.00 |
| Final balance | -38.86 | 0.00 | 0.00 | 0.00 | 0.00 | 7.60 | -1.92 | -0.30 | 0.00 | 0.00 | 0.00 | -2.14 | 0.00 | 0.00 | 0.00 | 0.00 |
| Blood flow correction (+10.7%) | | | | | | | | | | | | | | | | |
| Input balance | -43.36 | -6.08 | -0.03 | 0.30 | -4.49 | 0.09 | -0.67 | -0.47 | -0.17 | -0.24 | 0.36 | -5.44 | 5.72 | -1.91 | -0.34 | 0.56 |
| Final balance | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 11.89 | -7.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

¹ OAA, oxaloacetate; α KG, α -ketoglutarate; PYR, pyruvate; ACA, acetylCoA; GLC, glucose; G6P, glucose-6-phosphate; G3P, glyceraldehyde-3-phosphate; SER, serine.

objective of the remainder of this paper is to describe procedures that were used to incorporate the stoichiometry outlined above in a generic, compartmental model of cellular metabolism. It is not the purpose to describe in detail all aspects of metabolism. It may serve as a basis from which other, specialized model may evolve.

Pivots were all considered as compartments (state variables). Because the model may eventually serve to model nutrient fluxes between organs, a distinction was made between nutrients that can leave or enter the cell (e.g., lactate, individual amino acids, free fatty acids) and those than cannot (e.g., G6P). Additional pivots were included because of their implication in the regulation of intermediary metabolism (i.e., phosphoenolpyruvate, cytoplasmic and mitochondrial citrate, cytoplasmic acetylCoA, free fatty acid, and acylCoA). In its initial form, all compartments were considered so-called zero pools (Baldwin, 1995). The mathematical purpose of these pools is to maintain a zero content either by transferring all its content to the next pool (e.g., G6P → G3P) or pulling it from a preceding pool (e.g., a negative pool of NADPH will pull sufficient G6P to the pentose phosphate pathway in order to become zero again). Because of the generic structure of the model, the cell possesses the ability of opposing pathways (e.g., glycolysis and gluconeogenesis). In reality, these opposing pathways will seldom function simultaneously because of activation and deactivation mechanisms of the enzymes involved. In the model, enzymes functions were included as switches (having a value of either 0 or 1), which are set by the user. All compartments were initially empty and fluxes were described as first-order differential equations using the switches as fractional rate constants. In a later stage of model development, the switches may also take intermediate values and be piloted by cellular conditions (e.g., ATP/ADP ratio) rather than set by the user. The metabolic fate of a nutrient may be calculated by forcing a continuous entry of the nutrient into the system, setting the appropriate enzyme switches and awaiting steady-state conditions. Because all compartments are initially empty and most compartments transfer their contents to the next compartment, 40-50 integration steps are required before steady state conditions are attained. For example, when fatty acid is synthesized from glucose, the carbon-chain of glucose passes through nine compartments (glucose, G6P, G3P, pyruvate, mitochondrial acetylCoA, mitochondrial citrate, cytoplasmic citrate, cytoplasmic acetylCoA and the fatty acid). At each integration step size, 1 mol of glucose enters the systems and the first fatty acid will appear after nine integration steps. It is only at the last integration step (i.e., acetylCoA → fatty acid) that a requirement for NADPH is generated. Synthesis of NADPH by the pentose phosphate cycle then deviates G6P away from glycolysis (fatty acid synthesis). With time progressing, an equilibrium will be progressively attained between glycolysis and utilization of G6P in the pentose phosphate pathway.

Synthesis of NADPH can be potentially limiting synthesis of fatty acids. Apart from the pentose phosphate pathway, several other pathways exist that may provide NADPH. In the cytoplasm, isocitrate can be decarboxylated to α -ketoglutarate by isocitrate dehydrogenase yielding NADPH. In ruminants, this is a major pathway of NADPH synthesis. In the present model, this process can be seen as the conversion of citrate (cytoplasm) to α -ketoglutarate. Assuming that acetylCoA is the starting point, synthesis of NADPH by isocitrate dehydrogenase requires a full turn of the tricarboxylic acid cycle (TCA cycle) in order to regenerate oxaloacetate, where the synthesis a 1 (mitochondrial) NADH is replaced by synthesis of 1 NADPH. This implies that approximately 25% of the energy of acetylCoA is retained in NADPH and the remainder in FADH₂, mitochondrial NADH and ATP. Although this may be a feasible scenario for a cell with high energy (ATP) expenditure, for adipocytes this appears less likely. An alternative scenario is one in which the 75% of the remaining energy of acetylCoA is not used by the adipocyte itself, but exported to other tissues. If α -ketoglutarate were converted to glutamate or glutamine, excess energy from acetylCoA could become available for transport to other organs. Nevertheless, re-entry of a TCA-cycle intermediate would be required in order to avoid depletion of TCA-cycle intermediates. There are indeed indications that glutamine and glutamate are involved in adipocyte metabolism. Kowalski *et al.* (1997) observed a net export of glutamine in adipocytes, whereas

aspartate (a precursor for oxaloacetate) was taken up. This is of course not evidence of glutamine participating in the transport of excess energy from acetylCoA to other organs. Nevertheless, at the whole animal level, the contribution of adipose tissue to glutamine synthesis appears significant (Frayn *et al.*, 1991). This systemic approach illustrates that energy and protein metabolism are potentially intertwined. Non-essential amino acids may play an important role in the inter-organ transport of carbon-chain energy in different oxidation states.

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Investigations on energy and protein metabolism in fattening steers using different experimental techniques

I. Voicu, Doina Grossu, R. Burlacu, Dorica Voicu, A.G. Marinescu & Gh. Burlacu

Institute of Biology and Animal Nutrition, Balotesti, Romania

Summary

The paper shows a comparison between the experimental results of the energy and protein metabolism in fattening steers, with the results obtained with the mathematical model of energy and protein metabolism simulation in ruminant animals developed in our institute. A good agreement was observed between the two sets of data, as shown by the regression equations both for the gross and net gain, and for their components.

Keywords: steers, metabolism, mathematical model

Introduction

The aim of this study is to investigate diet efficiency in Romanian Spotted steers from 150 to 450 kg body weight using the method of energy and protein balance measured by comparative slaughtering and respiratory exchanges.

We used several types of diets which to provide different ratios of concentrate feeds to bulk forages (40 to 60%), using forages commonly used in Romanian animal farms: corn and alfalfa under different forms of conservation. Finally, we tried to make use of the experimental data by comparing them with the experimental model developed in the Laboratory of Physiology of the Institute of Biology and Animal Nutrition by the collective Burlacu et al. (1996) materialized as TAURUS software.

We considered it necessary to resume this study knowing the quite different results yielded by different researchers such as Daenicke et al. (1982), Beckenbauer et al. (1984), Richter et al. (1984), Neergaard et al. (1985), Varga et al. (1985), Ochrimenko et al. (1985), Schwartz et al. (1985).

Material and methods

The experiment used (half brother) Romanian Spotted steers brought from the elite farms from Prejmer - Brașov County. Initially, each experimental group had 10 animals. Two animals were slaughtered and the remaining eight were assigned to two groups by experiment, the first with 4 animals with an initial weight of 150 kg and the second with four animals with the initial body weight of 300 kg, shortening thus the experimental period, the steers from the first group being fattened from 150 to 300 kg and the steers from the second group being fattened from 300 to 450 kg. The experimental period was 120 days in average, with the longest of 200 days and the shortest of 63 days.

The dietary bulk feeds were corn, alfalfa and triticale preserved under different forms, which formed 8 diet variants, as shown in Table 1:

Diet 1 - corn silage + enzyme preparation (*Aspergillus*)

Diet 2 - corn silage + urea %

Diet 3 - corn silage + ammonia (2.5 kg / 100 l water)

Diet 4 - corn silage + Biocons (lactic acid bacteria) 1 kg / t

Diet 5 - triticale silage

Diet 6 - alfalfa hay

Diet 7 - alfalfa haylage (50.3% DM)

Diet 8 - alfalfa silage (38.8% DM)

The dietary concentrate feeds were barley, corn, sunflower meal and peas, and ranged from 32.3% (diet 6) to 48.5% (diet 2) for the steers 150 - 300 kg and from 27% (diet 6) to 52% (diet 8) for the steers 300 - 450 kg, as shown in Table 3.

The diets were balanced as energy and protein content at the level of norms (Burlacu, 1996) for each category.

During the experiments, the animals were kept in a house with 8 stands fitted to record individually the feed intake and excreta (faeces and urine). The forages and excreta were analyzed chemically (Weende) and calorimetrically (adiabatic calorimeter). The caloric energy was assessed both by comparative slaughtering and by respiratory exchanges in metabolism chambers (24h x 2) during the balance periods. When the caloric energy (CE) was assessed by comparative slaughtering, two animals from the spare group were slaughtered in the beginning of the experiment and the entire experimental group (8 steers) was slaughtered in the end of the experiment in order to determine the retained energy.

Table 1. Diet structure.

| Forage | Steers 150 - 300 kg | | | | | | | | Steers 300 - 450 kg | | | | | | | |
|-------------------------|---------------------|------|------|------|------|------|------|------|---------------------|------|------|------|------|------|-----|-----|
| | Diets % DM | | | | | | | | Diets % DM | | | | | | | |
| Forage | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Corn silage, enzyme | 48,6 | | | | | | | | 59,2 | | | | | | | |
| Corn silage, urea | | 45,5 | | | | | | | | 55,3 | | | | | | |
| Corn silage, ammonia | | | 43,2 | | | | | | | 50,3 | | | | | | |
| Corn silage, Biocons | | | | 51,1 | | 32,4 | | | | 50,3 | | | | | | |
| Triticale silage | | | | | 39,3 | | | | | | 50,7 | 45 | | | | |
| Alfalfa hay | 4,6 | 5,2 | | | 12,4 | 34,3 | | 3,6 | | | 8,0 | 27 | | | | |
| Alfalfa haylage | | | | | | 57,5 | | | | | | 60,3 | | | | |
| Alfalfa silage | | | | | | | 45,5 | | | | | 46,6 | | | | |
| Barley | 16,5 | 25,6 | 22,3 | 15,7 | 36,4 | | | | 13,9 | 29,1 | 31,1 | 27,3 | 29,4 | | | |
| Corn | | | | | | 18,7 | 36,2 | 42,0 | | | | 7,0 | 38,7 | 52,4 | | |
| Peas | | | | | | 13,6 | 5,3 | 2,4 | | | | 20,0 | | | | |
| Sunflower meal | 29,3 | 22,7 | 33,5 | 32,2 | 10,9 | | | | 5,5 | 25,9 | 14,6 | 17,6 | 21,4 | 10,9 | | |
| Vit.-mineral premix | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| TOTAL % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Results and discussion

a) Tables 2 and 3 show the chemical composition of the bulk forages and concentrate feeds (g/kg DM).

Table 2.

| Specification | DM | OM | CP | CF | GE/MJ |
|-----------------------|-----|-----|-----|-----|-------|
| Corn silage + enzyme | 379 | 927 | 91 | 227 | 18.24 |
| Corn silage + urea | 372 | 946 | 100 | 230 | 18.43 |
| Corn silage + ammonia | 281 | 922 | 79 | 254 | 17.67 |
| Corn silage + Biocons | 254 | 920 | 67 | 251 | 17.65 |
| Triticale silage | 290 | 918 | 46 | 325 | 18.09 |
| Alfalfa hay | 851 | 898 | 171 | 330 | 18.47 |
| Alfalfa haylage | 503 | 904 | 208 | 276 | 18.37 |
| Alfalfa silage | 388 | 905 | 197 | 266 | 18.69 |

Table 3.

| Specification | DM | OM | CP | CF | GE/MJ |
|----------------|-----|-----|-----|-----|-------|
| Barley | 848 | 962 | 101 | 65 | 18.41 |
| Corn | 874 | 974 | 105 | 28 | 19.00 |
| Sunflower meal | 834 | 925 | 333 | 250 | 18.93 |
| Peas | 875 | 962 | 245 | 73 | 18.84 |

c) Animal performance (Figure 1 and 2).

The average daily gross gain for both steer categories was lower (850 g/steer/day and 906 g/steer/day) for the diets with triticale and with corn silage treated with urea (978 g and 1075 g respectively). These values are first of all the result of a poor bulk forage ingestibility, but also to the rather low proportion of dietary concentrate feeds.

Better steer performance was noticed for the groups treated with alfalfa haylage and silage as bulk forages and corn as concentrate feed: 1365 g and 1432 g respectively, 1403 g and 1400 g/steer/day, respectively.

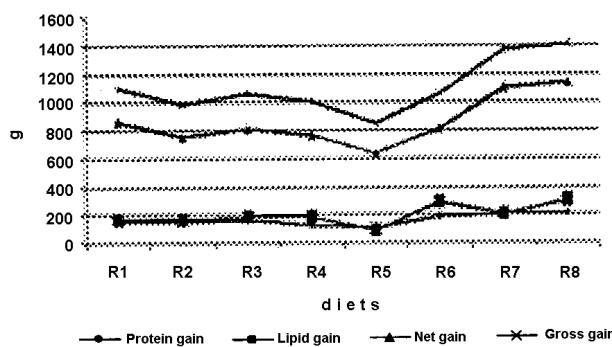


Figure 1. Steer performance (150-300 kg).

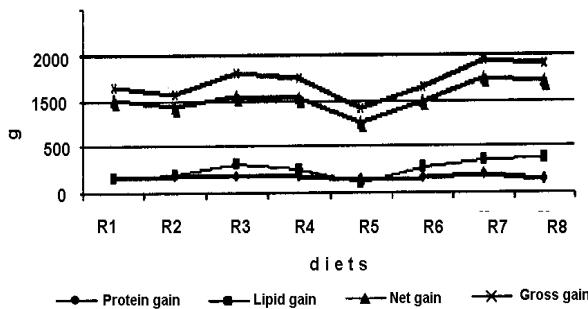


Figure 2. Steer performance (300-450 kg).

This time bulk forages ingestibility showed a higher value of satiety; the energy-protein balance (IDPN-IDPE) was achieved by mixing the nitrogen compounds rich alfalfa (as haylage or silage) with ear corn, with high energy level.

This performance is in agreement with the results of Daenicke et al. (1982), Beckenbauer et al. (1984), Stetter et al. (1988), Todorov et al. (1988) using diets with similar energy-protein levels

d) Nutrient digestibility (Fig.3 and 4) varied according to the type of diet as follows:

- organic matter digestibility ranged between 69% and 73% in the corn silage-based diets (1, 2, 3 and 4) and between 70% and 76% in the alfalfa-based diets. OM digestibility ranged between 68% and 66% in the diet based on triticale silage.
- protein digestibility in the corn silage-based diets was slightly in the 150 - 300 kg steers (68% and 70%) compared to the 300 - 450 kg steers (66% to 68%).

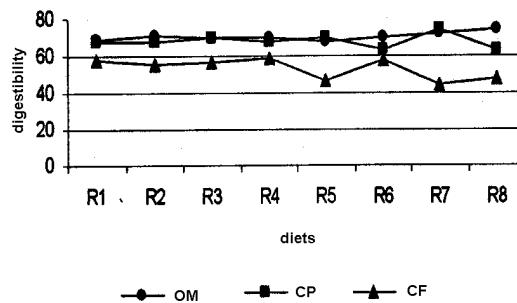


Figure 3. Nutrient digestibility (%) (150-300 kg).

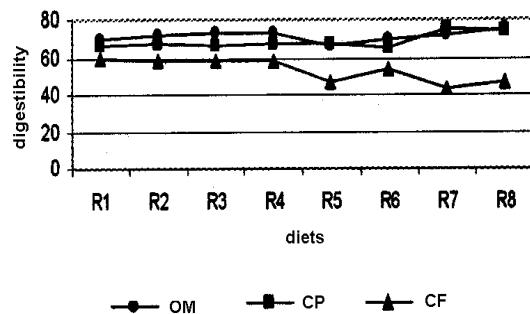


Figure 4. Nutrient digestibility (%) (300-450 kg).

- in the alfalfa-based diets (6, 7 and 8) these coefficients ranged between 63% and 75%.
- fibre digestibility was lower (44% and 48%) in the diets with alfalfa and triticale and higher (54% and 60%) in the diets with corn silage.

e) Feed conversion ratio in the first four (corn silage-based) diets ranged between 4.73 kg/kg and 5.22 kg/kg in the steers 150 - 300 kg and between 6.0 kg/kg and 6.49 kg/kg in the steers 300 - 450 kg. Higher values (8.11 kg/kg) were observed in the diet based on triticale silage, particularly for the steers 300 - 450 kg, whose performance was lower.

In diets 7 and 8, which used alfalfa haylage and silage, feed conversion ratio ranged between 3.85 and 4.1 kg/kg in the steers 150 - 300 kg and between 5.38 and 5.9 kg/kg in the steers 300 - 450 kg.

f) Energy balance of the experimental animals (Fig.5 and 6) show gross energy values ranging between 81,929 kJ and 110,950 kJ in the 150 - 300 kg steers and between 122,800 kJ and 156,006 kJ in the 300 - 450 kg steers. The ratio of the digestible energy to ingested energy ranged between 66% and 70% and between 64% and 72% in the first and second categories of steers, respectively. The metabolisable energy related to the ingested gross energy, which also expresses the dietary energy concentration ($q = ME/ GE$) ranged between 56% and 61%, respectively between 53% and 62%.

The caloric energy corresponding to the energy loss for maintenance and production, determined both by respiratory exchanges and by comparative slaughtering ranged between 39,761 kJ/steer/day and 50,791 steer/animal/day in the first category and between 61,991 kJ and 79,986 kJ/steer/day for the second weight category, which represents 49 - 46%, respectively 49 - 50% of the ingested gross energy.

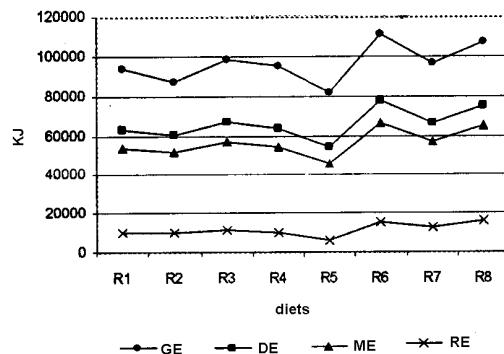


Figure 5. Energy balance (kJ/steer/day) (150-300 kg).

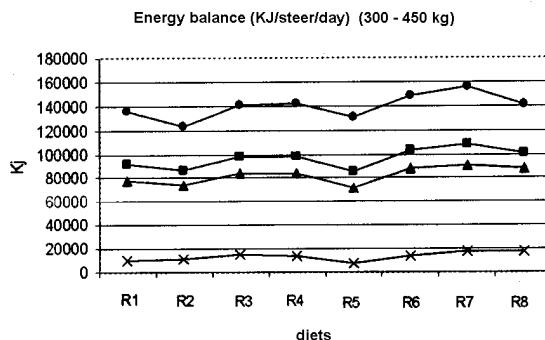


Figure 6. Energy balance (kJ/steer/day) (300-450 kg).

Comparing the values of the retained energy obtained by slaughtering or by the difference between the metabolisable energy and the caloric energy determined by respiratory exchanges, we noticed higher values with the latter technique, generally by 3 to 6% (Table 4).

The difference is due to the fact that when the respiratory chambers are used, the caloric energy may be underestimated, because the activity of the animal is lower, the energy losses are smaller, so the caloric energy has a lower value, which by difference compared to the metabolisable energy yields a higher value of the retained energy.

The problem of the discrepancy between the values of the caloric energy determined by comparative slaughtering and by respiratory exchanges was debated by many researchers, most of them supporting the lower values of the energy determined by respiratory exchanges (Larson, 1997; Johnson et al., 1997; Unswort et al., 1991; Steen et al., 1997).

Table 4. Comparison between the values of the retained energy determined by slaughtering and by respiratory exchanges (kJ/steer/day).

| 150 - 300 kg | | | | | 300 - 450 kg | | | | |
|--------------|-------------------|-------------------|-------------------|-------------------|--------------|-------------------|-------------------|-------------------|-------------------|
| ME | Caloric energy | | Retained energy | | ME | Caloric energy | | Retained energy | |
| | Comp. slaught. | Resp. exchange | Comp. slaught. | Resp. exchange | | Comp. slaught. | Resp. exchange | Comp. slaught. | Resp. exchange |
| 53726 | 43964 | 43574 | 9762 | 10152 | 78077 | 68171 | 67874 | 9906 | 10203 |
| 51451 | 41583 | 41003 | 9868 | 10448 | 73914 | 61991 | 61504 | 11923 | 12410 |
| 56772 | 44934 | 45211 | 11138 | 11561 | 84180 | 69605 | 68905 | 14575 | 15275 |
| 54297 | 44285 | 43884 | 10012 | 10413 | 84082 | 70056 | 69497 | 14026 | 14615 |
| 45790 | 40005 | 39716 | 5785 | 6074 | 71292 | 64116 | 63750 | 7176 | 7542 |
| 66265 | 50791 | 50296 | 15474 | 15969 | 87683 | 73739 | 79986 | 13944 | 14697 |
| 57169 | 44611 | 44084 | 12558 | 13085 | 90406 | 72664 | 72025 | 17742 | 18381 |
| 62222 | 48872 | 44929 | 16350 | 17298 | 87999 | 69645 | 70090 | 17354 | 17909 |

g) The retained energy as result of protein and lipid energy retained daily in the organisms of the animals, ranged in average between 5.785 kJ (105 g Pr + 86 g Lr) and 15.474 kJ (180 g Pr and 289 g Lr), which represented 7%, respectively 13.9% of the ingested gross energy in the 150 - 300 kg steers and between 7.176 kJ (140 g Pr + 101 g Lr) and 17.742 kJ (187 gPr + 343 g Lr), which represented 5.38%, respectively 11.4% of the ingested gross energy in the 300 - 450 kg steers.

Considering protein utilization by nitrogen balance, we observed daily amounts of ingested nitrogen between 85.12 g/steer/day and 154 g/steer/day in the 150 - 300 kg steers and between 126.88 g and 208.16 g in the steers 300 - 4540 kg.

Nitrogen digestibility was between 63% and 75%, respectively between 66% and 75% in the first, respectively the second weight category, while the retained nitrogen was between 14% and 22.3% and between 13% and 19%, respectively, in the two weight categories.

The papers of Beranger and Robelin (1978), Geay et all. (1987), Chillard et al. (188), Mc Cracken (1991) and Byers et al. (1997) were used in interpreting these results.

h) Comparison between the experimental performance and those calculated with the mathematic model

Using the resulting set of experimental results on the quantitative and qualitative (chemical composition) intake of food we input them into the software simulating the energy and protein metabolism in animals and compared the two sets of results.

Plotting these data into a graphic with the calculated data on (x) axis and the experimental data on (y) axis, their intersection yielded several points (related to the number of data) which, joined by a regression line, described the equation of type $y = ax$.

The gross gain for 150 - 300 kg steers measured experimentally (y) corresponds 97.7% with the calculated one, with the standard error of 0.033, while the net gain corresponded in a proportion of 94.1%, with the standard error of 0.036.

Regarding the retained protein, the relation between the calculated and the experimental data was given by equation $y = 0.934x$, with the standard error of 0.027; regarding the retained lipids, the relation was given by equation $y = 0.099x$ with the standard error of 0.036, both equations expressing a good agreement between the calculated and experimental data.

If at the first weight category of steers the experimental values were generally lower than the calculated ones, the shape of the equation being $y < 1.0$, at the second weight category, the equations show values $y > 1.0$, which shows, generally, slightly higher experimental values than calculated values, but quite close, however.

At the weight category 300 - 450 kg, the equation for the gross gain was $y = 1.049x$ with the standard error 0.02, for the net gain, $y = 1.025x$ with the standard error 0.023, for the protein, $y = 1.049x$ with the standard error 0.025, for lipids, $y = 1.04x$ with the standard error 0.035. All equations showed an agreement ranging between 96% and 98% between the calculated and experimental data.

The correlation between the mathematical model and the experimental one are also supported by the papers of Burlacu et al. (1993), who considered experiments presented in the literature: Daenicke et al. (1982), Beckenbauer et al. (1984), Richter et al. (1984), Neergaard et al. (1985), Varga et al. (1985), Ochrimenko et al. (1985), Schwartz et al. (1985), Burlacu et al. (1990), which show a ratio between the calculated and experimental gain given by equation $y = 0.9762x$, with the standard error of 0.094.

Conclusions

Analysing the energy balance represented by the retained energy ($Pr+Lr$) obtained either by the difference between the metabolisable energy (ME) and caloric energy (CE, respiratory exchanges), or by comparative slaughtering, we noticed values higher by 3% to 6% when the values were calculated by the difference between ME and CE.

The gross efficiency of these diets RE/ME ranged between 18% and 19.6% for the 150 - 300 kg steers and between 12.7% and 17.3% for the 300 - 450 kg steers, for the corn silage-based diets and between 22% and 25%, respectively between 15.9% and 19.7% for the alfalfa-based diets (hay, haylage and silage), which shows a higher efficiency of energy retention in the first weight category, with higher values in the alfalfa diets than in the corn silage diets; the respective values were 12% and 10%, respectively, lower for the triticale diets.

Comparing the experimental data with the data supplied by the mathematical model we observed a good agreement between the two, as shown by the regression equations both for the gross and net gain, and for their components ($Pr+Lr$).

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Can bypass starch reduce the need for essential amino acids in dairy cows?

M.R. Weisbjerg & C.F. Børsting

Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark

Summary

A substantial part of absorbed amino acids is normally used for gluconeogenesis. This raises the question whether increased supply of other glycogenic substrates can reduce the requirements for amino acids, and especially limiting essential amino acids. The hypothesis that bypass starch would spare amino acids for gluconeogenesis was tested in a production trial with 64 dairy cows, in a 2x2x2 factorial design with two forage types (grass silage vs. whole crop pea silage), two starch types (wheat vs. maize) and with or without supplementation of protected methionine. The forage type had a pronounced effect on milk yield (grass silage 34.1 vs. pea silage 30.0 kg/d). Starch type (grain) did not significantly affect milk yield and composition. Methionine supplementation increased milk protein concentration significantly independent of combination of forage and grain. However, due to a negative effect of methionine supplementation on milk yield, methionine supplementation also decreased energy corrected milk (ECM) production when wheat was the starch source. When maize was the starch source, methionine supplementation had an insignificant positive effect on ECM production.

Keywords: bypass starch, protected methionine, dairy cows, milk protein yield

Introduction

Amino acid protein is a major nutrient for ruminants, both for protein synthesis and for gluconeogenesis. In lactating cows 2-40% of the glucose can originate from amino acids (Danfaer et al., 1995). Therefore, supply of other glucogenic substrates might spare limiting amino acids, and thereby reduce the amino acid deamination and the urinary excretion of nitrogen.

Many starch sources are quickly degraded in the rumen, however, some starch sources are slowly degraded and therefore result in some bypass of starch to the small intestine. Starch digestion in the small intestine will result in glucose absorption, whereas starch fermentation in the rumen results in production of microbial matter and short chain fatty acids, which is less glucogenic compared to direct glucose absorption.

Based on this, we hypothesized that supply with starch with a low rumen degradability would reduce the need for essential amino acids. This was tested in an experiment with dairy cows where two starch sources (wheat vs. maize) were compared in a factorial design which also included two levels of methionine (no supplementation or supplementation with protected methionine) and two different forages (grass silage vs. pea silage).

Material and methods

Sixty-four Danish Holstein dairy cows were included in a 2x2x2 factorial design. Factors were two types of forage (grass silage vs. whole crop pea silage), two types of grain supplement (wheat vs. maize) and two levels of supplementation with protected methionine (0 vs. 1 g Smartamine M/kg dry matter (DM)).

Cows were blocked according to parity (half were primiparous) and expected calving date. For the first 3 weeks after calving, all cows were fed the same ration, for the next 12 weeks the cows were fed the eight experimental rations. Cows were fed and milked twice daily. Feed intake and milk yield were registered on individual cow basis. Feed offer and orts were registered daily, and daily milk yield and composition were recorded once per week. Milk was analysed for protein, fat and lactose concentration by Milkoscan.

Pea whole crop was cut when seeds were still undeveloped on the 3rd July (immediately after the end of flowering) with a starch concentration of 5% of DM. Perennial rye grass was cut as first cut on the 13th May. Both crops were previlted for 2 days. Pea silage organic matter digestibility measured in sheep digestibility trials was 75% which was considerably lower than grass silage with 83% digestibility.

Ration composition (% of DM) was as follows. Grass silage rations consisted of 63.5% grass silage, 36.0% concentrate and 0.5% lime. Pea silage rations consisted of 66.2% pea silage and 33.8% concentrate. Concentrate consisted of 72.3% grain (wheat or maize), 21.3% soybean meal products, 2.7% lipitec (palm fatty acid calcium soaps), and 3.7% mineral mixture. For wheat diets the soybean products were only Soypass (protected soybean meal), whereas for maize diets half was Soypass, and half unprotected soybean meal to equilibrate supply with rumen undegraded feed protein. Smartamine M was mixed in a premixture used directly in the total mixed rations (TMR) to avoid physical damage of protection. Forages, concentrate mixtures and methionine premixture were mixed in TMR, which were fed ad libitum.

Mean feed intake and milk yield for week 2 and 3 were used as covariates in the statistical analysis of feed intake and milk yield, respectively. For milk composition, however, only the results of week three were used as covariates, as composition changed drastically from week 2 to week 3. As fixed effects, block was included in the model together with type of forage, type of grain supplement and methionine supplementation and the interactions between these three experimental factors. Statistical analyses were performed using GLM procedure in SAS (SAS, 2001).

Results

The effect of forage on intake and yield has been discussed by Børsting & Weisbjerg (2002), and will not be discussed in detail here, as this paper will focus on the effect of grain type and methionine supplementation.

Starch degradation in the rumen was tested in nylon bag studies, and showed as expected a much lower rate of starch degradation for maize compared to wheat. Already after 2 h rumen incubation, 98% of wheat starch had disappeared from nylon bags, whereas for maize only 58% had disappeared after 8 h, and 90% after 24h. Protein degradation in nylon bags showed less difference between wheat and maize, and less difference between soybean meal and Soypass than expected; therefore protein degradability and protein value of the two concentrates were not similar as planned. Rumen degradation of individual amino acids showed that methionine degradation generally followed the trend for the other amino acids.

Results for feed intake, milk production and milk composition are shown in Table 1. P-values for the interactions forage x methionine and forage x grain x methionine are not shown in Table 1, as no significant effects were found ($P > 0.3$).

DM intake was considerably lower (1.4 kg DM/day) for pea silage than for grass silage, whereas none of the other treatments affected the DM intake. Inclusion of parity in the model showed that older cows had 2 kg higher daily DM intake than primiparous cows, however, parity did not interact with any treatment (results not shown).

Table 1. Daily feed intake, milk production and milk composition.

| Treatments | | | Feed intake | | | | Milk production | | Milk composition | | |
|-------------------|-----|-----|-------------|--------|------------|-----------|-----------------|-------------|------------------|----------------|----------------|
| F | G | M | DM (kg) | FU | AAT (g) | % AAT-MET | Milk (kg) | ECM (kg) | Fat (%) | Protein (%) | Lactose (%) |
| GS | WH | - M | 18.7 | 19.5 | 1768 | 1.96 | 34.7 | 34.1 | 3.99 | 3.06 | 4.97 |
| GS | WH | +M | 17.9 | 18.6 | 1688 | 2.62 | 32.1 | 31.6 | 3.85 | 3.14 | 4.95 |
| GS | MA | - M | 19.0 | 20.0 | 1910 | 1.93 | 34.6 | 33.6 | 3.79 | 3.06 | 5.05 |
| GS | MA | +M | 18.6 | 19.5 | 1865 | 2.56 | 34.5 | 33.8 | 3.83 | 3.10 | 5.04 |
| PS | WH | - M | 17.1 | 16.3 | 1600 | 1.84 | 31.3 | 31.2 | 4.06 | 3.07 | 5.04 |
| PS | WH | +M | 17.4 | 16.6 | 1630 | 2.47 | 29.2 | 30.3 | 4.27 | 3.12 | 5.02 |
| PS | MA | - M | 17.1 | 16.5 | 1701 | 1.82 | 30.1 | 29.7 | 3.94 | 2.99 | 5.03 |
| PS | MA | +M | 17.0 | 16.4 | 1695 | 2.42 | 30.4 | 29.9 | 4.01 | 3.11 | 4.94 |
| <i>Statistics</i> | | | | | | | | | | | |
| SEM | | | 0.6 | 0.6 | 55 | - | 0.7 | 0.7 | 0.13 | 0.05 | 0.04 |
| P | F | | 0.001 | 0.0001 | 0.0003 | - | 0.0001 | 0.0001 | 0.02 | 0.6 | 0.8 |
| | G | | 0.7 | 0.4 | 0.003 | - | 0.2 | 0.9 | 0.09 | 0.3 | 0.6 |
| | M | | 0.5 | 0.5 | 0.5 | - | 0.02 | 0.1 | 0.6 | 0.04 | 0.2 |
| | FxG | | 0.4 | 0.4 | 0.3 | - | 0.2 | 0.07 | 0.7 | 0.7 | 0.03 |
| | GxM | | 1 | 1 | 1 | - | 0.01 | 0.05 | 0.9 | 0.8 | 0.6 |

FU = Scandinavian feed units; AAT = amino acids absorbed in the intestine; ECM = energy corrected milk; F = forage type; G = grain type; M = methionine supplementation; GS = grass silage; PS = pea whole crop silage; WH = wheat; MA = maize

Concentrates were similar in energy concentration, therefore feed unit (FU) intake was only affected by forage type. Amino acid supply measured as AAT (amino acids absorbed in the intestine) followed DM intake. Maize rations were higher than wheat rations in AAT supply, showing that we had not fully succeeded to equilibrate AAT supply. Starch intake was slightly higher on pea silage than grass silage rations, and slightly higher on maize rations than wheat rations (results not shown). Methionine content (g/16 g N) was 1.44 for grass silage, 1.09 for pea whole crop silage, 1.34 for wheat concentrate and 1.47 for maize concentrate (results not shown). AAT-MET in % of total AAT intake was 1.96 for grass-wheat, 1.93 for grass-maize, 1.84 for pea-wheat and 1.82 for pea-maize rations. For methionine supplemented diets AAT-MET in % of total AAT were approx. 0.6 higher than for unsupplemented diets. This is based on the producer information that Smartamine M contains 75% DL methionine with 10% rumen degradability and 90% intestinal digestibility of rumen undegraded methionine.

Yield of milk and of energy corrected milk (ECM) showed similar trends for effects of treatments. Beside the considerably lower milk and ECM yield on pea silage compared to grass silage, there was a significant interaction between grain type and methionine supplementation, and a tendency for an interaction between forage type and grain type for ECM. For both forage types, methionine supplementation resulted in a decrease in ECM yield when wheat was the grain type fed, whereas methionine supplementation resulted in a slight increase in ECM yield when maize was fed. This was due to an effect on milk yield in kg, as no significant interaction between grain type and methionine supplementation on milk composition was seen. Inclusion of parity in the statistical analysis showed that the only interaction between parity and treatment was to forage type. The lower yield of ECM for pea silage than grass silage was much more pronounced for older cows (4.1 kg/day) than for first lactation cows (2.2 kg/day) (results not shown). Pea silage compared to grass silage resulted in a higher fat concentration in the milk. Milk protein concentration was

only affected by methionine supplementation, and methionine consistently resulted in increased protein concentration. As a mean the increase was 0.7 g per kg milk. When grass silage was fed, maize concentrate increased milk lactose % compared to wheat, whereas the opposite was found with pea silage. Daily production of fat, protein and lactose in kg was only affected significantly by forage type. Numerically, methionine supplementation resulted in slight insignificant reductions in the daily production of fat, protein and lactose (results not shown).

Discussion

We expected that by pass starch from maize would spare amino acids for gluconeogenesis. Therefore we also expected a higher positive effect of methionine supplementation to methionine deficient rations when wheat than when maize concentrate was fed. Results were fully opposite, maize rations were not affected by the methionine supplementation as expected, but wheat rations were negatively affected. It cannot be explained by methionine supply as shown above. However, if cows can discriminately use non-essential amino acids for gluconeogenesis, sparing amino acids for gluconeogenesis would affect methionine supply less. The reduced rumen fermentability of maize compared to wheat could also result in reduced microbial protein synthesis, counteracting the eventually positive effect of by pass starch. Further, the digestibility of maize bypass starch in the intestine could have been low. However, the negative effect of methionine supplementation on ECM production for wheat rations cannot be explained. The numerically highest decrease in ECM production due to methionine supplementation was seen for the grass-wheat combination, where also the AAT to FU ratio (90.5 g AAT/FU) was lowest. Although no or even negative effects of methionine supplementation on milk production, methionine supplementation resulted in increased protein concentration in milk for all ration combinations, which implies a direct effect of methionine on milk protein concentration. The 0.7 g protein per kg milk increase in milk protein concentration, which was the mean effect of methionine supplementation, was about half of the increase expected based on a literature review by Misciattelli et al. (2002).

The present study indicates that substituting wheat with maize does not alter starch supply to the intestine in a way that reduces the need for essential amino acids.

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Leptin plasma levels and pancreatic secretion in rats fed on high or low energy diets

T. Zebrowska¹, B. Pastuszewska¹, R. Matyjek¹, Maria Babelewska¹ & M. Switonska²

¹ *The Kielanowski Institute of Animal Physiology and Nutrition, Instytucka Str. 3, 05-110 Jabłonna, Poland*

² *Poznań University of Agriculture, Department of Animal Physiology and Biochemistry, Faculty of Animal Husbandry, Wołyńska Str. 35, 60-637 Poznań, Poland*

Summary

We investigated the leptin plasma levels, fat and energy content in the body, and pancreatico-biliary juice (PBJ) secretion in growing male Wistar rats fed low- vs. high-metabolisable energy diets. The results indicate that in growing rats, a high-energy diet resulting in large body fat and energy contents increases the leptin level in the blood and the protein content in PBJ.

Introduction

Leptin, a 16 kDa hormone, is produced and secreted by adipose tissue, muscles and most other tissues and organs. However, it is the white adipose tissue that is the principal site of production and the major determinant of the level of the circulating hormone. This fact is evident from the correlation between plasma leptin and body fatness indices in both humans and animals. It is also implicit in the observation that transgenic mice with little or no adipose tissue have very low circulating leptin levels (Trayhurn & Beattie, 2001). An increase in adiposity leads to an increase in circulating leptin concentrations, reducing the animal's appetite and increasing energy expenditure (Fruhbeck, 2001; Ahren et al., 1997).

Besides the energy balance regulation model there are number of studies pointing to a regulatory role of leptin in gastrointestinal (GI) tract functioning. Leptin receptors have been found in the stomach (Bado et al. 2001), small intestine (Lostao et al. 1998), pancreas on β -cells (Kieffer et al. 1996) and acinar cells (Jaworek et al. 2002). It was recently demonstrated that exogenous leptin in physiological doses inhibits the pancreatic enzyme protein output in anaesthetized rats (Matyjek et al. 2003), in contrast to a previous study by Guilmeau et al. (2002) who used a similar rat model and observed stimulation of PBJ secretion following *iv* leptin at doses 16 to 160 times higher.

Nonetheless, no data have been published yet concerning the ratio of circulating leptin originating from fat tissue and that from the GI tract in whole leptin pool. The contribution of fat-originated leptin in the regulation of GI tract functions, particularly pancreatic exocrine secretion, is also unknown.

Thus, in this study we assessed the long-term effect of fat content in rats fed low- and high-metabolizable energy diets on pancreatic juice secretion and circulating leptin levels.

Materials and methods

Animals, diets and experimental procedure

Forty-six male Wistar rats from IFZJaz outbred colony, aged 23 days and with an initial body weight of ~60 g, were divided according to litter and weight into two groups of 20, and one group of 6 animals, killed and analyzed for initial body composition as the "zero group". The rats were housed in an environmentally controlled room, in individual cages allowing for control of feed intake and collection of faeces and urine. High- (HE) and low- (LE) energy diets (Table 1),

containing 25 and 4 % fat, respectively, were fed during 30 (HE) and 40 (LE) days. The LE diet was offered in amounts restricted to ad libitum feed intake of the HE diet during the previous 24 h.

In ten rats from each group total collection of faeces and urine was performed during the whole experiment to determine the metabolizable energy (ME) value of diets and ME intake. At the end of the experiment, the rats from the balance groups were killed by exposure to CO₂, and blood was taken immediately from the heart for leptin analysis. Total body was autoclaved in glass jars, homogenized and analyzed for dry matter, protein, fat and energy contents. Deposition of these components was calculated as the difference between their contents in experimental and zero animals.

In the remaining 10 rats from both HE and LE groups, pancreatic secretion was determined. Pancreatic-billary juice (PBJ) was collected under pentobarbiturate anaesthesia. in 15 min intervals starting from standard conditions (*iv* infusion of 0.9% NaCl, 2ml/h per rat, 30 min) and thereafter during continuous infusion of CCK-8 solution (*iv*, 40 pmol/kg*h, Sigma, 60 min).

Table 1. The composition of high energy (HE) and low energy (LE) diets.

| Ingredient | Amount [g/kg] | |
|----------------|---------------|-----|
| | HE | LE |
| Casein | 185 | 185 |
| DL-Methionine | 1.8 | 1.8 |
| Starch | 373 | 583 |
| Saccharose | 100 | 100 |
| Soya oil | 40 | 40 |
| Lard | 210 | -- |
| Cellulose | 40 | 40 |
| Mineral premix | 30 | 30 |
| Vitamin premix | 20 | 20 |

Analysis

In the carcass of the rats, the contents of dry matter, protein, ash, fat and energy were analyzed according to AOAC, (1990); N was determined in the urine and faeces of rats using a Kjeltec apparatus, Tecator AB, Sweden. Serum leptin concentrations were determined using Leptin RIA kits (Linco Research, USA). The pancreatic juice was analyzed for total protein content by the Lowry method modified to be performed in 96-microwell plates, using BSA as the standard. The trypsin content was analyzed according to the Erlanger method using BAPNA as a substrate after activation of the juice with enterokinase.

Results and discussion

The mean daily gains of HE and LE rats were 7.2 and 4.8 g, respectively. The chemical body composition of rats differed between the groups: the HE rats contained more dry matter (38.2 vs. 34.5) and fat (18.3 vs. 11.9) and less protein (16.3 vs. 19.2 g per 100 g final live body weight) than the LE animals (Table 1); the energy content in the body of HE rats was greater than in LE rats (Table 2). Energy and fat digestibility were 91.5 vs. 94.2 and 91.1 vs. 95.7 in rats fed on HE and LE diets ($P < 0.001$), respectively, while protein digestibility was similar. In the HE group daily ME intake was 29% greater and deposition 87% greater than in the LE group (73.1 vs. 56.7

and 22.5 vs. 12.0 kcal, respectively), hence the overall efficiency of ME utilization for energy deposition was considerably greater on the HE than the LE diet (0.31 vs. 0.21, respectively). The serum leptin concentration was much higher ($p < 0.001$) in HE than in LE rats (mean values 8.0 vs. 2.3 ng/ml, respectively), and was positively correlated with energy ($r = 0.84$) and fat content ($r = 0.88$) in the body. The volume of PBJ and trypsin output did not differ, while total protein output was significantly ($p < 0.05$) higher in rats on the HE than on the LE diet, as related to basic secretion (Table 2).

HE rats had a higher body mass, higher leptin serum levels, but their pancreas mass equaled that in the LE group. No differences were found in water (Figure 1, volume) or protein output in PBJ

Table 2. Selected parameters (values and SEM) in high energy (HE) and low energy (LE) groups.

| Parameter | Group | |
|--|--------------------|------------------|
| | HE | LE |
| Final body weight [g] | 282.0 ± 16.6 | 252.0 ± 7.0 |
| Pancreas weight [g] | 0.9 ± 0.1 | 1.1 ± 0.1 |
| Body gain [g/day] | 7.2 ± 1.2 | 4.8 ± 0.2 |
| Total feed intake [g] ¹ | 452.8 ± 24.3 | 590 |
| Total energy intake [MJ] | 10.45 ± 0.6 | 10.57 |
| Total fat intake [g] | 115.1 ± 6.2 | 28 |
| Dry matter body content [g/100g] | 38.2 ± 3.0 | 34.5 ± 1.2 |
| Protein body content [g/100g] | 16.3 ± 1.0 | 19.2 ± 0.4 |
| Fat body content [g/100g] | 18.3 ± 2.7 | 11.9 ± 1.4 |
| Energy body content [KJ/100g] | 1188.5 ± 120.5 | 989.9 ± 55.3 |
| Leptin serum levels [ng/ml] | 8.0 ± 2.1 | 2.3 ± 0.6 |
| Ratio of basic to CCK stimulated secretion of PBJ total protein [%] ² | 167.4 ± 19.0 | 113.0 ± 22.4 |

¹ HE and LE daily intakes: the LE rats were restricted according to the mean daily HE ad libitum intake on the previous day. The HE balance experiment lasted 30 days, the LE experiment, 40 days. For the last 10 days the LE rats received the same amount as on day 30 of the HE group.

² Means from 30 min before and 30 min after CCK-8 iv stimulation

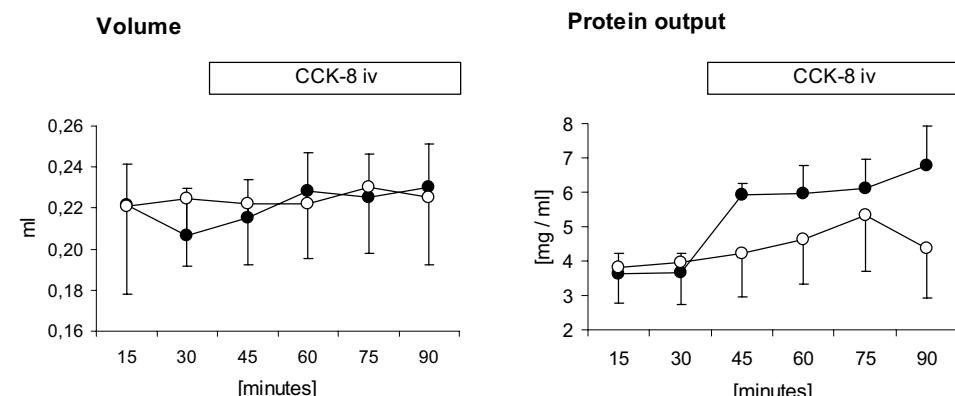


Figure 1. The kinetics of PBJ volume and protein secretion in high energy (HE) and low energy (LE) groups.

during baseline secretion, indicating that leptin decreased HE PBJ protein output (Matyjek et al. 2003) in this phase, since due to the higher energy intake the HE group was more likely to have higher basic PBJ secretion. However, after stimulation by CCK, the high level of leptin in the HE group (Table 2) did not influence PBJ protein. The reason is that leptin could not overcome the stimulating mechanisms (Deng & Whitcomb, 1998) developed during pancreatic adaptation to the HE diet.

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Joint session 3

Nutrition and Immunity

Handling of dietary antigens - nutritional interactions with the gut immune system

J. Zentek

Institute of nutrition, Veterinary University of Vienna, Veterinärplatz 1, A-1210 Vienna

Summary

The gut mucosa is exposed to numerous exogenous factors and has differentiated regulatory mechanisms, that enable selective permeability for nutrients and certain macromolecules but also exclusion of potentially harmful dietary, environmental or bacterial antigens. Discrimination of absorption and exclusion, tolerance and reactivity is resulting from complex regulatory processes that are depending on the age of the individual, the functional and regulatory mechanisms of the immune system and the influence of exogenous factors. The interaction between luminal factors of dietary or bacterial origin and the gut wall is of particular importance. Exogenous food antigens, e.g. peptides, glycoproteins and lectins, but also microorganisms have the ability of interacting with the gut wall and to induce reactions and regulatory and counter regulatory processes. The interaction of luminal factors with the gut wall influences digestion (secretion, absorption, motility), immunological mechanisms (exclusion of antigens, regulation of the GI immune system, on the other hand antigen processing, sensitivity, allergy), and neuro-endocrine processes and integration. Nutrition has significant impact on the gastrointestinal tract of young animals and is of special importance for the function of the gut and the associated immune system during early growth phase and later in adulthood.

Keywords: *antigens, nutrition, mucosa, immune system, proteins*

Introduction

Development of the digestive system in young animals is subjected to distinct patterns of appearance and change of transport capacities for carbohydrates, amino acids and peptides, lipids and many other substances including chemically different macromolecules (Buddington 2002a). Rates of qualitative and quantitative nutrient digestion and absorption match changes of diets, beginning with amniotic fluid, subsequently colostrum and milk and with species specific differences solid food at different times during ontogeny (Buddington 2002b). Significant influences affect the digestive tract and the gut associated immune system during early development and in adulthood. This is including prenatal factors as the exposure of the developing organism to immunoglobulins E and G, amniotic fluid cytokines, allergens and maternal immune cells in the feto-maternal environment, but also quality and quantity of maternal fat intake, e.g. the relation of n6 and n3 fatty acids (Fritzsche et al. 1993; Warner and Warner 2000; Zusman et al. 2000; Zhou et al. 2000). After birth exogenous factors affect the immune system, among those food or feed antigens and environmental or intestinal micro-organisms that are of specific importance for the development and stability of the complex digestive, immunological, endocrine and nervous processes in the gastrointestinal tract (Guy-Grand and Vassalli 1993; Tyler et al. 1994; Pacha 2000; Cross and Gill 2001; Kelly et al. 2001). The exposure to dietary, bacterial and environmental antigens is necessary for the development of a stable immune system (Bailey et al. 2001), although many questions have to remain open at present due to the extremely complex relationship between the microflora, dietary factors and the gut. Protein intake seems to be of major importance because it is providing the major load of dietary antigens. The particular

understanding of the interaction between intraluminal digestive processes and the absorption of amino acids, peptides and macromolecules and the effects of diet composition on the intestinal microflora has increased in the past years. Bacteria that colonize the intestinal mucosa can elicit a strong mucosal immune response, whereas food antigens are often only weakly immunogenic (Dahlgren et al. 1991). The explanation may be the physical and chemical properties of bacterial substances compared to food proteins from animals and plants, and specific stimulating properties of bacteria. Immune response of the intestinal mucosa can be reinforced by simultaneous challenge with protein and microbial antigen (Porter et al. 1987). Dietary protein intake has been shown to have distinct effects on the composition and the metabolic activity of the intestinal microflora (Amtsberg et al. 1989; Zentek 1995; Steen et al. 1997), but the significance of a change in the composition of the microecology for the intestinal immune response has not yet been elucidated.

Developmental aspects of protein digestion

Proteins are of major importance as nutrients especially in the young, providing amino acids for protein synthesis and tissue accretion. Digestive capacity has been investigated in different species and distinct age related patterns have been described. The small and large intestine of piglets gain rapidly in weight and length especially during the first 24 h after birth, the rate of growth slowing down during the next 9 days. In the jejunum the weights of both muscular and especially mucosal tissue increases (Widdowson et al. 1976) and the total activities of lactase and acid phosphatase in the jejunal mucosa increase during the first 24 h in suckled pigs because of the synthesis of mucosal tissue. Total activity of digestive enzymes (chymotrypsin, trypsin, amylase and gastric proteases) is increasing depending on age, either due to higher tissue weight or to higher enzyme activity per tissue weight (Lindemann et al. 1986). The activities of sucrase, isomaltase, maltase, lactase, trehalase and pancreatic alpha-amylase show age related patterns in piglets and a characteristic distribution along the intestine (Kidder and Manners 1980), approaching the adult status by the age of 8 weeks. Glucocorticoids seem to evoke elevation of the carbohydrazes enzymes necessary for initiating the hydrolysis of starch. The normal decrease in lactase activity can be accelerated by glucocorticoid administration (Chapple et al. 1989). In suckling rats, corticosteroid administration increases levels of proteases in the gastric mucosa, pancreas and small intestinal contents (Britton and Koldovsky 1988), indicating that steroids influence also protein digestive capacity during the perinatal period. Enzymatic digestive capacity is not only increased for proteins, but also for carbohydrazes when glucocorticoids are administered (Chapple et al. 1989). Protein digestive capacity is subjected to distinct changes during the development of the young. Prochymosin is present in the foetal gastric mucosa from about 3 weeks before birth. After birth, mean chymosin activity decreases. Prochymosin has been demonstrated in the gastric mucosa of newborn piglets (Foltmann et al. 1978), while pepsinogen was absent from the gastric mucosa at less than or equal to 5 days of age, but increases after the first week of life (Foltmann et al. 1981). With age the total activity of chymotrypsin, trypsin, and gastric proteases increases, mainly due to the increase of the organ weights (Lindemann et al. 1986). Liquid milk formulas containing either intact bovine milk, hydrolyzed bovine milk, or isolated soybean protein as source of protein did not affect the activities of pepsin, intestinal trypsin and chymotrypsin and pancreatic chymotrypsin, except that piglets fed the bovine milk had a lower activity of pancreatic trypsin (Moughan et al. 1990). Piglets fed on milk or a dry starter diet did not show clear differences in the intestinal enzymatic activities (Pluske et al. 1996b).

Developmental aspects of protein absorption

The intestinal tract is capable of absorbing amino acids, di- and tripeptides and larger molecules depending on influences by age, species and physiological condition. Young piglets are absorbing dietary proteins efficiently. Luminal perfusion with predigested and bile acid-solubilized sow's

milk of jejunum and ileum demonstrated increased mucosal permeability in one day old piglets compared to perfusions in older animals. This increased permeability is probably not linked to a higher metabolic turnover, for the intestinal oxygen uptake remained unchanged (Crissinger and Burney 1996). The intestinal transmission of macromolecular markers, with similar molecular weight but different susceptibility to proteolytic digestion, was investigated in newborn piglets or during the first week of life. When piglets were given a mixture of bovine serum albumin and fluorescein-isothiocyanate labelled dextran (70 kDa) by stomach tube, a rapid decrease in the transmission of the markers was observed during the first day of life in suckled piglets and intestinal macromolecular closure was well developed after 18-36 h of life. After that time, only small amounts of the markers were transmitted to the serum. Molecules having a molecular weight greater than 3000 Daltons were excluded upon macromolecular closure, independently if they had protein structure or not. Smaller molecules were transmitted across the intestinal barriers unrelated to closure (Westrom et al. 1984). Transport of porcine or human immunoglobulin G across the small intestine of the newborn piglet is mainly related to endocytosis. Preferential transport of porcine over human immunoglobulin G was demonstrated when both were given as a single solution, but the degree of preference was small. Colostrum generally stimulates transport processes mainly by increasing the amount of endocytosis (Burton and Smith 1977). Trypsin inhibitors have an important role for they evidently increase the efficiency of absorption of undegraded colostral proteins (Carlsson et al. 1980). Sow milk contains a trypsin inhibitor that was suspected to be of specific importance for these effects. But, as large quantities of proteins are still absorbed even when inhibitor-free colostrum was fed, other factors must also be involved in the regulation of intestinal protein absorption. The serum concentrations of immunoglobulins G, M, and A, and agglutinating antibodies for *B. bronchiseptica* were higher in piglets fed trypsin inhibitors in their diets, although, even if no trypsin inhibitors were fed, a considerable immunoglobulin absorption was found (Jensen and Pedersen 1982). Administration of iodinated immunoglobulin G within 3 hours after birth resulted in the appearance of the immunoglobulin in the piglet circulation in unaltered form. Immediate feeding of bovine colostrum followed by administration of iodinated immunoglobulin G after 3 days resulted in the appearance of fragments only. Administration of 15 g of lactose during the first 24 hours reduced absorption of immunoglobulin G by 26% compared to controls, while treatment with 54 g of lactose reduced absorption by 94%. On the contrary, when bovine colostrum was administered immediately after purified immunoglobulin G, the amount of swine immunoglobulin G absorbed was 50-70% greater than in controls. Mature milk failed to have the same influence. When the amount of purified swine immunoglobulin G administered varied from 1 to 8 g, absorption was directly proportional to the amount administered (Werhahn et al. 1981). Piglets are obviously less selective with regard to the transport of macromolecules from the gut to the blood, compared to other species (rats, mice and hamsters). Enterocytes in the proximal part of the small intestine transport more proteins than those in the distal part. Bovine albumin or porcine immunoglobulin G were transported at about the same level. Mixtures of these proteins behaved differently, immunoglobulin G was preferentially transported and albumin enhanced immunoglobulin G transport, probably by a micropinocytotic mechanism (Leary and Lecce 1979). The lymphatic system is of major importance for the transport and processing of absorbed proteins. By collection of thoracic duct lymph from non-suckled newborn pigs it was demonstrated, that bovine colostral proteins are absorbed from the intestine into lymph (Kiriyama et al. 1988). The gut wall is not only permeable for proteins, it may also allow cells to cross the intestinal barrier. In 7 day old piglets the intestinal absorption of labelled colostral maternal lymphoid cells via the lymphatic vessels and the mesenteric lymph nodes was demonstrated. Electron microscopy revealed that absorption took place intercellularly. Obviously, this way was restricted to colostral cells of the own mother sows, for lymphocytes from other sows were only detected in the epithelial layer of the mucous membranes. Lymphoid cells isolated from the sows' blood and heat-treated colostral lymphoid cells were not detected (Tuboly et al. 1988).

The uptake and the handling of dietary antigens by the piglet is modified by the feeding of the sow. When sows were fed ovalbumin (OvA) as a novel protein antigen either throughout gestation and lactation or during lactation only, the uptake of OvA into blood, colostrum and milk along with a specific Immunoglobulin G response was demonstrated independently from that treatment. In piglets from sows fed the antigens from gestation, OvA and antibodies to OvA were detected in the serum after ingestion of colostrum. In a large proportion of these piglets OvA was still detected at 3 weeks of age. A significant proportion of piglets responded to OvA whilst suckling from sows fed ovalbumin from lactation. At 3 weeks of age all piglets were weaned onto an egg-based diet. A similar uptake of OvA was seen in all piglets but there was no response to OvA in the piglets from sows fed ovalbumin from gestation. In piglets from sows fed only during lactation, a rapid Immunoglobulin G anti-OvA response and signs of diarrhoea were seen. The results were interpreted in a way that factors of immunological importance are passed over from mother to offspring and that immunological experience of dietary antigens by the mother is important for a tolerance induction in her offspring (Telemo et al. 1991).

Antigen handling and immune response

The normal status of the intestinal immune system is active tolerance with the option to develop active response when exposed to potential pathogens. The gastrointestinal immune system is highly developed and compartmented in adults, but in young animals the degree of organization is much less developed (Bailey et al. 2001). The intestine acts as a barrier preventing uncontrolled entry of dietary antigens into the gut wall and into the immune system. For this purpose, different mechanisms have been developed evolutionary, among those the luminal digestive processes, mainly the proteolytic activity of the stomach and of the small intestinal and pancreatic secretions, the mucus layer with different unspecific mechanisms, e.g. pH, negative charge, and non specific proteolytic activity, and the secretory immunoglobulin A antibodies. On the other hand, the intestine must allow the uptake of macromolecules, that are important for growth and development (Sanderson 1999; Sanderson and Walker 2002). As long as antigens are transported across the intestine in physiological amounts, immunosurveillance is maintained and the reactivity of the gut immune system is regulated in a sense of active tolerance. Passage of small quantities of dietary antigens is an important prerequisite for the interaction of food or feed antigens with B-cells, inducing secretion of immunoglobulins and T-cells via binding of peptides to MHC-molecules and interaction with T-cell receptors. When pathological transport occurs, especially when the mucosal barrier is breached, intolerance, allergy or chronic diseases may result. Development affects these mechanisms and changes in gene regulation are important for giving informations from the intestinal lumen to the mucosal immune system. Some transport processes are related to specific receptors and by this the molecules are shuttled through absorptive cells. Unspecific transport mechanisms have also been characterized related to the formation of vesicles and the transport to the basolateral side of the cell. Alternatively, antigens pass the epithelium via specialized M cells located in the area of Peyer's patches (Sanderson and Walker 2002). Uptake of macromolecules by receptors has been characterized for the immunoglobulin G from the intestinal lumen, allowing young animals a certain discrimination between species specific and foreign immunoglobulins and a more or less selective uptake of species specific antibodies (Werhahn et al. 1981; Staley and Bush 1985). In very young piglets a nonselective and massive absorption of macromolecules occurs for up to 3 days and decreases after that (Mehrazar et al. 1993) with sow colostrum having an enhancing effect. A selective absorption of immunoglobulins with small quantities of ingested bovine serum albumin (0.02% to 0.1%) was found in 5 day old piglets. Gut closure was delayed after feeding foreign antigens to immature rat pups. Cow milk antigens interfere with the maturational process of gut closure and jejunal permeability to macromolecules was higher in rat pups fed by gavage with cow milk on day 14 or even when cow milk was fed to the dams. Jejunal eosinophilic infiltration was found in association with the

increased jejunal permeability in the pups fed with cow milk and pups from dams fed with cow milk, the number of antibody secreting cells in peripheral blood against beta-lactoglobulin was significantly higher. Mucosal barrier function can obviously be impaired due to a local hypersensitivity reaction to foreign antigens, irrespective of the protection of maternal milk or maternal antigen processing (Arvola et al. 1993).

The Fc receptor is able to cross cells and seems to be transported by membrane transport mechanisms. The neonatal Fc receptor for immunoglobulin G, an MHC class I-related molecule, functions to transport immunoglobulin G across polarized epithelial cells and to protect immunoglobulin G from degradation (Zhu et al. 2001). Transport of immunoglobulin to the newborn mammal is important for immune defence during the first weeks of life and receptors for the Fc portion of immunoglobulin G isolated from intestinal epithelial cells of suckling rats bear a resemblance to class I histocompatibility molecules (Gastinel et al. 1992). The expression of the Fc receptor gene seems to be linked to the uptake of mother milk, for a decrease in expression was described after weaning.

Additionally to these mechanisms, enterocytes can act as antigen presenting cells and are regarded as non professionals compared to the classic presenting cells like dendritic cells, B cells and macrophages. All of those cells are able to express a glycoprotein, the major histocompatibility complex II, that interacts with the T cell receptor. Non specific transport through enterocytes can also occur in older animals after binding of macromolecules to receptors on the apical cell membrane and transport to the basolateral part of the gut cell (Sanderson and Walker 2002). The alternative to Fc receptor mediated transport is that macromolecules are engulfed in vesicles apically and transported through the intestinal cell. An important question is how antigens are processed in the gut epithelium. Peptide fragments could be generated during epithelial transport or antigens could be processed from whole food or feed protein that has traversed the epithelium and reached the antigen presenting cells. By intracellular proteolysis, the antigenicity of proteins may decrease and result in a condition, where antigen presenting cells and lymphocytes would not be able to recognize and process the ingested proteins.

Specialized cells, called M cells or microfold cells, are overlaying lymphoid tissue in the Peyer's patches. They have a specific cell surface and the mucous overlay is less compared to the adjacent gut areas. Macromolecules can enter via M cells and come into contact with immune cells located in close relation to the M cells. Macromolecules enter non specifically into the cells by engulfment into vesicles and can be released into the areas where lymphoid cells and antigen presenting cells are located. M cells have a high capacity for transcytosis of a wide range of microorganisms and macromolecules, they are believed to act as an antigen sampling system. M cells provide functional openings of the epithelial barrier, but normally, there seems to be a balance between antigen uptake and immunological response (Kucharzik et al. 2000).

Practical implications

At weaning, dietary and often also environmental changes can stress young animals severely. In practice, diarrhoea or systemic disease is a common problem in many species. Economic impact is obvious in piglet production and weaning associated disorders are of specific importance.

The villous height in the small intestine is high in unweaned piglets and decreases subsequently to weaning. Nutrition has profound effects on intestinal morphology and function. A significant decrease in villus height was seen in piglets weaned on a dry diet on days 8 and 11 post-weaning, whereas in the piglets receiving a liquid diet villus heights remained stable. Weaned pigs showed an increase in crypt depth and an increase in the complexity of villus morphology with a reduction in villus height (Hampson 1986a). Villus height and crypt depth were maintained by feeding cows' milk after weaning (Pluske et al. 1996a). Villus height was greater in a group of piglets fed high amounts of milk than in either a weanling diet or low milk group. Apparently, villous atrophy was due more to the level of feed intake than to the composition of the diet (Beers-Schreurs et al.

1998). Villous height and crypt depth were significantly correlated with dry matter intake after weaning in milk-fed and starter-fed piglets (Pluske et al. 1996b).

A general depression in pancreatic enzymatic activities, but not in gastric proteolytic activity, was found during the first week after weaning. Following increases in activity of lipase and chymotrypsin were due to the higher pancreatic weight after weaning. Amylase, trypsin and gastric protease increases were due to increased weight and increased activity per g tissue (Lindemann et al. 1986). The activities of lactase decline along the small intestine at weaning, while sucrase activity declines temporarily and then recovers. Minimum values were recorded about four to five days after weaning. The large loss of digestive enzyme activities at brush borders in weaned animals coincided with a reduced xylose absorption (Hampson and Kidder 1986). The activities of pepsin, intestinal trypsin and chymotrypsin and pancreatic chymotrypsin were not influenced by dietary protein source (intact bovine milk, hydrolyzed bovine milk, or isolated soybean protein), but piglets receiving the bovine milk-based formula had a lower level of activity for pancreatic trypsin (Moughan et al. 1990). Total absorption of galactose and glucose, adjusted for live weight and plasma volume, increased after weaning, although galactose index (Gal:AUC for galactose ingested as lactose divided by the AUC for the same dose of galactose ingested as the monosaccharide) and fructose index (Fruc: AUC for fructose ingested as sucrose divided by the AUC for the same dose of fructose ingested as the monosaccharide), both decreased after weaning (Pluske et al. 1996a). Switching from milk to cereals increases some mucosal enzyme activities, intestinal sodium dependent glucose absorption, and response to secretagogues. Alkaline phosphatase- and sucrase-specific activities were higher in cereal-fed piglets than in milk-fed piglets, while dipeptidylpeptidase activity was higher in wheat-fed piglets. Sodium dependent glucose absorption was 1.7-fold higher in cereals-fed piglets than in milk-fed piglets (Boudry et al. 2002). Aminopeptidase A activity increases compared with values at birth after weaning, while sucrase, maltase, lactase and aminopeptidase N activities increase but develop diet dependent (Jensen et al. 2001). Spray dried porcine plasma or casein as protein source had no effect on lactase-, sucrase- or maltase-specific activities of the small intestine (Van Dijk et al. 2002).

The weaning of piglets on diets containing soya meal is common practice. Active response to the dietary protein was demonstrated by the appearance of serum anti soya immunoglobulin G. The response of piglets to soya in the weaning diet was lower, when one gram of soya protein was given at birth. Interestingly, similar amounts of protein itself could be detected in the serum. The response of piglets primed orally with soya at birth to injected soya was not significantly reduced, indicating that the regulation of responses to fed and systemic antigens is largely separate (Bailey et al. 1994). Hypersensitivity response to dietary antigen might be a predisposing factor in the aetiology of post weaning diarrhoea. Small amounts of feed antigens given to baby pigs before weaning significantly increased the severity and accelerated the onset of post weaning diarrhoea (Miller et al. 1983). Diets based on either skinned-milk powder, soya-bean-protein concentrate, soya-bean meal or fish meal affected post-weaning feed intake, pancreatic weight, gastric pH and gastric protein breakdown, and pancreatic and jejunal trypsin and chymotrypsin activities (Makkink et al. 1994). In piglets, weaned at 28 days of age, immunoblots of serum were made to detect both residual antigenic storage proteins of the seeds of dietary legumes used and Immunoglobulin G specific to those storage proteins. Antibodies against beta-conglutin of *L. luteus*, vicilin of *V. sativa* and vicilin of *L. cicera* were detected 28 days after feeding the diet in the sera of piglets, but no storage protein was found in the same animals. The presence of antibodies against feed proteins indicates an immune response in the weaned piglets (Seabra et al. 2001). Piglets fed on protease treated soya bean meal or skim milk powder and fish meal did not show significant differences between diets with respect to piglet serum anti-SBM specific antibodies. There were no consistent changes in small intestine enzyme-specific activities, histopathological examination of the small intestine, villus height or crypt depth (Rooke et al. 1998). Hydrolysed casein had a protective effect on the gut structure and function, probably related to the low level of antigenicity of the diet (Hampson 1986b). Macromolecule transport was shown

to be affected by the dietary protein source. Newborn piglets were bottle-fed with porcine colostrum, bovine colostrum, porcine plasma, porcine milk, bovine colostrum containing porcine plasma or a milk replacer to test the potential effects of the protein source on the absorption of bovine serum albumin. The percentage of absorbed BSA just after birth was highest for piglets fed porcine colostrum (30-50%), and was reduced to 23-30 % when bovine colostrum without or with porcine plasma and to 7-20 % for the piglets fed with porcine plasma or milk, all relatively to those fed colostrum (Jensen et al. 2001).

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Tissue amino acid fluxes and valine kinetics in lambs with a *Trichostrongylus colubriformis* infection

E.N. Bermingham^{1,2*}, W.C. McNabb¹, G.W. Reynolds², G.C. Waghorn¹, I.A. Sutherland^{1#}, D.K. Revell³ & N.C. Roy¹

¹ AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand

² Massey University, Palmerston North, New Zealand

³ Department of Animal Science, The University of Adelaide, Adelaide, Australia

* Present address: CSIRO Livestock Industries, Perth, Western Australia

Present address: CSIRO Livestock Industries, Queensland, Australia

Summary

This study investigated the effects of parasite infection on amino acid (AA) flux in lambs fed fresh salla (*Hedysarum coronarium*). Lambs were prepared with an arterio-venous preparation across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and hind limbs. The lambs were infected with 6000 *Trichostrongylus colubriformis* L3 larvae per day for 6 days (n=6) or kept as parasite-free controls (n=6) according to a completely randomised block design. On day 48 post-infection, the lambs were continuously infused for 8 h with [$3,4\text{-}^3\text{H}$]-valine into the jugular vein (7.6 MBq/h), ρ -aminohippuric (PAH) into the mesenteric vein (0.14 mmol h^{-1}) and indocyanin green (ICG) into the abdominal aorta (14.6 mg h^{-1}) to measure plasma valine kinetics and flow across the TSP and hind limbs, respectively. Blood was continuously collected from the mesenteric, portal and hepatic veins, mesenteric artery and vena cava for 2 h periods throughout the infusion. Plasma was harvested and processed to measure AA, PAH and ICG concentration and valine specific radioactivity to calculate the corresponding net flux of AA and irreversible loss rate (ILR) of valine across the tissue beds. Parasite infection resulted in a reduction ($P<0.15$) in feed intake and liveweight gain (LWG), but did not affect ($P>0.15$) nitrogen (N) retention or valine ILR across the tissue beds. Parasitic infection resulted in less total AA (TAA) being released from the MDV and PDV ($P<0.05$). There was no effect of parasite infection on TAA utilisation by the liver ($P>0.15$). However, a net release of TAA by the TSP was observed in the control lambs, as opposed to a net uptake in the infected lambs ($P<0.10$). Parasite infection had no effect on the net flux of TAA across the hind limbs ($P>0.15$). Valine ILR across all tissue beds was similar between treatments. Decreased TAA appearance in the portal drainage of infected lambs could be explained by the reduction in feed intake, as PDV protein turnover (estimated from valine ILR) was similar between treatments. Consequently, less TAA was released by the TSP into the peripheral circulation in the infected lambs and this could explain why LWG was impaired in these animals. However, N retention and hind limb net AA flux and protein turnover was not affected 48 days post-infection.

Keywords: parasite infection, net amino acid flux, tissue beds

Introduction

Metabolic events that occur during parasite infections may result in the catabolism of muscle protein to meet the additional metabolic demands placed on the gastrointestinal tract (GIT) and liver. Increased amino acid (AA) requirement in these tissues may be responsible for initiating changes in tissue catabolism (Coop & Kyriazakis, 1999). The repartitioning of AA from the muscle to the GIT and liver may explain the reduction in live weight gain (LWG; van Houtert *et al.*, 1995) and wool production (Steel *et al.*, 1982) that has been observed following parasite

infection. Increased GIT protein turnover during a trickle sub-clinical *Trichostrongylus colubriformis* infection resulted in less leucine being available to other tissues (Yu *et al.*, 2000). There has been no attempt to quantify the changes in net AA flux across tissue beds in the infected animal. Moreover, there is little data available on the effects of an established infection on estimation of tissue protein turnover in lambs fed fresh forages.

Our hypothesis is to verify if the presence of an established *T. colubriformis* infection in the small intestine will result in an increased utilisation of AA by the mesenteric-drained viscera (MDV), portal-drained viscera (PDV; stomachs, small intestine, large intestine, pancreas, spleen), liver and total splanchnic tissues (TSP; PDV+liver) and if this will be achieved by an increased release of AA from the hind limbs (muscle, skin, fat). This repartitioning of AA is likely to involve a change in protein turnover in these tissues. Therefore, our aim was to quantify the net flux of AA and irreversible loss rate (ILR) of valine (estimate of protein turnover) across the MDV, PDV, liver, TSP and hind limbs in the lamb fed fresh sulla.

Materials and methods

Twelve wether lambs (33 kg) were weaned from their dams, housed in individual metabolism crates and offered fresh sulla (*Hedysarum coronarium*; 800 g DM/d) at hourly intervals. Permanent indwelling catheters were placed in the mesenteric artery, and the mesenteric, portal and hepatic veins (Huntington *et al.*, 1989) and vena cava (Ortigues & Durand, 1995) for blood sampling. Permanent catheters were also placed in the mesenteric vein (upstream from the sampling site) and abdominal aorta for infusion of p-aminohippuric acid (PAH) and indocyanin green (ICG), respectively to measure MDV, PDV, liver, TSP and hind limb plasma flows. A temporary catheter was inserted into the jugular vein two days before the infusion of [3, 4-³H]-valine. One week after surgery (day 1 of the experimental period) six sheep were given 6000 *T. colubriformis* L3 larvae per day orally for 6 consecutive days (parasite treatment) while the remaining six sheep were kept as controls (control treatment). A completely randomised block design was used. Faecal egg counts from each sheep were determined every second day from day 20 to 45 and intestinal worm burdens were measured at slaughter (Birmingham *et al.*, 2000).

Infusions and blood sampling

On day 48, the lambs were continuously infused with [3,4-³H]-valine (7.6 MBq/h; Amersham Life Science, Buckinghamshire, UK; containing 1.68 mg/L cold valine) into the jugular vein for 8 h. In order to measure plasma flow across the MDV, PDV, liver and TSP, a sterile solution of PAH (723 mg/h; 0.14 mmol/L Na form) was continuously infused into the mesenteric vein for 8 h. The lambs also received a continuous infusion of ICG for 8 h into the abdominal aorta (14.6 mg/h; 0.83 mmol/L ICG) to measure plasma flow across the hind limbs (Wester *et al.*, 2000). To prevent blood clotting during the continuous sampling, 6000 iu heparin/h was infused into the jugular vein with the [3,4-³H]-valine over the 8 h period. Sampling lines and syringes were kept in an ice-water bath for minimising the degradation of blood constituents. Blood was continuously withdrawn every 2 h from the mesenteric artery, the mesenteric, portal and hepatic veins and the vena cava over the infusion period. After each two-hour collection period, the syringes were removed from the collection lines and carefully mixed by gentle rotation. Blood was centrifuged (4(C; 3270 g for 15 min), plasma harvested and either processed as described below or stored at -85°C.

Analytical methodologies

Amino acid concentration in plasma (0.5 mL) was determined as described in Birmingham *et al.* (2000). To measure the specific radioactivity (SRA) of valine, 2 mL of plasma was mixed with 1

mL of a solution containing 0.75% w/v SDS, 9 mM EDTA and 200 µL DTT (80 mM). Norleucine (100 µL; 3 mM in 0.1% phenol) was added as an internal standard. The samples were left at 20°C for 15 min before adding 1 mL of TCA (30% w/v) to precipitate protein and then centrifuged (3270 g, 15 min at 4°C). The resulting supernatant was filtered (0.45 µm) and stored at -85°C. Total ³H-valine counts in deproteinised plasma was determined by β-radioactivity using a HPLC with an in-line detector (Lee *et al.* 1995). p-aminohippuric acid dye dilution was used to determine the plasma flow through the MDV, PDV and TSP tissues (Katz & Bergman, 1969; Lobley *et al.*, 1995). Plasma flow across the hind limbs was calculated using the ICG concentration measured in plasma (Wester *et al.*, 2000).

Calculations and statistical analysis

Plasma flow, net flux of AA and ILR of valine across the MDV, PDV, liver, TSP and hind-limbs were calculated as described by Lobley *et al.* (1995) and Harris *et al.* (1992). The SRA of plasma valine was calculated by dividing its radioactivity by its concentration (Bermingham *et al.*, 2000). Statistical analysis was done using a General Linear Model (SAS version 8, 1999). Least squares means and associated pooled standard deviation are reported. Probabilities lower than 0.10 indicate a significant change and values between 0.10 and 0.15 to indicate a trend.

Results

Dosing lambs with infective L3-*T. colubriformis* larvae resulted in a parasite burden (245 vs. 17600 (SD 7000) worms; P<0.01). Faecal egg counts peaked at day 26 post infection (1800 eggs/g wet faeces) but by day 48 they had fallen to 600 eggs/g (P<0.0001). DM intake over the experiment (769 vs. 689 (SD 47) g DM/d) and the LWG over the last 20 days of the experiment (50 vs. -50 (SD 70) g/d) were lower in the parasitised lambs (P<0.15).

Plasma flow through the MDV, PDV, TSP and hind limbs were unaffected by the presence of parasitic infection (data not shown). Infection decreased the net fluxes of Total AA (TAA) across the MDV and PDV (P<0.15; Table 1). However, there was no effect of treatment on net flux of TAA across the liver. The TSP of infected lambs utilised TAA while in the control lambs TAA were released (P<0.15; Table 1). There was no effect of treatment on the net flux of TAA across the hind limbs or the ILR of valine across all tissues (Table 1).

Discussion

The presence of an established parasite infection did not increase the utilisation of TAA by the MDV, PDV, liver or TSP, nor was there an increase in the release of TAA from the hind limbs associated with altered protein turnover. The pattern of net TAA flux and valine ILR across these tissues are within the range reported in the literature (e.g., Harris *et al.*, 1992; Lobley *et al.*, 1995; Yu *et al.*, 2000; Hoskin *et al.*, 2001).

The MDV of parasitised lambs released less TAA, which is likely to be due to the reduction in intake observed in these lambs. However, more AA may also have been utilised within this tissue for increased endogenous protein losses and/or the repair of damaged tissue. Yu *et al.* (1999, 2000) indicated that a trickle infection increased the endogenous protein loss, protein turnover and AA oxidation in the MDV. However, in the present study the ILR of valine in MDV was unaffected, which suggests that there was no effect of an established parasite infection on AA utilisation for oxidation and protein synthesis. Infection decreased the release of TAA from the PDV. The fate(s) of the AA within the PDV are not quantified in this study and they could be utilised for oxidation and/or protein synthesis. As ILR in PDV was unaffected by infection it seems unlikely that there was any alteration in these processes, and it is probable that the decrease in MDV TAA release was responsible for the reduction observed in the PDV.

*Table 1. The net flux of total amino acids (TAA) and irreversible loss (ILR) of valine across the mesenteric-drained viscera (MDV), portal-drained viscera (PDV), liver, total splanchnic tissues (TSP) and hind limbs of lambs fed fresh sulla (Hedysarum coronarium) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls. Positive values represent a net utilisation by the tissue, while a negative value represents a net release. Results are presented as LSmeans and associated pooled standard deviation (SD).*

| | | Control n=6 | Parasite n=6 | SD | P |
|---|------------|----------------|-----------------|-----|------|
| TAA flux ($\mu\text{mol}/\text{min}$) | MDV | -539 | -146 | 234 | 0.11 |
| | PDV | -286 | -70 | 113 | 0.03 |
| | Liver | 120 | 127 | 109 | 0.46 |
| | TSP | -91 | 65 | 81 | 0.06 |
| | Hind limbs | -27 | -13 | 86 | 0.83 |
| Valine ILR (mmol/h) | MDV | 1.4 | 1.3 | 2.0 | 0.95 |
| | PDV | 2.3 | 1.9 | 1.1 | 0.69 |
| | Liver | 1.7 | 1.3 | 1.3 | 0.63 |
| | TSP | 4.1 | 3.3 | 0.9 | 0.58 |
| | Hind limbs | 1.5 | 0.9 | 0.8 | 0.32 |

Despite a decreased release of TAA into the portal drainage, there was no effect of infection on the utilisation of TAA by the liver, nor was valine ILR affected. This suggests that there was no increase in AA requirement in the liver during infection. The TSP showed a significant effect of infection, with AA being released in the control lambs compared to net utilisation in the infected lambs. However, TSP valine ILR was unaffected by infection indicating no changes in protein turnover. Therefore the effects on the TSP were mainly due to less AA being released by the PDV as hepatic TAA utilisation was similar between treatments. Despite less TAA being released by the TSP into the peripheral blood, there was no effect of infection on the utilisation of TAA across the hind limbs which is similar to results reported by Bermingham *et al.* (2002). The LWG was lower in the infected lambs, however, there was no change in net AA flux and valine ILR across the hind limbs.

Conclusions

An established parasite infection reduced intake in lambs, and this had a negative impact on the appearance of AA in the mesenteric, portal and hepatic drainages. Despite lowered supply of AA to the hind limbs there was no apparent repartitioning of AA from this tissue to the GIT or liver.

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Energy and protein metabolism and liver activity changes in dairy cows treated with *E. coli* endotoxin 3 days after calving

G. Bertoni, E. Trevisi, A. Ferrari & M. Bionaz

Institute of Zootechnics, Faculty of Agriculture, U.C.S.C., 29100 Piacenza, Italy

Summary

To investigate the effects of an inflammatory model (lipopolysaccharides treatment that causes a cytokine release) on energy-protein metabolism and on liver activity, 5 multiparous cows received 2 i.v. injections of endotoxin (ETX), in their 3rd day after calving. Other 5 cows, in analogous conditions, were used as control (CTR). Cows were routinely monitored for feed intake, health status, metabolic profile, BCS, milk yield and liver biopsy (trigly-cerides). Blood was withdrawn more frequently after ETX treatment, for metabolic-endocrine profile; contemporary rectal temperature was measured. ETX caused typical animal responses (i.e. fever, reduction of feed intake and milk yield) and several metabolic-endocrine changes (i.e. reduction of Ca, Zn, T₃ and increase of NEFA, enzymes, insulin, glucagon and cortisol). Interestingly, it was also observed an initial decrease of glucose and a raise of triglycerides. With concern to the liver activity responses, haptoglobin was increased and the usual protein synthesis were reduced (i.e. decrease of albumin), while liver TG content was increased. The raise of globulin confirmed the activation of immune system. Although our model to mimic inflammatory phenomena has determined short-lived effects, it seems in principle able to impair the liver TG excretion and, likely, to promote the liver lipidosis worsening.

Keywords: *lipopolysaccharides, hormones, liver lipidosis*

Introduction

An inflammatory condition is promoted by eicosanoids (prostaglandins, tromboxans etc.) and by some cytokines which are released after various stimuli acting upon immune system (i.e. trauma, bacteria, virus, endotoxin, digestive and metabolic disorders etc.). The cytokines, such as TNF, IL-1 and IL-6, typical cause of acute phase response, have a lot of different functions on whole metabolism (Elsasser *et al.*, 2000). This event modify the homeostasis, particularly at liver level (Bode & Heinrich, 2001), where acute phase proteins (i.e. haptoglobin and ceruloplasmin) are synthesized while typical proteins (i.e. albumins, carriers of vitamins, several apolipoproteins) are partially suppressed. In addition, these cytokines reduce feed intake by several mechanism (Ingvarstsen & Andersen, 2000) and induce lipolysis directly (Zhang *et al.*, 2002) and indirectly (Qi & Pekala, 2000).

These modifications in the liver functions could be particularly harmful at the time of a very marked lipomobilization. In fact, hepatocytes are normally able to efficiently reutilize blood NEFA, either for energy metabolism or triglycerides (TG) and lipoprotein synthesis (VLDL). Conversely, the activation of immune system increases adipocytes NEFA release and, simultaneously, induces the production of acute phase protein in the liver while impairs the synthesis of the usual proteins (i.e. albumin, lipoproteins). As previously showed by Cappa *et al.* (1989), after calving it is often observed a reduction of usual liver proteins in cows showing symptoms of inflammations; furthermore the impairment of liver activity, associated to acute phase response around parturition, has been related to a reduction of fertility (Trevisi *et al.*, 1998; Bertoni *et al.* 2001). For this reason, as previously suggested by Bertoni *et al.* (1998a), a release of cytokines (i.e. uterus involution, stress-trauma for calving, metabolic or infectious diseases,

etc.) could be a primer of liver lipidosis in early lactating dairy cows particularly if a large amount of NEFA is contemporary released. Aim of our experiment was to investigate the effects of an inflammatory model, i.e. the lipopolysaccharides (LPS) treatment, that causes a cytokine release, on energy and protein metabolism as well as on liver activity of dairy cows at the beginning of lactation.

Material and methods

The research was carried out on 10 multiparous cows (696 ± 55 kg of b.w. immediately after calving) in an experimental barn, with almost constant climate conditions, daylight interval and feed-diet characteristics. Cows were individually fed *ad libitum*; forages (maize-silage, alfalfa and grass hay) were offered in two meals (7:00 a.m. and p.m.), while concentrate in 2 (dry) to 8 (lactation) meals by auto feeder. After calving concentrate were gradually increased (about 0.3-0.5 kg/d, till the maximum level of 1 kg/3 kg of milk).

On 3rd day after calving, 5 cows received 2 i.v. injections of endotoxin (*E. coli* 055:B5, Sigma), respectively 1 and 7 hours after morning forage meal (ETX). The dosage of each injection was 0.1 µg/kg body weight. The other 5 cows were used as control (CTR). Cows were routinely monitored for: dry matter intake and health status (daily), metabolic profile (2 times a week), body weight and BCS (once every 14 days) and milk yield (at each milking). In addition, blood samples for metabolic profile plus T₃, cortisol and insulin determinations were taken before 1st endotoxin treatment then 1.5, 3, 6, 9, 12, 24, 36, 48 and 72 hour after. In coincidence of these samplings, rectal temperature was also measured. The measurements on blood were done according to Bertoni *et al.* (1998b). Liver biopsy was taken 1 and 3 weeks before calving as well as 1 day before ETX treatment, then 5 and 12 days after it; the TG content was determined according to Rukkwamsuk *et al.* (1999).

Data were statistically analysed as repeated measures using the PROC MIXED of SAS [version 8 (TS M0)], including in the model cow, treatment, day (or hour) from treatment and interaction treatment X day.

Results and discussion

The cows treated with endotoxin showed the usual symptoms, i.e. an increase of rectal temperature (+0.8 °C at 3rd hour after 1st injection, followed by a quick decrease after 9th) and tachypnea. Dry matter intake was slightly reduced only in the day of treatment (-4% *vs* the day before), while it increased in CTR (+6%). Milk yield also showed a transient reduction in ETX (-22% the day of treatment *vs* the day before; P<0.01), but the theoretical recovery was quick and completed in one week, while later the production was higher than CTR group. At blood level, endotoxin treatment determined immediately the typical effects: dramatic changes of some minerals (P<0.05), reduction of Ca, Zn and P from 6 to 24 h; at the same time an increase of Na (from 6 to 72 h) and Cl (from 24 to 72 h) has been observed. Also expected were the significant increases of NEFA after 1st injections (fig. 1) as well as urea, creatinine, alkaline phosphatase γ-glutamil transferase and aspartate transaminase (P<0.05). These changes were more or less relevant, but never at pathological level. In this case, a significant increase of TG was also observed (P<0.05).

Interestingly, glucose showed a transient decrease (till 9th h) after ETX treatment, followed by significant increase (P<0.01 at 24th h); this behaviour differs from previous trial realized on dairy cows in late lactation (Lombardelli *et al.*, 1998), when glucose was suddenly increased. About hormones, our previous results have generally been confirmed (Bertoni *et al.*, 1991); in fact the cortisol showed a quick and marked increase (2.14, 58.32 and 12.03 ng/ml respectively at 0, 1.5 and 9 h; P<0.01) after endotoxin treatment as well as glucagon (fig. 1, P<0.05). Insulin also showed an increase in ETX group (fig. 1, P<0.01), but it peaked quite later at 12 h. The endotoxin treatment caused also a marked reduction of T₃ (fig. 1) since 6th h.

With concern to the liver activity responses, the acute phase protein haptoglobin (0.77 vs 0.59 g/l, $P<0.05$) was significantly increased the day after endotoxin treatment, while ceruloplasmin increased the day after without significance (3.12 vs 2.86 $\mu\text{mol/l}$ of the day before). On the contrary, a lower liver synthesis of usual proteins seems to be demonstrated by albumin reduction at 36 and 72 h from treatment (32.83 and 33.14 g/l respectively in comparison to 35.30 g/l at 0 h, $P<0.01$). Nevertheless, plasma cholesterol (lipoproteins) did not show any reduction, otherwise previously observed in a longer period and in more advanced lactation (Bertoni *et al.*, 1989). However liver TG content showed a significant increase only after calving in both groups, the peak was observed around one week of lactation (about 10 % on wet weight). ETX group demonstrated slightly higher values of liver TG 5 day after treatment (10.49 vs 9.10 %, but not significant) and a slower decrease in the following week. Finally, an immune system activation has been confirmed by the increase of globulins after 24 h from treatment (37.43 vs 34.22 g/l, $P<0.05$).

Conclusions

The LPS treatment of dairy cows at the beginning of lactation has caused a typical inflammatory situation that negatively affected milk yield and feed intake with a quick recovery. At metabolic level, a strong mobilization effect has been observed (lipolysis and muscle proteins breakdown, while glycogenolysis seemed very small).

Nevertheless, an increased risk of ketosis could be excluded as β -hydroxybutyrate was slightly reduced, as well as a serious cytolytic damage of liver does not appear possible for the relatively small and short-lived increase of liver enzymes.

Otherwise a reduced liver synthesis of usual proteins (i.e. albumin and lipoproteins) has been demonstrated, but perhaps the inflammatory event was too short to significantly impair the TG excretion and therefore the liver lipidosis status (anyway higher in the treated cows).

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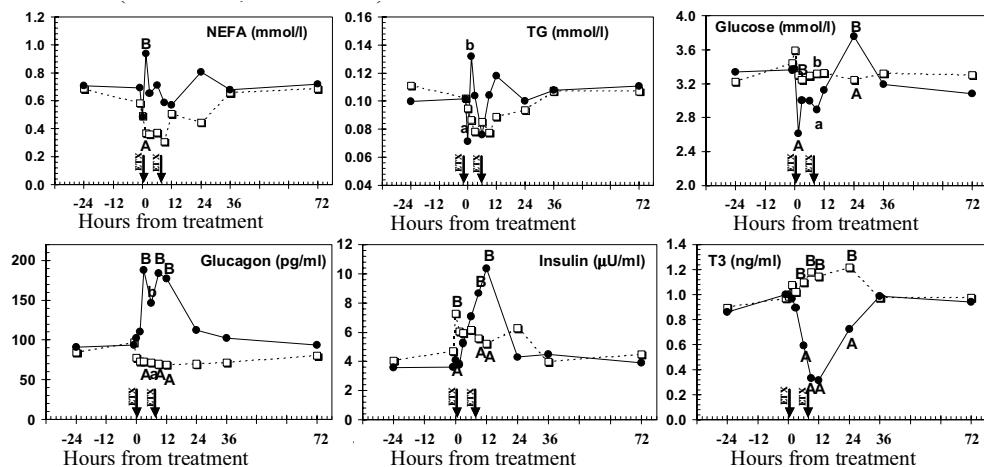


Figure 1. Pattern of changes of plasma NEFA, TG, Glucose, Glucagon, insulin and T3 in dairy cows treated (●) or not (□) with endotoxin (↓) at their 3rd day of lactation (a-b $P<0.05$; A-B $P<0.01$).

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Pancreatic juice protects gut from pathogenic bacteria

Danuta Kruszewska¹, Åsa Ljungh¹ & Stefan G. Pierzynowski²

¹Dept Med Microbiology, Dermatology and Infection, Lund University, Sölvegatan 23, SE-223 62 Lund, Sweden

²Dept Cell and Organism Biology, Animal Physiology, Lund University, Helgonavägen 3b, SE-223 62 Lund, Sweden and Marine Fisheries Institute, Kollataja 1, 82-332 Gdynia, Poland

Summary

The role of the exocrine pancreas in regulating gut microflora colonization is unclear. The main objective in the present study was to assess the affect of pancreatic fluid on the growth of pathogenic bacteria and fungi. Pancreatic juice samples used in the study were obtained from nine eight-week old weaned pigs in which catheters were implanted in the pancreatic duct. The antibacterial activity of pure pig pancreatic juice collected from healthy, conscious and also anaesthetized pigs was investigated with multi-resistant microbial isolates and non-pathogenic strains. Studies were performed on 23 bacterial and 2 *Candida albicans* isolates, including 4 lactic acid bacteria (LAB) and 3 reference strains. Pancreatic juice was effective ($p<0.01$) against multi-drug resistant bacterial pathogens, whereas other strains had only moderate sensitivity ($p<0.05$) to its antibacterial action and furthermore LAB were insensitive. The antibacterial action was independent of pancreatic juice proteolytic activity and stable when measured before and after enterokinase activation of trypsinogen. We demonstrated *in vitro*, that the antibacterial properties of pancreatic juice last for several hours. Our data suggests that broth composition may modulate the intensity of pancreatic juice antibacterial activity *in vitro*; this can have implications for digestive related antibacterial activity *in vivo*. Thus, pancreatic juice antibacterial activity may be an important factor in limiting the colonization of pathogenic bacteria in the gastrointestinal tract, in both the small and the large intestines. We postulate that observed antibacterial activity of the pancreatic juice could play an important role as one of the factor of innate immunity.

Keywords: pancreas, innate immunity

Introduction

The exocrine pancreas is regulating gut microflora colonization upper small bowel since this part of the intestine is usually low loaded with bacteria: The invasion of microorganisms is immediately opposed by innate defense mechanisms e.g., the amplification of levels of the pancreatic cationic antimicrobial peptide beta defensin-1 (hBD-1), detected as expression of hBD-1 mRNA in pancreas acini (Schnapp et al, 1988). It is postulated that the antimicrobial activity of the pancreas is responsible for the resistance of this organ to primary bacterial infections (Gyr et al, 1985) e.g., in animals no pathology has been described relating to primary pancreatitis and in humans pancreatic infections are rare and most likely the result of bacterial translocation. The objective of these studies was to explore the effect of pancreatic fluid on the growth of pathogenic microorganisms.

Material and methods

The antibacterial activity of pig pancreatic juice obtained from healthy, conscious and from anaesthetized pigs was investigated against resistant microbial isolates and comensal strains. Studies were performed on 23 bacterial isolates and 2 *Candida albicans* isolates, including 4 lactic acid bacteria (LAB) and 3 reference strains.

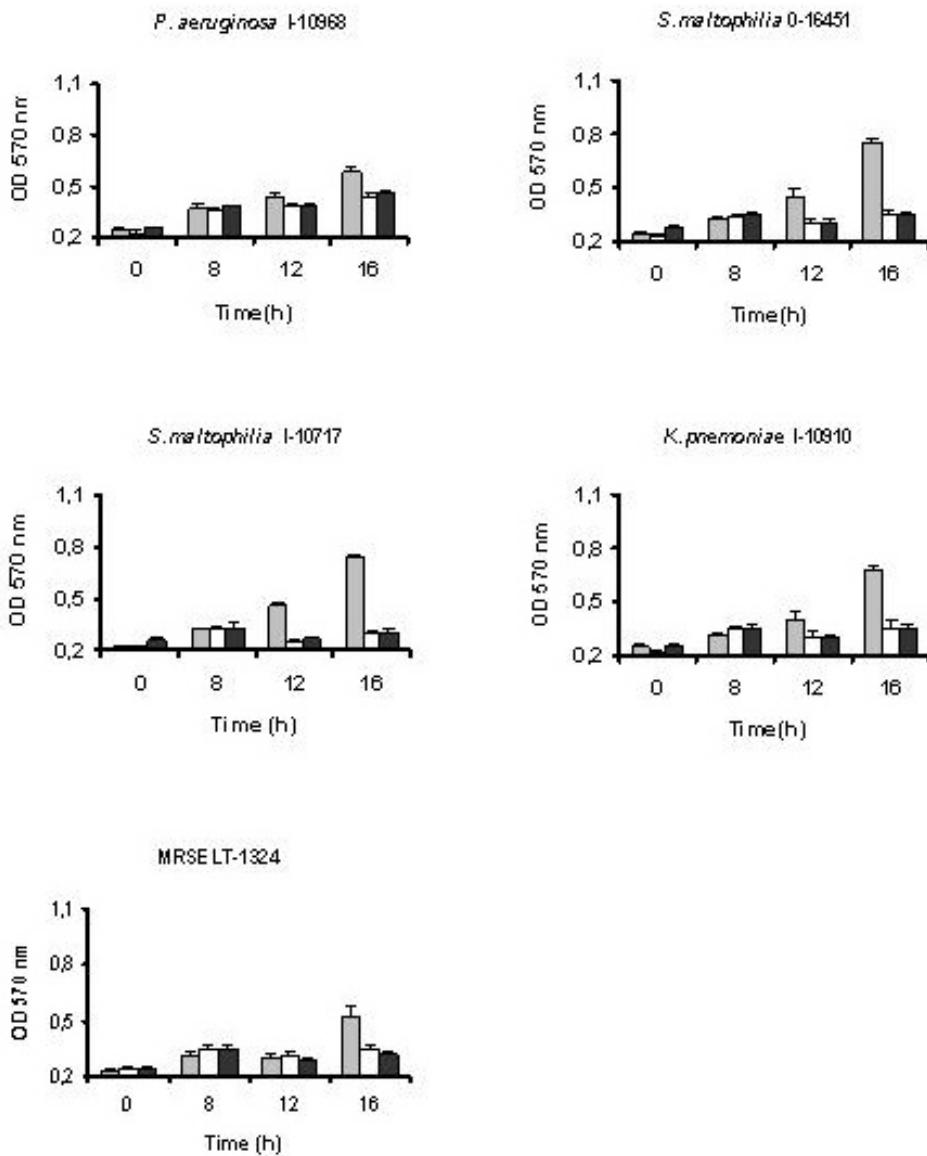


Figure 1. Antibacterial activity of the fresh pancreatic juice and juice processed together with broth ($n=6$, means \pm SD). Absorbance (OD_{570}) changes during bacterial growth are presented as bars where: gray bars indicate OD in control conditions; white bars indicate OD corresponding to bacterial growth in MHB enriched with fresh PJI, and black bars represent the OD of bacterial growth in MHB previously overnight preincubated together with PJI at 37°C.

Results

Pancreatic juice was effective against multi-drug resistant pathogens, whereas nonpathogenic bacteria were moderately sensitive to its action and LAB were insensitive. The antibacterial action is independent of proteolytic activity, lasts for several hours and remains stable and independent of enterokinase activation of trypsinogen to trypsin.

The growth patterns of the selected bacteria were produced following 16 hours incubation in the presence of pure PJ1. The effect of antibacterial activity on pathogenic bacteria was varied from bacteriostatic to bactericidal as presented on Figure1.

Discussion

Data obtained clearly shown the existence of a host regulatory mechanism of antimicrobial factors present in pancreatic juice, as postulated previously (Gyr et al., 1985, Pierzynowski et al., 1993). The gut mucosal barrier is one of the factors protecting the host from pathogenic microflora. This role is mainly realised via a broad spectrum of antibiotic acting peptides of different origin. This may include a pancreatic juice antibacterial protein component, which bathes the epithelial surface of intestine. Zhao et al. (1996) localised alpha-defensins HD5 and HD6 mRNA in Paneth cells. The human pancreas expresses high levels of hBD-1 mRNA and it is postulated that focus should be applied to the pancreas as an innate immunity organ (Porter et al., 2002).

From the clinical point of view, it is important to elucidate the involvement of pancreatic juice related antibacterial action (Rubinstein et al., 1985; Pierzynowski et al., 1992; Pierzynowski et al., 1993) on the development of gut bacterial infections in animals and humans. The participation of pancreatic juice component in the innate immunity to protect microbial ecological niches from pathogenic multi-resistant bacteria is fascinating, specially if we consider that LAB strains colonizing the GIT are only slightly inhibited by these factors ($p<0.05$), as compared to clinical microbial strains (data not shown). Thus, under *in vivo* conditions, pancreatic juice may have a impact on GIT bacterial colonization and animal health.

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Glutathione changes in blood and amino acid concentrations in plasma in response to nematode infection in Merinos selected for parasite resistance

S.M. Liu¹, T.L. Smith¹, P. Young¹, D.G. Palmer², L.J.E. Karlsson² & R.B. Besier²

¹ CSIRO Livestock Industries, Private Bag 5, PO Wembley, WA 6014, Australia

² Department of Agriculture of Western Australia, Locked Bag 4, Bentley Delivery Centre, WA 6983, Australia

Summary

Blood glutathione (GSH), plasma amino acid concentrations and production were examined using 40 parasite resistant and 20 susceptible Merino hoggets without or with infection of *Trichostrongylus colubriformis* and *Ostertagia circumcincta*. Resistant sheep had significantly lower GSH concentration ($P < 0.05$) during eighteen weeks of infection compared with susceptible animals. Infection did not affect GSH or the total amino acid concentrations, but altered the amino acid profile with Phe, Tyr, Thr, Ala and Glu significantly reduced ($P < 0.05$). For both genotypes, feed intake and body weight change were not affected by the parasite challenge.

Keywords: immune response, sheep, FEC

Introduction

Increasing resistance to anthelmintics by internal parasites is a major problem in the sheep industry. Selecting animals for resistance to parasites is an effective approach to help combat the problem. The Department of Agriculture of Western Australia has been selecting sheep (Rylington Merino), based on a measurement of faecal egg count (FEC) for increased resistance to sheep worms for the past decade. Field tests have shown the resistant line has significantly lower FEC compared with the control line (Greeff & Karlsson, 1997). Some aspects of the mechanism of worm resistance are poorly understood. This indoor experiment was conducted to further characterize the mechanisms for resistance.

Materials and methods

40 resistant and 20 susceptible (as indicated by low and high FEC in the field) male Merino hoggets (about 19 month old) were used in this study. The sheep were kept in individual pens and fed *ad libitum*. The diet consisted of 760 g/kg oaten hay, 110 g/kg barley, 110 g/kg lupin seeds and 20 g/kg Siromin (mineral mix), providing an estimated 8.6 MJ/kg of metabolizable energy and 50 g/kg dry matter of metabolizable protein. Daily intakes ranged from 2.6% to 3.1% of their body weights, and the energy intake was approximately $1.6 \times$ maintenance.

The animals were treated with anthelmintics (abamectin, levamisole and oxfendazole) upon arrival at the animal house and again three weeks later, aiming for complete removal of parasites from the digestive tract. The experimental period then began and continued for twenty-four weeks. All the animals were maintained in a parasite-free condition during the first six weeks, confirmed by zero egg counts in the faecal samples collected in weeks three and six. Half the sheep (20 resistant and 10 susceptible) were then orally administered larvae of *Trichostrongylus colubriformis* and *Ostertagia circumcincta*, three times per week from week seven until week twenty-four (the infection period). The weekly dosage was 10,000 larvae from each species. The remaining sheep (20 resistant and 10 susceptible) were used as controls.

Faecal samples from infected sheep were collected weekly from week 9 until week 24, and FEC in the samples were measured within 24 hours of collection. Blood samples were collected from all sheep every fortnight during the first 6 weeks, and then weekly for the remainder of the experiment. Approximately 0.5g of blood was immediately weighed and processed for analysis of glutathione concentration according to the method used by Mata *et al.* (1995). Plasma was harvested from the blood samples and stored at -20°C. Amino acid concentrations were measured using plasma collected in week eighteen (infection period) from six sheep with the lowest FEC in the resistant line (0 to 5 eggs per g) and six sheep with the highest FEC in the susceptible line (590 to 1870 eggs per g), using an Amino Acid Analyser (Biochrom 20). Plasma samples collected in week six (pre-infection period), from the same sheep, were also analysed to examine the effect of parasite infection on amino acid concentrations.

The glutathione results were statistically analysed with repeated ANOVA procedure with genotype and infection as factors. The amino acid concentration results were analysed with ANOVA procedure with genotype and infection (before and after infection) as factors. The concentration ratio of after to before the infection was also calculated and a t-Test applied to examine genotype difference in response to the infection. All analyses were performed using Genstat 6.1 (VSN International Ltd, 2002).

Results

Average FEC in the parasite resistant sheep from week nine to week twenty-four of the experiment was 76 eggs per g, substantially lower than the average of 716 eggs per g in the susceptible sheep (Figure 1). There were no significant differences between genotypes in daily voluntary feed intake or weekly live weight changes throughout the experimental period.

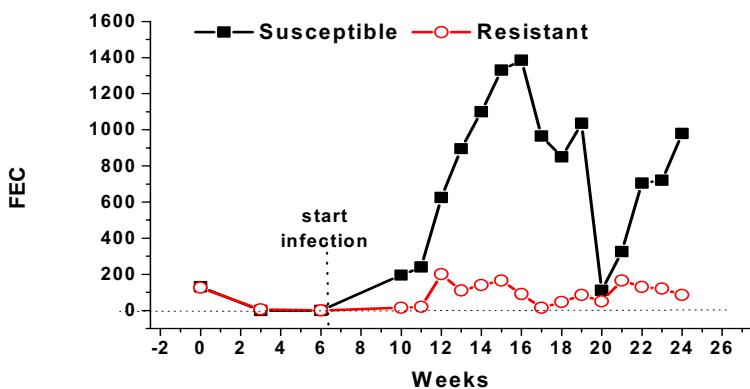


Figure 1. Faecal egg counts (FEC, eggs per g) in nematode resistant and susceptible Merinos infected with *T. colubriformis* and *O. circumcincta*.

There was no significant difference ($P > 0.05$) in GSH concentration during the pre-infection period between the genotypes. Post-infection, the concentration of GSH in resistant sheep was considerably lower ($P < 0.05$) compared with susceptible sheep. Infection itself did not have a significant effect ($P > 0.05$) on GSH concentrations compared with uninfected sheep.

Nematode infection reduced plasma concentrations of Phe, Tyr, Thr, Ala, Glu and Taurine, whereas Gly concentration increased ($P < 0.05$, Table 1). There were no significant differences ($P > 0.05$) between genotypes in amino acid concentrations, or in the relative changes in concentrations in response to infection. There were no significant differences in the total concentration of essential (1367 vs 1280 μ M) and non-essential (2385 vs 2443 μ M) amino acids between the control and infected sheep ($P > 0.05$).

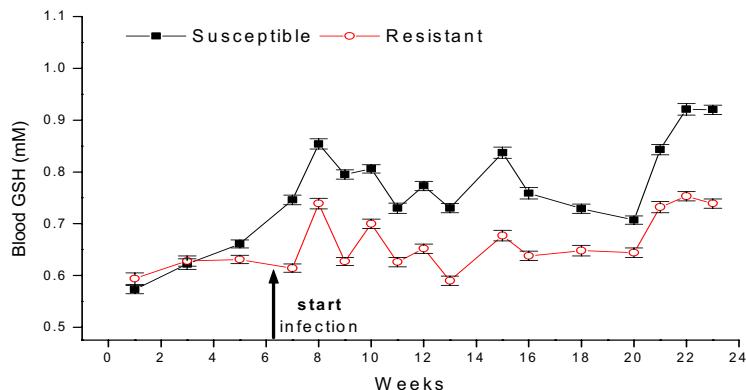


Figure 2. Glutathione concentration in whole blood of Merinos infected with nematodes.

Table 1. Amino acid concentrations ($\mu\text{mol/L}$) in plasma of Merino lambs with ($n=12$) or without ($n=12$) infection with *T. colubriformis* and *O. circumcincta*.

| Amino acids | Uninfected | Infected | Significance (P value) | Amino acids | Uninfected | Infected | Significance (P value) |
|-------------|------------|----------|------------------------|-------------|------------|----------|------------------------|
| His | 69 | 64 | ns | Ala | 300 | 258 | 0.024 |
| OH-Pro | 25 | 21 | ns | Arg | 162 | 132 | 0.08 |
| Ile | 88 | 95 | ns | Asp | 287 | 257 | 0.09 |
| Leu | 111 | 105 | ns | Cit | 174 | 189 | ns |
| Lys | 189 | 211 | ns | Gly | 826 | 998 | 0.01 |
| Met | 25 | 28 | 0.08 | Glu | 173 | 109 | 0.001 |
| Phe | 60 | 52 | 0.02 | Gln | 357 | 376 | ns |
| Pro | 147 | 136 | ns | Orn | 84 | 84 | ns |
| Thr | 197 | 141 | 0.007 | Ser | 154 | 174 | ns |
| Tyr | 122 | 104 | 0.04 | Taurine | 29 | 18 | 0.046 |
| Val | 167 | 193 | ns | | | | |

ns = not significant, $P > 0.1$.

Discussion

Selection for parasite resistance successfully reduced FEC as expected, and worm burden was lower when compared with susceptible sheep as measured when the animals were slaughtered. This confirms the existence of a strong resistance to nematodes in the selection line. As there were no significant differences between genotypes in the voluntary feed intake, dry matter and N digestion (data not shown), body weight change (data not shown) and amino acid concentration in the plasma, the nematode resistance in Rylington Merinos did not result in any obvious overall changes in energy and protein metabolism and utilization.

Nematode resistance in this line of Merinos could partly be due to variations in some metabolic pathways. The significantly lower GSH concentration in the resistant line under parasite-infected conditions seems to support this hypothesis. GSH is an antioxidant, it provides cysteine to tissues and affects the redox potential of the tissue (Grimble 2002). The immune system is particularly sensitive to oxidative stress (Hughes 2002), and there is a correlation between tissue GSH and immune functions (Grimble 2002). If the resistance to nematodes is related to a strong immunity

in these sheep, the lower GSH concentration in blood suggests that resistant sheep could have a high rate of GSH use by tissues.

Parasite infection did not affect the total concentrations of amino acids in the plasma, though intake (as % of body weight) was reduced by 6% ($P < 0.05$), but amino acid profiles were altered. The reduction in Phe, Tyr, Thr, Ala and Glu suggest that these amino acids could be involved in host-parasite interactions. Glu is the single largest contributor to intestinal energy generation. In piglets, 95% of the dietary Glu presented to the mucosa was metabolized in the first pass in intestinal tissue, 50% of which was oxidized to CO_2 (Reeds *et al.*, 2000). Higher consumption of Glu would result from increased oxidation rate of amino acids in the intestinal tissue during parasite infection (Yu *et al.*, 2000), necessary for repairing tissue damage. Glu is the substrate for biosynthesis of Gln, which in turn is used at a high rate by cells of the immune system (Calder & Newsholme, 2002). In rats, Glu supplementation improved immune status of subjects immunized with an injection of methotrexate (Lin *et al.*, 1999), indicating a higher demand for Glu. The functions of other amino acids in an immune response to parasite infection are not clear, and metabolism in both the host and parasite needs further consideration.

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Does chronic lung inflammation induce an increase in tryptophan catabolism through IDO (indoleamine 2,3- dioxygenase) pathway in pigs?

D. Melchior, N. Mézière, B. Sèvre & N. Le Floc'h

Institut National de la Recherche Agronomique, Unité Mixte de Recherches sur le Veau et le Porc-35590 St-Gilles, France

Summary

In a preliminary study we observed that piglets suffering from chronic lung inflammation induced by an i.v. injection of complete Freund's adjuvant (CFA) showed a marked decrease in plasma tryptophan (Trp) concentration suggesting increased Trp utilization. During inflammation, Trp provided by food and accelerated muscle protein catabolism could either be catabolized to kynureneine under activation of a cytokine-induced enzyme called indoleamine 2,-3 dioxygenase (IDO) or serve for specific molecules synthesis such as acute phase proteins. IDO pathway has been documented in mammals mainly for clinical issues but has never been explored in pigs. In order to test if the decrease in Trp following chronic lung inflammation in pigs was due to an increase in Trp catabolism under IDO activation, we compared IDO activity in lung and tracheo-bronchial lymph nodes from 7 CFA challenged piglets with 7 pair-fed littermate healthy controls. After catheterisation of the jugular vein, one littermate received i.v. CFA whereas the other littermate was injected with a sterile saline solution (CON). Piglets within a litter were pair-fed in order to avoid the confounding effect of feed intake on plasma Trp and kynureneine concentrations. Blood sample were taken at 0, 2, 5, 7 and 10 days following CFA injection to measure Trp, kynureneine and haptoglobin concentrations. At day 10, pigs were slaughtered and there lung and tracheo-bronchial lymph nodes were removed for IDO activity assay. IDO activity in tracheo-bronchial lymph nodes ($P < 0.05$) and plasma haptoglobin ($P < 0.01$) were higher in challenged pigs than in controls. Plasma Trp and kynureneine were not significantly affected by CFA injection. The lack of response of plasma Trp may be due to high level of Trp relative to lysine in the food. Our data show that IDO is activated under chronic lung inflammation in pigs but the contribution of IDO pathway to total Trp fluxes is still unknown. Besides, the roles and functions of IDO pathway remain to be explored.

Keywords: tryptophan, indoleamine 2,-3 dioxygenase, inflammation

Introduction

During inflammation and stimulation of the immune system, amino acids (AA) are deviated from growth and normal physiological issues towards tissues and cells involved in inflammatory and immune responses. Little is known about AA requirements caused by inflammation and immune response in pigs. Improved knowledge of these processes will provide opportunities for nutrition intervention to cover nutritional requirements for both growth and animal defenses. In a preliminary study we observed that piglets suffering from chronic lung inflammation had significant lower plasma tryptophan (Trp) concentrations than pair-fed healthy piglets suggesting increased Trp utilization (Melchior et al. 2002). Two main hypotheses could explain these observations. Reeds et al. (1994) proposed that Trp could be incorporated in Acute Phase Protein (APP). Secondly, Trp catabolism through the kynureneine pathway could be increased. Two known enzymes catabolize Trp to kynureneine. Tryptophan 2,3 dioxygenase (TDO) is expressed predominantly in the liver and regulates systemic Trp concentrations. A second enzyme, with a

larger tissue distribution, indoleamine 2, 3 dioxygenase (IDO) is inducible by inflammatory mediators such as INF- γ and other cytokines. Activation of IDO pathway has been reported in various cases of immune system activation (Brown et al., 1991) and may play crucial roles in the regulation of the immune response and body defenses (Christen et al., 1990; Mellor and Munn, 1999; Pfefferkorn, 1984). Yet it has never been explored in pigs. The objective of this study was to determine if the decrease in plasma Trp concentrations previously observed in piglets suffering from chronic lung inflammation can be explained by the induction of IDO activity.

Material and methods

All procedures were performed according to current legislation on animal experimentation in France (authorization N ° 7719 delivered by the French Ministry of Agriculture and Fisheries). Eighteen days post-weaning, seven pairs of littermate piglets were selected on the basis of their body weight. One littermate received 3 mL of CFA via a jugular catheter whereas the other littermate was injected with an equal volume of sterile saline solution (CON). CFA is a mineral oil that contains inactivated *Mycobacterium tuberculosis* cells. Its intra-venous injection induces interstitial pneumonia (Edwards and Slauson, 1983). The piglets were fed with a commercial phase II post-weaning diet. The daily ration was calculated to be 40 g of food /Kg of body weight. Because inflammation can induce anorexia, CON pigs were pair-fed the intake of the CFA pigs. Blood sampling were taken 0, 2, 4, 7 and 10 days after CFA injection after an overnight fast. Plasma Trp and kynurenine concentrations were measured by an HPLC method (Widner et al., 1999). Plasma haptoglobin was quantified using a commercial kit (Haptoglobin Assay, Tridelta, Ireland). Haptoglobin is a major APP in swine used as an indicator of inflammation. At day 10, piglets were slaughtered. Because lung are the target tissue of CFA challenge, IDO activity was measured in lung and lymph nodes removed after slaughter and kept at - 80° C until analysis. Tissues were ground in liquid nitrogen and homogenized in a ice cold potassium buffer, pH 7.0 with a polytron homogenizer (Kinematica, Suisse) at 9500 rotations per minute. The homogenates were centrifuged at 14,000 g for 30 min at 4 °C and IDO activity in the supernatant was determined according to the method described for mice by Lestage et al. (1999). Data were analyzed with the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The effect of inflammation was always tested using inflammation x pair as the error term.

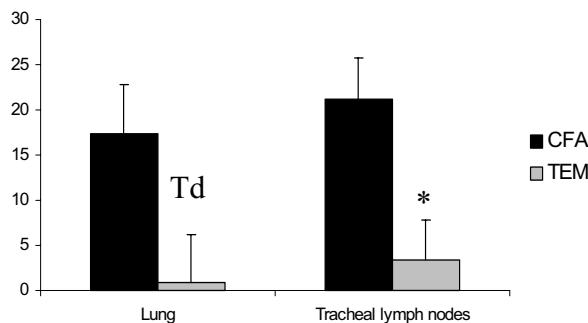
Results

Plasma haptoglobin was higher in challenged than in control pigs ($P < 0.05$, Table 1). Plasma Trp and kynurenine were not significantly affected by CFA injection (Table 1). At slaughter, macroscopic granulous pulmonary lesions were observed in CFA pigs. IDO activity in tracheo-bronchial lymph nodes was higher in challenged than in control pigs ($P < 0.05$, Figure 1). IDO activity in the lung tended to be higher in CFA pigs than in CON pigs ($P < 0.07$, Figure 1) because IDO was not induced in two piglets from the CFA group.

Discussion

Lung lesions observed at slaughter and the higher plasma haptoglobin concentrations in CFA pigs indicate that CFA injection induced a lung inflammation. For the first time we have shown that IDO is induced in lung and broncho-tracheal lymph nodes following lung inflammation in pigs. IDO, a rate limiting enzyme for the catabolism of Trp to kynurenine, can be induced by interferon- γ (Pfefferkorn, 1984) and other cytokines (Liebau et al., 2002). In pigs as in other species, the roles of IDO pathway remains to be understood. IDO activation could be an inducible host defense mechanism (Pfefferkorn, 1984), a cell proliferation modulator (Mellor and Munn, 1999) and a free-radical protector mechanism (Christen et al., 1990). This latter role is attributable directly to

IDO activity nmol Kynurenine/mg protein/H



*Figure 1. Indoleamine 2,3 dioxygenase (IDO) activity in the lung and tracheo-bronchial lymph nodes in healthy pair-fed pigs (CON) or in pigs injected i.v. with 3 mL of complete Freund's adjuvant (CFA). Data are least squares means \pm SEM, n = 14. Td indicates p < 0.1 and * indicates p < 0.05.*

Table 1. Plasma Trp, kynurenine and haptoglobin concentrations in healthy pair-fed pigs (CON) or in pigs injected i.v. with 3 mL of complete Freund's adjuvant (CFA) measured after an overnight fasting. Data are least squares means calculated from the whole experimental period \pm SEM, n = 14.

| | CFA | CON | SEM | C ¹ |
|----------------------|-------|-------|------|----------------|
| Trp (nmol/mL) | 36.79 | 38.77 | 2.91 | NS |
| Kynurenine (nmol/mL) | 1.08 | 1.12 | 0.05 | NS |
| Haptoglobin (mg/mL) | 2.01 | 1.24 | 0.18 | * |

¹ C indicated the CFA challenge effect, NS indicated that P-value > 0.05 whereas the sign * indicated significant effects of CFA challenge P < 0.05.

IDO that removed superoxyde radicals using them in its catalytic process. It would act also indirectly via the production of metabolites that may function as free-radical scavengers and antioxidants (Christen et al., 1990). IDO induction often results in decreased plasma Trp and increased plasma kynurenine concentrations (Widner et al., 2000). In the present study, plasma Trp and kynurenine concentrations were not affected by the lung inflammation despite an increase in IDO activity. The lack of kynurenine accumulation may be explained by the fact that kynurenine can be hydrolyzed in other active metabolites as it is suggested above. Yet the apparent regularity of plasma Trp concentrations in CFA pigs is intriguing and contrast with our previous results (Melchior et al., 2002). Analysis of dietary AA composition showed a ratio Trp/Lysine (0.25) higher than the requirement for growing piglets as determined by Chung and Baker (1992) and higher than the ratio measured in our previous study (Melchior et al., 2002). Therefore Trp was probably not limiting in the present experiment. In other words, Trp utilization through the IDO pathway may have been largely covered by the excess Trp dietary supply. On this basis, we question the meaning of plasma data as indicator of metabolic fluxes. The contribution of IDO pathway to total Trp fluxes needs to be evaluated by other techniques like tracer infusion. Moreover, because the IDO pathway is involved in the control of various immune and body defense functions, the activity of this enzyme and the associated metabolic pathway deserve further studies in pigs.

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Energy session 1

Environmental Aspects of Energy Homeostasis

Regulation of the energy metabolism: nutritional, environmental and molecular aspects

B. Löhrke¹, W. Jentsch² & M. Derno²

¹ Research Institute for the Biology of Farm Animals, Department of Genetics and Biometry,
„Oskar Kellner”, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

² Research Institute for the Biology of Farm Animals, Department of Nutritional Physiology
„Oskar Kellner”, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

Summary

Whole body thermogenesis increases with higher intake of metabolizable energy. Beyond the energy required for maintenance, the energy assimilated in form of body growth or milk production is linearly correlated with intake of metabolizable energy. Therefore, regulation of this intake and heat production at rest are of pivotal interest to develop strategies for the improvement of animal's productivity. Metabolic control analyses determining the strength of the control that individual processes exert over cellular fluxes and metabolic levels revealed that control is shared within a system with no single rate determining step being present. In turn, mitochondria and the regulation of their activity appear to be central for the thermogenic control. Biogenesis of mitochondria has been reported recently to be dependent on NO. In consequence, transcription factors are of interest, which influence the expression of enzymes that catalyse the synthesis of NO. Among those, PPAR γ regulates negatively the expression of NO synthetase (NOS). PPAR γ has been found to be diet-responsive in ruminants and non-ruminants. High intake of metabolizable energy decreases PPAR γ protein in bovine cardiomyocytes. This response may allow higher NOS expression and NO production, in consequence an inhibition of electron transport chain but a stimulation of mitochondrial biogenesis. Given, these data are confirmed and are true also in other tissues important for whole body heat production at rest, they explain both the increases in oxygen consumption with higher intake of metabolizable energy and dietary-digestive-metabolic-interactions observed at the whole body level.

Keywords: *thermogenesis, mitochondrial biogenesis*

Introduction

Interactions between nutritive potential and metabolic processes

Productivity of farm animals depends on the intake of metabolizable energy. An essentially linear positive correlation exists between an intake of metabolizable energy beyond the requirement for maintenance and the assimilated energy occurring as tissue growth in growing animals (Schiemann et al., 1976, 1987) or milk output in dairy cows (Schiemann et al., 1970) as shown in Figure 1.

In high-productive dairy cattle, the strongly improved overall efficiency of feed conversion appears therefore largely accountable by a simple dilution of maintenance needs (Table 1).

Calculations of the requirement of metabolizable energy for maintenance relative to the performance may suggest that differences in feed use seem to mirror altered partitioning of nutrients while the efficiencies of partial processes appear to remain effectively unchanged (Oldham, 1999). The ranges seem to be consequent on differences in body composition, the size and metabolic rate of the visceral organs (Taylor & Murray, 1991). However, the efficiencies of metabolic partial processes may become important with the view of considering the low fertility

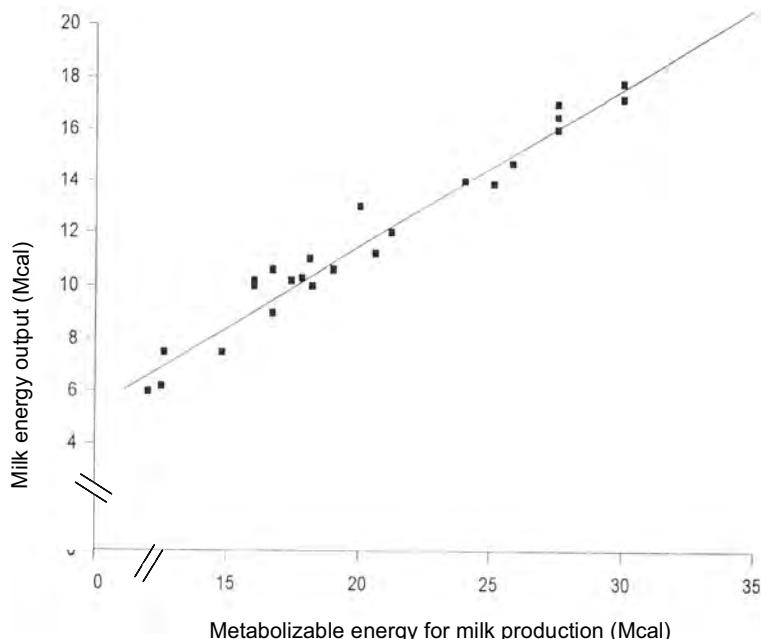


Figure 1. Relationship between milk energy output and intake of metabolizable energy.

Table 1. Commonly accepted values for the requirement of metabolizable energy (ME) for maintenance in thermoneutral conditions.

| | MJ ME/kg ^{0.75} | Relative net energy for maintenance |
|----------------------------------|--------------------------|-------------------------------------|
| Dry beef cows | 0.40 - 0.55 | >70 % |
| Lactating beef cows | 0.50 - 0.65 | 45-55% |
| Lactating dairy cows | 0.65 - 0.77 | 35-55% |
| Dairy cows > 20.000 kg milk/year | | < 25 % |

of modern high genetic merit dairy cows (Robinson, 1999). Currently, high productivity in ruminants can be achieved only through use of large amount of dietary grains.

This is acceptable if grain is available and can be produced in a sustainable manner, i.e. with a low environmental impact (Satter et al., 1999). However, adverse effects of high-protein diets on fertility of modern high genetic merit dairy cows have been drawn attention on the nutritional management. The mechanisms underlying associations between nutritional and reproductive regulations are poorly understood, but excess in ruminally degradable protein decreases the pH of uterine secretions. Particularly, ammonia impairs ion transport of the endometrium during the luteal phase (Elrod & Butler, 1993, Butler et al., 1996) and exerts direct effects on the metabolic activity and viability of embryos (Mc Evoy et al., 1997). Impaired fertility decreases the overall efficiency of feed conversion and cannot be explained only by altered partitioning of nutrients.

In light of recent reports, dietary-digestive-metabolic interactions have also to be considered. With regard to fermentation, ruminal efflux of metabolizable protein correlates with metabolizable energy intake (kJ/kg body weight) as shown in Figure 2.

In addition, a strong relationship exists between the microbial crude protein efflux from the rumen (kg/d) and the flux of fermented carbohydrates (kg/d). In contrast, Figure 2 demonstrates that daily

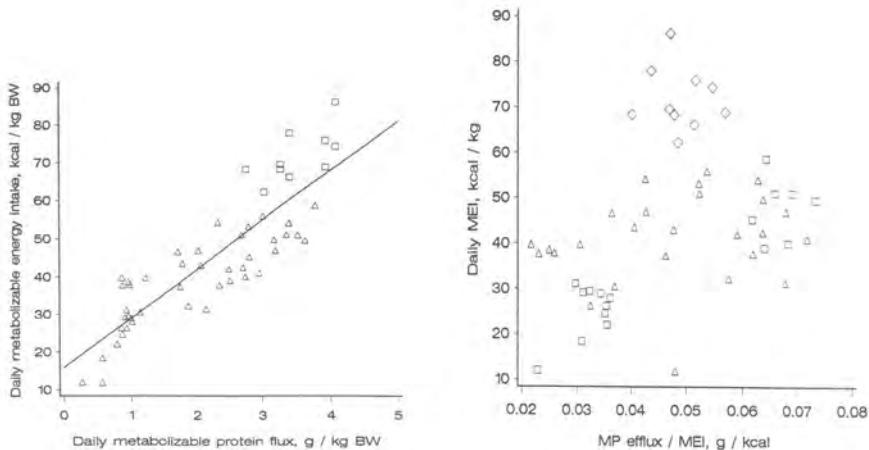


Figure 2. Daily metabolizable energy intake (MEI) is related to ruminal efflux of metabolizable protein (MP) but not to MP efflux / MEI in growing (triangle) or lactating (square) cattle fed forage (Ellis et al. 1999).

voluntary metabolizable energy intake (kJ/kg body weight) fails to correlate with ruminal metabolizable protein efflux per ME intake (Ellis et al., 1999).

Thus comparable variables yield different relationship, perhaps, because variation among animal's nutritional requirements is included in the voluntary metabolizable energy intake. Individual nutritional factors, such as anabolic and catabolic utilizations of amino acids, have been proposed to be most important, regulating voluntary intake within the physical constraints imposed on nutrient acquisition (Ellis et al., 1999).

Figure 3 shows that the escape of neutral detergent-soluble fiber can inversely vary with a daily metabolizable protein flux from the rumen dependent on loading with neutral detergent - soluble fiber. This yields interactions that are not explainable without reference to the intake - driving force of nutritional status of the ruminant's tissue metabolism. In turn, details of the mechanism determining ruminant's metabolism regulated by diets are largely lacking.

Results and discussion

Adaptive whole body thermogenesis

A common, non-invasive technique to measure oxidative metabolism of whole body uses respiration chambers to record the gas exchange in response to nutritional variants and ambient temperatures. In the idealised resting state an animal does not net work on its environment and there is no net synthesis of cellular macromolecules. Then all of the energy arising from oxidation of fuels is released as heat regardless of particular substrate turnover.

With the view of particular pathways, oxidations in mitochondria are coupled or not with ATP production. Uncoupled reactions are linked to so-called thermoregulatory heat. This heat is induced by cold ambient temperature. The ambient temperature that triggers thermoregulatory heat is defined as critical temperature. However, the mechanism underlying these regulations are poorly understood. There are differences among species regarding the induction of heat without ATP production. In cattle, swine and dog, the induction depends on the level of intake of metabolizable energy. With higher intake, oxidative processes rise in an extent that additional heat is not needed to maintain body core temperature. At high energy intake, the heat required to maintain constant body temperature appears to be produced without marked induction of

Ruminal neutral detergent- soluble fiber loading (NDFL)

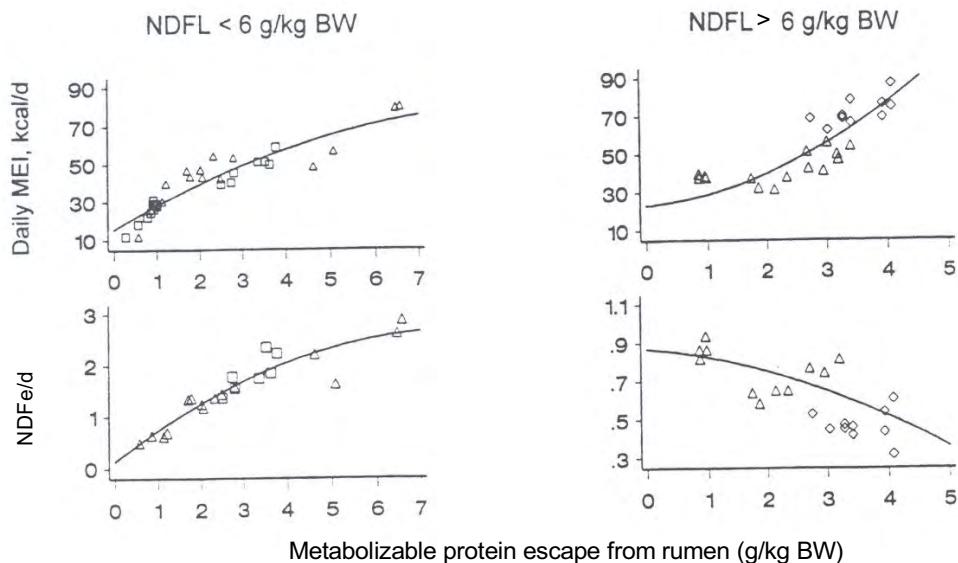


Figure 3. Ruminal neutral detergent- soluble fiber (NDF) loading (NDFL) alters the relation between metabolizable protein escape from rumen and metabolizable energy intake (MEI) as well the escape of NDF from total ruminal digesta (NDFe) in growing (triangle) or lactating (square) cattle fed forage (adapted from Ellis et al. 1999).

uncoupling the respiration chain in decreasing the ambient temperature. As a result, the critical temperature can decrease. This regulatory type has been previously called heat compensation (Rubner, 1902).

In light of recent studies on the regulation of heat production, this regulatory response is of considerable interest. It does not occur in species with brown fat, such as rodents, or with developing a high muscle mass, such as broiler chicken. Heat production seems to be stronger controlled by uncoupling the respiration chain in these animals. Therefore, heat is induced in a fashion, which is directly related to the ambient temperature with contributing to the whole body heat production in an additive fashion.

Table 2. High intake of metabolizable energy shifts heat production ($\text{kJ}/\text{kg}^{0.75}\text{d}$) in young bulls exposed to cold ambient temperatures.

| 1.0-times MEm | | 2.0-times MEm | |
|---------------|----------|---------------|----------|
| 18°C | 4°C | 18°C | 4°C |
| 466 ± 15 | 593 ± 38 | 564 ± 29 | 567 ± 24 |

Energy balance

Assimilation of forage energy is based on ATP production by the mitochondrial respiration and the cytosolic glycolysis. ATP equivalents are then used in body's syntheses. Cellular ATP turnover has historically been argued to be controlled by ATP consumption. In contrast, recent studies on muscle, hepatocytes and liver have suggested that mitochondrial function controls ATP turnover (Soboll et al., 1998). In turn, metabolic control analyses, determining the strength of the control that individual processes exert over cellular fluxes and metabolite levels, have indicated that control is shared within a system with no single rate determining step being present (Kacser & Burns, 1973, Ainscow & Brand, 1999). Processes that consumed ATP accounted for about 35 % of the control over the rate of ATP consumption. Reactions that produced ATP exerted the most control (49 %) over ATP consumption rate, particularly the mitochondrial phosphorylation (30 % of control) and glycolysis (19 %). Glycolysis had little control over the rate of ATP production by mitochondria while mitochondrial phosphorylation and proton leak processes exerted significantly negative control over glycolysis (Ainscow & Brand, 1999). Studies on the production of ATP by mitochondria with regard to the control of oxygen consumption in rat hepatocytes showed that control over respiration was shared between the reactions that produced mitochondrial membrane potential and those that dissipated it (Harper & Brand, 1993).

One of the most important energy-dissipating cycles is the futile cycle of proton pumping and proton leak across the inner mitochondrial membrane. This natural uncoupling of mitochondria allows oxygen consumption without ATP synthesis (Figure 4).

Mitochondrial proton leak contributes significantly to the respiration rate. In resting hepatocytes about 26 % of the respiration is used to drive the proton leak pathway. Hepatocytes stimulated to produce glucose and urea double their respiration. Then the proportion accounting for proton conductance drops to 22 %. In perfused resting rat hindquarter, the proton leak reaction accounts for roughly 50 % of respiration but drops to about 34 % if muscles are working. Calculations based on these observations indicate that at least 20 % of the whole body heat production can be attributed to mitochondrial leak (Brand et al., 1999).

The mechanism of the conductance is not fully understood. Historically, the good coupling of oxygen consumption to ATP synthesis led to the implicit view that the basal leak of protons across the mitochondrial membrane was a preparation artefact that did not occur in cells. This was concluded from the observation that the proton leak current does not depend linearly on its driving force, the protonmotive force, i.e. the mitochondrial transmembrane potential, as described by Ohm's law, but proton conductance increases greatly at higher potentials. This is expected for non-catalysed ion diffusion across a phospholipid bilayer. However, several of the mitochondrial substrate carriers, including adenine nucleotide and glutamate/aspartate transporters, can catalyse a fatty-acid dependent proton leak (Samartsev et al., 1997). In addition, mitochondria from many tissues, particularly brown and white adipose tissue and skeletal muscle, have an inducible proton conductance catalysed by uncoupling proteins, UCP1 in brown fat, UCP2 and UCP3 in white adipose tissue, skeletal muscles, kidney, and non-parenchymal liver cells (Boss et al., 1997, Vidal-Puig et al., 1997, Kelly et al., 1998).

UCP requires activation to catalyse rapid proton leak across mitochondrial inner membrane. ATP and GDP inhibit proton conductance by binding to a region near the C-terminus of UCP. Fatty acids activate UCP-mediated proton conductance, however, the mechanism of activation remained unclear (Gonzales-Barraso et al., 1998, Brand et al., 1999).

Some arguments against the hypothesis that UCPs catalyse the basal proton conductance are based on the observation that the mRNA amount of UCPs does not correlate with the proton conductance in different tissues. In this regard most striking is liver. Hepatocytes contain no UCP1, UCP2 and UCP3 but their basal proton conductance is significant.

Thyroid hormones alter the proton conductance of hepatocyte mitochondria more than six-fold, but UCP2 mRNA levels in non-parenchymal liver cells remain unchanged. Conversely, thyroid

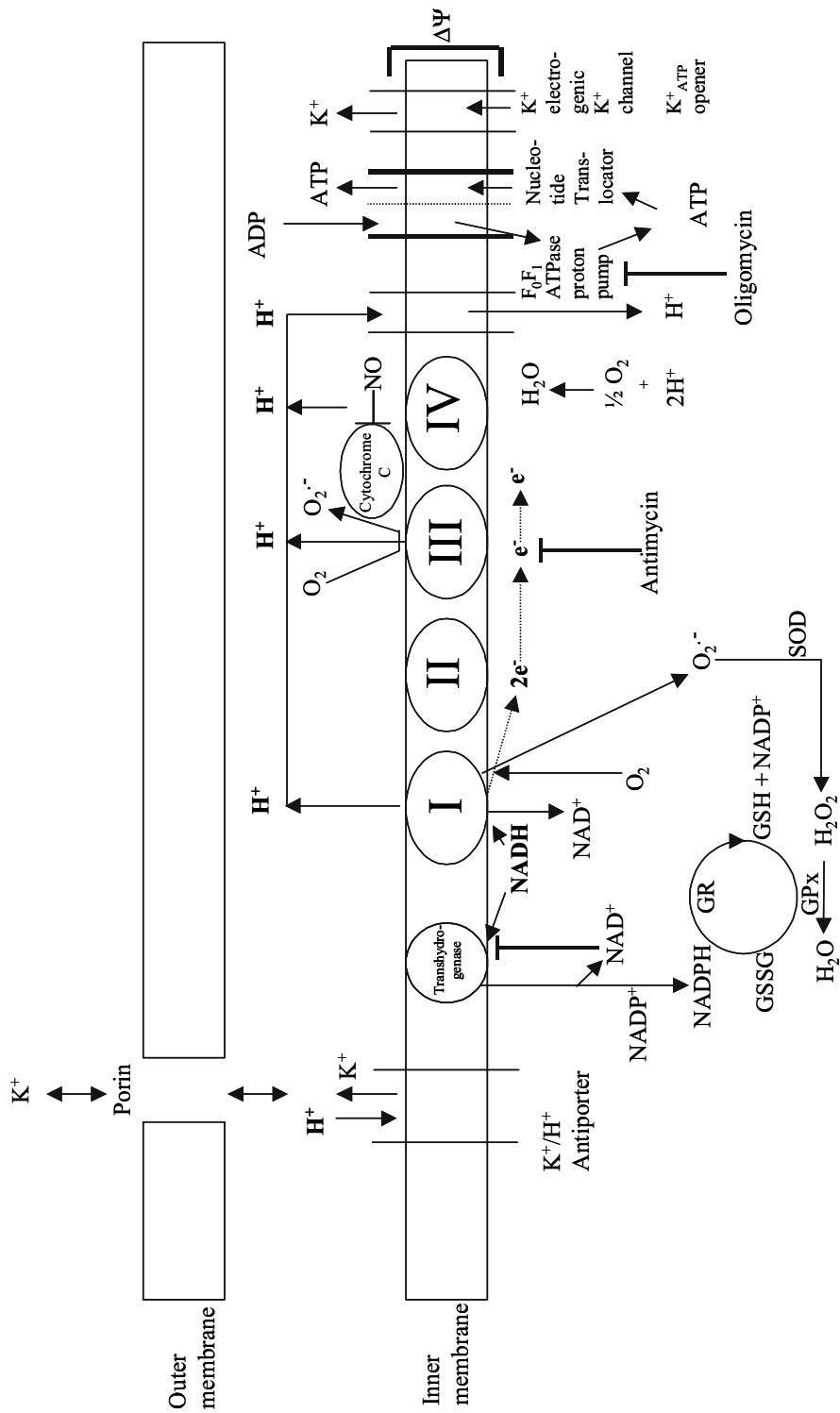


Figure 4. Proton pumping and proton leak across the inner mitochondrial membrane, effects of and defence against oxygen radicals.

hormones change substantially UCP3 mRNA levels in skeletal muscle (Gong et al., 1997) and UCP2 mRNA in rat heart (Lanni et al., 1997) but there is little effect of hypothyroidism on the basal conductance. UCP1 knockout mice over-express UCP2 mRNA in brown adipose tissue, but the mitochondria have the same basal proton conductance of controls where UCP1 is inhibited by GDP (Brand et al., 1999).

UCP gene expression is not only responsive to thyroid hormones. Rats subjected to cold or fasting have been also shown to have higher UCP2 in white adipose tissue (Boss et al., 1997). UCP3 in rodent muscle is induced by caloric restriction and leptin (Liu et al., 1998), in white adipose tissue by β 3 adrenergic agonists but not by cold exposure (Gong et al., 1997). Although induction of UCP2 and UCP3 gene expression seems to be a consistent metabolic adaptation to caloric restriction, it is unclear how this relates to their putative function as mediators of thermogenesis. Moreover, mRNA levels do not always report protein levels so that conclusions regarding function from differences in mRNA are not convincing. Nevertheless, it remains an exciting possibility that UCPs have a proton translocating function not only in brown adipose tissue.

Apart from control of respiratory chain by nitric oxide (NO), a stimulation of cellular pathways leading to higher activity of NO synthases, causes a transient inhibition of mitochondrial respiration through binding of NO to cytochrome c oxidase (Cleeter et al., 1994). Many effects of NO are mediated by an increase in the intracellular cGMP level, but the inhibition of respiration is cGMP-independent. In contrast, NO was recently found to trigger mitochondrial biogenesis in several cell types, such as brown adipocytes and precursors of mouse white fat cells. This is a more time-consuming and cGMP-dependent process which includes activities of the transcription factors peroxisome proliferator-activated receptor γ (PPAR γ) coactivator 1 and the nuclear respiratory factor (NRF-1) (Nisoli et al., 2003). PPAR γ coactivator-1 (PGC-1) is a master regulator of mitochondrial biogenesis in brown adipose tissue and in cardiac and skeletal muscle (Wu et al., 1999). PGC-1 stimulates expression of NRF-1, therefore the expression of nuclear and mitochondrial genes that encode mitochondrial protein, including proteins of the respiratory chain such as cytochrome oxidase and UCPs. Cold exposure and exercise trigger the expression of PGC-1 through activation of β 3-adrenergic receptors, increases in intracellular cyclic adenosine monophosphate and Ca^{2+} (Puigserver et al., 1998, Wu et al., 2002). These signals stimulate NO production in brown adipocytes (Nisoli et al., 2003), indicating that different stimuli may control mitochondrial number, thereby brown fat function, via a common pathway. Similar effects have been also reported for white fat cells. They did not differentiate to mature adipocytes and responded with an increase in PGC-1, NRF-1, mitochondrial DNA and cytochrome oxidase (subunit IV) protein when treated with NO (Nisoli et al., 2003). Combined treatment with NO and

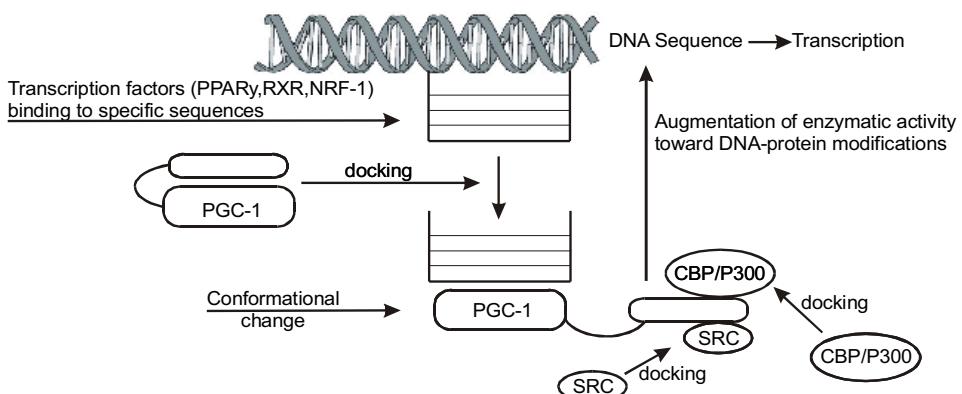


Figure 5. Model of interactions between proteins regulating the expression of genes important for mitochondrial biogenesis and activity.

a specific inhibitor of guanylyl cyclase blocked the response to NO, indicating that cGMP is essential for activating the PGC-1 mediated pathways.

PGC-1 is a relatively inactive molecule that requires docking to PPAR γ or NRF-1 in order to undergo a conformational change and affect transcriptional activity (Puigserver et al., 1999). PGC-1 has no domains with selective DNA-binding properties and only a latent histone acetyltransferase activity. PGC-1 exerts its effect through the assembly of a complex that includes transcription factors capable of binding specific DNA sequences, such as PPAR(and CREB (cAMP response element binding protein), and transcriptional coactivators with acetyltransferase activity, such as steroid receptor coactivators (SRC) and CREB binding proteins (CBP/P300). Thus, transcription factor docking switches on the activity of a coactivator protein enabling the transcription of specific genes with response elements recognized by DNA-binding transcription factors, such as C/EBP, NRF-1 and PPAR γ .

PPAR γ belongs to the family of nuclear receptors. It forms heterodimers with another transcription factor, the retinoid X receptor. Natural PPAR γ ligands include oxidized constituents of low density lipoprotein, prostaglandins of the D2/J2 types and NO (von Knethen and Brune, 2002). Synthetic ligands belong to the thiazolidine class of drugs and PPAR γ is a pharmacological target in the treatment of type II diabetes (Tontonoz et al., 1998, Barak et al., 1999). PPAR γ is abundantly expressed in both white and brown adipose tissues, but also in skeletal muscles, macrophages, and T-cells. PPAR γ deficiency in PPAR γ gene knockout mice interferes with terminal differentiation of the trophoblast and placental vascularization. This leads to severe myocardial thinning and embryonal death. PPAR γ exerts these effects by cooperation with PGC-1 and CEBP γ , which is responsible for the expression of tissue-specific glucose transporters. PPAR γ suppresses the expression of leptin (Kubota et al., 1999), NOS (Colville-Nash et al., 1998) and the secretion of endothelin, a endothelium-derived vasoconstricting peptide (Itoh et al., 1999), thereby activation of PPAR γ has been highlighted to improve insulin resistance as a common causal factor for hypertension, hyperlipidaemia, diabetes mellitus and obesity. In turn, heterozygous PPAR γ -deficient mice were protected from the development of insulin resistance due to adipocyte hypertrophy under a high-caloric diet (Kubota et al., 1999).

Normally, dietary increase in metabolizable energy enhances thermogenesis and circulatory activity measured by heart rate and blood flow. When these responses relate to the molecular mechanism described, differences between lower and higher nutritional levels are to be expected that may have consequences on expression of regulatory proteins. To address this, we carried out experiments with twelve young bulls (290 to 300 days old and 260-280 kg in weight at beginning the experiments). They were allocated to three groups fed 1.0-, 1.5-, and 2.0-times MEm. Following adaptation to the diet and to respiration chambers, gas exchange was measured and heart rate by Sport-Tester for at least three days. Then the animals received the diet until slaughtering. Samples from tissues were frozen in liquid nitrogen and stored at - 80°C. The protein concentration of three transcription factors (PPAR γ , C/EP α , and CREB) was measured in cardiomyocytes by immunocytochemistry using fluorescent secondary antibodies. Fluorescence was detected by flow cytometry to separate cell debris, present after preparation of single cells by digestion of the tissue by collagenase.

The results (Figure 6) indicate that PPAR γ and CREB in its activated form (phosphorylated CREB) co-varied and their intracellular concentrations increased with elevating the nutritional level from 1.0-times to 1.5-times MEm. However, in the group fed 2.0-times MEm, a level that corresponded to intake of ME ad libitum leading to an effective intake of 1.8- to 1.9-times the requirement of metabolizable energy for maintenance, PPAR γ and pCREB protein dropped below the 1.0-MEm level. In contrast, C/EP α protein steadily decreased with higher metabolizable energy intake. In the light of the functions of these transcription factors (regulation of genes encoding proteins involved in uptake of glucose and lipids, lipid accumulation as well lipid oxidation) the results may indicate an adaptation to high energy intake. This was associated with excess adipose tissue adjacent to the heart. Bovine heart seems to cope with excessive adipose

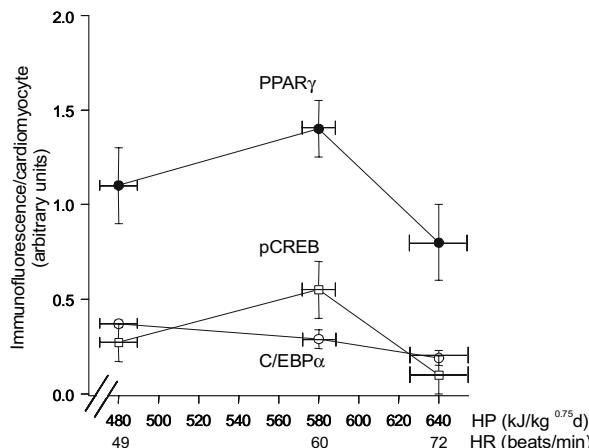


Figure 6. Induction of changes in immunoreactive transcription factor proteins in bovine cardiomyocytes and the heat production (HP) and heart rate (HR) by higher intake of metabolizable energy (from left to right, 1.0-, 1.5-, 1.9-time MEm).

development by down-regulation of PPAR γ and C/EBP α -regulated pathways. Glucose level in bovine circulation is very strongly controlled and did not significantly differ between MEm groups. Although the mechanism of maintaining bovine energy homeostasis requires further elucidation, the data may mirror interactions at molecular level corresponding to metabolic interactions at non-genomic levels in ruminants as discussed above.

Dietary impact on mitochondrial activity

Figure 7 shows that an intake of metabolizable energy ad libitum is associated with an increase in cytochrome oxidase reportedly used as an indicator of mitochondrial biogenesis (Nisoli et al., 2003) but with a decrease in Cu/Zn superoxide dismutase. To include a functional aspect, we measured the steady-state transmembrane potential of heart mitochondria. It is a consequence of proton pumping by the F0F1 ATPase that uses the proton gradient as energy source for ATP synthesis. Without effective pumping, H⁺ ions accumulate in the intermembrane space as they are

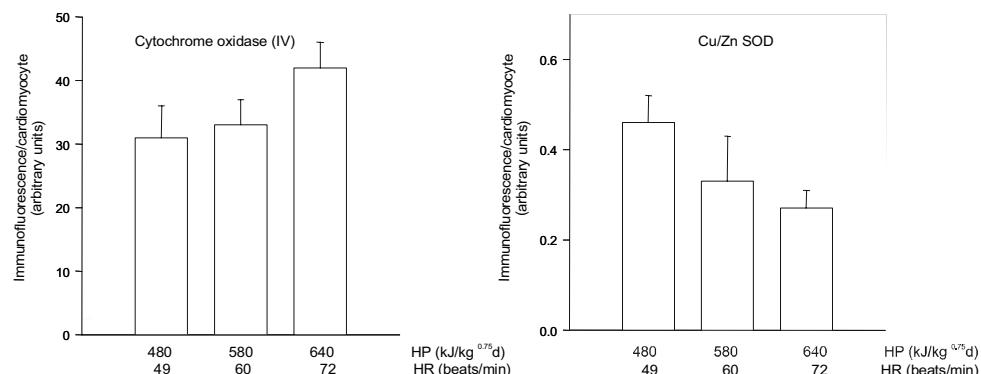


Figure 7 Response of immunoreactive cytochrome oxidase (IV) and Cu/Zn superoxide dismutase protein in bovine cardiomyocytes and of the heat production (HP) and heart rate (HR) to diets providing 1.0-, 1.5-, 1.9-times MEm.

extruded by the respiratory chain complex but fail to flow down the gradient back into the matrix. Intermembrane proton accumulation in intact mitochondria induces hyperpolarization.

Surprisingly with view of the increase in cytochrome oxidase protein, an intake of metabolizable energy ad libitum was associated with a drop of mitochondrial transmembrane potential ($\Delta\psi_m$) compared to the mitochondria prepared from the heart of animals with restricted intake of metabolizable energy (Figure 8).

The observations shown in Figure 8 do not allow the conclusion that the drop of $\Delta\psi_m$ is a consequence of an increase in proton conductance of the inner mitochondrial membrane, because a similar response may arise from the inhibition of the respiration chain. To address this, the basal level of oxidized FAD/FMN was measured by the fluorescence of the flavoids excitable only in the oxidized state. The results indicated that reduced flavoids were predominant in cardiomyocytes from the group fed MEm ad libitum, suggesting an inhibition of the electron transport. This contrasts with data from measuring the oxygen consumption of whole body. To address this, the cells were treated with dinitrophenol, uncoupling the respiratory chain (Figure 9). FAD/FMN fluorescence increased but remained below the level of the cells from animals fed 1.0- or 1.5-times MEm. This suggests that uncoupling of the respiratory chain is not sufficient to cope with inhibition at least in vitro. Therefore, an increase in the number of mitochondria seems to mediate higher oxygen consumption in feeding MEm ad libitum.

To gain more specific information about the putative inhibition of the electron transport, the mitochondria were exposed to openers of ATP/ADP-regulated potassium channels. Potassium ions are the major determinant of mitochondrial matrix volume. The inner mitochondrial membrane is impermeable, therefore the transport of ions and metabolites across the inner membrane can be controlled by several channels and transporters (Figure 4). They include an adenine nucleotide translocator, a K^+/H^+ antiporter and electrogenic K^+ channels of which ATP-sensitive potassium channels (KATP) play a key role in ischemic preconditioning of the heart (Murata et al., 2001). KATP are closed by high intracellular ATP- and low ADP-levels. Their activation causes hyperpolarization and is associated with ATP consumption (Bienengraeber et al., 2000). The response of $\Delta\psi_m$ to levromakalim, an opener of KATP, was most striking in the heart mitochondria from animals fed ad libitum (Figure 10).

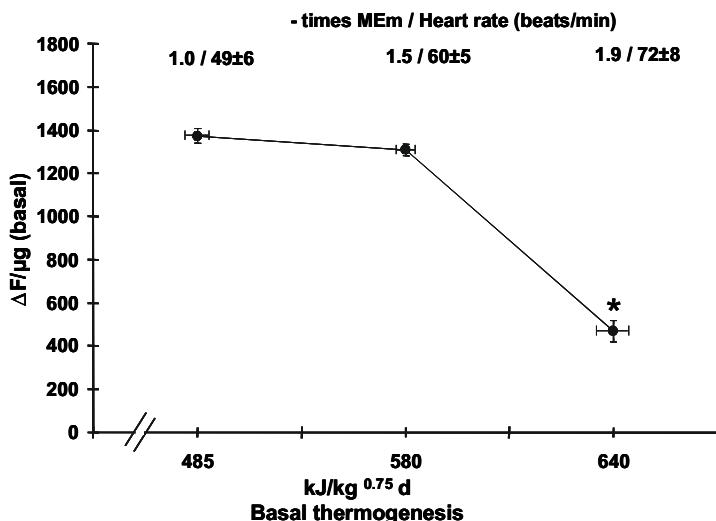


Figure 8. Changes in cardiomyocyte's mitochondrial transmembrane potential ($\Delta F/\mu\text{g}$ mitochondrial protein) thermogenesis and heart rate induced by differential intake of metabolizable energy in young bulls ($n=4$ in each group).

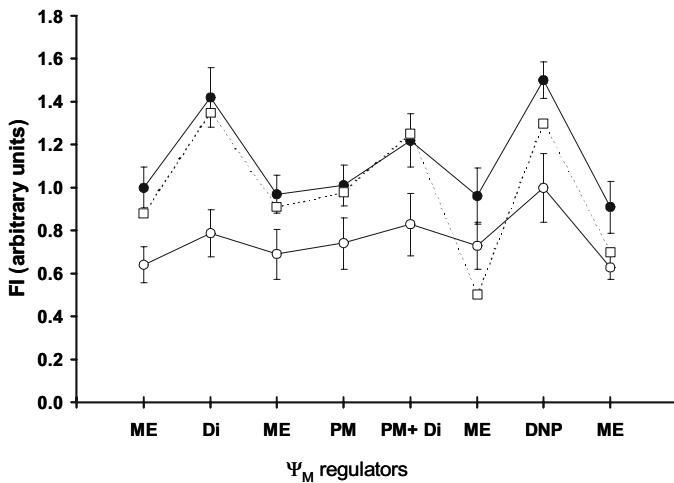


Figure 9. FAD/FMN fluorescence (FI) of bovine cardiomyocytes in response to dietary metabolizable energy levels (filled and empty circles, 1.0- and 1.9-times MEm; squares, 1.5-times MEm) and to energy dissipation by a KATP opener (diazoxide, Di) and by uncoupling the respiration chain (dinitrophenol, DNP).

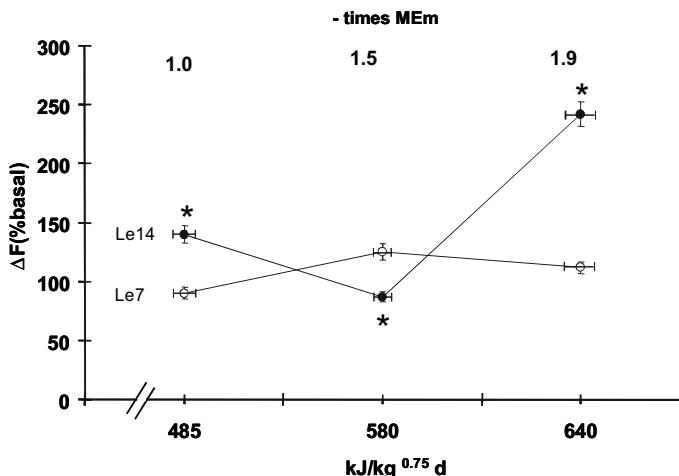


Figure 10 .Response of transmembrane potential difference (ΔF) expressed in terms relative to basal ΔF (Figure 8) in mitochondria from bovine cardiomyocytes to an opener of ATP/ADP-regulated potassium channels (levcromakalim, Le7 and Le14 μ M) depends on the level of metabolizable energy intake. Asterisks stand for significant difference to basal value.

In polarized mitochondria from animals fed a diet restricted to 1.5-times MEm, the opener induced depolarisation as expected due to energy dissipation. In contrast, depolarized mitochondria from animals fed MEm ad libitum responded to the opener of KATP with a strong increase in $\Delta\psi_m$ which reached the basal level of mitochondria from animals fed 1.5-times MEm. The findings that the responses were dependent on dose and the electron transport inhibition was reversible indicated specific results. Similar data were obtained using diazoxide as KATP opener.

In light of reports that NO reversibly inhibits respiratory chain at the complex III by binding of NO to cytochrome c (Cleeter et al., 1994), the expression of NOS and NO-caused protein modification

were studied as an index of NOS activity (Figure 11). The results showed an increase in nitric oxide synthase protein and nitrotyrosine content in cardiomyocytes. Therefore, they revealed a regulation of the expression of NOS and its activity as likely mediators of inhibiting the respiratory chain induced by a high intake of metabolizable energy.

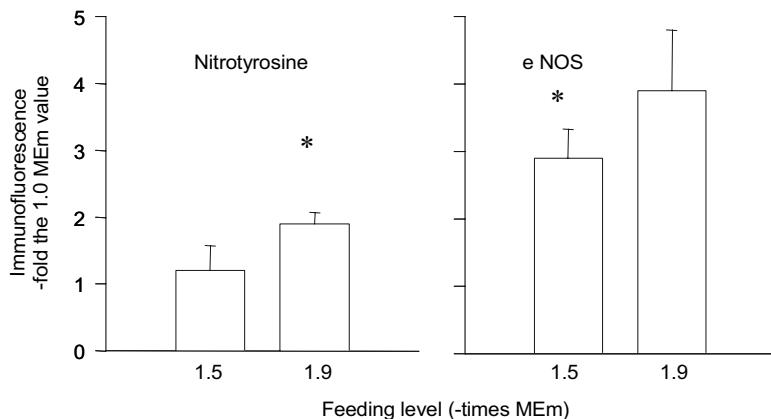


Figure 11. The nitric oxide synthase (eNOS) protein and nitrotyrosine content of proteins in bovine cardiomyocytes increases with higher intake of metabolizable energy. Asterisks stand for significant changes relative to 1.0-times MEm (four young bulls in each group).

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Low protein diets reduce greenhouse gas production in finisher pigs while maintaining animal performance

J.K.A. Atakora, S. Möhn & R.O. Ball

Swine Research & Technology Centre, 4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, AB, Canada, T6G 2P5

Summary

Reducing dietary protein maintained performance and reduced nutrient excretion of finisher pigs with wheat-barley based, but not corn based diets. Reducing dietary protein reduced N excretion and CH₄ production, but had little effect on CO₂ production. The CO₂ equivalents arising from the animals themselves (CO₂ and CH₄ production) tended to be lower with reduced protein contents. The further greenhouse gas production depends on the conversion of excreted carbon and N into CO₂, CH₄ and N₂O. Reducing dietary protein contents reduces the N excretion by pigs, which gives the potential to reduce greenhouse gas emissions by pigs by over 20%.

Keywords: pig, protein, protein retention, heat production, greenhouse gas

Introduction

Reducing dietary protein contents while supplementing free amino acids lowers the N excretion and maintains pig performance, although reduced performance of finisher pigs has been observed. Reducing dietary protein increases the content of carbohydrates and alters their composition. Carbohydrates have a lower C-content than protein and are used more efficiently in energy metabolism. This may lead to a reduction of CO₂ production. Altering the composition of the carbohydrate fraction may reduce CH₄ production. The objectives were to assess the effect of low protein diets on performance, N-excretion, CO₂ and CH₄ emissions of finishing pigs.

Materials and methods

Twenty-four female pigs of initially 50 kg were fed either wheat-barley (WB) or corn (C) based diets. Within each ingredient group, 12 pigs were offered either conventional (HP) or protein-reduced amino acid supplemented (LP) diets in a cross-over design. During a 7 d adaptation to diet and housing the pigs were periodically confined in the respiration chambers after which a 7-d N balance with quantitative collection of urine and faeces study was performed, followed by a 4 h respiration measurement. The pigs were then switched to the other diet to repeat measurements. Four pigs fed the C based diets were selected for 24-h respiration measurements.

Equipment

Respiration boxes approximately 2 m³ in volume were built around commercial farrowing crates, fitted with a feeder and drinker. Air was drawn through these boxes at a rate of 240 L min⁻¹. After passing through a cold water condenser, air flow was measured with commercial air meters. Part of the air was drawn off and delivered to the gas analyzers. The digitalized data were acquired at maximum rate to record 1-min averages of the gas concentration throughout the experiment.

Diets and feeding

The WB-HP and WB-LP diets had equal metabolizable energy (ME) and true ileal digestible contents of lysine, methionine, threonine and tryptophan. The C-HP and C-LP diets were formulated to achieve a ratio of lysine to ME similar to that of the WB diets. Nutrient contents of all diets at least met the recommendations of NRC (1998). The WB diets were fed at 90% of the ad-libitum feed intake according to NRC (1998). To achieve equal ME intake to the WB diets, the C diets were offered at a lower rate. Feed was offered twice daily, except for respiration studies, when half the daily allowance was offered in 8 equal hourly meals.

Analyses

Prior to each respiration study all gas analyzers were calibrated and the average reading for the standard gases recorded before and after each study, along with gas concentrations in ambient air. Proximate analysis was performed according to AOAC (1990). Gross energy in feed and feces was determined using an adiabatic bomb calorimeter. Carbon content in feed and feces was measured using an elemental combustion system. Dietary amino acid contents were determined by ion-exchange chromatography using post-column derivatisation with ninhydrin.

Calculation of results and statistical analysis

The average gas exchange was extrapolated to 24-h values using the factors derived from the comparison of 4-h and 24-h measurements. Lipid and energy retention were calculated using the C-N balance technique. The CO₂ equivalent was calculated based on the potency as a greenhouse gas, the factors being, per Mol, 1 for CO₂, 21 for CH₄ and 310 for N₂O. Effects of type of diet and protein level were estimated using the general linear models procedure (SAS, 1999).

Results

The daily gain of 766 (SE 18) g was not affected ($P > 0.3$) by type of diet or protein level. The N retention was similar ($P = 0.49$) for WB-LP and WB-HP at 26.5 (SE 0.7) g d⁻¹, but lower ($P = 0.03$) for C-LP than for C-HP, due to the lysine content in C-HP being greater than expected. WB-LP had similar ($P = 0.13$) fecal N, but lower ($P < 0.002$) urinary and total N excretion than WB-HP. Due to the depression in retained N, the N excretion for C-LP and C-HP were similar ($P > 0.1$). Lipid retention tended ($P = 0.08$) to be greater for the LP diets. Heat production tended to be lower ($P = 0.08$) for the LP diets indicating improved energy utilization.

During 24 h respiration, the pigs consumed 11.4% less O₂, produced 11.0% less CO₂, but 37.3% more CH₄ than during the 4 h measurement. The reduced O₂/CO₂ values for 24 h are probably the result of reduced activity at night (van Milgen et al., 2001). The CH₄ production increased after offering half the daily feed allowance in the afternoon, leading to greater 24-h values. The ratio of 4-h to 24-h CO₂ and CH₄ measurements was not affected by protein level.

The CO₂ production was lower for C diets and non-significantly lower for the LP diets. The CH₄ production was lower for LP than HP diets, but not different between C and WB diets. The CH₄ production was correlated ($P < 0.05$) to the dietary NDF and hemicellulose contents, which declined with reduced protein contents. The combined CO₂ and CH₄ production averaged a CO₂ equivalent of 3181 g d⁻¹, did not differ between diets ($P = 0.89$), but tended ($P = 0.10$) to be lower for LP diets. The carbon excretion averaged of 135.4 g d⁻¹, was lower for C diets, but similar for HP and LP diets. The excreted carbon has implications on greenhouse gas production from manure: the CO₂ equivalent would be 10428 g d⁻¹ if quantitatively converted to CH₄.

The CO₂ equivalent produced by pigs depend on the transformation of excreted N into N₂O. If quantitatively transformed, 17787 g d⁻¹ CO₂ equivalent could be produced. When aerating manure, 5% to 30% of the N in manure may be lost as N₂O (Béline et al., 1999). Assuming these rates of N₂O loss leads to two-fold difference in CO₂ equivalent of 4070 (SE 115) g d⁻¹ vs 8517 (SE 218) g d⁻¹, and affects data interpretation: the greater rate shows a significant protein effect, but the lower does not. However, reducing dietary protein contents reduced the production of CO₂ equivalent even at the low rate of conversion.

Conclusions and implications

Reducing dietary protein maintained performance and reduced nutrient excretion with wheat-barley based diets, but not with corn based diets. Reducing the dietary protein reduced the average N excretion and CH₄ production, but led to only a marginal reduction of CO₂ production. The CO₂ equivalents arising from the animals themselves - from CO₂ and CH₄ production - tended to be lower with reduced protein contents. The further greenhouse gas production depends on the conversion of excreted carbon and N into CO₂, CH₄ and N₂O. Reducing dietary protein reduces the N excretion by pigs, with the potential to reduce greenhouse gas emissions by pigs by over 20%. To realize the full potential, the conversion of nutrients in manure into greenhouse gases must be investigated further, along with means to influence this conversion.

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Dynamic responses of feeder cattle to simulated heat waves

T.M. Brown-Brandl¹, J.A. Nienaber¹, G.L. Hahn¹, R.A. Eigenberg¹ & A.M. Parkhurst²

¹ USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE U.S.A.

² University of Nebraska-Lincoln, Department of Biometry, Lincoln, NE U.S.A.

Summary

Heat stress reduces performance and, in the most severe cases, death of feedlot cattle resulting in major economic losses. A study was designed to evaluate the dynamics of thermoregulation and feed intake of feeder cattle when exposed to either a simulated heat waves or a standard cyclic environment. Nine crossbred steers were randomly assigned to individual pens in one of three environmental chambers. Three temperature regimes (heat wave simulation from Rockport, MO 1995; heat wave simulation from Columbia, MO 1999; and Cyclic heat stress treatment of $32 \pm 7^\circ\text{C}$) were administered to each of three chambers for a period of 18 days according to a Latin square treatment design, with a 10-day thermoneutral period separating treatment periods ($18 \pm 7^\circ\text{C}$). Respiration rate (RR), core body temperature (T_{core}), heat production (HP), and feed intake (FI) were measured on each animal for the duration of the experiment. From this study, it appears that RR and FI, observable parameters, give an accurate insight into the thermoregulatory status of the animal during heat challenges.

Keywords: respiration rate, core body temperature, heat production, heat waves

Introduction

Hot weather affects animal bioenergetics, and can have negative impacts on animal performance and well-being. Reductions in feed intake, growth, and efficiency are commonly reported in heat-stressed cattle (Hahn, 1995). The impact of heat load on these production losses are quite varied, ranging from little to no effect in a brief exposure, to death in vulnerable animals during an extreme event (Hahn and Mader, 1997).

Heat waves are a recurring phenomenon in many cattle-producing areas of the World. A heat wave is defined as “a period of abnormally uncomfortable hot and usually humid weather of at least one day duration, but conventionally lasting several days to several weeks...” (AMS, 1989). Hahn and Mader (1997) reported an operational definition of heat waves as “three to five successive days with maximum temperatures above a threshold, such as 32°C .” Several heat waves have occurred in the Midwestern United States in the last ten years causing the death of thousands of feedlot cattle (Hahn, 1999), and loss of millions of dollars in revenue to the cattle industry in direct cattle losses and indirect performance losses.

Typically, heat stress studies rely upon a high constant (e.g., 32°C) or a cyclic temperature (e.g., $32 \pm 7^\circ\text{C}$) to simulate the weather conditions that would induce heat stress in the field. However, heat waves do not have uniform temperatures from day to day, and the nighttime low has been found to directly impact the level of stress imposed on the animal (Hahn and Mader, 1997). A study was designed to evaluate the bioenergetic responses when feeder cattle were exposed either to simulated heat waves or with a standard hot cyclic environment.

Materials and methods

Nine crossbred steers (1/4 Angus, 1/4 Hereford, 1/4 Pinzgauer, 1/4 Red Poll), weighing 400.3 ± 10.0 kg at an average age of 452 ± 5.1 days, were randomly assigned to individual stalls in one of

three environmentally and photoperiod (14 h light 10 h dark) controlled chambers (three animals per chamber). A standard feedlot ration (high moisture corn plus silage) and water was fed ad libitum, with refusals removed and fresh feed provided daily.

Each chamber was subjected to each of three temperature treatments (TRT) for a n 18-day period representing: 1) conditions at Columbia, MO during July - Aug , 1999; 2) condition at Rockport, MO during July, 1995; 3) Standard Cyclic (dry-bulb temperature [T_{db}] = $32\pm7^{\circ}\text{C}$, fig. 1). In all heat-stress TRT, T_{dp} was held constant at 14°C . A Latin square treatment design was used, with all animals exposed to every TRT over three periods, separated by 10-day thermoneutral periods (TN, $T_{db} = 18\pm7$; $T_{dp}=7$). Animals were exercised and weighed prior to the beginning and after the end of every TRT period.

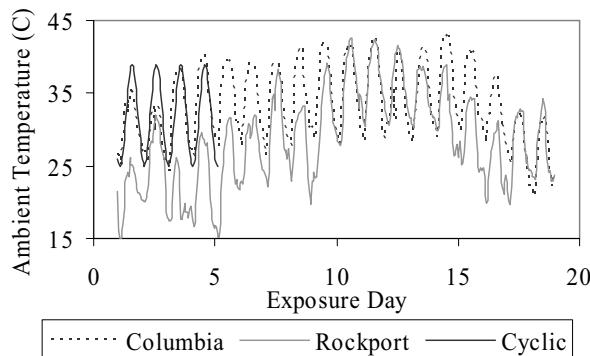


Figure 1. Ambient temperature patterns for the three treatments are shown (for clarity, only the first four-days of the cyclic condition are shown).

Total heat production (HP) was calculated using indirect calorimetry methods. Each of the environmental chambers containing three animals was used as an calorimeter chamber. Inlet airflow was measured using an electronic differential pressure transmitter, which was fitted inside the inlet air duct. The error in flow measurement was less than 2.5%. Calorimeter gas samples were analyzed for O_2 , CO_2 , and CH_4 once every 15 minutes. Oxygen and CO_2 were measured to the nearest 100 ppm, while CH_4 was measured within 10 ppm. Total heat production was calculated using the total inlet air added, and the average concentrations of O_2 , CO_2 , and CH_4 over a 24 hour period for fresh inlet air and chamber air.

Respiration rate (RR), core body temperature (T_{core}), and feeder weights were continuously recorded during both the imposed TRT and the TN periods. Daily feed intakes (FI) were calculated. Respiration rate was obtained using respiration rate monitors; the output signal from the RR sensor was recorded for one minute every 15 minutes at 10 Hz (Eigenberg et al., 2000). Core body temperature was measured at a frequency of one reading per minute using a telemetry system (HQ, Inc¹, Palmetto, FL). Prior to the experiment, a licensed veterinarian implanted a transmitter in the abdominal cavity of each steer (Brown-Brandl et al., 2003). Rectal temperature was used as a backup measurement of core body temperature and was converted to T_{core} for analysis. The conversion used an equation develop specifically for each animal using data collected from both method simultaneously.

Daily averages (THP, T_{core} , RR, and FI) were analyzed using repeated measures in PROC MIXED, with an autoregressive order error structure (SAS, 1996). The model included chamber,

¹Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

period, and animal nested within chamber and period as random effects, and TRT, day of exposure (DAY), and TRT*DAY interaction as fixed effects. Response differences were considered to be significant when the probabilities were less than 0.05. Daily average for all TRT and TN periods were analyzed using the general linear model procedure in SAS was used to evaluate the linear and quadratic effects of T_{db} on RR and T_{core} and the effects of FI, T_{db} , and DAY on THP.

Results and discussion

The bioenergetic responses (RR, T_{core} , FI, and HP) measured during each of the three TRT (Cyclic, Rockport, and Columbia), along with the responses during TN (although not statistically compared) are shown in fig. 2. It appears that the steers' response to the cyclic TRT shows signs of acclimation, whereas the response to the other two TRT appear to follow the temperature change.

Under cyclic heat stress, the response showed a three to four day acclimation period. The RR increased the first three days, then decreased on day four, where it remained for the rest of the TRT period. Core body temperature seemed to take a day longer to acclimate than RR, increasing over the first three days, then a large decrease over the next two days, and then levels off (even though it appears to decrease slowly until day 14 there is no significant difference). Feed intake and HP follow similar patterns, decreasing the first three days and then leveling off.

The other two TRT show responses correlated with T_{db} . Respiration rate and T_{core} track with temperature, although days 10 - 12 where the temperature pattern for these two TRT are similar, might indicate slight acclimation in the Columbia TRT. Feed intake and heat production show a similar trend on days 10 - 12; however, there is no significant difference.

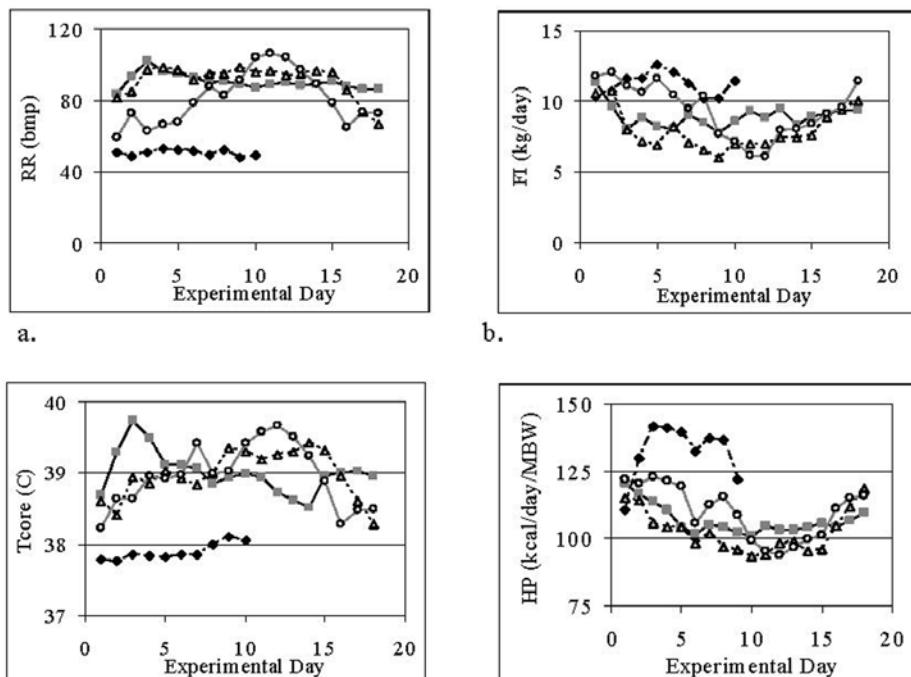


Figure 2. Bioenergetic responses of feed cattle to simulated heat waves: a. respiration rate (RR, breathes per minute [bpm]); b. feed intake (FI, kg/day); c. core body temperature (T_{core} , C), d. heat production (HP, kcal/day/MBW).

Simple regression analysis revealed RR was highly correlated with T_{db} ($r^2=0.79$), while T_{core} and FI were only moderately correlated with T_{db} ($r^2=.29$ and 0.30, respectively). When animal was added to the model the r^2 increased only 0.04 for RR, while r^2 increased substantially for FI to 0.47 and doubled for T_{core} ($r^2=0.59$). With these results it was concluded that RR makes an excellent parameter for a producer to monitor.

In the regression analysis of HP, it was found that T_{db} , DAY, and FI accounted for 63.4% of the variation. In an analysis of standardized estimates, it was shown that t_{db} and FI account for almost equal amount of variation (55.5% and 40.6%, respectively) leaving only 3.6% to be accounted for by DAY.

Conclusions

In response to cyclic heat stress, crossbred steers took three to four days for acclimation in all parameters measured. There appears there was little acclimation in the simulated heat wave from Columbia, MO, and almost no acclimation in the Rockport TRT. This study suggests that RR and FI, observable parameters, provide an accurate insight into the thermoregulatory status of the animal. The response variable T_{core} tends to follow a similar response patterns as RR, and HP closely follows FI.

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Energy and protein (AA) metabolism of high productive laying hens in dependence on exogenous factors

A. Chudy, W.B. Souffrant, S. Kuhla & H. Peters

Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Forschungsbereich Ernährungsphysiologie "Oskar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf

Summary

Results of respiration trials, including complete protein (amino acids) and nutrient balances, with two strains of high productive laying hens (Lohmann BROWN and Lohmann LSL) were described with recommendations and conclusions for requirements and feeding strategies.

Keywords: laying hens, energy metabolism, protein, amino acids, heat production

Introduction

The aim of this investigation was to determine the energy and nutrient metabolism of two high productive strains of laying hens (Lohmann Brown (LB) and Lohmann White (LSL)) in dependence on exogenous factors (feed composition, environmental temperature, lighting programmes,) as fundamentals for feed strategies.

Materials and methods

Respiration experiments (climatically controlled respiration chamber with 4 cages á 3 hens (n=12), 27 measuring periods of 4 - 18 days) were carried out in two series with two groups, in each 12 or 8 laying hens - LB (BW 1840±106 g) and LSL BW 1530±82 g) in series 1 with 6 or only LB (BW 1888±43 g) in series 2 with 10 different treatments; both in the course of laying period from 18th to 77th week of age. The hens were fed ad libitum. The variations of exogenous factors were:

Feed composition: Protein 16.1 - 26.9 %, fat 2.7 - 9.1 % and starch+sugar 32.2 - 49.8 % of DM;

temperature 5 - 30 °C; light program 12h /12 h or 3h/3h and 8h/10h light/dark per day.

The heat production (HP) (indirect calorimetry over 24 h, measurement of O₂ consumption and CO₂ production continuously (air input, chamber gas concentration every 20 sec.), HP calculated according to BROUWER (C- and N-balance)), was measured daily. The excrements were collected totally, mixed, fresh (N) and frozen dried analysed, as well as the feeds and eggs, by standard methods: N, C, energy (E), amino acids (AA) and feed analyses (WEENDE): (dry matter (DM), ash (A), crude protein (CP), crude fat (CF), starch (ST), sugar (SU) and in difference to organic matter (OM) the N-free residue (NFR). The differences between total N and amino acids N in excrements are calculated as urine N. Therefore we get complete N-, C-, energy-, protein and amino acid as well as nutrient balances.

Results and discussion

The feed intake was relatively low and depends significantly (p<0.01) on environmental temperature: It was up to 14 % higher at 10 °C and up to 23 % lower at 30 °C, compared to at 22 °C. The lighting programs are summing-up over 24 h without influence, but the differences in gas exchange and heat production are between light (135 %) and dark periods (75 %) 60 % of the daily average by influence of different physical activity. The egg production (EP) was high (over 90 %) and very stable. LB tended to have higher feed intake and egg production; but in average

the feed conversion is equal to LSL. The lower temperature declined the feed conversion up to 2.0 or 1.82 kg DM/kg egg mass in LB and LSL, respectively; higher temperature improved the feed efficiency for both strains to 1.45 kg DM/kg egg mass. But this result is also caused the low feed intake and therefore the utilisation of body nutrients as energy source. The analysis of body composition revealed that the majority of the 300 g BW-differences between LB and LSL is metabolic inactive fat (100 g from 184 g DM-difference!). The basic metabolism of egg production is between the strains, expressed in absolute dates, equal. Therefore is it for the compare of metabolic dates not useful to relate these dates to metabolic body size. In the following are discussed only relationships of fundamental importance.

Amino acid metabolism

The metabolism of AA-protein ($\text{g} \cdot \text{d}^{-1}$) in dependents on intake is shown in figure 1. The metabolizable AA-protein (line 1) and the intake were correlative ($0.74\text{g} \cdot \text{g}^{-1}$, $R^2=0.76$), as well as the oxidized AA-protein (line 2) admittedly with lower dependence ($0.60\text{g} \cdot \text{g}^{-1}$, $R^2=0.55$). The AA-protein secretion in the eggs shows also a small dependence on the intake of (metabolisable) AA, but the efficiency is very low ($0.17\text{g} \cdot \text{g}^{-1}$, $R^2=0.21$). Hence, in the commercial use one should concern the costs. These relationships are very different for the single AA. The graduation responses of essential AA to intake is: threonine > lysine > cystine > methionine > tryptophane. Concerning the AA balancing one should have a special feature in poultry because the AA balance includes the AA which are retained in body and/or oxidised. Summarised, the oxidised AA one can calculate out of the AA balance - N-balance*6.25 (protein balance). In dependence on energy balance (feed intake) and feed composition AA were desaminated (oxidised) for energy supply or fat synthesis in the amount of 30 - 146 % of AA balance, that means 43 to 91 % of urine N (= N-excrements - AA_N) comes from desamination of AA. Generally, higher AA intakes as the requirements for maintenance function and egg production are not efficient for egg production.

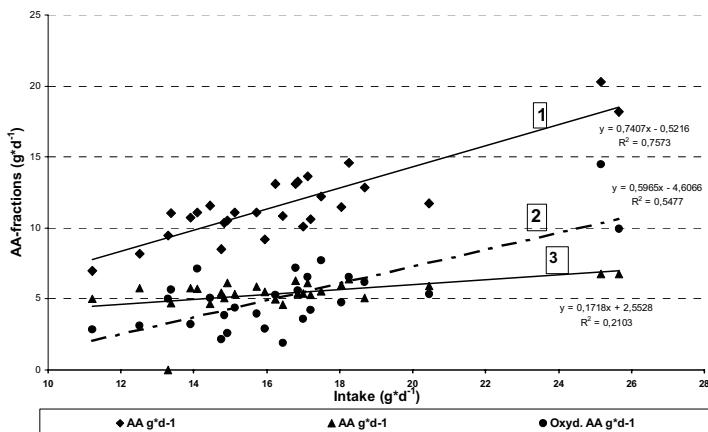


Figure 1. Metabolism of AA-protein ($\text{g} \cdot \text{d}^{-1}$).

Fat metabolism

Fat is the second important nutrient for poultry. The fat metabolism is summarised depicted in figure 2. The digestible fat is positively linear with the intake ($0.88\text{g} \cdot \text{g}^{-1}$, $R^2=0.97$) (line 1). The fat secretion in eggs is practically constant ($4.1 \pm \text{g/d}$) (line 4). Therefore the fat balance (line 3) related directly to the digestible fat intake but with a lower increase rate ($0.78\text{g} \cdot \text{g}^{-1}$), which reflect a little higher fat content in the eggs ($0.1\text{g} \cdot \text{g}^{-1}$) in the case of high fat feed. The amounts of

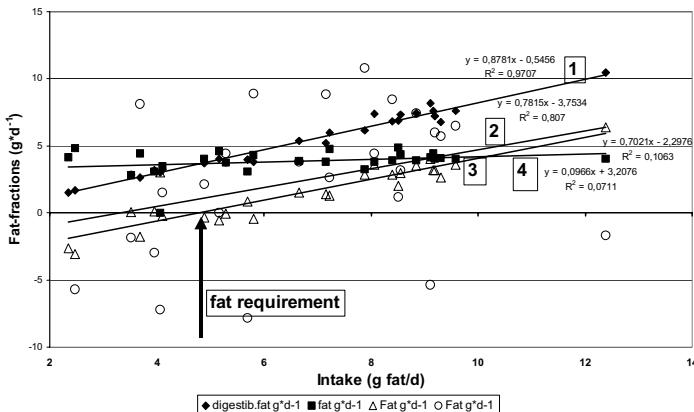


Figure 2. Fat metabolism (g^*d^{-1}).

oxidised fat (line 2), calculated from gas exchange dates (CO_2 , O_2), were (in average) higher as the fat balance, but with very high variation between the treatments. This can be an indication of oxidised fat from “turn over” with replacement over fat syntheses from carbohydrates. The fat requirement (balance = 0) derived to ≈ 5 g digestible fat*day $^{-1}$.

Carbohydrate metabolism

The metabolizable and balanced carbohydrates (figure 3, line 1 and 2) increased with intake of carbohydrates (starch, sugar inclusive NFR)($0.71\text{g}^*\text{g}^{-1}$, $R^2=0.83$), but the oxidised amounts increased slower because they are - if energy surplus is reached - used for fat syntheses. The amounts of oxidised carbohydrates decreased with increasing of environmental temperature ($0.77\text{g}^*\text{1}^\circ\text{C}^{-1}$) whereas the amounts of oxidised protein and fat remained unchanged. The retention of carbohydrates in eggs or body is infinitesimal (line 4).

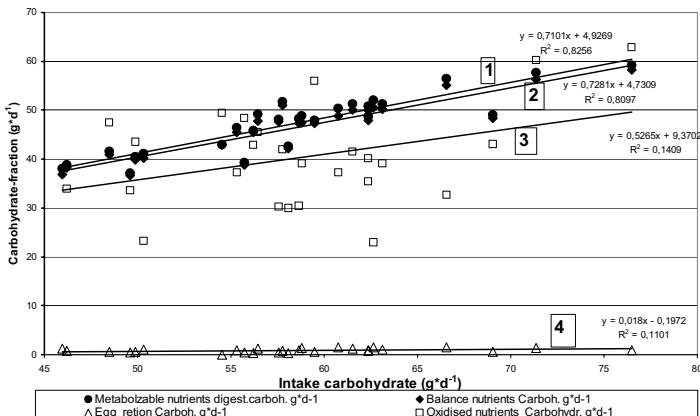


Figure 3. Carbohydrate metabolism (g^*d^{-1}).

Energy metabolism

The relationships between ME intake and heat production, egg energy secretion and body energy retention are shown in figure 4. The heat production (line 1) is positively linear with ME intake (0.54 kJ/kJ ME , $R^2= 0.66$). The egg energy secretion is $317\pm34 \text{ kJ} \cdot \text{d}^{-1}$ (line 2), which is nearly constant and therefore independent of energy supply. Differences between supply and requirements resulted only in gains or losses of body (fat) energy (line 3).The efficiency is with 0.38 kJ/kJ ME low and the relationships with $R^2=0.3$ casually.

The influence of environmental temperature on energy metabolism (figure 5) is characterised by a decreasing of heat production ($-13.65 \text{ kJ/1 } ^\circ\text{C}$, $R^2=0.58$) (line 1) and maintenance energy requirement ($-13.45 \text{ kJ/1 } ^\circ\text{C}$, $R^2=0.64$) (line 2) through increasing of environmental temperature. The influence on egg energy secretion is insignificant (line 3) and low on body energy retention (line 4), which decreased also because feed (ME) intake decreased. The energy requirement for maintenance (MR), calculated by assumption of energetic utilisation of 64 % for egg production (Hoffmann, 1973) and 73 % for body gain, is averagely $675 \text{ kJ ME} \cdot \text{hen}^{-1} \cdot \text{d}^{-1}$ at the environmental temperature of 20°C (line 5). The graduation of decreasing of maintenance indicates the effect of heat requirement and heat losses as well as the possible extent of heat compensation, totally between 5 and 30°C $335 \text{ kJ ME} \cdot \text{hen}^{-1} \cdot \text{d}^{-1}$. This is a volume of 50% of the maintenance requirement at 20°C ! These facts are to consider in all energy metabolism investigations.

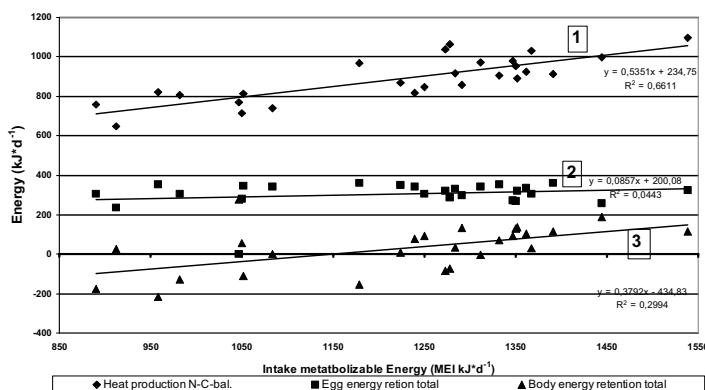


Figure 4. Energy metabolism in dependence of ME intake($\text{kJ} \cdot \text{d}^{-1}$).

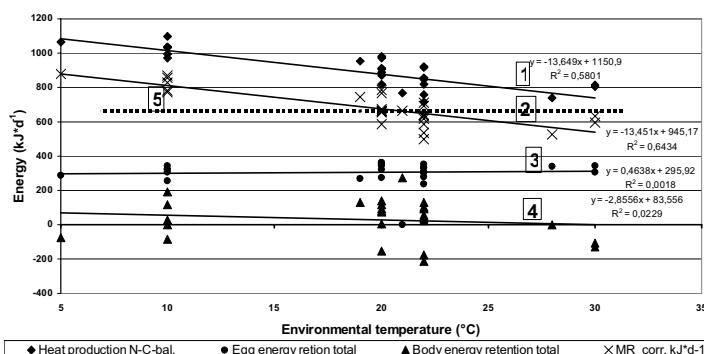


Figure 5. Energy metabolism ($\text{kJ} \cdot \text{d}^{-1}$) in dependence on environment temperature($^\circ\text{C}$).

Conclusions

We recommend the requirements of the high productive strains of laying hens to yield effective egg production as following:

Requirement of amino acids (g/*hen⁻¹* d⁻¹)

| | THR | LYS | CYS | MET | TRP | AA-Protein |
|--------------------------|------|------|------|------|------|------------|
| Egg-Sekretion | 0,27 | 0,38 | 0,12 | 0,16 | 0,07 | 5,5 |
| Feed - gross amino acids | 0,65 | 0,85 | 0,30 | 0,30 | 0,17 | 16,0 |
| - digestible amino acids | 0,46 | 0,56 | 0,21 | 0,22 | 0,13 | 11,7 |

Energy requirements (kJ metabolizable Energy)

| Category | Dimension | BROWN | LSL | |
|---|---|--|-------------|-------------|
| | | 1800 g BW | 1500 g BW | |
| Maintenance and activity | KJ*kg BW ⁻¹ | 345/390 ¹⁾ | 400 | |
| | KJ*[kg BW ^{0,75}] ⁻¹ | 400/450 ¹⁾ | 440 | |
| Egg production | KJ*g ⁻¹ | 9,4 | 9,6 | |
| Gain | KJ*g ⁻¹ | 21 | 21 | |
| Homiothermy (thermal Requirement) | < 22 °C > 22 °C | KJ*[kg BW*°C] ⁻¹ KJ*[kg BW*°C] ⁻¹ | 7,8 4,6 | 6,8 2,5 |
| | < 22 °C > 22 °C | KJ*[kg BW ^{0,75*°C}] ⁻¹ KJ*[kg BW ^{0,75*°C}] ⁻¹ | 7,2 6,15 | 7,65 4,5 |

T = environmental temperature - °C 1) floor keeping

The range of optimal temperature for high productive laying hens is 15/18 - 22 °C, whereas lower temperatures < 18 °C are better tolerated as the higher over 22 °C.

Lighting programmes have no recognizable influence on energy and protein metabolism.

Acknowledgement

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Heat production and substrate oxidation in rats during feeding, starvation and re-feeding

A. Chwalibog¹, A-H. Tauson¹, K. Jakobsen², R. Barea³ & G. Thorbek¹

¹ Department of Animal Science and Health, The Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark

² Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, P.O. Box 50, 8830 Tjele, Denmark

³ Unidad de Nutrición Animal, Estación Experimental del Zaidín (CSIC), Camino del Jueves s/n, 1810 Armilla, Granada, Spain

Summary

Energy metabolism and substrate oxidation were measured in 12 growing rats by means of indirect calorimetry and nutrient balances. The measurements were carried out during 5 days on *ad libitum* feeding, immediately followed by 3 days of starvation and 5 days on *ad libitum* re-feeding. The measurements, carried out in accordance with a model applied for pigs, demonstrating energy transfer between protein, carbohydrate and fat pools, showed that the model is valid for rats. The pattern of protein metabolism was identical in the feeding and re-feeding periods, with 57 % of energy from digested protein being retained and 33 % released as heat. However, the pattern of carbohydrate and fat metabolism was different in the two periods with about 22 % of energy from the carbohydrate pool being transferred to the fat pool during feeding and only 8 % during re-feeding. The total heat production was covered by 83 % from oxidation of carbohydrate and 17 % from oxidation of protein. No oxidation of fat was measured during the feeding periods. The pattern changed completely during starvation in which the main fuel was energy from body fat oxidation.

Keywords: energy expenditure, nutrient oxidation, fasting, rats

Introduction

A model introduced by Chwalibog et al. (1992) describing the pathways of energy flow from digested protein (DP), carbohydrate (DCHO) and fat (DFAT) to energy retained in protein (RP) and fat (RF) or released as heat (HE) was applied on pigs as described in detail (Chwalibog et al., 2003). The model and way of calculation have in the present investigation been used on data from experiments with growing rats during periods of feeding, starvation and re-feeding. The quantitative energy metabolism was calculated in the traditional way, while the energy metabolism of the individual macronutrients and the contribution of heat from oxidized substrates to the total HE was calculated in accordance with the model. The purpose of this study was to quantify changes in substrate metabolism between feeding, starvation and re-feeding.

Materials and methods

Twelve male Wistar rats delivered in three batches with an initial live weight (LW) around 65 g were used in the experiment comprising five days on *ad libitum* feeding, immediately followed by three days of starvation and five days on *ad libitum* re-feeding. A commercial feed compound with 209 g crude protein, 582 g crude carbohydrate, 33 g crude fat and 16.2 MJ gross energy per kg was applied in the feeding periods. The rats were placed in individual metabolic crates for daily collection of faeces, urine and feed residuals. Oxygen consumption and carbon dioxide production

were measured individually each day from 11⁰⁰ to 9⁰⁰ (recalculated to 24 hours values) by means of a respiration unit "Micro-Oxymax Gas Respirometer", Columbus Instruments, Ohio, USA. The ambient temperature was 28 °C and the relative humidity was 65 %, and the rats had free access to water during the whole experiment. The experiments were performed in accordance with Danish legislation.

Results and discussion

The mean LW was 76.5 (SEM 1.55) and the feed intake 10.4 (SEM 0.28) g/d, corresponding to a gross energy intake of 1155 kJ/kg^{0.75} during the 5 days of feeding, in which the LW gain was 4.9 (SEM 0.21) g/d. In earlier experiments with Wistar rats (Thorbek et al., 1982) the same LW gain was obtained, but energy intake was 1420 kJ/kg^{0.75}. The 20 % higher efficiency in the present investigation may be caused by differences in ambient temperature, being kept constant at 28 °C, considered to be in the thermoneutral zone (Hoffmann et al., 1982) while it was only 20 °C in the previous experiment.

Table 1. Energy metabolism. Metabolizable energy (ME), heat production (HE) and energy retained in protein (RP) and fat (RF), kJ/d, during five days of feeding and the three last days of re-feeding

| n = 12 | Feeding | | Re-feeding | |
|--------|---------|------|------------|------|
| | Mean | SEM | Mean | SEM |
| LW, g | 76.5 | 1.55 | 103 | 2.0 |
| ME | 130 | 3.6 | 142 | 4.6 |
| HE | 75.9 | 1.78 | 95.1 | 2.80 |
| RP | 23.9 | 1.42 | 26.1 | 1.31 |
| RF | 29.8 | 2.29 | 20.9 | 3.00 |

The energy metabolism during feeding and re-feeding calculated in the traditional way (Table 1) showed in relation to metabolic LW a higher ME intake of 891 kJ/kg^{0.75} in the feeding period against 783 kJ/kg^{0.75} in the three last days of re-feeding. With HE being identical in both periods with 521 and 523 kJ/kg^{0.75}, the total energy retention (RE) was highest in the feeding period (37 vs 28 %) and the efficiency (RE/ME) was higher (42 vs 33 %). In the previous experiments (Thorbek et al., 1982) the efficiency was only 30 %, caused by the lower temperature and consequently higher requirements for maintenance. In the feeding period energy retained in protein and fat was 24 and 30 kJ/d, corresponding to 1.0 g protein and 0.75 g fat. Calculating with 25 % protein in "meat" gain, the total gain of "meat" + fat was 4.8 g/d, similar to the observed LW gain of 4.9 g/d, indicating a high precision of the measurements.

The energy flow between nutrients during feeding and re-feeding, calculated in accordance with the model is shown in Table 2. The pattern of protein metabolism was identical in the feeding and re-feeding periods with about 57 % of energy from DP being retained and 33 % oxidized. The values of DP and RP in relation to metabolic LW were 282 and 164 kJ/kg^{0.75} in the feeding period. In the previous experiments (Thorbek et al., 1982) DP was much higher with 372 kJ/kg^{0.75}, caused by the lower temperature, but RP was identical, indicating that the level of RP in the present experiment corresponding to 1.0 g/d, must have been near maximum protein gain for the LW in question.

Table 2. Protein, carbohydrate and fat metabolism, kJ/d, during five days of feeding and three last days of re-feeding. Digested protein (DP), retained protein (RP), oxidized protein (OXP), urinary energy from nitrogenous components (UE_N), energy transfer from protein to carbohydrate pool (GLUC). Digested carbohydrate (DCHO), oxidized carbohydrate (OXCHO), urinary energy from N-free components (UE_{N-free}), energy transfer from carbohydrate to fat pool (LIPO). Digested fat (DF), oxidized fat (OXF), retained fat (RF).

| n = 12 | Feeding | | | Re-feeding | | |
|--------------------------------|---------|------|-----------|------------|------|-----------|
| | Mean | SEM | % of pool | Mean | SEM | % of pool |
| <i>Protein metabolism</i> | | | | | | |
| DP | 41.0 | 1.12 | | 45.7 | 1.47 | |
| RP | 23.9 | 1.42 | 57.7 | 26.1 | 1.31 | 57.2 |
| OXP | 13.2 | 0.49 | 32.6 | 15.1 | 0.75 | 33.1 |
| UE _N | 2.6 | 0.10 | 6.4 | 2.9 | 0.15 | 6.4 |
| GLUC | 1.3 | 0.05 | 3.3 | 1.5 | 0.08 | 3.3 |
| <i>Carbohydrate metabolism</i> | | | | | | |
| DCHO | 83.3 | 2.20 | 98.3 | 90.1 | 3.01 | 98.4 |
| GLUC | 1.3 | 0.05 | 1.6 | 1.5 | 0.08 | 1.7 |
| CHO-pool | 84.7 | 2.19 | | 91.6 | 3.02 | |
| OXCHO | 62.7 | 2.05 | 74.3 | 80.0 | 2.45 | 87.9 |
| UE _{N-free} | 3.6 | 0.13 | 4.3 | 4.1 | 0.20 | 4.5 |
| LIPO | 18.4 | 2.14 | 21.5 | 7.5 | 2.85 | 7.6 |
| <i>Fat metabolism</i> | | | | | | |
| DF | 11.4 | 0.28 | 38.3 | 13.4 | 0.35 | 64.1 |
| LIPO | 18.4 | 2.14 | 61.7 | 7.5 | 2.85 | 35.9 |
| FAT-pool | 29.8 | 2.29 | | 20.9 | 3.00 | |
| OXF | 0 | | | 0 | | |
| RF | 29.8 | 2.29 | 100 | 20.9 | 3.00 | 100 |

The pattern of carbohydrate and fat metabolism was different between feeding and re-feeding periods. The intake of DCHO was 15 % lower during re-feeding (497 vs 573 kJ/kg^{0.75}), but OXCHO was identical (441 vs 431 kJ/kg^{0.75}). Thereby the energy flow from carbohydrate to fat metabolism was lowest in the re-feeding period (41 vs 126 kJ/kg^{0.75}). A restoration of the glycogen depots after the foregoing period of starvation has probably taken place, but body analyses are necessary for verification. There was no significant difference ($P>0.05$) between intake of DF in the two periods (76 kJ/kg^{0.75}), but with the low contribution from LIPO and no OXF, fat retention was lowest in the re-feeding period (115 vs 205 kJ/kg^{0.75}), the difference being highly significant ($P<0.001$). The values obtained for RP and RF, in accordance with the model, are in agreement with the values obtained by calculating metabolism in the traditional way.

The mean heat production was 528 kJ/kg^{0.75} in the five days of the feeding period, decreased by about 25 % on the 1st d of starvation, with further decline (40 %) to 323 kJ/kg^{0.75} for all rats on the 3rd d of starvation. A similar decrease of HE to 321 kJ/kg^{0.75} after 2 to 4 days of starvation was measured by Chwalibog et al. (1998). It is interesting to note that in the present investigation four rats from the last batch were near dying on the 3rd d of starvation and their HE was 15 % lower than from the other eight rats (293 vs 338 kJ/kg^{0.75}). However, on the 1st d of re-feeding the 4 weak rats were not significantly different ($P>0.05$), indicating a fast recovery.

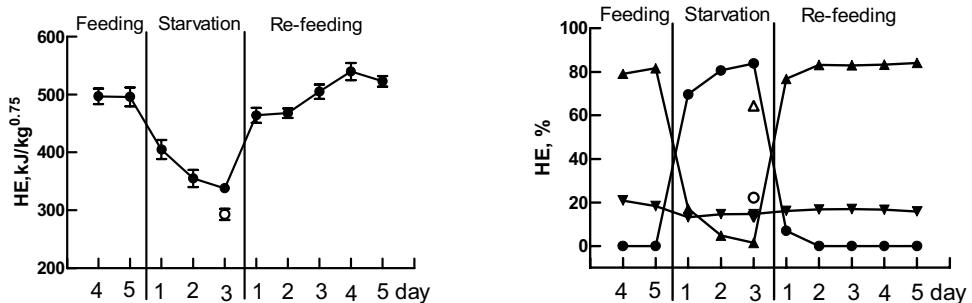


Figure 1. Left: Heat production (HE). Mean and SEM values of 12 rats except for the 3rd d of starvation with 8 and 4 rats (open symbols). Right: Heat from oxidation of protein ▼, carbohydrate ▲ and fat ●, during feeding, starvation and re-feeding.

The contribution of heat from OXP, OXCHO and OXF to the total HE was identical during the periods of feeding and re-feeding with about 83 % from OXCHO, 17 % from OXP and no OXF. During the 1st d of starvation the pattern changed completely with only 17 % from OXCHO, while OXF was the main fuel covering 70 % of HE. On the 2nd d a further reduction of OXCHO took place, being near zero, and on the 3rd d of starvation no OXCHO was measured in the eight rats. The four rats from the last batch showed a remarkable contrast by increasing OXCHO/HE to 64 % and decreasing OXF/HE to 22 %. It may be assumed that the body fat in these rats has been depleted to such an extent that the animals were forced to use energy from their glycogen depots. A reduction of the glycogen depots may increase the risk for low blood glucose concentration followed by coma and death. A slaughter experiment in order to quantify glycogen levels in the liver during feeding and starvation has been started to verify the assumption.

The present results demonstrate that the oxidative hierarchy is directly dependent on nutrient supply, hence during the feeding periods the main source of energy was OXCHO followed by OXP with no OXF, while during the starvation period the main fuel was OXF with no OXCHO as long as body fat reserves could cover energy requirements.

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Diurnal rhythm in heat production and oxidation of substrates in pigs during feeding, starvation and re-feeding

A. Chwalibog, A-H. Tauson & G. Thorbek

Department of Animal Science and Health, The Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark

Summary

Energy metabolism and substrate oxidation was measured in 12 growing pigs divided into two LW ranges (Group A: 20-25 kg and Group B: 30-40 kg) by means of indirect calorimetry and nutrient balances. The measurements were carried out during 6 days of feeding followed by 4 days of starvation and 4 days of re-feeding. The diurnal rhythm of heat production (HE) showed the same pattern during all measurements, but with strongly reduced level during starvation. During the time interval 4⁰⁰-8⁰⁰ the level of HE was lowest and considered as a basic metabolic rate (BMR). During feeding the BMR was 25% lower than the average HE between 12⁰⁰-4⁰⁰ (A: 315, B: 418 kJ/h). The lowest BMR was measured on the 4th d of starvation (A: 217, B: 304 kJ/h). During the first 16 h after feeding carbohydrate oxidation (OXCHO) was able to cover energy requirements without contribution from fat oxidation (OXF). However, about 40 h after feeding OXF became the main fuel and no OXCHO was measured. During the 1st d of re-feeding there was an immediate increase in OXCHO, and from the 2nd d of re-feeding the level of HE was re-established with the major contribution from OXCHO and no OXF.

Keywords: energy expenditure, nutrient oxidation, fasting, basal metabolic rate, pigs

Introduction

Measurements of energy metabolism in rats have shown a pronounced decrease in metabolic rate from the fed state to periods of starvation, being attributed to cessation of food processing in the digestive tract, nutrient assimilation and storage (Forsum et al., 1981; Munch et al., 1993; Chwalibog et al., 1998). In an experiment with pigs the 24 h energy metabolism and oxidation of nutrients during feeding, starvation and re-feeding has shown the same pattern (Chwalibog et al., 2003). By using data from this experiment the aim of the present investigation was to evaluate the diurnal rhythm in HE and the contribution of heat from oxidation of carbohydrate and fat to the total heat.

Materials and methods

The experiment included twelve castrated male pigs of Danish Landrace with six pigs being measured in the LW range 20-25 kg (Group A) and six pigs in the range 30-40 kg (Group B). All pigs were kept in individual metabolic crates, and the data included measurements on the last day of 6 d of near *ad libitum* feeding, immediately followed by 4 d of starvation and 4 d of near *ad libitum* re-feeding.

The gas exchange was measured for 22 hours from 11⁰⁰ to 9⁰⁰. Feeding took place once a day at 11³⁰ and data from the gas exchange measurements between 12⁰⁰-8⁰⁰ were divided in four hours periods, considering the interval between 4⁰⁰-8⁰⁰ as a basal metabolic rate (BMR). For further information see Chwalibog et al. (2003).

Results and discussion

The diurnal rhythm in heat production (HE) during the last day of the feeding period (6th d) demonstrated higher values for Group B, caused by higher LW and feed intake, but the pattern was identical for both groups (Figure 1).

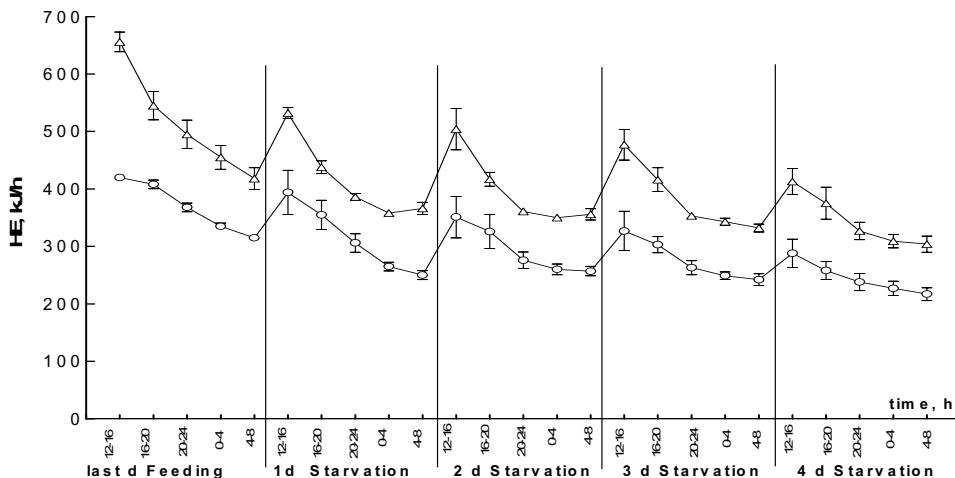


Figure 1. Diurnal rhythm in heat production (HE) on the last day of feeding followed by four days of starvation in Group A \circ and B Δ . Mean and SEM.

The HE values were highest between 12⁰⁰-16⁰⁰, caused by the dietary thermogenesis and animals' activity, and declined gradually to the lowest level between 4⁰⁰-8⁰⁰(BMR). The mean HE for 16 h between 12⁰⁰-4⁰⁰ was 22% higher than BMR for Group A (383 vs 315 kJ/h) and 29% higher for Group B (538 vs 418 kJ/h), indicating a substantial diurnal reduction of HE.

The same pattern of diurnal rhythm in HE was measured during starvation, but on a pronounced lower level, being in accordance with measurements on rats showing a lower energy expenditure by starvation (Forsum et al., 1981; Chwalibog et al., 1998). The lowest BMR was measured on the 4th d of starvation (A: 217, B: 304 kJ/h). The still existing peak between 12⁰⁰-16⁰⁰, even on the 4th d of starvation, may be explained by a higher activity because the pigs were probably looking for feed. The pattern of diurnal rhythm in HE during re-feeding (Figure 2) demonstrated a sharp increment during the 1st d of re-feeding, and then a similar pattern and magnitude as during the last day of feeding.

The contribution of heat from oxidation of carbohydrate (OXCHO) and fat (OXF) to the diurnal rhythm of HE were calculated from the hourly measurements of gas exchange, but without correcting for the urinary nitrogen (UN) excretion, as urine was only collected in 24 h intervals. The lack of UN values in the diurnal estimation will cause an over-estimation in HE, OXCHO and OXF, but as discussed in details by Chwalibog et al. (1998) the overestimation in HE is negligible and has no effect on the pattern of changes between the rates of OXCHO and OXF.

The pattern of OXCHO during the last day of feeding (Figure 3) showed that between 12⁰⁰- 4⁰⁰ OXCHO decreased with 20% in Group A (420 vs 335 kJ/h) and with 30% in Group B (656 vs 455 kJ/h) while no OXF was measured, indicating that digested carbohydrates were able to cover the energy requirements during the first 16 h after feeding. In the following interval 4⁰⁰-8⁰⁰ a slight OXF started with 42 and 31 kJ/h for Group A and B respectively, corresponding to about 10% of HE.

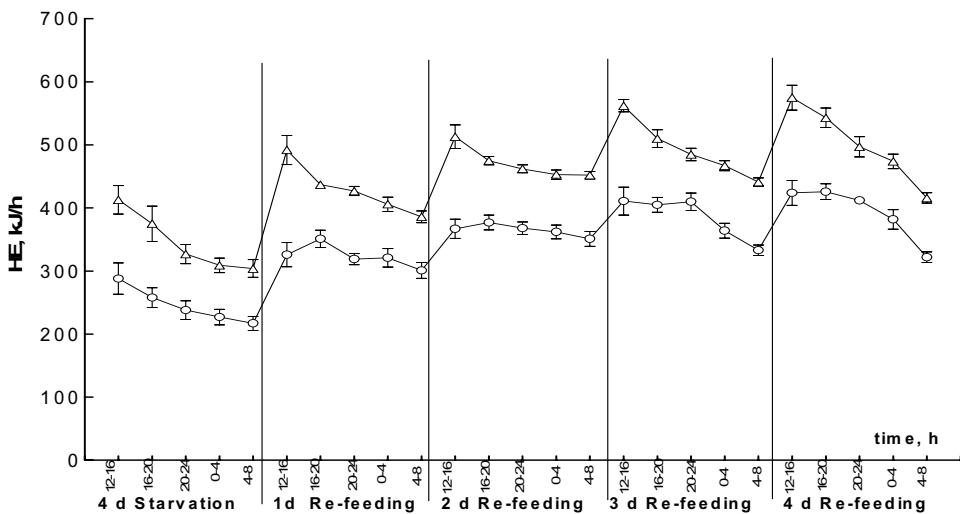


Figure 2. Diurnal rhythm in heat production (HE) on the 4th d of starvation followed by four days of re-feeding in Group A \circ and B Δ . Mean and SEM.

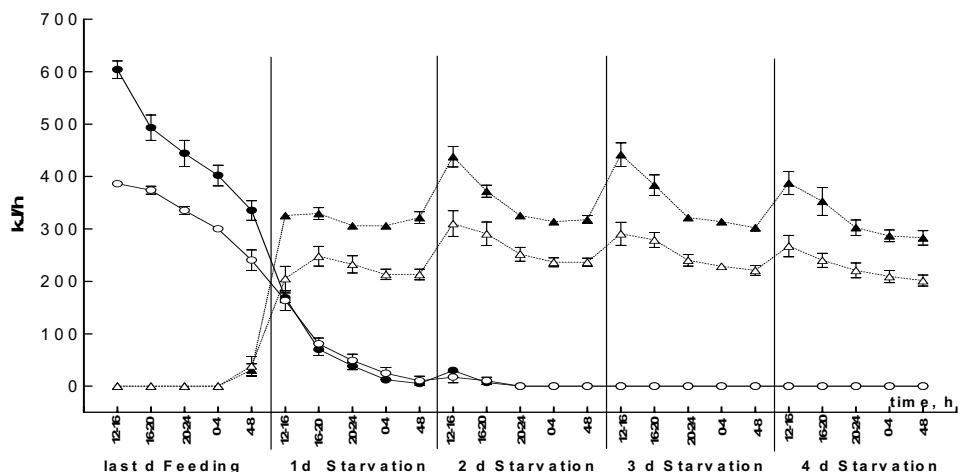


Figure 3. Diurnal rhythm in oxidation of carbohydrate in Group A \circ and B \bullet and fat in Group A Δ and B \blacktriangle on the last day of feeding followed by four days of starvation. Mean and SEM.

During the 1st d of starvation OXF increased reflecting the decrease of OXCHO, being near zero about 40 h after the last feed intake. From the 2nd d of starvation and during the following days OXF covered 92% of HE. Since there was no dietary fat, body fat must have been mobilized and oxidized in order to cover the energy requirements, as previously measured in starving rats (Munch et al., 1993; Chwalibog et al., 1998).

It is interesting to note that on the 2nd d of starvation a slight increment in OXCHO occurred between 12⁰⁰-16⁰⁰, probably caused by a release of glycogen from muscles in connection with physical activity by looking for feed. However, from the 3rd d of starvation OXCHO was zero except for one pig in Group A, being observed as extremely restless.

On the 1st d of re-feeding (Figure 4) a sharp increment in OXCHO took place immediately after feeding (12⁰⁰-16⁰⁰), which continued for the following 8 h (16⁰⁰-24⁰⁰) and then declined.

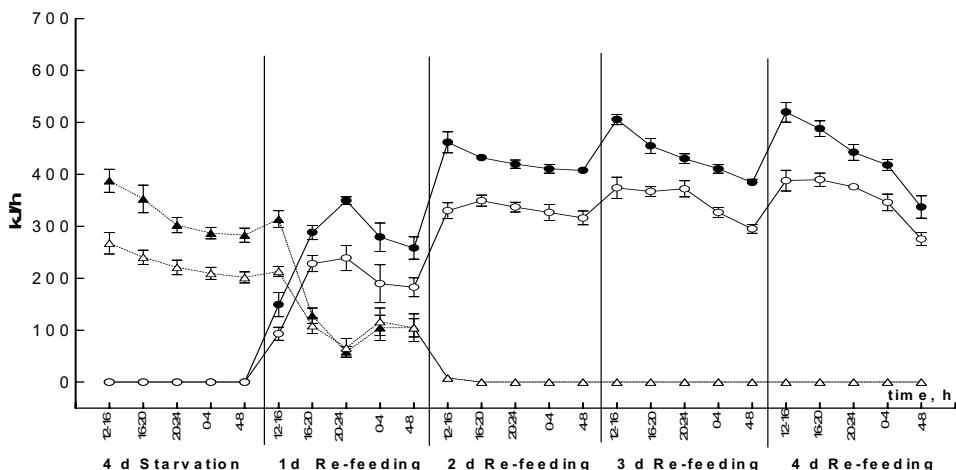


Figure 4. Diurnal rhythm in oxidation of carbohydrate in Group A (○) and B (●) and fat in Group A (Δ) and B (▲) on the 4th d of starvation followed by four days of re-feeding. Mean and SEM.

Simultaneous with the decline in OXCHO fat oxidation increased, covering about 28 and 38% of HE in Group A and B respectively, indicating that digested carbohydrates were not sufficient to cover the energy requirement during the 1st d of re-feeding. From the 2nd d no OXF was measured and the pattern of OXCHO was identical with the pattern from the last day of feeding.

This study demonstrates that the diurnal rhythm of HE, reflecting the diurnal rhythm of energy requirement, is similar during feeding, starvation and re-feeding. However, the contributions of carbohydrate and fat oxidation to the total HE are closely related to the supply of nutrients. As long as the supply of dietary carbohydrates is sufficient to cover energy requirements no OXF occurs, but the situation changes after about 16 h from the last feeding with initiation of OXF and a further decrease in OXCHO. About 40 h after feeding OXF becomes the main fuel. During re-feeding there is an immediate increase in OXCHO, however there is still a substantial contribution from OXF. From the 2nd d of re-feeding the level of HE is re-established with the major contribution from OXCHO and no OXF.

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Jules Reiset (1818-1896): Energy metabolism and environmental research pioneer

William P. Flatt¹ & William J. Payne²

¹ University of Georgia, Departments of Foods and Nutrition, Athens, GA 30602-3632 USA

² University of Georgia, Departments of Microbiology, Athens, GA 30602-3632 USA

Summary

Jules Reiset (1818-1896) was a truly outstanding research scientist who conducted numerous energy metabolism and environmental research studies at his own expense in his privately funded laboratory on his estate (Chateau d'Ecorcheboeuf; Anneville-sur-Scie) near Dieppe, France. Using a closed-circuit respiration system to measure gaseous exchange of decaying organic matter (fermenting manure or putrefied meat) he observed the release of fixed nitrogen, thus demonstrating that N₂ is truly cycled in nature. The nitrogen cycle, so commonly accepted today, should have been known as the **Reiset Cycle**. A few of Reiset's many scientific contributions, including environmental and agricultural studies are summarized in this paper.



Keywords: nitrogen cycle, closed-circuit apparatus

Introduction

Jules Reiset (1818-1896) and his senior colleague Henri-Victor Regnault (1810-1878) at the College of France, have been recognized internationally for more than 150 years for first developing closed-circuit chambers for use in measuring respiratory gas exchange in animals over extended periods. Their classic 220-page publication (Regnault & Reiset, 1849) described the chambers, method of generating oxygen, apparatus for measuring CO₂ production and gas analysis techniques for analyzing oxygen, hydrogen, methane, nitrogen and other gases. There was a particularly ingenious set of devices in light of the necessity they faced of generating free oxygen, a feat they accomplished by heating potassium chlorate and manganese dioxide together. They stored quantities of the gas in reservoirs connected to the airtight enclosures, that were connected in turn to the chambers, thus providing enough oxygen to sustain the experimental animals for prolonged periods. Carbon dioxide production was measured by providing potassium hydroxide within pipettes attached to the respiration chambers, to absorb the gas which they quantified by determining the resulting gain in weight. Simultaneous measurements of gas volumes consumed and released, gas composition, temperature, relative humidity, and barometric pressure were required for precise determination of the amounts of oxygen consumed, of carbon dioxide produced and of any changes in atmospheric nitrogen that might occur in the closed system. It is impressive to recall that these studies were carried out decades before cylinders of compressed

oxygen and other gases, electrical air pumps, automatic gas analysis equipment and other helpful devices were available.

Closed-circuit respiration trials

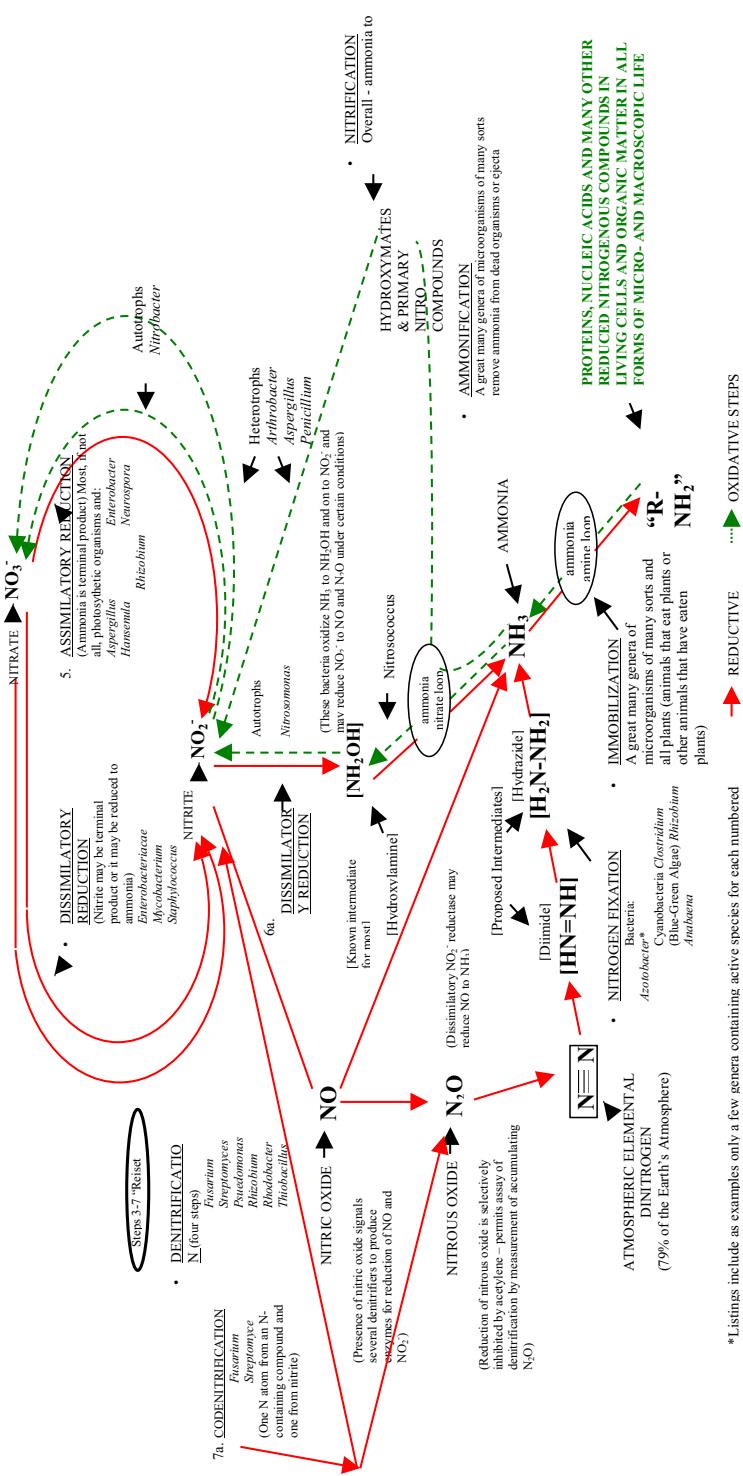
The species studied in the Reiset & Regnault collaboration included warm-blooded animals, such as dogs, cats, and rabbits as well as hibernating and non-hibernating marmots. Cold-blooded animals such as frogs, salamanders, reptiles and lizards were also studied. Hens, ducks, sparrows, greenfinches and crossbills were among the birds studied, along with insects, such as beetles, silkworms, silkworm pupae and may-bugs. In over 100 experiments their assays included the effects of temperature, season, diet, level of nutrition (inanition), composition of air, sex, hibernation, age, body weight, individual variability, oil coating and other variables on respiratory exchange and nitrogen exhalation or absorption. The relationship between oxygen absorbed and carbon dioxide excreted became known as the respiratory quotient (R.Q.). R.Q. = Vol. CO₂/Vol. O₂. The R.Q. values determined by Regnault & Reiset varied from 0.64 to 1.04, depending on the type of feed being eaten by the animals. They concluded that the type of diet consumed affected the R.Q. more than did the class of animal.

It was acceptance and use of the apparatus and of the methods developed by these colleagues that enabled scientists the world over to gain some understanding of utilization of energy by many different species of animals. Authors of several textbooks on animal nutrition (Lusk, 1928; McCollum, 1957; Blaxter, 1962; Kleiber, 1975) written in the twentieth century repeatedly cited the classic paper from 1849 or Reiset's subsequent publications (Reiset, 1863; 1863b) that described a larger closed circuit chamber for use in studying the respiration of sheep, calves, pigs, turkeys and geese.

Other publications by Reiset and his colleagues on topics related to animal physiology, gas analysis techniques, rural economy and nitrogen metabolism provided significant contributions to the scientific literature of his era, but none was cited as extensively and often as those describing the closed circuit respiration system for animals. In fact the inspiration for the space age science-fiction novel by Jules Verne (1865), "De la Terre à la Lune. Voyages Extraordinaires" was based on the methods described by Reiset and Regnault to provide oxygen and remove carbon dioxide produced by men, dogs and chickens in the "projectile". Reiset and Regnault were specifically cited several times (pages 123-124; 174-175; 205; 263) in this famous novel. (Flatt *et al.*, 2002, Wisniak, 2001).

Nitrogen cycle observation

Strangely enough, Reiset's most telling experiment in basic science was very little noticed when first reported, or was dismissed and thus remained uncelebrated. In 1856 he reported (Reiset, 1856) observing that during the complex set of phenomena called putrefaction the decaying organic matter released a portion of its fixed nitrogen into the atmosphere in the free, gaseous, elemental state as N₂, called "azote", Az, by French scientists at that time. For these studies, he replaced the experimental animals with strips of putrescent meat as the "respiring" material. He then perceptively asserted that azote is truly cycled in nature (i.e., after fixation into organic material, the gaseous "balance" in the atmosphere is restored by the process he observed and we now know that, just as importantly, nitrate, NO₃, the source of the nitrogen released by denitrification, does not accumulate to deleterious levels in soils and waters). Reiset's monumental observation might have been expected to bring him accolades from the scientific community, but it was essentially passed over for several decades, until others performed confirmatory experiments. And, even Reiset himself failed to appreciate the full implication of his demonstration. Payne (1988, 1990) noted that when Reiset died in 1896 his eulogizers took no note in his obituary of his significant contribution to the conceptualizing of nature's management of nutrients. The **Nitrogen Cycle**,



*Listings include as examples only a few genera containing active species for each numbered

Figure 1. The nitrogen cycle: A microbiological perspective (W.J. Payne 2003).

which later investigators have now described in such detail and which is so commonly accepted today, should have been designated then and even now should be known as the **Reiset Cycle** (Payne *et al.* 2002). In this regard, as in others, he was truly a man ahead of his time.

Agricultural and environmental studies

In 1850 Reiset established his 200 hectare estate (Chateau d'Ecorcheboeuf; Anneville-sur-Scie, near Dieppe, France) as a personally funded agricultural experiment station where he was active the rest of his life. His methodical approach to solving practical problems helped improve the welfare of the French farmers and citizens, thus dispelling certain myths and superstitions. By collaborating with many of the foremost scientists in Europe in mid nineteenth century, he succeeded in addressing such issues as insect control, soil moisture and drought resistance, sugar beet fermentation, nature of gaseous products of fermentation and putrefaction, use of aged manure as fertilizer, bloat in cattle, milk composition, and effects of rations on mutton quality (Reiset, 1853, 1863b, 1868, 1889). He also recorded over a period of many years the composition of air in different locations in France, including densely populated Paris, open fields, livestock pens, and woodlands (Reiset, 1893). These environmental studies provide additional justification for highlighting the contributions of Jules Reiset.

Civic and philanthropic contributions by Jules Reiset

Reiset also sought to serve the rural community by living and working among his neighbors. In doing so he earned the respect of the citizens of Seine-Inferieure, in particular those residing in the town of Anneville-sur-Scie, whom he served as Mayor for 32 years. He was Deputy of the Legislative Assembly (1858-1863) and was appointed Knight, the Legion of Honor in 1851 and Officer, the Legion of Honor in 1868. His generosity extended to the local Roman Catholic Church and to construction of a school for the boys and girls of the region. Duclaux (1896) commented on Reiset's generosity at his colleague's funeral.

Acknowledgements

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Effect of coping strategy and housing system on energy metabolism of breeding gilts

M.J.W. Heetkamp¹, N.A. Geverink², H. van den Brand¹ & J.W. Schrama³

¹ *Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O.Box 338, 6700 AH Wageningen, The Netherlands*

² *Ethology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O.Box 338, 6700 AH Wageningen, The Netherlands*

³ *Fish Culture and Fisheries Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O.Box 338, 6700 AH Wageningen, The Netherlands*

Summary

Effects of coping strategy and housing system (group vs. individual) on energy partitioning of gilts were studied after environmental changes. Thirty-six high resisting (HR) and 36 low resisting (LR) female piglets were selected on the basis of their response in two successive backtests at 10 and 17 days of age. During backtests piglets were restrained in a supine position for sixty seconds and the number of escape attempts was used to classify HR en LR piglets. After weaning, piglets were group-housed in 12 pens with three HR and three LR pigs per pen. From 7 months of age onwards, gilts out of six pens were housed in individual crates; the other gilts remained group-housed. At 13 months of age, in a 2 x 2 factorial design, 24 groups consisting of three gilts (HR or LR) were housed in one of four climatic respiration chambers, with housing conditions similar to their previous housing conditions (group or individual), for an experimental period of 7 days. On a weekly basis, heat production parameters were not affected by coping strategy (HR or LR), although differences in heat production (Q) between LR and HR gilts were demonstrated in the course of the experimental period, suggesting differences in adaptation. No effects of treatments were found in the change of activity heat production (Q_{act}) and activity corrected heat production (Q_{cor}) during this period. Housing system affected Q and Q_{act} . Q_{cor} was not affected, indicating that only physical activity raised Q. This higher Q and Q_{act} in individual housed animals were present throughout the day.

Keywords: coping strategy, housing system, gilts, energy metabolism

Introduction

Studies in a number of species, including the pig (Hessing et al., 1994), describe two distinct behavioural and physiological response patterns, a proactive or a reactive coping strategy. According to Hessing et al. (1994), an indication for the behavioural and physiological strategy of pigs can be obtained early in life by assessing the degree of resistance displayed in a manual restraint test, the backtest. Several studies in growing pigs (Bolhuis et al., 2002; Ruis et al., 2000), and in gilts (Geverink et al., 2003) have shown that the extreme responders in the backtest, the high resisting animals (HR) and low resisting animals (LR), differ in various behavioral and physiological variables. Furthermore, behavioural and physiological features can be affected by type of housing (Bolhuis et al., 2002).

In this study, effects of coping strategy and housing system (group vs. individual) on energy partitioning, with special emphasis on the change of heat production and activity heat in time, were studied in gilts. The coping style concept implies that HR animals are advantageous in stable situations, whereas LR animals easier adapt in a changing environment. When animals are moved from their customary environment to climate respiration chambers the HR animals probably have more problems to adapt. This is possibly reflected in a decrease of heat production in time.

Materials and methods

Female piglets were selected, out of a large pool of crossbred piglets (Pietrain x ((Large White Duroc) x British Landrace)) bred at a commercial farm, on the basis of their response in a backtest. During a backtest, at 10 and 17 days of age, a piglet was gently removed from its pen and put on its back on a table. It was restrained in this supine position for sixty seconds. The number of escape attempts (i.e. series of struggles of the hind limbs) was scored. A piglet making more than two escape attempts in each test was classified as a High Resister (HR) and when it made less than two escape attempts in each test it was classified as a Low Resister (LR). A total of 36 HR (3.7 ± 0.2 escape attempts) and 36 LR (0.4 ± 0.1 escape attempts) piglets were selected. After weaning, pigs were group-housed in 12 pens with three HR and three LR pigs per pen.

From 7 month of age onwards, six groups of gilts were housed in individual cages and the other six groups remained group-housed. Gilts were housed under thermo-neutral conditions, had free access to water by means of nipple drinkers and had a biting/playing chain available for behavioural studies. Artificial lights were on from 0700 until 1900, and during the night (1900 - 0700) small light bulbs created dimness. Twice a day, the animals were fed a commercial pelleted diet. From 8 months of age onwards, the amount fed was $2 \text{ kg} \cdot \text{gilt}^{-1} \cdot \text{d}^{-1}$.

At 13 months of age a total of 24 groups, containing three HR or three LR gilts, were housed in one of four climatic respiration chambers, for an experimental period of 7 days. The formerly individual-housed gilts were housed in individual cages ($0.85 \times 2.5 \text{ m}$) in one of two large identical, open-circuit, indirect climatic respiration chambers. The formerly group-housed gilts were group-housed in one of two medium-sized, identical open-circuit, indirect climatic respiration chambers measuring $3.5 \times 1.5\text{m}$. During the experimental period, gilts were fed the same diet and amount as before and were kept under the same environmental conditions as before. Pigs received their daily feed in two similar portions at 0800 and 1430. After morning feeding, straw was supplied in the food troughs ($0.03 \text{ kg} \cdot \text{gilt}^{-1} \cdot \text{d}^{-1}$). During a period of 3 days before the experimental period, gilts already received straw in these exact portions and were deprived of straw as bedding on the floor. From 3 days before the experimental period until the end of the experimental period, altrenogest (Regumate Pig, Hoechst Roussel Vet, Brussels, Belgium) was added to the morning feed ($20 \text{ mg} \cdot \text{gilt}^{-1} \cdot \text{d}^{-1}$) to suppress oestrus. All gilts were weighed at the start and end of the experimental period.

Summarizing, a 2×2 factorial design, implemented in 3 identical successive batches of 24 gilts each, with in total 24 experimental units was set-up to study energy partitioning. Energy and nitrogen balances per unit were measured during 7d. Exchange of oxygen, methane and carbon dioxide was determined at 9-min intervals as described by Verstegen et al. (1987) and used to calculate heat production (Q). Physical activity was monitored continuously with radar devices and recorded in the same 9-min intervals as Q. The heat production related to physical activity (Q_{act}) was calculated by means of linear regression. The heat production not related to physical activity (Q_{cor}) was derived by subtracting Q_{act} from Q. Data, first calculated as daily averages, was used to calculate the daily change in time from d1 to d7 of Q, Q_{act} and Q_{cor} . Weekly averages of Q, Q_{act} and Q_{cor} and data on change in time of Q, Q_{act} and Q_{cor} were analysed according to the following model: $Y_{ijk} = m + T_i + H_j + T_i * H_j + B_k + e_{ijk}$ where Y_{ijk} =dependant variable; m=mean; T_i =backtest Type (i=HR,LR); H_j =Housing system (j=group,individual); $T_i * H_j$ =interaction backtest Type and Housing system; B_k =effect of 3 identical successive batches; e_{ijk} =residual error

Results

Energy balance data are presented in Geverink et al (submitted). In short some of their observations were: LR gilts demonstrated a higher metabolizability ($m(E)$), resulting in a higher ADG and RE. Individual housed gilts showed lower $m(E)$, lower RE, higher ME_m and higher Q_{act} . In Table 1 the effect of treatments on heat production parameters is shown.

Table 1. LSMeans of heat production parameters as affected by Type and Housing¹.

| Trait | Group housing | | Individual housing | | | P value | |
|---|---------------|-------|--------------------|----------------|-------|---------|---------|
| | HR | LR | HR | LR | SEM | Type | Housing |
| No. of groups | 6 | 6 | 4 ² | 4 ² | - | - | - |
| Initial body wt, kg | 177.3 | 173.6 | 166.2 | 163.7 | 3.38 | 0.269 | <0.001 |
| ADG, kg/day | 0.79 | 1.14 | 0.02 | 0.29 | 0.140 | 0.039 | <0.001 |
| Heat production, kJ.kg ^{-.75} .d ⁻¹ | | | | | | | |
| Total (Q) | 412 | 407 | 454 | 437 | 7.5 | 0.154 | <0.001 |
| Activity related (Q _{act}) | 69 | 64 | 110 | 100 | 7.9 | 0.374 | <0.001 |
| Activity corrected (Q _{cor}) | 343 | 343 | 344 | 336 | 4.3 | 0.343 | 0.566 |
| Change in time of Heat production, kJ.kg ^{-.75} .d ⁻² | | | | | | | |
| Total (Q) | -0.4 | -3.2 | 1.1 | 0.4 | 0.79 | 0.041 | 0.009 |
| Activity related (Q _{act}) | -1.1 | -2.6 | 1.0 | -0.2 | 1.26 | .271 | .117 |
| Activity corrected (Q _{cor}) | 0.7 | -0.6 | 0.1 | 0.7 | 1.25 | .792 | .827 |

¹ No significant interactions were found and therefore interactions are not presented

² Because of technical problems with some of the activity meters in the first batch we excluded data from 4 groups (2 HR and 2 LR) in the "Individual housing" treatment.

As a 7 day average, Q and Q_{act} were higher in individual housed gilts (P<0.001) but no effect of coping strategy was found. Q_{cor} was not affected by housing system or coping strategy. All changes in time developed rather linear. During the 7 day experiment, looking at changes in time, Q was affected by type of animal and by housing system. In LR gilts Q decreased 1.4 kJ.kg^{-.75}.d⁻¹ every day while HR gilts showed an increase of 0.4 kJ.kg^{-.75}.d⁻¹ every day. Group housing reduced Q by 1.8 kJ.kg^{-.75}.d⁻¹ day by day whereas individual housing increased Q by 0.7 kJ.kg^{-.75}.d⁻¹ day by day. Analyzing data by day showed a housing effect (P<0.01) on Q and Q_{act} every day and a Type effect on Q on d 7 (434 vs. 416 kJ.kg^{-.75}.d⁻¹ for HR and LR, respectively).

Finally, as hourly averages of 7 days, diurnal rhythms of Q, Q_{act} and Q_{cor} are presented in Figure 1. Throughout the day Q and Q_{act} in nearly every hour, were affected (P<0.05) by housing system while Q_{cor} during the day was very similar between treatments. Type of animal only affected Q and Q_{act} between 18:00 and 21:00 hour and in the hour after feeding at 8.00 a.m.

Discussion

ADG of LR gilts is higher so they seem to have an advantage in terms of energy efficiency when environment is changed. Effects of different coping styles in gilts using heat production data on a weekly basis are not present. Demonstrating the coping style theory using daily data on Q and Q_{act} indicate increasing differences in Q between LR and HR gilts in the coarse of the first week after environmental changes.

This indicates that LR gilts adapt faster than HR gilts. Housing system had an effect on Q and Q_{act}. On the other hand Q_{cor} was not affected indicating that only physical activity raised Q. Higher Q and Q_{act} in individual housed animals seems to be present throughout the day. Geverink et al. (2003) already demonstrated in the same animals, a higher level of oral stereotypies in the individually housed gilts, which probably explains the higer Q_{act} of these animals.

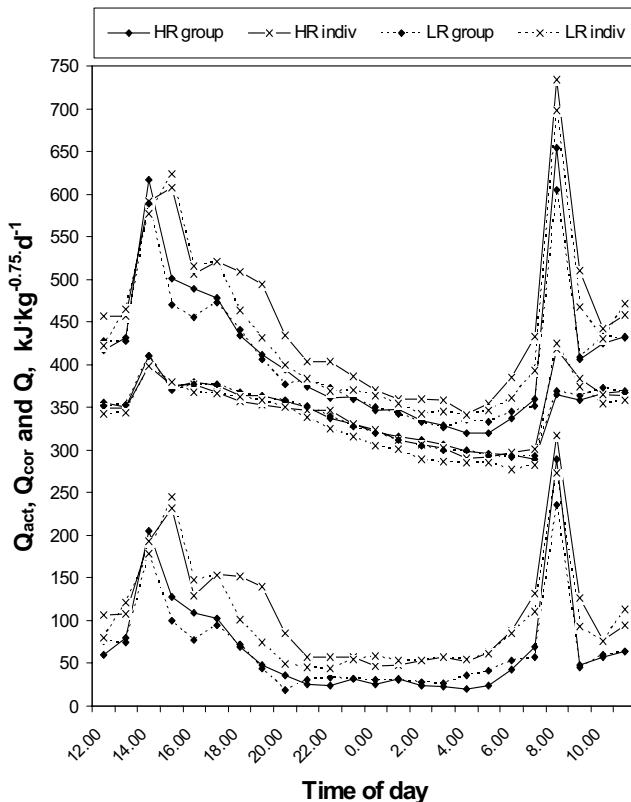


Figure 1. Circadian rhythms in Q , Q_{cor} and Q_{act} (sets of 4 lines from top till bottom, respect.).

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Methane emission by charolais cows under minimised condition for selective grazing

C.S. Pinares-Patiño¹, K.A. Johnson² & C. Martin¹

¹ *Equipe Digestion et Valeur des Aliments, Herbivore Research Unit, INRA Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France*

² *Department of Animal Sciences, Washington State University, Pullman 99164, USA*

Summary

In order to examine the relationship between methane (CH_4) emission and herbage quality under minimised conditions for selective grazing, six 8-yr old, dry and early-pregnant charolais cows (712 ± 22.7 kg LW) were grazed on a mono-specific pasture of timothy at four phenological stages: early vegetative, booting, full flowering and senescence. Daily CH_4 production was measured during seven days using the sulphur hexafluoride (SF_6) tracer-gas technique. Organic matter intake (OMI) was determined from fecal output estimated by Yb_2O_3 dosing and feed digestibility (OMD) estimated using fecal N index. OMD (%) decreased ($P < 0.05$) from 77.6 at early vegetative to 56.2 at senescence, but no cow effect upon OMD was observed. OMI (14.8 g kg^{-1} LW) and CH_4 production (273 g d^{-1}) at booting were higher ($P < 0.05$) than the corresponding values at the other stages. However, the proportion of gross energy intake lost in CH_4 , did not differ with phenology (mean 6.4% of GEI). Cow effect upon OMI and CH_4 emission was significant ($P < 0.05$). CH_4 production (g d^{-1}) was not related to OMD, but had a strong correlation with digestible NDF intake (kg d^{-1}) ($r = 0.61$, $P < 0.01$). At all the phenological stages the between-cow variation explained a large proportion (54 to 70%) of the total variation in CH_4 production.

Keywords: methane, cattle, timothy

Introduction

Under controlled conditions, Blaxter & Clapperton (1965) observed that both feed intake and digestibility were important factors determining CH_4 emission from ruminants. However, under grazing conditions, McCaughey et al. (1999) failed in predicting CH_4 emission on the basis of these factors and suggested that selective grazing was the reason for the failure. Variation between animals in selective grazing may also contribute to the larger between-animal variation in CH_4 emission observed in sheep (Ulyatt et al., 1999) compared to that commonly found in calorimetry chambers (see, for example, Blaxter & Clapperton, 1965).

Variation in diet quality resulting from differences in selective grazing between animals is not accounted for by the available techniques for herbage intake estimation. However, it is well established (e.g. Demarquilly et al., 1995) that due to chemical and physical changes in the plants, forage quality decreases with advancing phenological development. Thus, if homogeneous herbages differing in phenological development are offered under conditions which minimise selective grazing, a relationship between herbage quality and CH_4 emission may be established and the resulting between-animal variation in CH_4 emission may be attributable to factors other than differences in diet selection. This study was planned to examine the above relationships with cattle grazed on timothy at four stages of phenology.

Material and methods

The study was carried out during the 2001 grazing season on a 4-ha mono-specific pasture of timothy (*Phleum pratense* L. cv. Climax) located at Laqueuille (1250 m.a.s.l.; Auvergne, France). Six 8-yr old, dry and early-pregnant charolais cows (712 ± 22.7 kg LW) were grazed at controlled herbage allowances (HA) during four consecutive periods corresponding to four phenological stages of the pasture: early vegetative, booting, full flowering and senescence. Cows were strip-grazed controlled by electric fences. Daily HA was calculated to offer twice the cows intake capacity (Geay & Micol, 1988). At each period, animals were acclimatised for seven days (d 1-7) before a 7-d measurement phase (d 8-14) began.

Herbage mass (HM) was measured daily, before the animal occupation. Four quadrats (40×50 cm) were randomly positioned over the strip and the herbage cut 5 cm above the ground level. Samples of the herbage eaten by the cows were collected immediately before the cows were offered the strip, by cutting to the height grazed on the previous day. Organic matter (OM) intake (OMI) by individual cows was determined from faecal OM output and OM digestibility (OMD) of the herbage eaten. Faecal OM output was calculated using ytterbium as a marker. Gelatin capsules containing 0.9 g of Yb_2O_3 were orally dosed twice daily over days 3-14. Daily samples of faeces corresponding to each cow were proportionally collected from dung pats. For this purpose, coloured plastic particles were orally dosed (80 g cow^{-1}) once daily over days 6-14. OMD was estimated from faecal nitrogen (N) contents, using the equation of Chenost (1985). CH_4 production was measured daily (over days 7-13) using the SF_6 tracer technique (Johnson et al., 1994) as described by Ulyatt et al. (1999).

Samples of herbages (both, on offer and eaten) were oven-dried (60°C , 48 h), later pooled within the measurement periods, ground (1-mm) and used for OM, gross energy (GE), N, neutral detergent fibre (NDF) and acid detergent fibre (ADF) analyses according to the procedures described by Pinares-Patiño et al. (2003). Dried faecal samples (60°C , 96 h) were ground, pooled within individual cows and analysed for OM, N, NDF and Yb contents. Yb was determined by atomic absorption spectroscopy using a nitrous oxide/acetylene flame.

Data for mean daily feed intake, apparent feed digestibility and CH_4 emission were analyzed by ANOVA using the model: $Y_{ij} = \mu + C_i + P_j + e_{ij}$, where Y_{ij} is the variable under consideration; μ is the overall mean; C_i is the i th cow; P_j is the j th phenological stage and e_{ij} is the experimental error term. Within each phenological stage, the proportion (R^2) of the total variation in CH_4 production (g d^{-1}) due to between-cow and between-day sources were calculated by fitting the effects of cow and day in the ANOVA.

Results and discussion

HM (kg DM ha^{-1}) of the vegetative sward was the lowest (1167), whereas HM at full flowering was the highest (7261). As a consequence of decay, HM decreased by 12% from full flowering to the senescent stage.

Predictably, herbage CP declined, and NDF and ADF contents increased, with advancing phenological stages (Table 1). Consequently, OMD (%) decreased ($P < 0.05$) with advancing phenological development, but no cow effect upon OMD was observed. The latter was most likely due to the conditions which minimised selective grazing.

Daily GEI and OMI observed at early vegetative, flowering and senescent stages were similar from each other (Table 2), whereas the corresponding intakes at booting were significantly ($P < 0.05$) higher than that at the other three stages. Intake of forages is affected by rates of digestion and passage, which are closely related to the NDF contents (Demment et al., 1995). In the present study we aimed to achieve an uniform intake of NDF by limiting the HA. In fact, except at the early vegetative stage, the daily intakes of NDF were maintained (Table 2). The early vegetative

Table 1. Chemical composition (DM basis) and organic matter digestibility (OMD) of the herbage eaten at each phenological stage.

| | Vegetative | Booting | Flowering | Senescent |
|---------------------------|------------|---------|-----------|-----------|
| OM (%) | 93.40 | 93.88 | 95.51 | 96.57 |
| CP (%) | 31.38 | 13.22 | 7.84 | 4.41 |
| NDF (%) | 52.63 | 59.84 | 68.43 | 75.35 |
| ADF (%) | 24.86 | 32.31 | 40.26 | 45.34 |
| GE (MJ kg ⁻¹) | 19.82 | 18.93 | 19.10 | 18.78 |
| OMD (%) | 77.6 | 74.8 | 63.8 | 56.3 |

Table 2. Effects of phenology and cow on daily feed intake and CH₄ emission (n=24).

| | Phenology effect ¹ | | | Cow effect | | |
|------------------------------|-------------------------------|---------|-----------|------------|------------------|---------|
| | Vegetative | Booting | Flowering | Senescent | SEM ² | P value |
| LW (kg cow ⁻¹) | 723a | 761b | 770b | 800c | 5.6 | <0.01 |
| Feed intake: | | | | | | |
| GEI (kJ kg ⁻¹ LW) | 266a | 299b | 264a | 244a | 10.3 | 0.03 |
| OMI (g kg ⁻¹ LW) | 12.5a | 14.8b | 13.2a | 12.5a | 0.51 | 0.03 |
| NDFI (g kg ⁻¹ LW) | 7.1a | 9.5b | 9.4b | 9.8b | 0.35 | 0.03 |
| CH ₄ emission: | | | | | | |
| g d ⁻¹ | 204a | 273b | 232a | 228a | 9.5 | 0.04 |
| % of GEI | 5.9a | 6.7a | 6.6a | 6.5a | 0.27 | <0.01 |
| g kg ⁻¹ OMI | 22.6a | 24.4a | 23.6a | 22.8a | 0.97 | <0.01 |

¹ Values with different letter in the same row differ significantly between phenological stages ($P < 0.05$).

² Standard error of mean.

HM was around 1000 kg DM ha⁻¹ and this probably limited intake by affecting bite weight (Peyraud et al., 1996).

Cows differed ($P < 0.05$) from each other in their daily feed intakes (Table 2). The mechanisms of these differences are unknown. Jarrige et al. (1995) reported that social dominance did not affect feed intake when grazing conditions were not limiting. However, under the conditions of the present study social dominance may had influenced intake.

The effect of phenology stage upon daily CH₄ production (g d⁻¹) was significant ($P < 0.01$) (Table 2). CH₄ productions at vegetative, flowering and senescent stages were similar from each other, but CH₄ production at booting was higher than that at the other stages. Neither CH₄ yield (% of GEI), nor the CH₄ production per unit of OMI (g kg⁻¹) differed with phenological stage (Table 2). The mean CH₄ yield found in this study (6.4 % of GEI) was within the range (6.2-7.7) found by Ortigues et al. (1993) in calorimetry chambers for charolais cows fed on grass hay.

Neither CH₄ production (g d⁻¹) nor CH₄ yield (% of GEI) was correlated with feed digestibility. CH₄ production (g d⁻¹) was positively correlated with LW (kg cow⁻¹) ($r = 0.40$, $P = 0.05$), GEI (MJ d⁻¹) ($r = 0.49$, $P = 0.02$) and OMI (kg d⁻¹) ($r = 0.53$, $P < 0.01$). However, a much higher correlation was observed between CH₄ production and the intake of the digestible fraction of NDF (DNDFI, kg d⁻¹) ($r = 0.61$, $P < 0.01$). A relationship similar to the latter was also found on small ruminants (Pinare-Patiño et al., 2003), confirming the concept that CH₄ production is mainly a function of the amount of cell walls digested (Moe & Tyrrell, 1980).

Despite the minimised conditions for selective grazing, cow effects upon CH₄ emission parameters were significant (Table 2). At all the phenological stages the between-cow source explained a large proportion (54-70 %) of the total variation in CH₄ production (g d⁻¹). This suggests that there exist intrinsic animal differences associated with CH₄ production.

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Housing conditions and carbohydrate source affect within-day variation of energy metabolism in growing pigs

M.M.J.A. Rijnen¹, J.J.G.C. van den Borne², J.W. Schrama³ & W.J.J. Gerrits^{2, 4}

¹ Nutreco - Hendrix UTD, P.O. Box 1, 5830 MA, Boxmeer, The Netherlands

² Animal Nutrition Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands

³ Fish Culture & Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands

⁴ Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands

Summary

In the present study, effects of housing conditions (i.e. individual vs. group housing) and carbohydrate source (i.e. sugar beet pulp vs. maize starch) on energy metabolism and circadian rhythms in energy expenditure and physical activity were studied in growing pigs in a 2×2 factorial design. No interactions between housing conditions and diet composition were present. Digestibility and metabolisability of dietary energy was higher for individually housed pigs than for group-housed pigs. Circadian rhythms in energy expenditure and energy expenditure for physical activity were clearly affected by housing conditions and diet composition. Total energy expenditure, as well as activity related energy expenditure, was increased during the dark phase of the day in individually housed pigs when compared with group-housed pigs. Pigs fed the sugar beet pulp based diet had a reduced energy expenditure on physical activity, which mainly occurred during the night.

Keywords: energy metabolism, circadian rhythm, pigs

Introduction

In feed evaluation studies, growing pigs are mostly housed individually, whereas in commercial conditions growing pigs are group-housed. Various studies reported effects of housing conditions on behaviour, feeding patterns, performance and faecal digestibilities (e.g., De Haer & De Vries, 1993; Gomez et al., 2000). Little is known, however, about the effects of housing conditions on energy metabolism in growing pigs. Research on energy metabolism of group-housed growing pigs and sows has showed that pigs spend less energy on physical activity, when the intake of dietary fibre from sugar beet pulp (SBP) increases (Schrama et al., 1998; Rijnen et al., 2003). These findings are not always consistent with literature data. In this paper, it is hypothesized that compared with group-housed pigs, individually housed pigs have little opportunity for altering their physical activity and lack the presence of social interactions with other pigs. Therefore, diet induced reduction in physical activity may depend on (experimental) housing conditions. This paper addresses this hypothesis, with special emphasis in circadian rhythms in energy expenditure and physical activity.

Material and methods

The effects of housing conditions (i.e. individual vs. group-housing) and carbohydrate source (i.e., SBP vs. starch) on energy metabolism and circadian rhythms in energy expenditure and physical activity was studied in a 2×2 factorial design. Therefore, two diets were composed: a control

diet that consisted of 75% basal diet supplemented with 20% gelatinised maize starch and 5% potato protein and a SBP diet in which 15% of the gelatinised maize starch and 1% of the potato protein from the control diet was replaced by 17.5% SBP. Pigs on both diets were fed similar amounts of the basal diet, therefore feeding level (in g/d) of pigs fed the SBP diet was 1.5% higher than pigs fed the control diet. Pigs were fed restricted ($2.5 \times$ maintenance) in two equal portions at 0800 and 1530.

In total, 10 groups of 14 pigs and 10 individually housed barrows (44 kg) were studied in five trials. The experiment consisted of a 13-d preliminary period and a 13-d experimental period. At the start of the experimental period, pigs were housed, either individually or in groups, in one of four climatically controlled respiration chambers. In the chambers, ambient temperature, relative humidity and air velocity were fixed at 20°C, 65% and <0.2 m/s, respectively. Lights were on from 0700 until 1900. During the experimental period, nitrogen and energy balances were measured weekly. Energy expenditure (EE) was measured using indirect calorimetry in 9-min intervals. Physical activity was recorded using radar-Doppler devices according to the method used by Wenk & Van Es (1976). Per respiration chamber, the 9-min data on EE were related to physical activity and energy expenditure for physical activity (EE_A) was calculated. Resting energy expenditure (REE) was calculated by difference of EE and EE_A . All traits were analysed for the effects of housing conditions, diet composition and their interaction using a two-way ANOVA.

Results and discussion

The effects of housing conditions and diet composition on energy partitioning are shown in Table 1. No interactions between housing conditions and diet composition were present for energy metabolism traits. Metabolisability (ME:GE) and digestibility (DE:GE) of dietary energy were higher ($P < 0.001$) for individually housed pigs than for group housed pigs. Total EE and EE_A were not affected by housing conditions, whereas REE was higher ($P = 0.04$) for individually housed pigs than for group-housed pigs. Metabolisability and digestibility of dietary energy was higher ($P < 0.001$) for pigs fed the control diet than for pigs fed the SBP diet, due to a difference in digestibility. Total EE and REE were unaffected by dietary treatment, whereas EE_A tended to be higher ($P = 0.08$) for pigs fed the control diet than for pigs fed the SBP diet, which is similar to the results of Schrama et al. (1998).

Circadian rhythms in EE and EE_A were dependent on housing conditions (Figure 1). At the start of the light period (at 0700) and during morning feeding (from 0800 to 0900) EE and EE_A were not affected by housing conditions. From 1000 until the afternoon feeding (from 1530 to 1630), EE_A was lower ($P < 0.05$) for individually housed pigs than for group-housed pigs, which caused differences in EE. The first hour after afternoon feeding, EE_A was lower ($P < 0.05$) for individually housed pigs than for group-housed pigs. From 1900 until midnight, EE_A was higher ($P < 0.05$) for individually housed pigs than for group-housed pigs, which caused differences in EE. During the rest of the dark period, EE_A was not affected by housing conditions. The differences in circadian rhythms in EE and EE_A are also shown in Table 1. During the light period, EE tended to be lower ($P = 0.07$) for individually housed pigs and during the dark period, EE was higher ($P < 0.001$) for individually housed pigs than for group-housed pigs. During the light period, EE_A was lower ($P = 0.006$) for individually housed pigs than for group-housed pigs, but during the dark period, this was reversed ($P < 0.001$). The mechanism for these differences is not clear, but may be related to stress and/or stereotypic behaviour in individually housed pigs, isolated in respiration chambers. Circadian rhythms in EE and EE_A were similar for both diets (Figure 2). During the light period no effect of diet composition was found, but during the dark period EE_A was higher ($P < 0.001$; Table 1) for pigs fed the control diet than for pigs fed the SBP diet.

Table 1. Effects of housing conditions and diet composition on energy partitioning in pigs.

| | Housing conditions | | Diet | | SEM | P-value ^a | |
|---|--------------------|------------|----------------------|------|-----|----------------------|--------|
| | Group ^b | Individual | Control ^b | SBP | | H | D |
| GE intake, $\text{kJ}\cdot\text{kg}^{-0.75}\cdot\text{d}^{-1}$ | 1192 | 1168 | 1173 | 1186 | - | - | - |
| DE:GE, % | 87.6 | 89.9 | 90.6 | 87.1 | 0.1 | <0.001 | <0.001 |
| ME:GE, % | 81.3 | 85.0 | 84.9 | 81.4 | 0.3 | <0.001 | <0.001 |
| Energy expenditure, $\text{kJ}\cdot\text{kg}^{-0.75}\cdot\text{d}^{-1}$ | | | | | | | |
| Total (EE) | 646 | 657 | 657 | 646 | 8 | 0.330 | 0.356 |
| light period ^c | 716 | 690 | 707 | 699 | 10 | 0.072 | 0.592 |
| dark period ^c | 576 | 624 | 607 | 593 | 8 | <0.001 | 0.233 |
| Activity-related (EE_A) | 83 | 74 | 84 | 73 | 4 | 0.178 | 0.077 |
| light period | 127 | 87 | 110 | 103 | 8 | 0.006 | 0.558 |
| dark period | 38 | 61 | 58 | 42 | 2 | <0.001 | <0.001 |
| Resting (REE) | 562 | 583 | 571 | 573 | 6 | 0.038 | 0.778 |
| light period | 588 | 603 | 594 | 596 | 7 | 0.142 | 0.867 |
| dark period | 537 | 563 | 548 | 552 | 6 | 0.011 | 0.699 |
| Energy retention, $\text{kJ}\cdot\text{kg}^{-0.75}\cdot\text{d}^{-1}$ | | | | | | | |
| Total (RE) | 328 | 340 | 344 | 324 | 9 | 0.375 | 0.160 |
| Protein (REp) | 137 | 151 | 150 | 138 | 2 | <0.001 | 0.003 |
| Fat (REF) | 191 | 189 | 194 | 186 | 10 | 0.920 | 0.585 |

^a D = effect of diet, H = effect of housing conditions.

^b During one week of one group of pigs on the control diet, EE_A was not measured due to technical failure.

^c Light period from 0700 to 1900; dark period from 1900 to 0700.

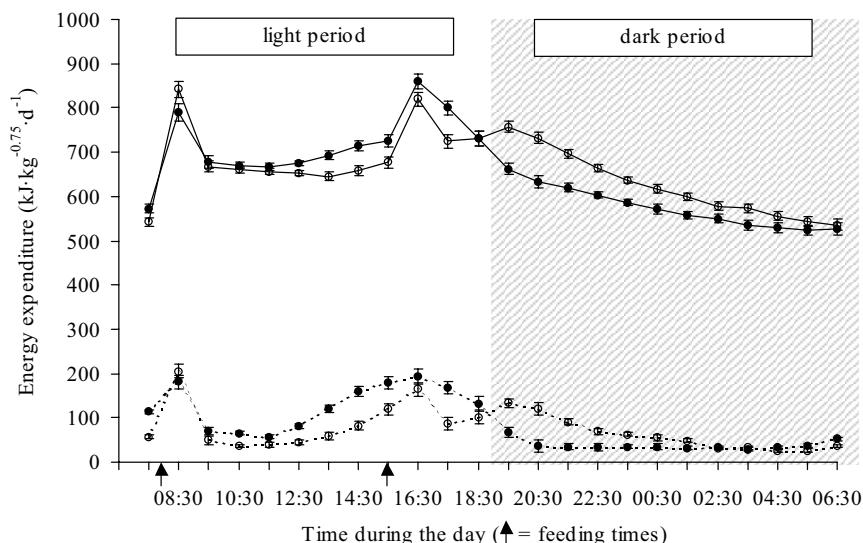


Figure 1. Circadian rhythms in energy expenditure (—) and energy expenditure for physical activity (---) for individually housed (○) and group-housed (●) growing pigs.

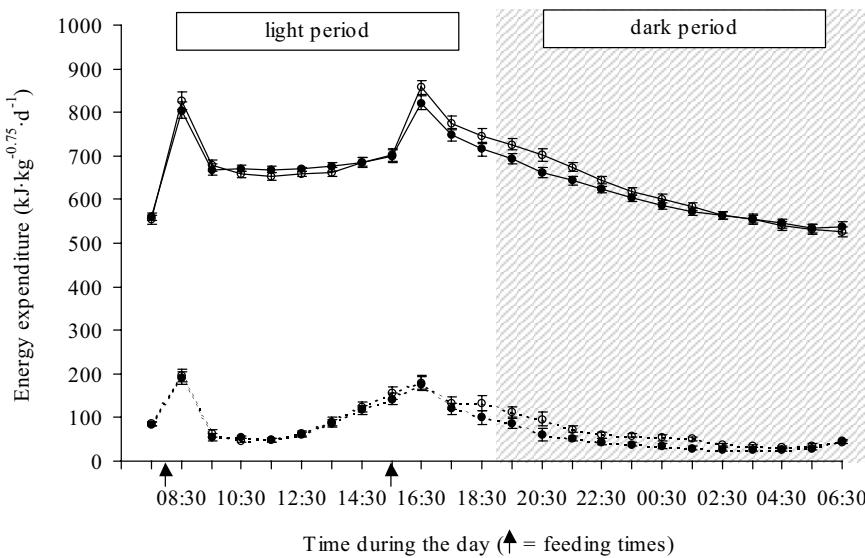


Figure 2. Circadian rhythms in energy expenditure (—) and energy expenditure for physical activity (---) for pigs fed the control diet (○) and for pigs fed the sugar beet pulp diet (●).

Conclusions

This experiment illustrates the absence of an interaction between housing conditions and the energy value of fermentable carbohydrates from SBP. The reduced physical activity, induced by fermentable carbohydrates from SBP, observed in earlier studies, was confirmed, and occurred mainly during the dark phase of the day. Interestingly, meal-fed, individually housed pigs exhibit a clearly different circadian rhythm compared with meal-fed group-housed pigs. When compared with the group-housed pigs, the individually housed pigs showed an increase in total, as well as activity related energy expenditure during the dark period.

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Control and energetic recycling of methane emitted from ruminants

J. Takahashi¹, Y. Gamo¹, B. Mwenya¹, B. Santoso¹, S. Chetral¹, K. Umetsu¹, H. Mizukoshi², K. Kimura² & O. Hamamoto³

¹ Department of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan

² Yakult Central Institute for Microbiological Research, Tokyo, 186-0011, Japan

³ Mitsui Engineering & Shipbuilding Co., Ltd. Tokyo 104-8439, Japan

Summary

To abate rumen methanogenesis with nutritional manipulation the *in vitro* and *in vivo* trials have been conducted to clarify the suppressing effects of nisin, some strains of lactic acid bacteria and yeast and/or β 1-4 galactooligosaccharide and *yucca schidigera* on methane emission compared with monensin. For *in vitro* trials, cumulative methane production were compared by using anaerobic methane production system inoculated the strained rumen fluid of rumen fistulated Holstein cow.

For *in vivo* trial eight sheep were used for two sets of 4x 4 Latin square designs with four sequential ventilated head cages to examine the manipulating effects of β 1-4 galactooligosaccharide, lactic acid bacteria (*Leuconostoc mesenteroides* subsp. *mesenteroides*), yeast (*Trichosporon sericeum*), nisin and *yucca schidigera* on rumen methanogenesis. Furthermore, energy as a biogas recycled from animal effluent was evaluated with anaerobic bioreactors. The utilization of the recycling energy for fuel of co-generator and fuel cell was tested in the thermophilic biogas plant system.

From the results of *in vitro* and *in vivo* trials, β 1-4 galactooligosaccharide, *Candida kefyr*, nisin and L-cysteine were suggested to possibly control rumen methanogenesis in the rumen. It is possible to simulate the available energy recycled through animal effluent from feed energy resources by making out total energy balance sheets from feed energy to recycled energy.

Keywords: methane, oligosaccharide, fuel cell

Introduction

Methanogens in the rumen are hydrogenotrophic bacteria which carbon dioxide is chiefly reduced with hydrogen though the methanogens generate the methane from the acetic acid, the formic acid, the methanol, and the methylamine, etc. The mechanism has a complex route by the enzyme reaction in seven stages (DiMarco, et al., 1990). The fiber bacteria that use structural carbohydrates such as cellulose and hemicelluloses produce this hydrogen. When the hydrogen accumulates in large quantities in the rumen, the proliferation and activities of rumen microorganisms are inhibited by high pressure of hydrogen. The generation of methane with methanogens has an important meaning of removal of harmful hydrogen for the rumen microorganism (Interspecies Hydrogen Transfer) (Hegarty & Gerdes, 1999; Miller, 1995). However, rumen methanogenesis becomes a big load in environmental preservation. Therefore, it is a global issue to control rumen methanogenesis, which is not only animal agriculture but also, should be solved from respect of the prevention of global warming.

On the other hand, methane included in the biogas generated from anaerobic fermentation of animal effluent is convertible to an alternative energy source of fossil fuel. Furthermore, hydrogen reformed from methane can be used to fuel cell power generation as fuel supply. Whilst rumen methanogenesis must be, therefore, reduced to abate greenhouse effect, the potential energy of

effluent should be recycled as a useful alternative energy source to reduce fossil energy consumption.

The present paper deals with some nutritional options using some probiotics and natural compounds compared with monensin to abate methane emission from farm animals, especially ruminants and the recycling rate of the potential energy of effluent as a fuel source of co-generator and fuel cell.

Materials and methods

In vitro continuous gas production system (Takahashi, 2002) was used to run these experiments. Rumen fluid harvested from two rumen fistulated cows was pooled and strained through woven nylon cloth. Then, 750 ml of strained rumen fluid was then mixed with 750 ml of autoclaved buffered artificial saliva. The buffer fluid was kept in four-neck incubation flask. The incubation was carried out anaerobically at 39 °C for 12 h with the addition of 5 g of orchardgrass silage.

To test the suppressing effects of β 1-4 galactooligosaccharide (GOS, 200 mg l⁻¹) and /or two different probiotics (two strains of yeast and lactic acid bacteria), and monensin, nisin and nisin with GOS on the cumulative methanogenesis. Control (CTR) incubations were conducted without any manipulators. The preparation of β 1-4 galactooligosaccharide (Yakult Central Institute for Microbiological Research, Tokyo, Japan) contains 49% galactooligosaccharide. Three strains of lactic acid bacteria (*Leuconostoc mesenteroides* subsp. *mesenteroides*: L1, *Leuconostoc lactis*: L2 and *Lactococcus lactis* subsp. *lactis*: L3) and three strains of yeast (*Candida kefyr*: Y1, *Saccharomyces pastorianus*: Y2 and *Trichosporon sericeum*: Y3) used as probiotics in this trial had been extracted from naturally-fermented milk "Laban" produced from sheep milk in Yemen. Lactic acid bacteria and yeast were prepared by growth on MRS broth and YM broth, respectively. Four rumen-fistulated wethers (51-55 kg) were allocated to four dietary treatments in each 4 × 4 Latin square design. All animals were individually maintained in metabolic crate equipped with a ventilated hood respiratory system to capture respiratory methane emission in experiment 1 and 2 (Takahashi, 1999). For experiment 1, animals were fed on a basal diet comprising timothy hay, lucerne hay cube and concentrates in a ratio 4:3:3 on DM basis at a maintenance level (55 g DM kg body weight^{-0.75}) and examined the suppressing effects of β 1-4 galactooligosaccharide (GOS, 20g), lactic acid bacteria (*Leuconostoc mesenteroides* subsp. *mesenteroides*: L1, 1g ADM kg⁻¹), yeast (*Trichosporon sericeum*: Y3, 1g ADM kg⁻¹) on the respiratory methane emission. The L1 was directly administered into the rumen via fistula as single dose 30 min after the morning feeding. GOS and Y3 were supplemented by sprinkling onto the feed and through rumen fistula, respectively. For the 2nd experiment, GOS (20g), *Yucca schidigera* (YS, 120 mg ADM kg⁻¹) and nisin (3 mg kg body weight^{-0.75}) was supplemented to the basal diet consisted of orchardgrass silage and concentrate (7:3 on DM basis) at a maintenance. Physiological saline (0.9% NaCl) was given as the control treatment (CTR) in both experiments.

To estimate the potential energy as methane recycled from animal manure excreted the anaerobic fermentation trials were conducted using the thermophilic biogas reactors. In this experiment cow manure was used instead of sheep manure due to the difficulty of promoting anaerobic fermentation. Furthermore, the efficiency of electric power use of methane generated from the anaerobic fermentation of animal effluent was measured in the thermophilic biogas plant with gas engine generator or proton exchange membrane fuel cell (PEMFC, Matsushita Electric Works, Ltd.).

Results and discussion

Figure 1 shows the modulation of *in vitro* cumulative methane production by GOS and/or lactic acid bacteria and yeast. Methane production decreased in GOS compared with CTR and lactic acid bacteria. The values of L1 and L2 were lower than CTR value. Higher value in L3 compared

with CTR declined remarkably by GOS. For yeast, Y1 and Y3 declined methane production compared with CTR. GOS decreased the values in Y1 and Y2.

Figure 2 shows the time course of changes in the cumulative methane production with supplementing monensin, nisin and nisin with GOS. Nisin, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, decreased methane production as well as monensin.

Table 1 and 2 show the results of respiratory methane emission in two Latin square designed respiratory trials. Supplementing YS and nisin decreased the respiratory methane emission significantly ($p<0.05$). However, the efficacy of GOS to depress the respiratory methane emission was different according to the constituent of feed. For probiotics, whilst Y3 significantly ($p<0.05$) decreased methane emission, the significant ($p<0.05$) increase in L1 was differed from the results in *in vitro*.

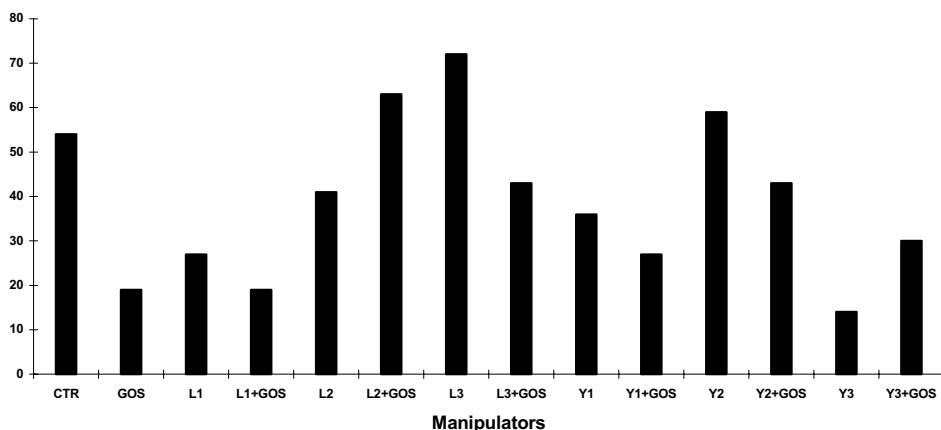


Figure 1. Effects of β 1-4 galactooligosaccharide and /or lactic acid bacteria and yeast on *in vitro* cumulative methane production.

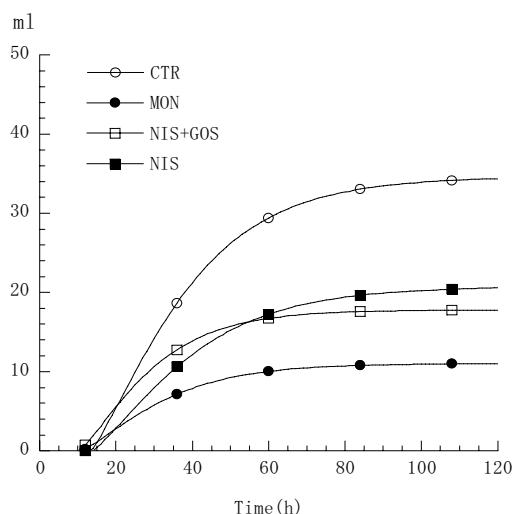


Figure 2. Effects of monensin, nisin and nisin with β 1-4 galactooligosaccharide on *in vitro* cumulative methane production.

Table 1. Effects of β1-4 galactooligo-saccharide, lactic acid bacteria and yeast on respiratory methane emission in sheep

| CH ₄ | CTR | GOS | L1 | Y3 |
|--------------------------|--------------------|-------------------|--------------------|-------------------|
| L kg BW ^{-0.75} | 1.88 ^a | 1.72 ^c | 2.02 ^{ab} | 1.73 ^c |
| L kg DMI ⁻¹ | 33.1 ^a | 30.1 ^c | 35.5 ^{ab} | 30.9 ^c |
| L kg DOMI ⁻¹ | 42.3 ^{ab} | 38.7 ^a | 45.5 ^b | 39.0 ^a |

Means with different superscripts in same row differ significantly ($p<0.01$). DMI: dry matter intake; DOMI: digestible organic matter intake

Table 2. Effects of β1-4 galactooligosaccharide, yucca schidigera, nisin on respiratory methane emission in sheep

| CH ₄ | CTR | GOS | YS | Nisin |
|--------------------------|--------------------|-------------------|--------------------|-------------------|
| L kg BW ^{-0.75} | 1.79 ^a | 1.75 ^c | 1.67 ^{ab} | 1.60 ^c |
| L kg DMI ⁻¹ | 23.4 ^a | 22.5 ^c | 22.1 ^{ab} | 21.0 ^c |
| L kg DOMI ⁻¹ | 34.0 ^{ab} | 32.6 ^a | 32.2 ^b | 31.4 ^a |

Means with different superscripts in same row differ significantly ($p<0.01$). DMI: dry matter intake; DOMI: digestible organic matter intake

From the results of anaerobic fermentation trials of cow manure in the biogas reactors, 300 L pure methane equivalent to 11.85 MJ can be collected per each kg DM of manure. In the measurement of power generation efficiencies from cow effluent in the thermophilic biogas plant, the ratios in power generation, heat and loss were 28%, 33% and 39% in gas engine generator, and 38%, 40% and 22% in PEMFC, respectively. As the DM intake of 600 kg cow at maintenance level is ca 7.5 kg d⁻¹, the cow excreted 2.6 kg DM d⁻¹ in her feces under 65 % digestibility. Thus, 31.1 MJ recycled energy can be withdrawn from the feces as methane. According to the power generation efficiencies, each electric power was estimated 8.7 MJ and 11.8 MJ in gas engine generator and PEMFC, i.e., 100 W and 136 W may be recycled from 7.5 kg DM intake per day.

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Energy session 2

Dietary and Genetic Aspects of Energy Metabolism

Gene expression and energy homeostasis

G. Murdoch, R.J. Christopherson & W.T. Dixon

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada

Summary

Activation of β adrenergic receptors increased heat production in sheep as much as 40 %, whereas α -2 receptor activation reduced heat production by up to 20 %. Expression profiles for genes related to cellular efficiency (Uncoupling protein-2,-3), whole animal orectic (Neuropeptide-Y) and satiety signaling (Leptin) as well as cell surface receptors involved in mediating metabolic activity of cells (adrenergic receptors, leptin receptors and neuropeptide-Y receptors) were examined in ruminant tissues (biceps femoris (BF) muscle, heart, liver, rumen, abomasum, duodenum, subcutaneous adipose (SA), peri-renal adipose (PA) and mesenteric adipose (MA)). Comparison of the gene expression profiles was made with measurements of heat production, metabolizable energy intake, weight gain and retained energy values. We found greater variation in heat production between animals within a breed than between breeds, and this was mirrored by variability in gene expression between individual animals. There was a positive correlation between expression of uncoupling protein -2 (UCP-2) mRNA in skeletal muscle, sub-cutaneous adipose and mesenteric adipose as compared to average daily gain (ADG). We documented expression of uncoupling protein-3 (UCP-3) in bovine cardiac muscle and found that this showed modest negative correlation with retained energy. It was also of note that cardiac muscle expression of neuropeptide-Y receptor message was negatively correlated with both efficiency of energy retention and maintenance ME requirement across all breeds and feeding levels. Expression patterns for leptin receptor mRNA in mesenteric and subcutaneous adipose are positively correlated with ADG. Acute cold exposure increased UCP-2 expression specifically in peripheral tissue (skeletal muscle and sub-cutaneous adipose) ($P < 0.01$), whereas, winter acclimatization enhanced expression of UCP-3 in muscle ($P < 0.05$). UCP-2, UCP-3, leptin receptor and NPY receptor genes appear to be good potential candidates as useful markers of energetic efficiency but future research is required to establish accuracy and reliability.

Keywords: *energy utilization, endocrinology, genes*

Introduction

Variations in energy requirements

The energy requirement for maintenance and growth represents a major component of the cost of production in the livestock industry. It has been common practice in beef cattle breeding to select animals for growth rate or weight at a given age without much attention being paid to feed requirements. Use of this selection approach has certainly resulted in increases in growth rate of slaughter cattle but has also typically led to an increase in mature body size in the breeding herd, which increases in the cow maintenance requirements. However, the maintenance requirements of breeding stock and the feed efficiency of growing animals can vary tremendously among animals of a similar size within a breed or within a herd even when receiving the same diet. In one of our recent studies there was more variation in heat production among individuals within a breed than between the different breeds with coefficients of variation for HP among animals within each breed of 24.6%, 24.9% and 16.7% for Angus, Brahman-Angus and Charolais groups, respectively.

Genetic studies with mice indicate that the heritability of energetic characteristics is high (Nielsen, 1997). A high heritability in cattle is also expected. Development of tools for early detection of genetic variations in cattle energetics could be of enormous benefit in the cattle industry if successfully used as part of a marker-assisted selection program.

Our recent studies have followed two general approaches: 1. We have examined impacts on energy expenditure in response to activation or inhibition of specific endocrine receptors. 2. The second approach has been to measure gene expression in animals and at the same time record physiological indices of energy metabolism and assess the strength of association between gene expression and energy metabolism. We have chosen to evaluate genes associated with systems with which we have had experience, although these represent only a fraction of potentially important elements of energy regulation. Candidate genes for many other physiological processes, such as those related to GH/IGF-I and ion transport, for example, are undoubtedly of critical importance in the context of energy metabolism and have been the subject of intensive studies in other laboratories (Crooker et al., 2000; Gill et al., 1989).

Candidate genes

Adrenergic receptors vs heat production in sheep

We have shown that beta adrenergic receptors, when stimulated by agonists, mediate increases in energy expenditure in ruminants by as much as 30 - 40 % in various tissues and that this can be reversed by beta blocking agents (Miaron et al., 1997). On the other hand, we have also shown that activation of alpha-2 adrenergic receptor sub-types appears to reduce energy expenditure (and therefore maintenance requirements) in cattle by 12 - 20 % in thermoneutral and cold environments (Miaron et al., 1995), whereas, blocking the alpha-2 receptors increases energy expenditure (Hidari et al., 1991). Potential, therefore, exists for reducing maintenance requirements by manipulation of these receptors and by identifying animals that naturally express these receptors to greater or lesser degrees.

We have found relationships between adrenergic receptor density in skeletal muscles, (biceps femoris, semitendinosus and gastrocnemius) and liver and heat production, measured by respiration calorimetry, which, although significant, were generally negative (Table 1). On the otherhand, heat production was positively related to beta receptor density in heart tissue. Although these correlations were significant, the R^2 values indicated that they explained only 22 to 30 % of the variation in heat production.

We conclude that these relationships resulted primarily from responses of the animals to increased feed intake. Increasing feed intake reduced receptor density in skeletal muscle and liver but increased adrenergic receptor density in heart tissue. Of course, heat production increased with increased intake, resulting in the correlations shown in Table 1. It is difficult to reconcile the inverse relationship between receptor density in large tissues such as liver and skeletal muscle and heat production. However, receptor expression in muscle, kidney and liver, but apparently not in heart muscle, may have become down regulated as a feedback response, in circumstances where there is increased activity of the sympathetic nervous system (increased secretion of catecholamines, which act on adrenergic receptors). Therefore, the tissue receptor responses seen in the sheep experiments may indicate a relatively higher activity of the sympathetic nervous system in association with increased energy intake and heat production. Clearly, the adrenergic receptor regulation of energy metabolism is very complex as a result of the existence of receptor sub-types and interactions with other hormones.

Table 1. Relationship between heat production and the density of Beta Adrenergic Receptors (fmol mg⁻¹ protein) in different tissues of sheep.

Regression of Heat production (watts/kg) on tissue beta adrenergic receptor (BAR) density or plasma T3 or Insulin concentrations.

| Independent variable | intercept | Regression coefficient | r value | Probability |
|-------------------------------|-----------|------------------------|---------|-------------|
| Heart beta BAR density | 2.12 | 0.008 | 0.55 | 0.01 |
| Biceps femoris BAR density | 4.01 | -0.019 | -0.34 | 0.05 |
| Semitendinosus BAR density | 4.21 | -0.032 | -0.40 | 0.05 |
| Gastrocnemius BAR density | 5.11 | -0.055 | -0.47 | 0.05 |
| Liver BAR density | 4.52 | -0.081 | -0.38 | 0.05 |
| Kidney BAR density | 4.45 | -0.034 | -0.31 | NS |
| Plasma T3 conc. (ng/dL) | 2.42 | 0.005 | 0.32 | NS |
| Plasma insulin conc. (uIU/mL) | 2.28 | 0.070 | 0.54 | 0.01 |

¹ Data from Ekpe et al., 2000.

Relationship of HP and energy parameters in cattle to expression of candidate genes at two feeding levels and across three breeds:

Neuropeptide Y (NPY), leptin and their receptors in various tissues

Activities of the sympathetic/adrenergic system are linked with other hormone receptor systems including leptin and neuropeptide-Y (NPY) which may influence both energy expenditure and regulation of feed intake (Wolf, 1997; Ahima et al., 1996); Thomas and Palmiter, 1997).

Analyses revealed that mRNA for NPY was not detectable in any of our bovine tissue samples at our criteria of 40 maximum PCR amplification cycles, except for hypothalamic tissue, where the RT-PCR analyses on total RNA demonstrated presence of NPY mRNA. Thus, lack of message in other screened tissues was very likely an accurate representation of sub-physiological or complete lack of expression of this gene in these other tissue types under these treatment conditions.

The RT-PCR analyses of leptin and leptin receptor and NPY receptor in bovine tissues have demonstrated wide spread expression of these genes. We have detected message for both leptin receptor and NPY receptor in cardiac muscle, liver, rumen papillae and duodenum, where we have further discovered that leptin message is inherently absent in all of these tissues. The screening of abomasum failed to detect any appreciable leptin or NPY receptor signal, but did reveal that leptin receptor mRNA is expressed. The absence of leptin transcript in bovine abomasum was somewhat surprising since it has been shown to be present in the fundus of both rat and human stomachs (Sobhani et al, 2000). Perhaps this serves as further emphasis of the differences in the digestive tracts of ruminants and monogastrics, and emphasizes the need to perform bovine specific research. The presence of message for leptin receptor in these tissues would infer that these tissue types may be somewhat influenced by circulating leptin. Leptin itself is often referred to as the satiety hormone but we also know that it is involved in the partitioning of nutrients (Scarpase et al, 1998 and Rouru et al, 1999). Leptin mutation results in obesity (Verpoegen et al, 1997), and elevates muscle fatty acid oxidation (Muoio et al, 1997). Future research is needed to determine the physiological role conferred by fluctuating leptin receptor presence, in various bovine tissue types.

We expect that changes in leptin receptor will influence the sensitivity of these tissues to leptin. We have observed high levels of specific mRNA message, for leptin, within the three adipose depots and lower expression rates were observed in skeletal muscle. The presence of message for

leptin receptors in both adipose tissue and skeletal muscle suggests an autocrine role for leptin. Further examination of the expression and release of leptin in both adipose and skeletal muscle will be required in order to ascertain the role of this peptide hormone in the bovine. The presence of leptin receptors in various regions of the bovine digestive tract begs the question as to the physiological relevance of such a distribution. Leptin may mediate the manner by which these digestive tract regions process and partition post-prandial substrates. The distribution of both the leptin and NPY receptors, in liver and cardiac muscle, suggests that serum leptin and NPY levels may be important mediators of whole animal metabolism in cattle. Moreover, individual animal receptor variability may in the future help to explain more of the cause for variability between animals offered the same nutritional plane and composition.

The expression of NPY receptor in cardiac muscle was found to be negatively correlated with both efficiency of energy retention (DMER/DMEI, $r=-0.41$) and ME requirement for maintenance ($r=-0.33$). These are modest negative correlations, but are significant with our degrees of freedom (33). If we accept the principle that more receptor message would elevate the sensitivity of such tissue to the NPY ligand, then given that NPY reduces fatty acid oxidation in cells the effective catabolism of the cardiac muscle may be reduced resulting in improved energy retention efficiency. Moreover, if increased NPY receptor expression in cardiac muscle can reduce the energy expenditure of said tissue then we may conceive that this could reduce the animal's basal maintenance requirements. However, there is a moderately positive correlation between the NPY receptor expression in liver and heat production ($r=0.40$). It is possible that in animals that are obtaining reasonable and consistent nutrient supplies, that the liver which is directly exposed to high levels of post-prandial nutrient supply via the portal vein needs to maintain its sensitivity to NPY signaling that is important for inducing food intake. The hypothalamic release of NPY, may serve to prepare the liver for the onset of nutrient intake, and thus the level of receptor expression in liver may be elevated in animals that have higher intakes, which would also correlate to higher heat production measurements.

Adipose tissue profiles for leptin, leptin receptor and NPY receptor

The expression of leptin message in perirenal adipose shows a small correlation with whole animal heat production ($r=0.31$), and a negative relationship to efficiency of energy retention($r=-0.35$). It was hypothesized that expression of this gene may be a reflection of an individuals intake. The modest positive correlation observed between the heat production and perirenal expression of leptin is the first indication that in young growing steers leptin gene may be associated with the animals metabolic heat production. Since leptin can increase fatty acid oxidation in muscle cells, systemic release of leptin from adipose would be expected to elevate heat production values. Overall though, our steers were quite lean and this may have reduced the amount of leptin expression that we characterized in our adipose tissues, somewhat reducing our observed correlations of this gene with indices of energy expenditure.

The leptin receptor expression in the mesenteric and subcutaneous adipose samples indicate a positive correlation with ADG ($r=0.43$ and $r=0.30$ respectively). Both of these adipose depots are considered as primary sources of leptin hormone in adult animals. The expression of leptin, especially by central adipose depots such as mesenteric, is believed to play a role in hypothalamic signalling of whole body adiposity. In lean animals the leptin released from adipocytes is the source of serum leptin that functions as an endocrine peptide, but expression of receptors by adipose tissue also indicates an autocrine role for this peptide. As with other cell surface receptors, low exposure to a ligand may induce increases in receptor expression (up-regulation). If a growing animal partitions more resources to muscle growth then body weight increases would be greater than if it were depositing more adipose tissue since adipose tissue is more energetically dense. The leaner body mass animals may thus increase in body weight and produce less leptin than animals that are less lean. The net effect may be observed as an increase in leptin receptor

expression in adipose of leaner animals, which would correlate with higher ADG values. A positive relationship between expression of both leptin and NPY receptor mRNA in subcutaneous adipose tissue and the metabolizable energy for maintenance (Lrec $r=0.32$, nrec $r=0.38$) was also observed. The subcutaneous adipose in young growing animals would be expected to have a high level of responsiveness to both indices of satiety and stimuli initiating nutrient intake (reflective of anabolic or catabolic states). As such, the level of receptor expression in these animals should vary and may be related to its maintenance requirements.

Leptin and NPY receptor expression in skeletal muscle

An unexpected but very significant correlation between leptin receptor and NPY receptor in muscle was observed in this study. The analyses of expression levels of the Leptin receptor gene and the NPY receptor gene in cattle Biceps femoris tissue has demonstrated that these two genes are synchronously expressed in individual animals at varying levels. The expression of these genes or, regulation of their respective expressions, are highly linked given their correlation of $r=0.91$ in 35 steers. These results suggest that, in the skeletal muscle of growing cattle, the sensitivity of the peripheral muscle to Leptin and NPY may be regulated in a coordinated fashion. If we accept the doctrine that receptor expression is associated to ligand sensitivity then this finding suggests that there are individuals with high levels of sensitivity to serum leptin and NPY and other animals that are less sensitive to both. Better understanding of muscle physiology in growing steers and differences between individual animals may facilitate informed decisions in future management or selection strategies.

Table 2 : Correlation between genes and indices of efficiency.

Correlations between energy parameters and Gene expression

| Gene | tissue | HP1.2M | MEm | Eff. ER | ADG/ME | HP2.2M |
|------------|-----------|--------|--------|---------|--------|--------|
| Leptin Rec | SA | 0.32* | 0.32* | 0.10 | 0.26 | 0.11 |
| | MA | 0.06 | 0.10 | 0.18 | 0.41* | -0.14 |
| | PA | 0.15 | 0.09 | 0.17 | 0.13 | 0.41* |
| | BF biopsy | -0.57* | -0.57* | -0.51* | -0.11 | 0.07 |
| | BF (post) | -0.08 | 0.12 | -0.13 | 0.20 | 0.37* |
| NPY Rec | SA | 0.35* | 0.38* | 0.21 | 0.25 | -0.04 |
| | MA | 0.01 | 0.15 | 0.02 | -0.34* | -0.14 |
| | PA | 0.26 | 0.22 | 0.06 | 0.26 | 0.17 |
| | BF biopsy | -0.32* | -0.48* | -0.34* | -0.04 | 0.01 |
| | BF (post) | -0.07 | -0.01 | -0.21 | 0.17 | 0.23 |
| Leptin | BF | 0.09 | 0.02 | 0.07 | -0.11 | -0.08 |

HP1.2M = heat production for that animal at 1.2M feeding

MEm = ME requirement for maintenance

Eff .ER = efficiency of energy retention above maintenance.

ADG/ME = average daily gain (g/MJME)

HP2.2M = heat production for that animal at intake of 2.2M

The expression of leptin receptor and NPY receptor in biceps muscle biopsies are highly and negatively related to HP measured at 1.2M, MEm and efficiency of ME intake, but are not related to ADG or HP at the 2.2M level of feeding. This may mean that the gene expression in the biopsy (taken early in the growth period) might be a good reflection of energy expenditure at maintenance

and slow growth but not a good reflection of maximum growth or energy metabolism later on. Relationships such as this are essential in the pursuit of reliable genetic markers that may serve in marker-assisted selection criteria. Further, the perirenal leptin receptor mRNA and post-mortem skeletal muscle expression of leptin receptor mRNA, are positively related to HP at 2.2M level of feeding. The mesenteric adipose leptin receptor mRNA is positively, whereas the mesenteric adipose NPY receptor mRNA is negatively related to ADG/ME. This suggests that Lrec and NPY rec are not closely related in adipose tissue which is very different from the situation observed for the skeletal muscle gene expression profiles.

Uncoupling proteins

Uncoupling protein-2 (UCP-2) and uncoupling protein -3 (UCP-3) may have roles in influencing energetic efficiency and maintenance (Fleury et al.,1997; Vidal-Puig et al.,1997; Boss et al., 1997). UCP-2 and UCP-3 are linked to proton-conductance pathways in mitochondria of cells. UCP-2 is a recently discovered gene that is expressed in many tissues in mice and humans, but has not yet been studied in cattle (Fleury et al.,1997). UCP-3 has also been studied recently in rodents and humans and is expressed in both skeletal muscle and brown adipose tissue but not other organs (Vidal-Puig et al.,1997; Boss et al., 1997). It has also not been studied in cattle. These genes could each have an influence on maintenance requirements and energy expenditure / efficiency in animals (Wolf, 1997). They may be of particular importance in ruminants since, in these species, the energetic efficiency of growth is lower than in monogastric animals.

UCP-2, is the most ubiquitously expressed subtype of uncoupling protein, and was present in all tissues examined except abomasum and duodenum. UCP-3 was detectable in three of our tissues, biceps femoris skeletal muscle, peri-renal adipose tissue and cardiac muscle. Although UCP-3 is predominantly expressed in skeletal muscle (Vidal-Puig et al, 1997), its expression in other metabolically important tissues such as adipose (perirenal) and cardiac muscle may be indicative of a role in adaptive thermogenesis.

The measurement of UCP-2 mRNA across our experimental samples, revealed little if any difference in expression profiles correlated to either feeding level or between breeds. More importantly, the large standard deviations within breed and feeding level illustrate the variability between individual animals in expression of UCP-2 message. Other studies indicate that expression of UCP-2 is influenced by catecholamines such as norepinephrine (Thomas and Palmiter, 1997), thyroid hormones (Obregon et al, 1996), peroxisome proliferation activating receptors (PPAR's) (Aubert et al, 1997) and leptin (Zhou et al, 1997), all of which are known to vary substantially between individuals as well as under differing physiological conditions. The highly significant increase ($P < 0.01$) that we have observed in UCP-2 expression in acutely cold exposed steers versus controls suggests that this gene may be of importance for health of cattle in cold climates. The capacity of UCP-2 to be up-regulated during conditions of cold stress supports the hypotheses that this gene may have a role in adaptive thermogenesis. UCP-2 is influenced by fasting in humans (Millet et al, 1997). However, we did not observe changes with level of feeding, as our lower level of intake was insufficient to induce similar changes in the expression profile as that induced by fasting. Although speculative, the variability observed in the UCP-2 gene expression between individuals, within and between breeds, may ultimately address some of the physiology behind similar variability's observed in individual animal's energetic efficiencies.

The expression of UCP-3 gene was less consistent across tissues than that of UCP-2, and no differences were attributable to either breed or level of feed intake. The expression of UCP-3 gene in skeletal muscle, cardiac muscle, and perirenal adipose again showed substantial individual animal variability. Individual variability was proportionally less in cardiac muscle than in the mixed fiber type skeletal muscle (biceps femoris) but it is yet unknown whether this is consistent over other skeletal muscle types. Skeletal muscle is typically considered the primary site of expression for UCP-3 (Boss et al, 1997). UCP-3 has been shown to be regulated in rat skeletal

muscle by thyroid hormone (Larkin et al, 1997), leptin (Liu et al, 1997) and catecholamines (Gong et al, 1997). Skeletal muscle is a suitable target tissue for investigating varied efficiencies in cattle since it represents as much as 50% of the animal weight. Furthermore, skeletal muscle is very metabolically active and dynamic in varied conditions, be they catabolic or anabolic states.

The detection and measurement of UCP-3 in bovine cardiac muscle is the first report of such presence but is supported by observation of this gene's expression in both rats and humans. UCP-3 mRNA has been observed in piglet skeletal muscle and adipose, as reported by Damon et al (2000), and is implicated in affecting the energy metabolism of these tissues in rats under fasting states. The lack of changes in UCP-3 expression levels with our reduced feeding level is most probably a facet of the modest degree in restriction that our treatment represents. We observed an increase in expression of UCP-3 mRNA in biceps femoris of cattle housed outdoors during winter ($P < 0.05$), suggesting a potential role in acclimatization to winter.

The expression of UCP-2 mRNA in skeletal muscle, subcutaneous adipose and mesenteric adipose were all positively correlated with ADG. Given that the uncoupling proteins are believed to uncouple the mitochondrial proton gradient from ATP synthesis, hence reducing efficiency of energy conversion, this trend may seem surprising. It is possible that the level of expression of these genes is indicative of the nutrient availability to these tissues. Given such a hypothesis, animals that show higher rates of average daily gain may represent animals that partition their nutrients to anabolic tissues such as the skeletal muscle and subcutaneous and mesenteric adipose. These same animals may also chronically adapt to express higher levels of uncoupling proteins on the basis of ultimately having higher established and more persistent proton gradients within their mitochondria associated with greater energy release. This may also be supported by the positive correlation ($r=0.44$) between UCP-2 expression in mesenteric adipose and MEI ($MJ/kgbw^{0.75}$). In this instance the expression of UCP-2 and UCP-3 may be representative of useful markers of animals that inherently show better degrees of body weight gain on the same nutritional plane.

A modest though significant correlation ($r=0.33$) was observed between the expression profile of UCP-2 mRNA in mesenteric adipose and heat production across all animals regardless of treatment. This correlation might be more attributable to the capacity of UCP-2 to uncouple the oxidative phosphorylation pathway away from energy storage in terms of ATP synthesis. This correlation represents the first such evidence of a link between whole animal heat production and uncoupling protein expression in bovine species. If we consider that the mesenteric adipose depot represents the long-term storage of lipids that accumulates in an animal provided with sufficient and/or abundant nutrients for growth, then the uncoupling of ATP synthesis in these animals should result in increased heat production. Support for this concept is provided by the demonstration that UCP is up-regulated by the presence of fatty acids (Klingenberg, 1993), as would be the case in nutrient abundance in cattle. Further, a significant negative correlation ($r=-0.34$) was observed between the expression of UCP-3 in cardiac muscle and energy retention, which could suggest that the expression of this gene in the heart is inversely associated with the animal's whole body energy retention. Research in humans by Cortright et al (1999), has demonstrated a positive correlation between UCP-3 gene expression in muscle and exercise, which may support the interpretation that elevations in metabolic rate, as are typical in exercise, result in elevated UCP-3 and reduced energy storage or retention. If we assume that cardiac fitness is related to whole animal fitness, then reduced energy retention may indicate reduced sedentary behaviour or, conversely, increased energy expenditure, and the elevation of UCP-3 in cardiac muscle may be representative of these more active though less efficient cattle.

Protease systems, protein turnover and energy metabolism

Genes that code for intracellular protease systems (including lysosomal, Ca^{2+} - dependent, and ATP/ubiquitin - dependent systems) (Baracos et al., 1995) and extracellular matrix protease which contribute to the processes of protein turnover in the animal's tissues, may also be linked to

differences in maintenance requirements and efficiency of growth. Regression analysis of results with cattle, revealed some positive correlations, which indicate a possible influence of the energy and protein intake on the expression of certain elements of the muscular proteolytic systems (D. Balcerzak, V.E. Baracos, W. Dixon and others, unpublished).

The expression of both μ -calpain and m -calpain large subunit was down regulated ($P < 0.03$) under feed restriction, whereas the calpastatin expression was unchanged. Moreover the expression of the gene of the two calpains was correlated to the level of heat production and nitrogen intake, indicating regulation in response to the physiological conditions. For the ATP-ubiquitin dependent proteolytic system, the ubiquitin gene was also down regulated ($P < 0.001$) under feed restriction and its expression showed a positive correlation with the average heat production, indicating energy savings during restriction (D. Balcerzak, V.E. Baracos, W. Dixon and others, unpublished). This response differed from studies on starved or fasted rats, which actually showed an increase of ubiquitin expression related to food deprivation and concomitant to an activation of the proteasome (Medina et al., 1991; Wing and Goldberg, 1993). However, in the study of Balcerzak the animals were not fasted but were only moderately restricted. Clearly, the degree of restriction modifies the gene expression response.

For the extracellular proteolytic system, a positive correlation was observed between the expression of uPa, TIMP-3, MMP-2 and MT3-MMP genes and the nitrogen intake, average daily gain and average heat production. In parallel with these observations, a positive correlation appeared between the excretion of 3MH and the heat production ($r = 0.39$, P value = 0.04) and between the excretion of hydroxyproline and the heat production ($r = 0.51$, P value = 0.006).

One of the physiological effects of the feed restriction is a significant reduction of heat production, which may reflect an adaptive saving of energy. The reduction of the average daily gain by almost 60% is certainly a big component associated with the energy saving and the decrease of heat production. A large decrease in protein turnover is another phenomenon occurring to save some energy in the feed-restricted condition. Collagen and myofibrillar proteins are the most abundant proteins in the whole body, and the excretion of hydroxyproline and 3 methyl-histidine in the urine (marker indicators of their degradation) both decreased under feed restriction by respectively 23.6% and 30.4%. Lobley et al. (2000) obtained similar results concerning the level of urinary excretion of 3MH in restricted calves (ADG: 1kg/d) versus full fed calves (ADG: 1.4 kg/d).

This result shows a general reduction in the whole body protein turnover, which would conserve energy.

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Energy metabolism and oxidation of substrates in pigs during feeding, starvation and re-feeding

A. Chwalibog, A-H. Tauson & G. Thorbek

Department of Animal Science and Health, The Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark

Summary

Energy metabolism and substrate oxidation was measured in 12 growing pigs by means of indirect calorimetry and nutrient balances. The measurements were carried out during 6 days of feeding followed by 4 days of starvation and 4 days of re-feeding. During the feeding period the energy transfer between protein, carbohydrate and fat pools, and partition between nutrient oxidation and retention were quantified in accordance with the model, showing the same magnitude as in previous experiments with sufficient supply of nutrients and energy. The main source of energy was oxidized carbohydrate and no oxidation of fat was measured, indicating that all digested fat was retained in the body. Starvation reduced the total heat production and affected the oxidation pattern by shifting from carbohydrate to fat oxidation with the latter being the main source of energy. Re-feeding again changed the relative contribution of each nutrient to heat production. On the 2nd day of re-feeding the heat production reached the same level as during the feeding period with similar contribution from oxidized carbohydrate (90 %), protein (10 %) and no fat oxidation. The results indicate that the pigs were able to re-establish the metabolic rate and oxidative patterns within 2 days after re-feeding.

Keywords: *energy expenditure, nutrient oxidation, fasting, pigs*

Introduction

A model describing the pathways of energy transfer from digested protein (DP), carbohydrate (DCHO) and fat (DF) to energy retained in protein (RP) and fat (RF) or released as heat (HE) was proposed by Chwalibog et al. (1992). The refined model (Fig. 1) has been applied in several investigations with growing pigs demonstrating that pigs on high feed level ($ME > 1.2 \text{ MJ/kg}^{0.75}$) and with DP between $11\text{-}15 \text{ g/kg}^{0.75}$ and $DCHO > 45 \text{ g/kg}^{0.75}$ covered their energy requirements for maintenance and growth by 80-85 % of HE from oxidation of carbohydrate (OXCHO) and by 15-20 % from protein (OXP), while no oxidation of fat (OXF) occurred (Chwalibog et al., 1992; Chwalibog and Thorbek, 2000). Later it was shown that OXF occurred when DF exceeded 7 $\text{g/kg}^{0.75}$ and DCHO was less than $44 \text{ g/kg}^{0.75}$ (Jakobsen et al., 2001).

During starvation in animals as in humans (Owen et al., 1998) HE is reduced and the relative importance of different metabolic fuels changes. Mobilized body fat becomes the main source of energy followed by body protein oxidation. However, the situation changes during re-feeding from utilizing body reserves to using dietary nutrients. The purpose of this investigation was to describe changes in quantitative substrate metabolism during feeding, starvation and re-feeding in pigs in accordance with the model.

Materials and methods

Twelve castrated male pigs of Danish Landrace were equally divided in two LW-groups 20-25 kg (Group A) and 30-40 kg (Group B). All pigs were kept individually in metabolic crates during the whole experiment and measured in a six days balance experiment on near ad lib. feeding,

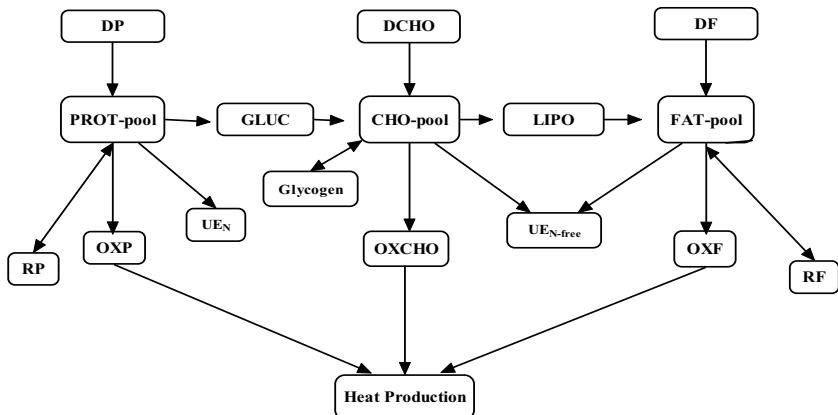


Figure 1. Model of energy flow. Digested protein (DP), protein retained or mobilized (RP), oxidized protein (OXP), urinary energy from nitrogenous components (UE_N), energy transfer from protein to carbohydrate pool (GLUC). Digested carbohydrate (DCHO), carbohydrate retained or mobilized (Glycogen), oxidized carbohydrate (OXCHO), urinary energy from N-free components (UE_{N-free}), energy transfer from carbohydrate to fat pool (LIPO). Digested fat (DF), oxidized fat (OXF), fat retained or mobilized (RF) and total heat production.

immediately followed by four days of starvation and four days on near ad lib. re-feeding. The feed compound consisted of 75 % maize with protein supply from soybean meal and with minerals and vitamins in accordance with Danish allowances. The chemical composition was 154 g protein, 656 g carbohydrate and 33 g fat with 16.5 MJ gross energy per kg. There was free access to water. Gas exchange was, by means of an open-air-circuit respiration unit, measured on the last three days of the balance experiment and each day in the following periods. The temperature was kept constant at 22 °C with humidity around 60 %. The metabolic crates were removed from the respiration chambers for a short period at 09.00 for collection, and feeding took place once a day at 11.30. The gas exchange was measured for 22 hours from 11.00 to 09.00 (recalculated to 24 h). Heat production and oxidation of nutrients were calculated in accordance with Chwalibog et al. (1992). The experiment was performed in accordance with Danish legislation.

Results and discussion

The results concerning classical measurements of energy metabolism (Table 1) demonstrated that HE, RP and RF were highest in Group B caused by the higher ME intake, but identical in relation to metabolic LW with a mean ME intake of 1259 (SEM 11.8) and a HE of 845 (SEM 12.7) kJ/kg^{0.75}. The energy retained in protein and fat was 164 (SEM 4.3) and 250 (SEM 15.0) kJ/kg^{0.75} respectively. The data were then used to calculate the quantitative metabolism in accordance with the model (Fig.1) as shown in Table 2.

DP was, in relation to metabolic LW, identical for both groups with 263 (SEM 4.6) kJ/kg^{0.75}, corresponding to 11 g/kg^{0.75}. The energy transfer from DP was 62 and 29 % to RP and OXP respectively, with a minor contribution of 3 % to the CHO-pool. DCHO was 960 (SEM 8.5) kJ/kg^{0.75} for groups A+B corresponding to about 55 g/kg^{0.75}. The energy flow from CHO-pool was around 80% to OXCHO and 18 % through lipogenesis to FAT-pool, in accordance with Chwalibog and Thorbek (2000). With the CHO-pool being above the lower limit of 44 g/kg^{0.75} and DF below the upper limit of 7 g/kg^{0.75} the total HE was covered by 91 % from OXCHO and 9 % from OXP. With no OXF the FAT-pool was retained in body fat with 2816 and 3435 kJ/d in Group

Table 1. Energy metabolism. Metabolizable energy (ME), heat production (HE) and energy retained in protein (RP) and fat (RF), kJ/d

| | Group A | | | Group B | | |
|--------|---------|------|-----------------------|---------|------|-----------------------|
| | Mean | SEM | kJ/kg ^{0.75} | Mean | SEM | kJ/kg ^{0.75} |
| LW, kg | 23.4 | 0.17 | | 35.9 | 1.28 | |
| ME | 13358 | 178 | 1255 | 18475 | 338 | 1260 |
| HE | 8879 | 174 | 835 | 12528 | 351 | 855 |
| RP | 1661 | 68 | 156 | 2510 | 111 | 171 |
| RF | 2818 | 198 | 265 | 3437 | 312 | 234 |

Table 2. Protein, carbohydrate and fat metabolism, kJ/d

| | Group A | | | Group B | | | A+B |
|--------------------------------|---------|------|-----------|---------|------|-----------|-----------|
| | Mean | SEM | % of pool | Mean | SEM | % of pool | % of pool |
| <i>Protein metabolism</i> | | | | | | | |
| PROT-pool, | 2673 | 44.0 | | 4027 | 64.1 | | |
| RP | 1661 | 68.3 | 62.1 | 2510 | 111 | 62.3 | 62.1 |
| OXP | 781 | 28.8 | 29.2 | 1172 | 56.4 | 29.1 | 29.2 |
| UE _N | 152 | 5.6 | 5.7 | 228 | 11.0 | 5.7 | 5.7 |
| GLUC | 79 | 2.9 | 3.0 | 118 | 5.7 | 2.9 | 2.9 |
| <i>Carbohydrate metabolism</i> | | | | | | | |
| DCHO | 10282 | 103 | 99.2 | 13955 | 225 | 99.2 | 99.2 |
| GLUC | 79 | 2.9 | 0.8 | 118 | 5.7 | 0.8 | 0.8 |
| CHO-pool | 10361 | 101 | | 14073 | 221 | | |
| OXCHO | 8144 | 192 | 78.6 | 11469 | 354 | 81.5 | 80.1 |
| UE _{N-free} | 211 | 7.8 | 2.0 | 316 | 15.2 | 2.3 | 2.2 |
| LIPO | 2006 | 187 | 19.3 | 2287 | 303 | 16.2 | 17.8 |
| <i>Fat metabolism</i> | | | | | | | |
| DF | 810 | 31.5 | 28.8 | 1147 | 44.0 | 33.4 | 31.1 |
| LIPO | 2006 | 187 | 71.2 | 2287 | 303 | 66.6 | 68.9 |
| FAT-pool | 2816 | 198 | | 3435 | 312 | | |
| OXF | 0 | | | 0 | | | |
| RF | 2816 | 198 | 100 | 3435 | 312 | 100 | 100 |

Abbreviations cf. Figure 1

A and B respectively, corresponding to the values obtained in the traditional way of calculating energy metabolism (Table 1),

The same pattern of heat production in relation to metabolic LW was measured in groups A and B during feeding, starvation and re-feeding (Fig. 2). During feeding HE was 845 (SEM 12.7) kJ/kg^{0.75} and decreased already on the first day of starvation with 20 % to 679 kJ/kg^{0.75} and with a slower decrease to 592 (SEM 21.1) kJ/kg^{0.75} or 30 % of the feeding value on the 4th day of starvation.

The same pattern of substrate contribution to HE was measured in Group A and B (Fig. 2). During the feeding periods OXCHO contributed with 91 % and OXP with 9 % to HE while there was no

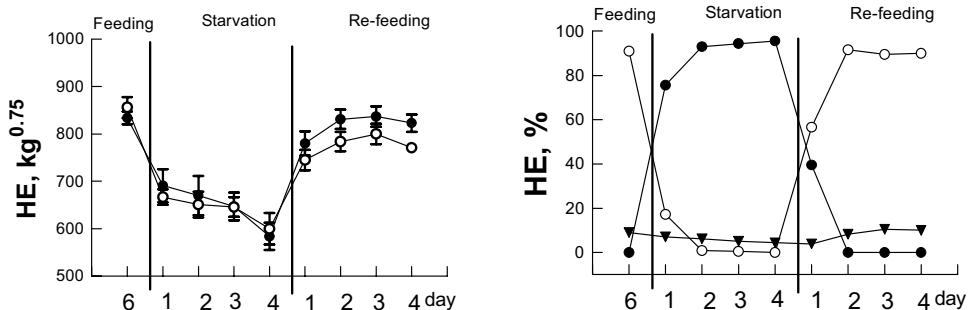


Figure 2. Left: Heat production per metabolic live weight ($\text{kg}^{0.75}$) during feeding, starvation and re-feeding periods in Group A● and B○. Mean and SEM. Right: Oxidation of protein▼, carbohydrate ○ and fat ● in proportion to heat production (HE) in Group A+B.

OXF. During starvation the pattern of substrate oxidation switched from OXCHO to OXF. Already on the 1st day of starvation, with no feed since 11³⁰ at the last feeding day, the contribution from OXCHO decreased to 17 % while OXF increased to 76 % of HE. In the following three days of starvation no OXCHO was measured and HE was covered by OXF (95 %) and OXP (5 %). The substrate contribution to HE changed rapidly during the 1st day of re-feeding with OXCHO increasing to 57 % and OXF being reduced to 40 %. Thus, the supplied dietary carbohydrate was immediately utilized as energy source, however, there was still oxidation of fat which exceeded the amount of digested fat. Already from the 2nd day of re-feeding the pattern was identical with the feeding period, showing 90 % of HE from OXCHO, 10 % from OXP and no OXF, indicating that in order to saturate depleted fat reserves during starvation dietary fat was used entirely for fat retention. The results demonstrate that growing pigs are able to re-establish oxidative pattern within 2 days of re-feeding.

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Effect of conjugated linoleic acids (CLA) and selenium on growth performance, feed conversion efficiency and CLA level in rats

M. Czauderna, J. Kowalczyk, I. Wąsowska, K. Niedzwiedzka, M. Taciak & B. Pastuszewska

The Kielanowski Institute of Animal Physiology and Nutrition ,05-110 Jabłonna, Poland

Summary

The effect of dietary selenium (Se) and/or conjugated linoleic acid isomers (CLA) on growth performance and CLA isomers profile of femoral muscles was investigated in female rats. Rats fed Se and/or the CLA mixture responded by decrease body mass gain, whereas Se and the *trans-10,cis-12* isomer (t10c12) significantly increased both body mass gain and feed con-version efficiency. Only the CLA mixture, the t10c12 isomer decreased feed intake. The CLA isomers in diets significantly increased the isomers level in muscles, while Se and the CLA mixture or the t10c12 isomer considerably increased the isomers content in muscles. The con-centration ratio of the t10c12 isomer to the c9t11 isomer in examined tissues and organs de-creased compared to the concentration ratio of these isomers in the standard mixture of CLA isomers in the diets.

Keywords: CLA isomers, selenium, rats, organs, feed efficiency, CLA deposition

Introduction

Conjugated linoleic acids (CLA) refer to isomers with of conjugated diene double bonds and a combination of *cis* and/or *trans* spatial configurations. CLA have been demonstrated to have positive health effects in animal. One of the most biologically active and the most abundant CLA isomer is *cis-9,trans-11* (c9t11) possessing antidiabetic, anticarcinogenic, antiathero-genic properties (Sébédio et al., 1999). Recently, many authors found some beneficial effects of another CLA isomer, *trans-10, cis-12* (t10c12), on lipid metabolism such as body fat reduction with enhancement of lean body mass (Sébédio et al., 1999; Alasnier et al., 2002). Several studies support CLA's ability to increase fatty acids β -oxidation at the expense of fatty esterification. In agreement with these results, many other studies demonstrated that feeding diets enriched in the t10c12 isomer is the more potent antiobesity agent in rodents compared to other CLA isomers. Moreover, the t10c12 isomer improved feed efficiency, reduced excess body mass gain and lowered blood lipids. Fat metabolism is also affected by dietary selenium (Se), which is the component of glutathione peroxidases and is an important protective agent against un-saturated fatty acids peroxidation damage (Crespo et al., 1995).

The objective of the study was to assess the effects of CLA isomers mixture and two individual isomers, fed without or with Se supplementation, on feed conversion efficiency, body mass gain and levels of CLA isomers in muscles of rats.

Material and methods

The experiment was performed on female rats (Wistar, Ifz:BOA), partly depleted of body fat. Ten groups of rats at 8 weeks of age and ~200 g initial body mass, were housed individually and during a oneweek were fed a standard Labofeed diet (Pastuszewska et al., 2000), at a sub-maintenance level. Then they were fed *ad libitum* Labofeed diet enriched in a CLA mixture at 1 and 2% or *cis-9,trans-11* (c9t11) or *trans-10,cis-12* (t10c12) isomers at 1%. All diets were unsupplemented (-Se) or supplemented (+Se) with 2 ppm Se given as Na₂SeO₄. Feed intake and body mass gain were measured weekly. After the 4-weeks experimental period rats were killed by

CO_2 , their femoral muscles, heart, spleen, pancreas, liver, brain and kidney were dissected and weighed and adipose tissue sampled. Small intestines of rats, fed a control diet and enriched in a CLA (1%) both without extra Se, were cut along mesenterium, rinsed and treated with 10% acetic acid solution until the Peyer's patches were visible, intestinal surface covered by lymphoid tissue was determined using a projection microscope (Nikon 104) connected with computer equipped with image analysis system (Lucia, 3.51). Peyer's patches are a part of gut associated lymphoid tissue (GALT) and may be considered as the indicator of the immunological status of the animal (Zyla et al., 2000).

HPLC grade acetonitrile and n-hexane were purchased from Lab-Scan (Ireland), other re-agents were of analytical grade (POCh, Poland). The CLA mixture, the c9t11 and t10c12 isomers were supplied by Larodan Fine Chemicals AB (Sweden). All biological samples (~ 30 mg) were hydrolyzed and prepared for HPLC analyses according to Czauderna et al., 2003. Two Chrompac ChromSpher 5 μm Lipids columns for fractionation of free CLA isomers were used (Czauderna et al., 2003), while two Nova Pak C₁₈ columns (Waters) for determination of the sum of the isomers were applied (Czauderna and Kowalczyk, 2002). All data were statistically analyzed using Student's t-test.

Results and discussion

The greatest differences in feed intake were found during the first two weeks of *ad lib* feeding (Table 1). Feed intake and body mass gain tended to decrease due to addition of 1 or 2% CLA to Se unsupplemented (-Se) and supplemented (+Se) diets. Similarly, supplementation of the diet enriched only in the t10c12 isomer more efficiently decreased feed intake, body mass gain and feed conversion efficiency.

These results are supported by several other studies, which have indicated that the t10c12 isomer, due to geometric and positional structure, is the most potent CLA isomer in term of antiobesity activity, so, the most efficiently reduced feed intake, body mass, as well as, increased energy expenditure. Feeding the diet enriched only in Se to rats tended to minute decrease feed intake and

Table 1. Effects of CLA mixtures (1% and 2%) and individual isomers (1%) (i.e. c9t11 or t10c12) without Se (-Se) and with Se supplemented (+Se) diets on feed intake, body mass gain, feed conversion efficiency (g body mass gain/g feed intake)¹.

| Group | Feed intake for periods (days) ² | | | Total feed intake (g) ³ | Body mass gain (g) | Feed conversion efficiency |
|------------|---|---------------------|-------|------------------------------------|--------------------|----------------------------|
| | 1-7 | 8-14 | 15-21 | | | |
| 0 -Se | 125.8 ^{ABCDab} | 114.7 ^{Aa} | 100.0 | 435.3 ^{ab} | 59.4 | 0.1365 |
| 0 +Se | 124.3 | 116.2 | 96.3 | 432.8 | 52.8 ^a | 0.1220 |
| CLA,1% -Se | 118.2 ^B | 112.0 | 97.6 | 426.5 | 54.8 | 0.1285 |
| CLA,1% +Se | 118.0 ^a | 114.7 | 100.0 | 425.4 | 56.1 | 0.1319 |
| c9t11 -Se | 125.0 | 122.0 | 98.1 | 439.5 | 59.7 | 0.1359 |
| c9t11 +Se | 123.5 | 115.7 | 97.5 | 430.4 | 55.5 | 0.1289 |
| t10c12 -Se | 116.6 ^A | 114.1 | 94.6 | 420.2 | 54.1 | 0.1287 |
| t10c12 +Se | 120.2 ^b | 116.1 | 99.8 | 435.8 | 62.2 ^a | 0.1427 |
| CLA,2% -Se | 116.4 ^C | 108.5 ^A | 96.7 | 412.9 ^a | 56.8 | 0.1376 |
| CLA,2% +Se | 113.4 ^D | 109.8 ^a | 95.6 | 414.6 ^b | 58.4 | 0.1409 |

¹ means in columns with the same letter are significantly different: A,B - $P < 0.01$; a,b - $P < 0.05$;

² feed intake in 22-28 days was not included as it did not differ among group;

³ during the whole experimental period (26 days).

Table 2. Effect of experimental diets on rats (g) and organ mass (g) after 4 weeks of the study.

| Group | Rat mass | Heart | Spleen | Pancreases | Liver | Brain | Kidneys |
|------------|----------|----------------------------|--------------------|-----------------------|--------------------|-------|---------|
| 0 -Se | 241 | 0.775 ^{ABCDEFabc} | 0.464 ^a | 0.880 ^{ABCD} | 9.09 ^{Aa} | 1.76 | 1.79 |
| 0 +Se | 238 | 0.833 ^{ad} | 0.486 | 0.943 | 9.47 | 1.75 | 1.77 |
| CLA,1% -Se | 239 | 0.856 ^b | 0.490 | 0.940 | 9.33 | 1.75 | 1.77 |
| CLA,1% +Se | 238 | 0.859 ^D | 0.471 | 1.005 ^B | 9.15 | 1.76 | 1.72 |
| c9t11 -Se | 245 | 0.897 ^A | 0.479 | 0.889 | 9.36 | 1.72 | 1.73 |
| c9t11 +Se | 240 | 0.928 ^{Ed} | 0.512 | 0.940 | 9.52 | 1.75 | 1.85 |
| t10c12 -Se | 238 | 0.844 ^B | 0.496 | 1.006 ^A | 9.48 | 1.74 | 1.81 |
| t10c12 +Se | 246 | 0.876 ^c | 0.515 | 1.038 ^C | 10.09 ^A | 1.74 | 1.79 |
| CLA,2% -Se | 240 | 0.911 ^C | 0.520 | 0.897 | 9.44 | 1.75 | 1.75 |
| CLA,2% +Se | 241 | 0.921 ^F | 0.518 ^a | 1.023 ^D | 9.80 ^a | 1.78 | 1.73 |

¹ means in columns with the same letter are significantly different: A,B - P < 0.01; a,b - P < 0.05 (derived from fresh organs mass of rats normalized to 100 g of rat)

the most efficiently reduced body mass gain, consequently, Se in the diet (Group 0 +Se) is responsible for the lowest feed conversion efficiency. On the other hand, when the c9t11 isomer was added to the diet minute increase body mass gain of rats was found despite the highest total feed intake. It seems reasonable to assume that the presence of CLA isomers in Se supplemented diets resulted in smaller body mass gain depression and better feed conversion efficiency than rats fed Se only (0 +Se).

The experimental diets had significant effects on heart, spleen, pancreases and liver mass, whereas all diets had no influence on brain and kidneys mass (Table 2). Heart mass were significantly increased by all experimental diets and particularly by the addition of the c9t11 isomer or 2% CLA to Se unsupplemented and supplemented diet. The increased heart mass associated with the conjugated isomers, regardless of the presence of Se in the diets is probably due to increase protein content (West et al., 1998). The CLA isomers were found to act as a growth factor (Ostrowska et al., 1999), and have ability to reduce fat accretion with concurrent increases in lean tissue

Table 3. The CLA isomers content ((g/g lyophilised samples) of muscles and concentration ratio (R) of the c9t11 isomer to the t10c12 isomer¹ in tissues and organs of rats².

| Group | Total CLA isomer | c9t11 | t10c12 | Muscles R | Liver R | Pancreases R | Adipose tissue, R |
|------------|--------------------|---------------------|--------------------|--------------------|--------------------|--------------|---------------------|
| 0 -Se | 197 | 55 | 59 | - | - | - | - |
| 0 +Se | 217 | 94 | 47 | - | - | - | - |
| CLA,1% -Se | 4922 ^{Aa} | 1693 ^{ABa} | 1293 ^{Aa} | 0.763 ^a | 0.761 | 0.763 | 0.681 ^{AB} |
| CLA,1% +Se | 7373 ^a | 3022 ^B | 2146 ^a | 0.716 ^a | 0.775 | 0.716 | 0.799 ^B |
| c9t11 -Se | 6188 | 4472 | 195 | - | - | - | - |
| c9t11 +Se | 6854 | 5307 | 164 | - | - | - | - |
| t10c12 -Se | 5801 | 126 ^C | 4006 | - | - | - | - |
| t10c12 +Se | 8558 | 528 ^C | 6034 | - | - | - | - |
| CLA,2% -Se | 9309 ^{Ab} | 3410 ^{Aa} | 2560 ^{Ab} | 0.754 | 0.714 ^a | 0.771 | 0.769 ^A |
| CLA,2% +Se | 17391 ^b | 6830 ^a | 5085 ^b | 0.753 | 0.815 ^a | 0.753 | 0.750 |

¹ the concentration ratio of the c9t11 isomer to the t10c12 isomer in dosed CLA standard: 1.0242;

² means in columns with the same letter are significantly different: A,B - P < 0.01; a,b - P < 0.05.

deposition. Our results are similar to the recently reports in rats, where the main effect of CLA in a diet was to increase the mass of the soleus and gastrocnemius muscles (Poulos et al., 2001). The action of the t10c12 isomer in Se supplemented diet to the highest increase of pancreases and liver mass ($P<0.05$) is likely due to lipids accumulation in these organs. In the work of West et al. (1998), it was argued that liver lipid accumulation could be explained by dietary manipulations, including body mass loss, as well as modifications of protein and fat composition. As the CLA isomers modulated immune function, probably through cytokines, it is not surprising that spleen mass also increased in rats fed the CLA isomers. High increase of spleen mass were found in rats fed 2% CLA mixture, regardless of the Se presence, or the t10c12 isomer in Se supplemented diet. We supposed that the mixture of CLA isomers the most efficiently stimulated the immune function. However, there was not statistical difference in the area covered by Peyer's patches in rats with and without CLA mixture (1%) in the diet.

As expected, all CLA isomers enriched diets significantly increased the content of the CLA isomers, moreover, the level of the isomers was in proportion to their content in the diets. The response to CLA was substantially greater on Se supplemented than on unsupplemented diets. Feeding both individual isomers increased to similar extent concentration of CLA mixture in muscles, and enhanced also concentration of respective isomer; it did not significantly affect muscle level of other isomer. In our study, the concentration ratio of the t10c12 isomer to the c9t11 isomer in all examined samples significantly decreased compared to the concentration ratio of these isomers in the standard mixture of CLA isomers in the diets (Table 3). We could hypothesized that, the t10c12 isomer in rats is more efficiently metabolised than their 9,11 homologues.

In conclusion, the finding that Se and some CLA isomers fed to animals significantly increased the level of the CLA isomers in body of animals is valuable for nutritionists carrying out research to improve the nutritive value of food for human health. We could suggest that the interaction between Se and the t10c12 and c9t11 isomers or its metabolites exercises protective effect on the CLA isomers from peroxidation damage and/or catabolism.

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Soyhull energy availability when fed alone or in forage mixtures

B. Diarra, H. Zakaria, R. Uctuk & D. Johnson

Department of Animal Science, Colorado State University, Ft. Collins, CO, USA 80523

Summary

Three experiments were conducted to examine the digestibility and/or metabolizability of energy of soyhulls (SH) when fed as varying fractions of forage diets. In expt. 1, SH were fed ad libitum to mature ewes as 0, 25, 50, 75, and 100% of the diet with the balance as chopped millet straw &/or supplement in a replicated incomplete block design (2 ewes/treatment, each of 3 periods). Diet nutrient intakes and fecal outputs were measured by 7-d balance and methane with 2-d open-circuit chambers. In expt. 2, SH were fed ad libitum to 55 kg wethers in a randomized complete block design with 2-periods and 4 SH levels in the diets (0, 33, 66, and 100%), the balance being chopped alfalfa hay. Particle passage rates were determined by single dose of Yb (SH), Cr-mordant (alfalfa) and C0-EDTA (liquid) at the beginning of 7-d digestion trials. During expt. 3, the kinetics of SH digestion was measured in situ, by incubations in the rumen of cannulated steers fed either SH or alfalfa with or without buffer. When SH were fed with varying ratios of straw (expt 1) intake increased linearly ($P < 0.01$) from 43 to 89 g/BW^{.75} as SH level increased. Nutrient digestibilities and ME increased linearly ($P < 0.01$) to a maximum when the diet contained 75% SH, and then declined (quadratically, $P < 0.05$) as SH increased to 100%. Energy yielding nutrient digestibilities of the SH/alfalfa mixtures (expt 2) exhibited similar positive associative effects to those in expt. 1, with linear increases, then quadratic decreases in DE ($P < 0.05$) in response to SH level. Contrary to expectations, however, the particle passage rates of SH were not increased and did not explain the depressed digestibilities when the wethers were fed the 100% SH diet. When SH or alfalfa were incubated in the rumen of steers fed SH (expt. 3), the in situ rate of DM and NDF disappearance was markedly depressed ($P < 0.05$) as compared to incubation in the rumen of steers fed alfalfa. Inclusion of buffers only partially restored the digestion rates of these fibrous substrates in the rumen of steers fed SH. Thus, the cause of low availability of energy from SH fed alone is unexplained and not apparently due to accelerated particle passage and only partially due to depressed rumen pH. Additional factor(s) such as an anti-quality factor e.g., tannin, are likely involved.

Keywords: soybean hulls, digestibility, associative effects

Introduction

In a review of soyhull (SH) nutritive value in ruminant diets, Ipharrague and Clark (2003) concluded that the understanding of SH nutrient use is limited by the paucity of data on digestion and particle kinetics when this soybean processing byproduct is included in diets at levels above 30% of DM. This paper describes digestive interactions suppressing SH value when fed at high concentrations and at high levels of intake.

Materials and methods

Experiment 1. Conventional 7-d total fecal and urine collections and 2-d respiration calorimetry measurements of gaseous exchange provided 6 observations per treatment using incomplete latin square designs. The five dietary treatments to the 10 mature 40-kg ewes were SH:millet straw diets first fed ad libitum at ratios of 0:100, 25:75, 50:50, 75:25, and 100:0 and then at restricted

levels at ratios of 0:100, 50:50, and 100:0. Diets containing > 50% straw were supplemented with limestone and cottonseed meal to supply protein to a minimum of 8%.

Experiment 2. Similarly, six observations of nutrient digestion and passage were made by 7-d total fecal collections from 12-50 to 60 kg wethers in a randomized complete block design. The treatments were diets varying in SH:alfalfa ratios, 100:0, 67:33, 33:67, and 0:100; all fed ad libitum. Particle and liquid passage rates were measured concomitantly by single dose of ytterbium labeled SH, Cr-mordanted alfalfa, and Co-EDTA.

Experiment 3. The kinetics of SH digestion was measured in situ, by incubations in the rumen of four cannulated cattle fed either SH or alfalfa, with or without buffer. Duplicate 7-g SH or alfalfa incubations in 10 x 20 cm nylon bags were made for each of 12 time steps over 96 hr. The NLIN procedure of SAS was used to estimate the lag time, indigestible residue, and rate of in situ bag component digestion. Nutrient digestibility data were analyzed by GLM procedures of SAS (1996).

Results and discussion

The addition of successive levels of soybean hulls (SH) to either millet straw or alfalfa forage resulted in linear increases ($P < 0.05$) in diet DE (Figure 1), reaching maximums at the 3/4 and 2/3 levels of SH inclusion when the diets were fed ad libitum during experiments 1 and 2. The next increment of increase to 100% SH, however, resulted in a quadratic ($P < 0.01$) decrease in DE. Mixtures of forage with SH thus exhibited marked positive associative effects on energy digestibility as well as all dietary fiber components (data not shown) when these diets were fed to sheep ad libitum. Restricting the intake to 50% of ad libitum, experiment 1 only, did not change the DE of straw fed alone, moderately improved DE of the 50:50 mixture, and markedly improved the digestibility of the SH fed alone (figure 1) demonstrating a dramatic interaction of diet with level of intake effects on nutrient digestibility.

Digestibility of energy of SH were calculated by difference when fed as mixtures assuming constant forage digestibility and adjusting for supplement consumption. The DE of SH calculated in this manner (Figure 2) ranged from 76 to 81% for low to intermediate levels of SH in the 2 experiments. These DE's calculated from diet mixtures were considerably above ($P < 0.01$) the 61% DE found when SH were fed alone and ad libitum. The ME content of SH fed ad libitum averaged 3.2 when fed at lower fractions of the diet (as calculated by difference) but only 2.4 Mcal/kg DM when fed alone. When DE or ME was determined while restricting intakes to 50% of ad libitum the SH fed alone were digested similarly to those in mixtures fed ad libitum.

Previous researchers (Quicke et al., 1959; Hintz et al., 1964) have speculated that a rapid rate of passage from the reticulorumen may suppress nutrient digestibility of SH fed at high concentrations in the diet. Surprisingly, however, experiment 2 results did not confirm this expectation. The SH particle passage rates through the rumen averaged slightly less and total tract retentions higher ($P > 0.10$) from 100% SH as compared to passage from mixed diets (Table 1). SH passage estimated as $0.59 \times L_1$ from a non-linear age-dependent model, averaged 0.026 when SH were fed alone vs. .033 and .036 when fed in mixtures, also similar to alfalfa particle passage rates. The passage rates of liquid of ~ 0.06 were also unaffected by SH:forage ratio. Thus varying passage rates are unlikely to explain the depressed DE's of SH fed singly. Support for this notion was found when the digestibility of SH was measured in situ during experiment 3 where the substrates were held in the rumen for up to 96 h. The NDF of either alfalfa or SH was markedly depressed ($P < 0.05$) when incubated in the rumen of steers fed SH as compared to those fed alfalfa (Table 2). First order NDF digestion rates were only 0.01 to 0.02 when incubated in SH fed steers as compared to rates ranging from 0.05 to 0.13 when incubated in control alfalfa fed steers. In any case, digestion in the rumen of SH fed animals was depressed even with no particle passage.

A second reason for low digestibility of SH fed at high concentrations could be low ruminal pH, indeed the NRC (1996), level 2 system, predicts SH fiber to have a digestibility of zero in response

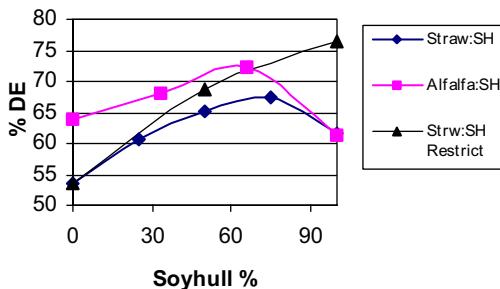


Figure 1. %DE of forage soyhull mixtures.

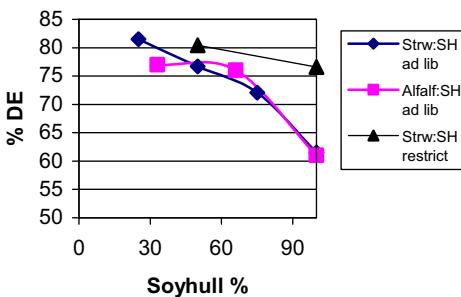


Figure 2. Soyhull DE % by difference.

to low effective NDF induced pH declines. The digestibility of NDF by sheep fed pure SH diets was 62% illustrating the gross inaccuracy of the NRC prediction system in this case. Nevertheless, low pH's of 5.6 to 6.3 were noted in the sheep and cattle fed 100% SH and undoubtedly contributed to depressions. The addition of buffer to the SH fed steers raised the pH from 5.6 to 6.1 but only partially overcame the low digestions rates (Table 2).

We conclude that the digestion of fiber and energy is depressed in SH when fed to ruminants in high dietary concentrations at high levels of intake. The cause is partially low rumen pH, unlikely to be the result of accelerated passage, and likely to be largely due to an inhibitory condition, yet undiscovered, such as tannin levels in this legume byproduct.

Table 1. Kinetics of soyhull (SH-Yb) and alfalfa (Alf-Cr) particle passage.

| Item | % soyhulls | | | |
|-----------------------|------------|------|------|------|
| | 0 | 33 | 66 | 100 |
| SH-Yb K ₁ | -- | .036 | .033 | .029 |
| Alf-Cr K ₁ | .027 | .035 | .026 | -- |
| Liq-Co K ₁ | .059 | .061 | .060 | .054 |
| TTRT-Yb, h | -- | 41 | 40 | 47 |
| TTRT-Cr, h | 37 | 24 | 25 | -- |
| TTRT-Co, h | 30 | 26 | 26 | 29 |

Table 2. Steer diet vs *in situ* NDF digestion rate.

| In situ bag substrate | Diet fed to steers | |
|--------------------------|--------------------|-------------------|
| | Alfalfa | Soyhulls |
| — NDF digestion, k_d — | | |
| Trial 1 | | |
| Alf-NDF | 0.05 ^a | 0.02 ^b |
| SH-NDF | 0.07 ^a | 0.01 ^b |
| Trial 2 | | |
| SH-NDF | 0.13 ^a | 0.01 ^b |
| — Buffer added — | | |
| SH-NDF | 0.13 ^a | 0.03 ^b |

^{a,b} P < 0.05

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Glucose metabolism by the gastrointestinal tract of sheep as affected by protein supply

S.W. El-Kadi, N.E. Sunny, M. Oba, S.L. Owens & B.J. Bequette

Department of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20742, USA

Summary

Gastrointestinal (GIT) metabolism of glucose was investigated in sheep ($n=6$, 35 ± 2.0 kg) fed a low protein diet (100 g CP/kg) to $1.6 \times$ maintenance. Catheters were fitted for duodenal infusion and flux measurements by the portal-drained viscera. Animals were given 10-d duodenal infusions of either glucose (50 g/d, Control) or Glucose + Casein (60 g/d) in a cross-over design. On days 7 and 10, [$1-^{13}\text{C}$]glucose was infused (6 h) into either a jugular vein or the duodenum to determine glucose utilization by the GIT. The GIT utilized 48-51% of glucose available from absorption and gluconeogenesis. Most of the glucose used by the GIT derived from the arterial circulation (65-82%) compared to that from the GIT lumen (18-35%). Compared to Control, Casein + Glucose increased ($P<0.05$) the contribution of luminal (first-pass) and decreased ($P<0.05$) that of arterial glucose utilization by the GIT, but total GIT utilization remained similar. Casein + Glucose infusion increased ($P<0.05$) total glucose availability, however, this derived mainly from an increase in gluconeogenesis (+27%).

Keywords: glucose, gastrointestinal, sheep

Introduction

Gastrointestinal tract (GIT) metabolism by ruminants places a major drain on amino acid (AA) and glucose for growth. Metabolism of intestinal essential AA supply is high (0.25-0.60, MacRae et al., 1997b), as is non-essential AA metabolism (80-100%, Heitmann and Bergman, 1981). Similarly, a significant proportion (0.40-0.60) of intestinal glucose is removed by the GIT (Cappelli et al., 1997). Reducing the catabolic losses of AA and glucose by the GIT is a goal towards improving N and energy efficiency of ruminants. In this respect, our aim is to determine the optimal pattern of substrates for productive processes, and this requires knowledge of the fuels selected by the GIT for metabolism.

Based on previous studies with ruminal and intestinal cells, where metabolic flexibility was demonstrated for AA and glucose oxidation (Okine et al., 1995; Oba et al., 2003), we hypothesized that if AA, and not glucose, are the preferential energy substrates of the GIT, then intestinal infusion of casein would lead to a reduction in glucose use by the GIT.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Maryland approved all animal use procedures. Six sheep (4 Katahdin and 2 Dorset x Polypay, 35 ± 2.0 kg) were fitted with chronic catheters placed into the duodenum, an artery, the hepatic-portal and two mesenteric veins. After recovery, sheep were placed in metabolic crates and fed a pelleted diet by automatic feeder (12×2 -h intervals). The diet was fed to $1.6 \times$ maintenance energy requirements (NRC, 1985), and was low in protein (100 g CP/kg) but energy adequate (7.5 MJ metabolizable energy/kg). Animals were arranged in a balanced cross over design with 10-d treatment periods. Treatments were duodenal infusion of either glucose (50 g/d, Control) or Casein (60 g/d) + Glucose.

On days 7 and 10 of each period, [1^{-13}C]glucose was infused (200 mg/h for 6 h) into either a jugular vein or the duodenum to determine the rate and proportion of glucose entry utilized by the GIT and to distinguish between luminal and arterial utilization of glucose. Plasma flow was determined by infusion (15 mg/min, pH 7.4) of p-aminohippuric acid into the distal mesenteric vein. During the last 4 h, blood was continuously withdrawn from each vessel over 1-h periods into sealed syringes submerged in an ice bath. Samples were mixed and processed for analysis.

Plasma glucose enrichment and concentration were determined by gas chromatography-mass spectrometry under electrical impact mode (Hannestad and Lundblad, 1997; Calder et al., 1999). Plasma concentrations of p-aminohippuric acid were determined as previously described (McRae et al., 1997b). Gravimetric procedures were used throughout to reduce error and increase precision. Data were analyzed using the MIXED procedure of SAS (2003), with sheep, period, breed, and treatment order considered as random effects. Differences were considered significant at $P \leq 0.05$. Plasma glucose entry rate was calculated for jugular vein (GE_{JV}) and duodenal (GE_{duo}) [1^{-13}C]glucose infusions employing standard isotope dilution principles. Fractional splanchnic removal was calculated as: $1 - (\text{GE}_{\text{JV}} / \text{GE}_{\text{duo}})$. Net flux (absorption or removal) of glucose by the PDV was calculated as the product of plasma flow (PF) and arterio-venous concentration difference. Fractional utilization of arterial glucose (f_a) was based upon jugular infusion of [1^{-13}C]glucose calculated by:

$$\frac{([\text{A}] \times \text{A}_E) - ([\text{V}] \times \text{V}_E)}{([\text{A}] \times \text{A}_E)}$$

where E is enrichment, and [A] and [V] are concentrations of glucose in artery (A) and portal vein (V). Arterial glucose utilization was calculated as: $[\text{A}] \times \text{PF} \times$ fractional utilization of arterial glucose. Luminal use of glucose (i.e. first-pass) was calculated from recovery of [1^{-13}C]glucose infused into the duodenum after correction for second-pass arterial removal, and converted to an absolute rate based on unlabeled glucose infusion rate into the duodenum:

$$1 - \left(\frac{([\text{V}] \times \text{V}_E) - ([\text{A}] \times \text{A}_E) + ([\text{A}] \times \text{A}_E \times f_a) \times \text{PF}}{\text{duodenal } [1^{-13}\text{C}] \text{glucose infusion rate}} \right) \times \frac{\text{duodenal glucose infusion rate}}{\text{infusion rate}}$$

Results

There were no significant differences detected in glucose metabolism between Katahdin and Dorsett \times Polypay sheep. Plasma glucose and glucose entry rates based on jugular and duodenal tracer infusion were not significantly affected by Casein + Glucose infusion compared to infusion of glucose alone (Control). Portal plasma flow tended ($P < 0.08$) to be lower with Casein + Glucose infusion. Arterial use of glucose by the GIT accounted for 65-82% and luminal use 18-35% of total GIT use of glucose. Total utilization of glucose by the GIT was not affected; however, infusion of Casein + Glucose shifted the proportion of total glucose use by the GIT leading to a reduced ($P < 0.05$) contribution from the arterial supply and an increased ($P < 0.05$) contribution from the gut lumen (first-pass). First-pass metabolism of glucose by the GIT accounted for 33 to 62% of the glucose infused into the duodenum, and this was reflected in a tendency ($P < 0.08$) for [1^{-13}C]glucose recovery across the PDV to be lower with Casein + Glucose. Total glucose availability (gluconeogenesis + luminal absorption) was greater ($P < 0.05$) with Casein + Glucose infusion, and after correction for luminal glucose absorption, indicated that gluconeogenesis increased with Casein + Glucose. Utilization of glucose by the GIT (arterial + luminal) accounted by 48 to 51% of glucose available from absorption plus gluconeogenesis, and this was not altered by treatment.

Table 1. Whole body and gastrointestinal tract metabolism of glucose in sheep given intraduodenal infusions of glucose (Control) or Glucose plus Casein.

| Item | Treatment | | SEM | P< |
|---|----------------------|-----------------------|-------|------|
| | Control (Glucose) | Glucose + Casein | | |
| Plasma glucose flux and metabolism | | | | |
| Arterial glucose (mM) | 3.84 (6) | 3.94 (6) ¹ | 0.490 | 0.59 |
| Glucose entry rate (mmol/h) | | | | |
| Jugular [1^{13}C]glucose infusion (A) | 37.6 (6) | 43.4 (6) | 3.01 | 0.13 |
| Duodenal [1^{13}C]glucose infusion (B) | 45.0 (6) | 48.2 (6) | 5.75 | 0.47 |
| Fractional splanchnic removal (1-(A/B)) | 0.21 (5) | 0.17 (5) | 0.100 | 0.83 |
| Gastrointestinal glucose metabolism | | | | |
| Portal plasma flow (g/min) | 1880 (5) | 1270 (6) | 217 | 0.08 |
| Glucose utilization | | | | |
| Net flux ² (mmol/h) | 81.3 (5) | -19.3 (6) | 55.00 | 0.16 |
| Arterial use (mmol/h) (C) | 13.6 (5) | 16.3 (5) | 3.72 | 0.62 |
| Luminal use (mmol/h) (D) | 3.9 (4) | 7.2 (5) | 0.79 | 0.01 |
| Total gut use (mmol/h) (E) | 21.0 (4) | 23.0 (5) | 5.49 | 0.78 |
| Fractional luminal tracer recovery | 0.65 | 0.45 | 0.134 | 0.15 |
| Fractional arterial use of total gut use ⁴ (C/E) | 0.82 (4) | 0.65 (5) | 0.071 | 0.03 |
| Fractional luminal use of total gut use (D/E) | 0.18 (4) | 0.35 (5) | 0.071 | 0.03 |
| Total glucose availability ⁵ (TGA; mmol/h) | 37.1 (5) | 47.7 (5) | 5.11 | 0.04 |
| Luminal use as % of TGA | 11.2 (4) | 15.6 (5) | 2.78 | 0.08 |
| Arterial use as % of TGA | 34.9 (5) | 34.0 (5) | 8.78 | 0.94 |
| Total gut use as % of TGA | 51.0 (4) | 48.0 (5) | 13.14 | 0.81 |

¹ Number of observations (n).

² Negative values indicate net removal and positive values net absorption.

³ For both treatments, fractional arterial use was greater than fractional luminal use (at least P<0.05).

⁴ The fractional contribution to total gut glucose use from arterial removal was significantly greater (P<0.01) than that from luminal use.

⁵ TGA = glucose entry rate (jugular [1^{13}C]glucose infusion) + first-pass luminal use.

Discussion

Many previous studies in ruminants have demonstrated that GIT metabolism places a major drain on AA and glucose availability for peripheral tissue anabolism. In this respect, there are two questions with regards to AA and glucose metabolism by the GIT: Is GIT metabolism of these nutrients obligate? To what extent are these nutrients metabolized from the gut lumen versus metabolism from the arterial circulation? We (Oba et al., 2003) and others (Okine et al., 1995) have demonstrated with ruminal and duodenal cells *in vitro* that the gut tissues of ruminants have some metabolic flexibility to oxidize either glucose or AA (glutamate, glutamine) for energy. In the present study, we tested, by increasing the supply of protein (casein) to the small intestines of sheep fed a marginally low protein but energy adequate diet, whether glucose metabolism by the GIT is 'spared' by provision of AA. Our results indicate that total GIT use of glucose is not affected by additional luminal AA supply, and so under these conditions glucose metabolism by the GIT was obligatory. However, this disguised the fact that casein infusion increased the amount and proportion of glucose use by the GIT that was derived from the gut lumen supply (3.9 vs. 7.2

mmol/h, 18% vs. 35%) and decreased the proportional use from the arterial supply (82% vs 65%). Further, casein infusion on this low protein diet increased total glucose availability from luminal absorption and gluconeogenesis, which after correction for luminal absorption indicated that casein infusion increased gluconeogenesis by 27%.

Nutrients are delivered to the GIT from luminal and arterial supplies, and thus the factors regulating use from these sources will differ. Our results indicate that glucose use by the ruminant GIT derives mainly (65-82%) from the arterial circulation, which is probably under hormonal control and suggests that the GIT may be in competition with peripheral tissues (eg. muscle, mammary gland) for glucose supplies. Partition predominantly from the arterial circulation has also been observed for essential AA, where for most AA (80% of GIT use of AA derived from the arterial circulation (MacRae et al., 1997a). This pattern of glucose and AA use also corresponds with the known distribution of luminal absorptive capacity of the ruminant GIT with only (20% of the luminal surface of the GIT capable of glucose and AA transport and absorption.

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Energy metabolism and cognitive performance in relation with postprandial metabolic changes after ingestion of different macronutrients in the morning

Karina Fischer¹, P.C. Colombani¹, W. Langhans² & C. Wenk¹

¹ Institute of Animal Science, Nutrition Biology, Swiss Federal Institute of Technology Zurich, 8092 Zurich, Switzerland

² Institute of Animal Science, Physiology, Swiss Federal Institute of Technology Zurich, 8092 Zurich, Switzerland

Summary

The effect of isoenergetic pure carbohydrate, protein or fat ingestion (1670 kJ) on simple as well as complex cognitive functions and the relation between the respective postprandial metabolic changes and changes in cognitive performance were studied in fifteen healthy male students using a repeated-measures, counterbalanced cross-over design. After the carbohydrate meal, there was an intense and significant linear increase ($P < 0.001$) in the respiratory exchange ratio (RER) whereas after the fat and protein meal the RER remained nearly at baseline values. Overall energy expenditure (EE) was significantly lower after the fat compared with the protein meal ($P < 0.05$). Most aspects of subjective performance and mood states did not differ between test meals. For all objective tasks postprandial cognitive performance was best after fat ingestion concomitant with relatively low EE and a relatively constant RER, as well as an almost constant glucose metabolism and constant state of metabolic activation measured by the glucagon to insulin ratio (GIR). In contrast, carbohydrate as well as protein ingestion resulted in lower overall cognitive performance, both together with partly marked changes ($P < 0.001$) in glucose metabolism and metabolic activation. They also differently affected specific cognitive functions ($P < 0.05$) in relation to their specific effect on metabolism.

Keywords: macronutrients, metabolism, cognitive performance

Introduction

The relation between postprandial metabolism and cognitive behaviour after macronutrient ingestion is only marginally characterised and outcomes are not conclusive. Furthermore, there is only little understanding of how the multitude of postprandial metabolic changes affects simple and complex cognitive functions as well as different mood states (Dye *et al.* 2000).

Generally, three targets must be considered that directly or indirectly influence acute cognitive functioning after macronutrient ingestion: energy supply to nerve cells (Gold, 1995), neurotransmitter and hormone modulations (Cahill & McGaugh, 1996) and activation or deactivation of the nervous system (Nielson & Jensen, 1994).

Since glucose is the predominant brain fuel and the state of metabolic activation is a basic index for subsequent changes in brain neurotransmitter or hormone modulation, we expected changes in energy metabolism in particular the glucose metabolism as well as the state of metabolic activation measured by the glucagon to insulin ratio (GIR) to be related to postprandial cognitive changes.

Subjects and methods

Seventeen healthy male students (mean age 26.5 (SD 3.3) years, non-smokers, not trained athletes, not colour-blind, mean body mass index of 21.9 (SD 1.7) kg/m², no medication, drugs or nutritional supplements) were tested according to a repeated-measures, counterbalanced cross-

over design in three sessions separated by exactly one week. After a 10-12 h overnight fast, baseline assessments were taken in the following order: 1. blood sampling, 2. respiratory measurements (15-min period, half open system, subjects walked slowly on a treadmill (2.5 km/h)), 3. questionnaires (mood and subjective performance) and 4. an objective computer based cognitive test (a demanding combi-test assessing accuracy and efficiency in short-term memory, peripheral attention and reaction times, Läubli *et al.* 1998). Subsequently, a 400 mL (1670 kJ) cream-like test meal (isoenergetic emulsions or suspensions of only one macronutrient with similar volume and sensory properties) was served. For repeated postprandial measurements within the next three hours, subjects had to rotate hourly through the same stations as under baseline conditions. Repeated-measures analysis of variance (ANOVA) was performed to detect overall meal and time effects as well as meal by time interactions. For significant overall differences between treatments, the data were further analysed with Tukey's post hoc comparisons. Polynomial contrast analysis was performed for significant meal by time interactions. Statistical significance was set at $P < 0.05$.

Results and discussion

For the fat meal, the plasma glucose, insulin and glucagon concentration as well as the GIR remained approximately constant over time (Figure 1). There were, however, significant changes over time for the same indices after the carbohydrate meal resulting in a quadratic decrease for the GIR. In contrast, after the protein meal, only the glucagon concentration changed significantly over time. It transiently increased compared to the temporary decrease after the carbohydrate meal resulting in a stepwisely rising higher GIR than after carbohydrate ingestion.

There was a significant overall meal as well as meal by time effect for the RER and a significant overall meal effect for EE (Table 1). After the carbohydrate meal, there was an intense and significant linear increase in the RER, which exceeded the value 1.0 in the second hour indicating that in addition to increased glucose oxidation, lipogenesis from carbohydrates might have been initiated. However, after the fat and protein meal, the RER remained nearly at baseline values suggesting that partially fatty or amino acids were oxidised. Overall EE was significantly lower after the fat compared with the protein condition.

For most data concerning the subjects' hourly subjective performance and mood states no significant overall meal effect or meal by time interaction were detected. For the accuracy and efficiency of the central short-term memory task as well as for the peripheral attention task of the combi-test subjects reached the highest scores after the fat meal. This was concomitant with a constant glucose metabolism and GIR as well as a low and constant RER, suggesting that plasma fatty acids probably had a sparing effect on liver glycogen allowing a constant glucose supply to the brain. For the accuracy in central short-term memory overall scores were significantly different (fat > carbohydrates > protein, $P < 0.05$). However, overall scores for the efficiency in central short-term memory as well as the peripheral attention task were in the order fat > protein > carbohydrates, with a significant difference between the fat and the carbohydrate meal ($P < 0.05$). Thus, carbohydrate ingestion resulted in relatively better short-term memory and accuracy of tasks concomitant with a relatively high RER and a low GIR, whereas protein ingestion resulted in better attention and efficiency of tasks concomitant with high relatively EE and a relatively higher GIR and therefore presumably with higher cognitive arousal.

In the first hour after test meal ingestion, reaction times of the combi-test improved after the carbohydrate meal and worsened after the protein meal, whereas after the second hour a worsened reaction for the carbohydrate meal and an improved reaction for the protein meal were seen ($P < 0.01$). This may be explained by the positive effect of glucose supply on brain function (Gold, 1995) immediately after carbohydrate ingestion and/or by delayed neurotransmitter synthesis after test meal ingestion.

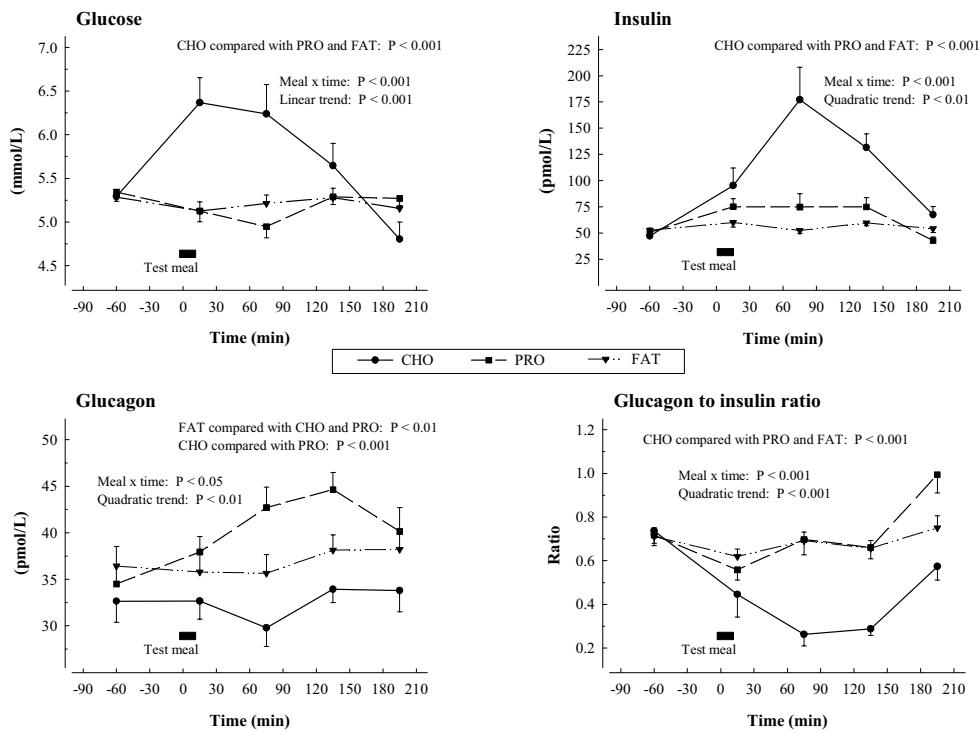


Figure 1. Temporal profiles for glucose, insulin and glucagon responses as well as for the glucagon to insulin ratio to pure carbohydrate (CHO), protein (PRO) or fat ingestion. Mean (SEM); n=15. Test meal ingestion (min 0-15) and postprandial temporal pattern of changes (min 15-195) are preceded by a baseline (min -60) measurement. Significant P-values are given for Tukey's post hoc comparisons of overall meal effects, for meal by time interactions and for polynomial contrast analyses (trends) of meal by time interactions.

In summary, in healthy young men, pure macronutrient ingestion in the morning influenced specific cognitive functions differently. Best and non-specific overall performance after fat ingestion was concomitant with a stable glucose metabolism and a high and constant GIR as well as with low EE and a constant RER. Carbohydrate and protein ingestion both resulted in lower overall cognitive performance, which was accompanied by changes in the RER or EE, respectively, and some marked changes in glucose metabolism and in the GIR. Our findings support the concept that good and stable cognitive performance is related to a balanced glucose metabolism and metabolic activation state.

Table 1. Temporal changes for the respiratory exchange ratio (RER) as well as for energy expenditure (EE) after pure carbohydrate, protein or fat ingestion in fifteen healthy young men.

| | Time ¹ of repeated measurements (min) | | | | | | | | Statistical significance of effects (repeated-measures ANOVA, P =) | |
|--------------------|--|------|------|------|------|------|------|------|--|-------------|
| | -45 | | 30 | | 90 | | 150 | | | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Meal | Meal x time |
| RER | | | | | | | | | | |
| Carbohydrates | 0.88 | 0.01 | 0.96 | 0.02 | 1.02 | 0.09 | 1.04 | 0.09 | | |
| Protein | 0.90 | 0.02 | 0.91 | 0.01 | 0.92 | 0.01 | 0.92 | 0.01 | 0.0001 ² | 0.001 |
| Fat | 0.88 | 0.01 | 0.91 | 0.01 | 0.89 | 0.01 | 0.90 | 0.02 | | |
| EE (kJ/min) | | | | | | | | | | |
| Carbohydrates | 14.3 | 0.52 | 14.5 | 0.49 | 13.8 | 0.44 | 13.7 | 0.41 | | |
| Protein | 13.9 | 0.59 | 14.3 | 0.68 | 13.9 | 0.53 | 13.8 | 0.54 | 0.01 ³ | NS |
| Fat | 13.8 | 0.41 | 13.5 | 0.51 | 13.1 | 0.51 | 13.2 | 0.47 | | |

¹ Test meal ingestion (min 0-15) and postprandial temporal pattern of changes (min 30-150) are preceded by a baseline (min -45) measurement.

² Tukey's post hoc comparisons for overall meal effects: carbohydrates > protein and fat ($P < 0.001$);

³ Tukey's post hoc comparisons for overall meal effects: protein > fat ($P < 0.05$).

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Effect of dietary cholesterol and phospholipid levels on the absorption and distribution rates of cholesterol in juvenile *Panaeus monodon* Tiger shrimp

E. Harrisil¹, D. Sastradipradja² & K. Sumawidjaja¹

¹ Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, IPB Darmaga Campus, Bogor, Indonesia

² Faculty of Medicine, Krida Wacana Christian University, Jakarta, Indonesia

Summary

An experiment was conducted to study the effect of cholesterol and phospholipid levels of diet on the absorption and distribution rates of cholesterol in juvenile *Panaeus monodon* tiger-shrimp. The experiment was designed using combinations of different levels of cholesterol and phospholipid in the diet. Dietary cholesterol level was 0, 0.25 or 0.5%, while phospholipid was 0, 2 or 4%. Juvenile shrimp of 0.5g BW were fed those diets labeled with ^3H -cholesterol. Zero, 3, 6, 9, 15 and 24h post feeding, all shrimps were analysed for evaluation of the hepatopancreas and hemolymph radioactivities. The results show that combinations of more than 0.6% cholesterol and less than 0.1% phospholipid in the diet resulted in high absorption rate ($>200\mu\text{g.}15\text{min}^{-1}$) and low distribution rate ($<10\mu\text{g.}15\text{min}^{-1}$) cholesterol in the body. Feeding with 0.69% cholesterol and 0.8 - 1.0% phospholipid in the diet has the best effect on growth, feed efficiency, protein and fat retentions, however, based on data on cholesterol absorption and distribution, chemical composition and structure of the hepatopancreas, growth response and survival, diet containing 0.41 - 0.47% cholesterol and 0.75 - 1.46% phospholipid is the best for juvenile tiger-shrimp.

Keywords: cholesterol and phospholipid, absorption, distribution, *Panaeus monodon* shrimp

Introduction

Shrimp is an important international trade commodity and therefore the competitive ability of shrimp culture should be improved. The efficiency of production, especially feed utilization plays a determining role. Despite of the fact that feed has been mass produced by the feed industry, there is still room to do basic research, especially on quantitative nutrition of typical nutrients essential for shrimp, due to the following reasons: (1). Quantitative nutrition of typical nutrients of shrimp, cholesterol and phospholipid in particular have been studied only on larvae and juvenile *Penaeus japonicus* and *P. penicillatus* (Teshima & Kanazawa, 1988; Chen & Jen, 1991). (2). The meat and hepatopancreas of *Panaeus monodon* tiger-shrimp reared in Indonesia contain less phospholipid compared to those of natural shrimp living in the Java sea (Leary & Mathews, 1990). (3). Cholesterol and phospholipid are relatively expensive feed components, thus the exact amounts needed for shrimp would determine economic efficiency of production. To gain basic information on the needs of tiger-shrimp for dietary cholesterol and phospholipid, the present research was carried out to study the effect of levels of combination of dietary cholesterol and phospholipid on the absorption and distribution rates of cholesterol in *Panaeus monodon* tiger-shrimp.

Materials and methods

The experiment involved dietary treatments consisting of 2 factors of which each covered 3 dose levels. The first factor was cholesterol (chol) levels of 0, 0.25 and 0.50%, while the second factor

was different levels of lecithin (phospholipid) at 0, 2 and 4% (Tabel 1). Cholesterol levels were based on the requirement of 0.5% for 0.25g *P.japonicus* shrimp (Teshima & Kanazawa, 1988). The levels of lecithin were taken according to the range of requirement between 1% and 3.5% for *P.japonicus* larva (Kanazawa, 1985).

Table 1. Nutrient composition of test diets.

| Ingredient | Dietary Treatment | | | | | | | | |
|--------------------------------|-------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | A | B | C | D | E | F | G | H | I |
| Casein (%) | 42.70 | 42.70 | 42.70 | 42.70 | 42.70 | 42.70 | 42.70 | 42.70 | 42.70 |
| Arginine (%) | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 |
| Glucose (%) | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 | 5.50 |
| α Cellulose (%) | 3.83 | 3.83 | 3.83 | 3.83 | 3.83 | 3.83 | 3.83 | 3.83 | 3.83 |
| α Starch (%) | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Polac liver oil (%) | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Sodium citrate (%) | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Sodium succ. (%) | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Mineral mix (%) | 8.65 | 8.65 | 8.65 | 8.65 | 8.65 | 8.65 | 8.65 | 8.65 | 8.65 |
| Vitamin mix (%) | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| CMC (%) | 7.42 | 7.42 | 7.42 | 7.42 | 7.42 | 7.42 | 7.42 | 7.42 | 7.42 |
| Cholesterol (%) | 0.00 | 0.00 | 0.00 | 0.25 | 0.25 | 0.25 | 0.50 | 0.50 | 0.50 |
| Lecithin (%) | 0.00 | 2.00 | 4.00 | 0.00 | 2.00 | 4.00 | 0.00 | 2.00 | 4.00 |
| Total (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Protein (%) | 45 | 45 | 45 | 45 | 45 | 45 | 45 | 45 | 45 |
| Cholesterol (%) ¹ | 0.20 | 0.16 | 0.12 | 0.45 | 0.41 | 0.37 | 0.69 | 0.65 | 0.61 |
| Phospholipids (%) ¹ | 0.08 | 1.36 | 2.64 | 0.08 | 1.36 | 2.64 | 0.08 | 1.36 | 2.64 |
| Digest. En. (kJ/g) | 26.06 | 26.06 | 26.06 | 26.06 | 26.06 | 26.06 | 26.06 | 26.06 | 26.06 |

¹ Due to the use of cod liver oil (2% cholesterol); cholesterol and phospholipid levels were properly corrected. The use of the lecithin source was only 65% pure.

Postlarval tiger shrimps originating from a single female parent and showing normal growth during the larval stadium were used. A total of 2000 postlarva-20 were reared in a large container and fed until they reached a size between 60 and 80 mg. Consecutively, the juveniles were divided into fibreglass containers of 20L capacity, each container consisted of 5 juvenile shrimp. Each container was supplied with a recirculation system, while water quality was kept at pH 7.5 - 8.2, temp. 30°C, NH₃ ≤ 0.1 ppm, O₂ ≥ 5 ppm and salinity at 24 ppt. An adaptation period of 20 days were applied before juveniles were being subjected to the dietary treatments for 56 days reaching a final body weight (BW) around 0.5 g. Feed was given at 20% BW.d⁻¹. Feed were offered at 6:00, 12.00, 18.00 and 22.00. On day 56 at 12.00, ³H-cholesterol labelled feed (66000 dpm/20 mg feed for the 0.25% chol; 92000 dpm/20 mg feed for the 0.50% chol.) were offered. Hepatopancreas (HP) and hemolymph (He) were collected at 0, 3, 6, 9, 15 and 24h post-label feeding. Altogether, 54 containers (units) were needed for 9 treatments times 6 sampling times. He samples were collected using a 1 ml syringe, HP were collected from each shrimp, weighted and placed into a scintillation vial for radioactivity counting. For the counting of He, 100 mg samples were used. HP 60-80 mg and 0.5 g shrimp were analysed for fat content by proximate analysis and fat composition by the method of Takeuchi (1988).

Cholesterol absorption and distribution rates were calculated as follows.

chol HP ($\mu\text{g chol}$) = dpm HP/Corr. Coef. HP; Cor. Coef. HP = (dpm/ $\mu\text{g feed chol}$)
 chol He ($\mu\text{g chol}$) = dpm He/Corr. Coef He; Cor. Coef. He = (dpm/ $\mu\text{g feed chol} \times (35\% \text{ BW}/100 \text{ mg})$); Total He is approximately 35% shrimp BW.

Feed chol t_1 = HP chol t_1 + He chol t_1 ; Feed chol t_2 = Feed chol t_1 - (HP chol t_2 - HP chol t_1) + He chol t_2 .

Absorption rate = (Feed chol t_1 - Feed chol t_2)/($t_2 - t_1$), expressed in $\mu\text{g chol.15min}^{-1}$.

Distribution rate = (He chol t_2)/($t_2 - t_1$), expressed in $\mu\text{g chol.15min}^{-1}$.

The mathematical model of cholesterol absorption and distribution rates follow a gamma function logistic model $Y_1 = a\text{Exp}(-cX)X^{(bc)}$, where Y_1 = radioactivity (dpm), X = time (h), a, b, c = constants. The data were statistically analysed using analysis of variance and to test the maximal response due to treatment, the polynomial orthogonal analysis was used (Steel & Torrie, 1980).

Results and discussion

Fat is transported in combination with cholesterol and phospholipid. By using ^3H -cholesterol labelled feed, the rate of fat transportation is reflected by the absorption rate of the ^3H -cholesterol into the HP and the distribution rate by the He. Based on the radioactivity data of HP and He, and the fat transportation pattern, cholesterol absorption and distribution rates were calculated. In particular, the initial radioactivity values are essential for this purpose, because during this period the degree of ^3H -cholesterol metabolism is relatively small, hence radioactivity values of HP and He can be considered as originating from intact ^3H -cholesterol molecules.

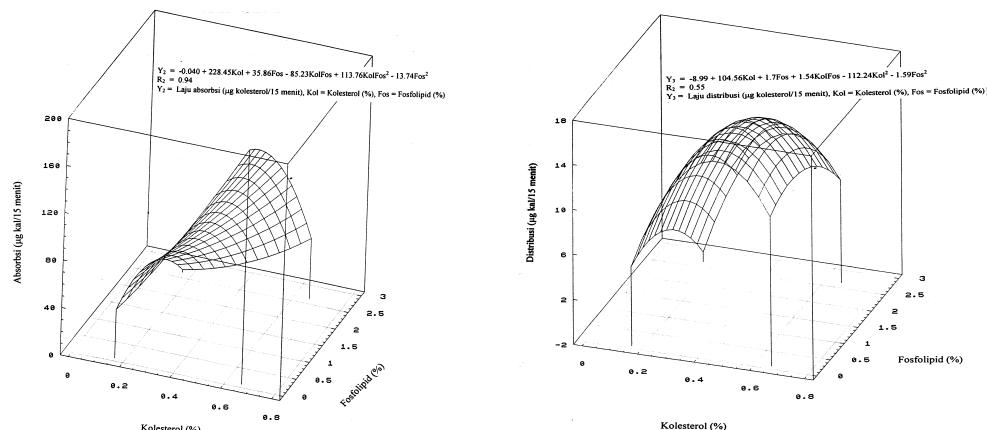


Figure 1. Cholesterol absorption rate (left) and distribution rate (right) in relation to cholesterol and phospholipid levels of diet during the first hour post feeding with ^3H -cholesterol labeled diet.

The data on cholesterol absorption and distribution rates as affected by the different dietary treatments are presented in Figure 1. The figure shows that maximal absorption occurred when the diet contains 0.69% cholesterol and 0.08% lecithin (treatment G), while the lowest absorption rate occurred with dietary treatment C (0.12% cholesterol and 2.64% lecithin).

Table 2 presents the ratios between values of distribution rates and their corresponding absorption rates, calculated at 15 min. intervals between 0 and 3 h post-label feeding. The data reveals that the maximum and the fastest distribution rate occurred with dietary treatment I (0.61% chol.; 2.64% lecithin), followed by treatment E (0.41% chol.; 1.36% lecithin) and treatment F. However, the absorption rates for I and F were lower than for E, thus treatment E gave the best results. High and fast absorption rates occurred with high cholesterol levels. This observation means that absorption

rate is not affected by the presence of the phospholipid which in turn indicated that the entrance of fat/cholesterol into HP in tiger-shrimp would not involve chylomicrons. On the other hand, the distribution rate is influenced by the presence of phospholipid. The magnitude of the difference between absorption and distribution rates indicated that the HP functions as the cholesterol pool. Low distribution rate also indicated that fat transportation in tiger-shrimp is not carried by LDL, but by HDL as have been reported by Teshima & Kanazawa (1986) for *P. japonicus*.

By considering absorption and distribution rates, and the ratio between distribution and absorption rates, it was concluded that a dietary treatment with 0.47% cholesterol combined with 0.1.46% lecithin would give the best result for rearing juvenile tiger-shrimp, i.e. cholesterol absorption rate $>120 \mu\text{g.}15\text{min.}^{-1}$, distribution rate $>16 \mu\text{g.}15\text{min.}^{-1}$ and [distribution rate]/[absorption rate] ratio equals 13.1%.

Tabel 2. Ratios between 15 min. distributed and absorbed cholesterol from 0 to 180 min. post-label feeding.

| Time (min) | Treatment | | | | | | | | |
|------------|-----------|------|------|------|------|------|------|------|------|
| | A | B | C | D | E | F | G | H | I |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 30 | 0.09 | 0.10 | 0.18 | 0.05 | 0.34 | 0.35 | 0.05 | 0.03 | 0.48 |
| 45 | 0.07 | 0.10 | 0.15 | 0.06 | 0.28 | 0.18 | 0.06 | 0.04 | 0.35 |
| 60 | 0.06 | 0.10 | 0.13 | 0.07 | 0.25 | 0.13 | 0.07 | 0.05 | 0.28 |
| 75 | 0.06 | 0.10 | 0.12 | 0.07 | 0.23 | 0.11 | 0.07 | 0.07 | 0.25 |
| 90 | 0.05 | 0.10 | 0.11 | 0.08 | 0.21 | 0.10 | 0.08 | 0.08 | 0.22 |
| 105 | 0.05 | 0.10 | 0.10 | 0.09 | 0.20 | 0.09 | 0.08 | 0.09 | 0.21 |
| 120 | 0.05 | 0.10 | 0.10 | 0.09 | 0.19 | 0.10 | 0.09 | 0.10 | 0.19 |
| 135 | 0.05 | 0.10 | 0.10 | 0.10 | 0.18 | 0.10 | 0.09 | 0.10 | 0.18 |
| 150 | 0.04 | 0.10 | 0.10 | 0.10 | 0.17 | 0.10 | 0.09 | 0.11 | 0.18 |
| 165 | 0.04 | 0.10 | 0.11 | 0.11 | 0.16 | 0.11 | 0.10 | 0.12 | 0.17 |
| 180 | 0.04 | 0.10 | 0.11 | 0.11 | 0.16 | 0.12 | 0.10 | 0.13 | 0.16 |

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Methane release and energy expenditure of dairy cows fed concentrates characterized by different carbohydrates

I.K. Hindrichsen¹, M. Kreuzer¹, A. Machmüller¹, K.E. Bach Knudsen², J. Madsen³ & H.-R. Wettstein¹

¹ Institute of Animal Science, Animal Nutrition, Swiss Federal Institute of Technology (ETH), ETH Centre/LFW, CH-8092 Zurich, Switzerland

² Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences (DIAS), Research Centre Foulum, PO Box 50, DK-8830 Tjele, Denmark

³ Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University (KVL), Grønnegårdsvej 2, DK-1870 Frederiksberg, Denmark

Summary

Six concentrates, differing in type and level of carbohydrates, were tested in an experiment with dairy cows. The roughage to concentrate ratio was 1:1 and the complete diets were isoenergetic and isonitrogenous. Feeding concentrate with lignified fiber decreased methane release per kg dry matter intake, which was accounted by a decreased fiber digestibility compared to the other five diets. Cellulose, sugar and starch had a significant stimulating influence on the daily methane release in decreasing order as listed. Existing regressions to predict methane emission from dietary parameters only to some extent described the actual measured methane emission in the present study.

Keywords: dairy cows, methane, carbohydrates

Introduction

Dietary carbohydrates have a major impact on the emission of methane (an important greenhouse gas) and energy utilization in ruminants. Most studies involving dietary carbohydrates focus on starch and fiber and, at the utmost, differences between fiber polysaccharides - hemicelluloses and cellulose. This approach neglects carbohydrates, which are present in less frequently consumed plant materials or being in low amounts in the diet for ruminants, i.e. pectin and sugars. Literature values, however, suggest that there can be contrasting effects of individual fiber polysaccharides particularly, particularly on the release of methane. Results of a preliminary study (Hindrichsen et al., 2002) performed with a rumen simulation system (RUSITEC) demonstrated that diets characterized by different easily-fermentable carbohydrates differ in their effect on methanogenesis even more than diets characterized by different cell walls carbohydrates, at least when the rumen fluid pH is kept constant. The aim of the present study was to investigate in dairy cows the effect of six diets varying in type and levels of dietary carbohydrates on methane release and energy expenditure.

Materials and methods

A total of twelve Brown Swiss dairy cows were fed six experimental diets consisting of roughage and concentrate in a ratio of 1:1. Roughage was composed of maize silage, grass silage and hay in the proportions 0.22, 0.45, 0.33 of DM. The six concentrates consisted of oat hulls (50%), soybean hulls (70%), apple pulp (54%), Jerusalem artichoke (68%), molasses (18%) or wheat (46%), thus modelling lignified fiber, non-lignified fiber, pectin polysaccharides, fructans, sugars and starch, respectively. All diets were formulated to have the same content of net energy lactation,

absorbable protein at the duodenum and nitrogen and were allocated in amounts which met requirements for maintenance and milk production. To achieve this, oat, crystalline fat, wheat straw meal, grass cubes, soybean meal or urea were completing the concentrate formulations. The chemical composition of the complete diets can be seen in Table 1.

Table 1. Chemical composition of the six experimental diets (g kg⁻¹ dry matter).

| Diets | 'Oat hulls' | 'Soybean hulls' | 'Apple pulp' | 'Jerusalem artichoke' | 'Molasses' | 'Wheat' |
|------------------|-------------|-----------------|--------------|-----------------------|------------|---------|
| Organic matter | 923 | 912 | 917 | 908 | 915 | 914 |
| Nitrogen | 22 | 24 | 26 | 27 | 24 | 23 |
| Ether extract | 54 | 49 | 42 | 22 | 23 | 51 |
| Glucose | 8 | 9 | 16 | 9 | 13 | 10 |
| Fructose | 20 | 22 | 30 | 23 | 25 | 22 |
| Sucrose | 9 | 7 | 8 | 92 | 53 | 13 |
| Total sugars | 37 | 38 | 55 | 110 | 91 | 44 |
| Fructans | 12 | 14 | 17 | 125 | 16 | 15 |
| Starch | 96 | 54 | 151 | 59 | 181 | 204 |
| Total uric acids | 24 | 53 | 37 | 31 | 23 | 23 |
| Crude fiber | 259 | 300 | 236 | 173 | 184 | 199 |
| NDF | 512 | 518 | 400 | 319 | 354 | 405 |
| ADF | 304 | 365 | 291 | 205 | 223 | 238 |
| ADL | 55 | 44 | 73 | 30 | 37 | 42 |
| Hemicelluloses | 209 | 152 | 110 | 114 | 131 | 167 |
| Cellulose | 249 | 322 | 218 | 175 | 186 | 196 |

The diets were fed over three periods were each cow received three different diets, which gave six replicates per experimental diet. After 14 days of adaptation to the respective feed, milk, faeces and urine were collected and sub-samples drawn and mixed over a period of eight days. On the fourth and fifth day additionally gaseous exchange was measured in open-circuit respiration chambers. Feed and faeces were analysed for gross energy, nitrogen and ether extract, and carbohydrates were analysed in detail for their composition. The fiber fraction of feed and faeces were both analysed with the common sequential procedure for ruminant feed in accordance to Van Soest et al. (1991) and with the non-starch polysaccharide method as described by Bach Knudsen (1997). Furthermore, starch, sugars and fructans contents were analysed in feed samples.

Results and discussion

The apple pulp diet had significant higher apparent cellulose digestibility compared to the wheat and oat hulls diet, while the soybean hulls diet had a significantly higher apparent hemicelluloses digestibility compared to the Jerusalem artichoke, oat hulls, and molasses diets (data not shown). The lowest apparent energy digestibility was found with the oat hull diet (56%), while Jerusalem artichoke (71%) had a significantly higher apparent energy digestibility compared to the wheat, soybean hulls, apple pulp and oat hulls diets. Jerusalem artichoke, on the other hand, caused the highest energy loss with urine, but metabolizability of gross energy was still lowest with oat hulls (Figure 1). Energy expenditure, energy retention and milk energy output was only numerically different among diets.

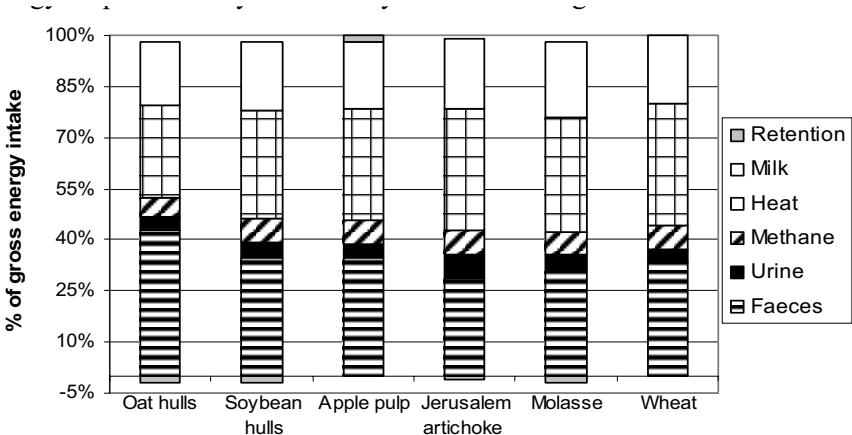


Figure 1. Energy balance of dairy cows fed different types of concentrates.

The use of hardly-fermentable oat hulls decreased ($P<0.05$) methane release per kg DM intake compared to the other five diets. Digestible fiber is commonly considered as the major contributor to methanogenesis (Moss et al., 2000). However, there were clear differences among diets in mmol methane emission per kg NDF digested, decreasing in the order of diets characterized by Jerusalem artichoke, molasses, apple pulp, wheat, oat hulls and soybean hulls, which is the same order as found in a preliminary study with the RUSITEC-system (correlation between *in vivo* and *in vitro*; $r = 0.99$), where the same diets were investigated (Hindrichsen et al., 2002).

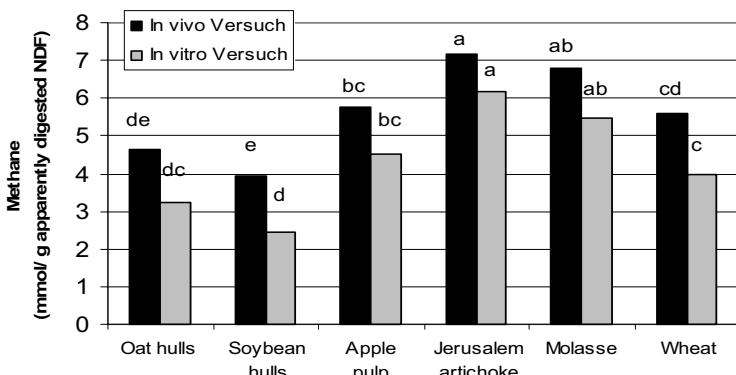


Figure 2. In vivo and in vitro methane emission using diets characterised by different types of carbohydrates.

The correlation between the measured values of methane emission from the present study (x-values) and the predicted value using often cited equations (y-values) is shown in Table 2. The best fitting equation is based only on DM intake of the cows. The second best fitting equation includes intakes of digestible fibre and digestible N-free extract. The equation using feed composition had all a lower coefficient of determination.

The data from the present study gave the following regression equation:
 $\text{CH}_4 \text{ (g/day)} = 94.5 + 59.5 \times \text{cellulose (kg/d)} - 17.8 \times \text{hemicelluloses (kg/d)} - 29.6 \times \text{ADL (kg/d)} + 40.5 \times \text{starch (kg/d)} + 54.2 \times \text{total sugar (kg/d)}$, ($R^2 = 0.82$, $P < 0.001$)

but it was only cellulose, total sugars and starch that had a significant influence ($P<0.05$) on methane emission.

Table 2. Gradient (α), intercept (β) and coefficient of determination (R^2) between measured values of methane production from the present study and predicted values using equations from literature.

| Equation | R^2 |
|---|-------|
| ${}^1)\text{CH}_4 \text{ (g/day)} = 10.9 + 0.121 \times \text{roughage (\%)} - 0.648 \times \text{DM intake (kg/d)} + 0.109 \times W^{0.75} (\text{kg})$ | 0.20 |
| ${}^1)\text{CH}_4 \text{ (g/day)} = 81 + 14.0 \times \text{DM intake (kg/d)}$ | 0.71 |
| ${}^1)\text{CH}_4 \text{ (g/day)} = 62.5 + 77.2 \times \text{crude fibre (kg/d)} + 10.8 \times \text{N-free extract (kg/d)} + 18.5 \times \text{CP (kg/d)} - 194.6 \times \text{EE (kg/d)}$ | 0.43 |
| ${}^2)\text{CH}_4 \text{ (g/day)} = 61.6 + 47.9 \times \text{cellulose (kg/d)} + 31.4 \times \text{hemicelluloses (kg/d)} + 9.2 \times \text{NfE (kg/d)}$ | 0.45 |
| ${}^2)\text{CH}_4 \text{ (g/day)} = 33.2 + 105.5 \times \text{dig. cellulose (kg/d)} + 38.8 \times \text{dig. hemicelluloses (kg/d)} + 20.7 \times \text{dig. NfE (kg/d)}$ | 0.55 |

Ref. ${}^1)$ Kirchgessner et al. 1991; ${}^2)$ Moe and Tyrell, 1979;

Conclusion

The results support previous *in vitro* results and suggest that diets characterized by easily-degradable carbohydrates such as sugars and starch do not necessarily result in a lower methane release in dairy cows compared with diets containing more fibrous feeds ingredients. This is probably due to lignifications of fiber and a positive effect of sugar on methane emission in the ruminant.

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Effect of specific structured triglycerides on energy metabolism in broiler chickens

H. Jørgensen, C.-T. Zheng, P.K. Theil & K. Jakobsen

Danish Institute of Agricultural Sciences, Research Centre Foulum, Department of Animal Nutrition and Physiology, P.O. Box 50, DK-8830 Tjele, Denmark

Summary

Structured triglycerides (STG) are a chemical mixture of fatty acids incorporated in the same glycerol moiety by hydrolysis and esterifications. STG have been found to have a positive effect on body weight gain, nitrogen balance in rats, and in human studies to show an increase in body fat oxidation. A study was designed to investigate the effects on nitrogen and energy metabolism of dietary inclusion of specific STG containing medium chain fatty acids in 1- and 3-positions and a long chain fatty acid in 2-position, which was prepared from rapeseed oil and capric acid (C 10:0) in broiler chickens. A total of 40 female broiler chickens (Ross 208) were kept at 37 °C in the first day and then temperature was decreased by 1 °C each day until 25 °C, after which the room temperature was kept constant. Relative humidity was 60-65 %. Light was on for 24 h during the first 12 days and 20 h (0300 to 2300) thereafter. The animals were at random allocated into five dietary treatments: A basal diet in meal form based on wheat, pea and soybean meal with graded level of STG of 0, 2, 4, 6 and 8% at the expense of rapeseed oil. At 12 day of age the chickens were placed pair-wise in metabolism cages. The grower period was divided into four consecutive balance periods of six days. Two 24 h measurements of gas exchange in two open-air circuit respiration chambers were performed during the second and third day of each balance period. The chickens were slaughtered at 36 d of age. Weight of the total empty digestive tract was relatively smaller when the chickens were fed STG compared with rapeseed oil. Furthermore feed efficiency, metabolizability and the relative protein retention were slightly improved when feeding STG. Also energy from oxidized fat increased with increasing level of STG in diets.

Keywords: interesterified fats, rapeseed oil, decanoic acid, indirect calorimetry

Introduction

Fats and oils are important dietary ingredients in poultry production owing to their high energy value. However, large amounts of dietary fats and oils may result in excessive fat deposition in the carcass. Medium chain triglyceride (MCT), which was composed of triglycerides with saturated fatty acids moieties of six to twelve carbon atoms, was reported to decrease fat deposition in rats, pigs and broiler chickens (Mabayo et al., 1993). Furthermore, they also found supplementing MCT to chicken diets improved weight gain and protein utilisation compared to a maize oil (a long chain triglyceride, LCT) supplemented diet when chickens were fed the same amount of feed. Lipids produced via interesterification of vegetable oils with MCT, with a regiospecific lipase, contain MCT in the sn-1/3 positions and LCT in the sn-2 position (specific triacylglycerol). Recently there has been great interest in structured triglycerides (Jensen and Jensen, 1992; Straarup and Høy, 2000). Most of structured triglycerides (STG) studied are the ones containing both medium chain fatty acids (primarily caprylic and capric acids) and long chain fatty acids. Preliminary studies suggested that such structured triglycerides might offer several advantages including superior nitrogen retention, preservation of reticuloendothelial system function and attenuation of protein catabolism, which may relate to their distinct absorptive properties. The intake of STG may result in higher fatty acid absorption. One reason for the higher absorption is

the rapid hydrolysis of the specific lipids, which is comparable to that of MCT. Another reason is that lingual and gastric lipases preferentially hydrolyse fatty acids in the sn-3 position with high activity towards MCT.

Dietary specific structured triglycerides (STG) have not been evaluated as a novel lipid source in poultry. Therefore, the present study was designed to investigate the effects of dietary inclusion of STG containing medium chain fatty acids in 1- and 3-positions and a long chain fatty acid in 2-position, which was prepared from rapeseed oil and capric acid (C 10:0) (Table 1), on growth performance and nitrogen and energy metabolism in broiler chickens.

Materials and methods

Forty female broiler chickens (Ross 208) were obtained from a commercial hatchery and allocated at random into five dietary treatments (see Table 2) in each of two series. They received a basal diet based on wheat, peas and soybean meal with 8% fat either as rapeseed oil or graded level of specific structured oil. In the starter period (from day 0 to day 12), the chickens were kept in two cages (replications) with four chickens each in each dietary treatment. In the subsequent grower period from day 13 to 36, the eight chickens in each dietary treatment were allocated into three metabolism cages, of which two cages held two chickens each, which were used for balance and respiration trials, and one cage held four chickens as extra ones for substitution if necessary. The grower period was divided into four consecutive balance periods of six days. The chickens had free access to the diets, which were given in mash, and water throughout the experiments. Light was on for 24 h during the starter period and 20 h (0300 to 2300) during the grower period. The room temperature was kept at 37 °C in the first day and then decreased by 1 °C each day until 25 °C, after which the room temperature was kept constant. Relative humidity was 60–65%. Feed intake and weight gain were recorded in each period. Droppings were collected daily from each cage and stored at -18 °C for analysis. Two consecutive 24 h measurements of gas exchange were carried out to estimate heat production in two open-air circuit respiration chambers (1.0 m × 1.0 m × 1.0 m each) in each group in the middle of each balance period. The concentrations of O₂, CO₂ and H₂, temperature, relative humidity, and rate of flow from each chamber were recorded automatically on-line every second minute, so that the composition of the gas from each chamber was measured fifteen times per hour. Chickens were slaughtered at day 36.

The measurements from the balances were calculated on cages containing two birds. Total heat production (HE) was calculated according to Brouwer (1965), while retention and oxidation of nutrients were calculated according to Chwalibog and Thorbek (1999). At slaughter the length of the small intestine, caecum and colon was measured. The gastrointestinal tract (GI) was removed and weighed, and the weight of the digesta-free empty body (EBW) and GI tract of each chick was recorded.

Results and discussion

The late professor Carl-Erik Høy at the Department of Biochemistry and Nutrition, The Technical University of Denmark delivered the rapeseed oil and specific structured oil used in the present experiment. The lipase-catalysed interesterification resulted in regiospecific triacylglycerols with C10:0 located in the sn-1/3 positions mainly at the expense of C18:1(n-9) (Table 1 and 2).

During the whole experiment, there was a cubic effect of the inclusion level of specific structured triglyceride on both average daily gain and average daily feed intake (Table 3). The feed conversion efficiency improved linearly with the inclusion level of specific structured triglyceride. However, when daily gain was adjusted to the mean daily feed intake there was a linear effect of the inclusion level of STG, which indicates that the weight gain was affected by both feed intake and the growth-promoting effect of specific structured triglycerides. That voluntarily feed intake decreased could indicate problems with palatability with higher inclusion level of STG above 2%.

Table 1. Major fatty acids in triacylglycerol and sn2- monoacylglycerol (mol/100 mol).

| | Triacylglycerols | | 2-monoacylglycerols | |
|-------------|------------------|--------------|---------------------|--------------|
| | Rapeseed oil | Specific oil | Rapeseed oil | Specific oil |
| C10:0 | ND ¹ | 28.1 | ND | 0.4 |
| C18:1 (n-9) | 56.4 | 38.4 | 49.8 | 47.7 |
| C18:2 (n-6) | 20.9 | 18.2 | 33.9 | 34.0 |
| C18:3 (n-3) | 8.5 | 8.1 | 13.4 | 14.9 |

¹ ND, not detectable.

Table 2. Dietary fat sources and chemical composition of the experimental diets.

| Diet | 1 | 2 | 3 | 4 | 5 |
|-------------------------------------|------|------|------|------|------|
| Specific structured triglyceride, % | 0 | 2 | 4 | 6 | 8 |
| Rapeseed oil, % | 8 | 6 | 4 | 2 | 0 |
| Chemical composition | | | | | |
| Protein (N x 6.25), g/kg DM | 219 | 228 | 225 | 226 | 225 |
| HCl-fat, g/kg DM | 113 | 109 | 106 | 105 | 102 |
| C10:0, mol/100 mol | 0.2 | 7.7 | 15.1 | 22.5 | 30.2 |
| C18:1 (n-9), mol/100 mol | 52.0 | 49.3 | 43.2 | 38.6 | 33.8 |

Table 3. Effect of specific structured triglycerides on performance, utilisation of dietary protein and metabolisable energy (ME) together with the effect on the gastrointestinal tract (GI) in broiler chickens.

| Diet | 1 | 2 | 3 | 4 | 5 | P-value |
|---------------------------------------|------|------|------|------|------|---------|
| Initial bodyweight, g | 37 | 37 | 38 | 38 | 37 | 0.51 |
| Final bodyweight, g | 1615 | 1694 | 1598 | 1446 | 1569 | 0.0001 |
| Daily gain, g/d | 43.1 | 45.5 | 42.2 | 38.0 | 42.1 | 0.0001 |
| Feed intake, g/d | 70.6 | 72.0 | 68.5 | 59.9 | 64.7 | 0.0001 |
| Feed/Gain, g/g | 1.64 | 1.58 | 1.62 | 1.57 | 1.54 | 0.0006 |
| Metabolisability (ME/GE), % | 75 | 76 | 77 | 76 | 77 | 0.36 |
| Retained protein, % intake | 64 | 63 | 64 | 65 | 65 | 0.13 |
| Heat Energy (HE), % ME | 56 | 53 | 56 | 58 | 57 | 0.47 |
| Retained energy (RE), % ME | 44 | 47 | 45 | 42 | 44 | 0.47 |
| RE-fat, % RE | 47 | 49 | 48 | 40 | 45 | 0.34 |
| RE-protein, % RE | 53 | 51 | 52 | 60 | 55 | 0.34 |
| RQ | 0.94 | 0.92 | 0.92 | 0.91 | 0.91 | 0.015 |
| Total GI-tract, g/kg EBW ¹ | 63.3 | 57.9 | 60.0 | 62.0 | 58.3 | 0.004 |

¹EBW, empty body weight.

In spite of this feed to gain ratio improved significantly showing positive absorption and/or metabolic properties that enhance the utilisation of nutrients.

The utilisation of dietary protein relative to intake showed a positive tendency although not significant. This together with a decreased utilisation of the supplied metabolisable energy (RE/ME) indicates a shift in the deposited amount of energy between protein (RE-protein) and fat (RE-fat). Thus showing what was also to be expected that relatively more of the included dietary fat be oxidised when more structured lipids were added. Furthermore, a linear decrease in RQ ($P=0.015$) also indicates increased oxidation with increasing inclusion of STG was found.

In accordance with more supplied ME being oxidised Figure 1 show that an increasing proportion of heat energy (HE) is from fat when the amount of specific structured triglyceride increased, although the total amount of fat in the diet was kept constant. Previous experiments with broiler chickens fed increasing amounts of rapeseed oil showed increasing oxidation of fat (Zhao et al. 2001).

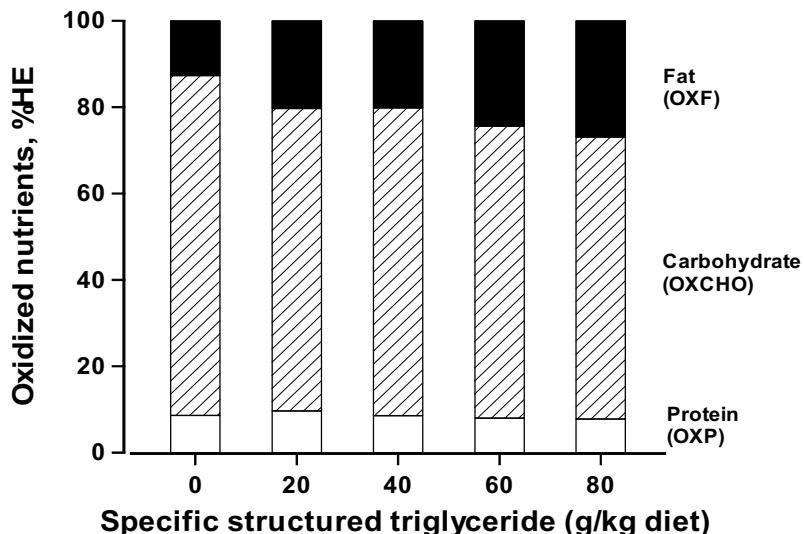


Figure 1. Contribution of oxidised protein, carbohydrate and fat to total heat production (HE).

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Effect of fat source and rapeseed lecithin on energy metabolism in pigs weaned at 4 weeks of age

H. Jørgensen, V. Danielsen & J.A. Fernández

Danish Institute of Agricultural Sciences, Research Centre Foulum, Department of Animal Nutrition and Physiology, P.O. Box 50, DK-8830 Tjele, Denmark

Summary

Two experiments were conducted to study the effects of 3 levels of additional fat (0, 5% rapeseed oil, 5% animal fat) and 3 levels of lecithin (0, 2 and 4%). In the first experiment, the production performance of ad-lib fed piglets (20 litters of 9 pigs each) was measured from weaning at 4 weeks to 8 weeks of age. In the second experiment, the metabolism of the above 9 diets was quantified by combined balance-slaughter studies with restrictively fed (60 % of ad-lib) weaned piglets (6 litters of 9 pigs). One extra pig from 5 of the 6 litters was sacrificed at the beginning of the experiment to provide initial values of body composition. The remaining 54 piglets were slaughtered, dissected and analysed at the end of the experiment 4 weeks later.

No interaction between fat level or sources and lecithin level in any of the experiments was detected. Fat sources, fat level or lecithin did not affect growth rate or feed conversion of restrictively fed pigs. Opposite, ad-lib fed pigs showed tendency to less intake of feed volume when fat was included and negative feed intake response to lecithin inclusion. Feed conversion of ad-lib fed pigs was improved both by fat and lecithin.

ME intake of pigs (MJ/d) in the balance trial was increased by fat level ($p>0.001$) and lecithin ($p>0.17$). Heat energy (MJ/d) tended to increase with fat level and with lecithin ($p>0.09$). Energy retention was increased both absolute (1.2 vrs. 1.5 MJ/d) and relative (24 vrs 27 % of ME) by fat inclusion. Similarly, protein and fat retention in piglet's body was also increased by fat addition. However, protein retention increased at a higher ratio than fat retention, which would lead to a better feed conversion as evidenced by the restricted fed pigs (NS) and specially by the ad-lib fed pigs ($p>0.002$). The partial efficiency of ME for energy retention was 0.65 and 0.62 for rapeseed oil and animal fat, respectively.

Keywords: piglets, rapeseed oil, animal fat, comparative slaughter technique

Introduction

Fats and oils are important dietary ingredients in animal production owing to their high energy value (Jørgensen et al. 2000ab). At weaning the activity of several digestive enzymes falls (Jensen et al. 1997) and a decline of the digestibility of nutrients is observed. Furthermore the gastrointestinal system has to adapt to considerable changes in the physiochemical properties of their feeds as well as to a change in the pattern of intake. Rapeseed-lecithin may act as an emulsifier and thus contribute to improved digestion of fats in weaned pigs. Fat of animal origin is regarded as less digestible than oil of vegetable origin especially due to a different fatty acid profile towards more unsaturated fatty acids in vegetable oil. Thus the effect of lecithin might be greater when used with an animal fat source. The objective of the study was to develop a diet for weaned piglets with high energy density and digestibility and utilization of nutrients by adding different dietary fat sources and lecithin.

Materials and methods

The experiment was arranged in a 3 x 3 factorial structure with three levels of additional fat (0, 5% rapeseed oil and 5% animal fat) and three level of lecithin (0, 2 and 4%) (Table 1). The rapeseed-lecithin oil source used contained about 55-60% phosphorlipids. The diets were used for digestibilty and balance experiments as well as in production experiments with early-weaned piglets. In the digestibility and balance experiments, 6 litters of 9 pigs were used. One pig from each of the five litter were killed and bleed at the beginning of the experiment to provide information about initial chemical composition. The pigs were housed individually in metabolic crates designed for separate collection of faeces and urine. In the first week the pigs were gradually adapted to the experimental diets while increasing feed intake from 100 to 250g/d. This was followed by three successive 7-d experimental periods with quantitative collection of faeces and urine from each pig during the last 5-d of each period. After the four weeks on the experimental diets piglets were killed three hours after feeding. The digestive tract was emptied. The pigs were frozen for subsequent mincing. After mincing representative samples of the carcass were autoclaved for 4 h at 121 °C and homogenized for subsequent analysis.

The production experiment comprised 20 blocks of 9 pigs, a total of 180 pigs (8.5 kg initial weight). Animals within the same block were littermates and distributed with one pig on each diet. Pigs were housed individually and feed and water was available *ad libitum*. The effect of lecithin and fat source on the feed intake, growth, feed utilisation and state of health was recorded weekly for four weeks.

Results and discussion

The content of metabolizable energy (MJ/kg DM) increased by 7.3 and 8.4 % when adding 5 % rapeseed oil or 5 % animal fat, respectively (Table 1). This illustrates the potential of increasing the energy density of diets by oils or fat addition. Addition of rapeseed-lecithin did not have any effect on digestibility or metabolizability on any of the two fat sources. This is in agreement with a study on weanling pigs by Øverland and Sundstøl (1995) in which they did not find any effect of adding lecithin to renderers fat.

There was a tendency for a higher daily gain for pigs fed on diets with added oil/fat, which together with a slight reduced feed intake resulted in a significant improved feed conversion. This was observed both under *ad libitum* feeding (feeding trial) and restricted feeding (balance-slaughter experiment). The enhancement of feed utilisation revealed significance when expressed as kg feed/kg gain. However, there was no significant difference when expressed as ME/kg gain. This show the importance of feed evaluation, when supplying equal amount of energy (here metabolizable energy) the utilisation is also expected to be of same magnitude. Addition of lecithin did not improve daily gain in either of the two experiments. On the contrary, a significant reduction in voluntary feed intake was evidenced in the growth trial, but as the reduction did not seem to affect daily gain, feed conversion was improved significantly both when expressed as kg feed/kg gain and as ME/kg gain.

Pigs were fed equal amount of ME in the balance-slaughter experiment (Table 3). Although there was no overall significant effect on feed utilisation, there was a tendency towards a negative effect on energy retention with increasing dietary addition of lecithin. This was more evidently expressed by an increase in heat energy with lecithin addition ($P=0.092$). The more ME is dissipated, as heat the less energy is available for growth.

The partial efficiency of ME for energy retention calculated for the two fat sources [(RE, MJ/kg DM)/(ME/ MJ/kg DM)] was found to be 0.65 for rapeseed oil and 0,62 for animal fat. This is in agreement with the hypothesis that energy in vegetable oil/fat is used more efficient than energy from animal fat. Similar results have been found by Just (1982) when feeding increasing amount of animal fat (4-24 % dietary fat) or by Jørgensen et al. (1996) when feeding graded level of

rapeseed oil. The two latter experiments were carried out on growing-finishing pigs and showed efficiencies for energy retention that were in the same range as those found in the present experiment, indicating that the efficiency for growth is similar for the two classes of pigs.

Table 1. Experimental design, main dietary ingredients and chemical composition of the experimental diets.

| Fat Source | 0 | Rapeseed oil | Animal fat | | | | | | |
|------------------------------------|-------|--------------|------------|-------|-------|-------|-------|-------|-------|
| Lecithin | 0 | 20 | 40 | 0 | 20 | 400 | 0 | 20 | 40 |
| Ingredients, g/kg | | | | | | | | | |
| Fat source | - | - | - | 50 | 50 | 50 | 50 | 50 | 50 |
| Wheat + rape-lecithin ¹ | - | 314 | 627 | - | 314 | 627 | - | 314 | 627 |
| Wheat + rape-oil ² | 590 | 290 | - | 590 | 290 | - | 590 | 290 | - |
| Chemical composition, g/kg DM | | | | | | | | | |
| Protein (N x 6.25) | 239 | 242 | 247 | 255 | 261 | 268 | 253 | 260 | 266 |
| HCl-fat | 80 | 89 | 98 | 133 | 137 | 147 | 133 | 144 | 148 |
| Energy concentration ³ | | | | | | | | | |
| ME, MJ/kg DM | 15.69 | 15.68 | 15.77 | 16.89 | 16.67 | 17.03 | 16.81 | 17.05 | 17.23 |

¹ Heat treated rolled wheat mixed with rapeseed lecithin/oil mixture (55-60% phospholipids) in the ratio 88.4 : 11.6.

² Heat treated rolled wheat mixed with rapeseed oil in the ratio 94.5 : 5.5.

³ ME estimated in a balance and digestibility experiment.

Table 2. Effect of fat source and/or lecithin on pig growth performance.

| | Fat source | | | P-value | Lecithin | | | P-value |
|-------------------------------------|------------|--------|-------|---------|----------|-------|-------|---------|
| | 0 | Rapse. | Anim. | | 0 | 20 | 40 | |
| Growth trial | | | | | | | | |
| No of pigs | 60 | 60 | 60 | - | 60 | 60 | 60 | - |
| Daily gain, g/d | 397 | 403 | 417 | 0.24 | 405 | 414 | 399 | 0.56 |
| Feed intake, g/d | 611 | 587 | 585 | 0.40 | 628 | 604 | 550 | 0.002 |
| Feed/Gain, g/g | 1.54 | 1.46 | 1.40 | 0.002 | 1.55 | 1.46 | 1.38 | 0.002 |
| ME, MJ/kg gain | 22.55 | 23.03 | 22.12 | 0.34 | 23.75 | 22.38 | 21.51 | 0.01 |
| Balance-slaughter experiment | | | | | | | | |
| No of pigs | 18 | 18 | 18 | - | 18 | 18 | 18 | - |
| Daily gain, g/d | 273 | 296 | 297 | 0.57 | 285 | 288 | 292 | 0.57 |
| Feed intake, g/d | 356 | 354 | 355 | 0.22 | 356 | 355 | 353 | 0.77 |
| Feed/Gain, g/g | 1.31 | 1.21 | 1.20 | 0.60 | 1.25 | 1.24 | 1.22 | 0.47 |
| ME, MJ/kg/gain | 18.70 | 18.62 | 18.72 | 0.91 | 18.75 | 18.60 | 18.70 | 0.37 |

The retention of protein and fat estimated by the comparative slaughter method was increased by dietary fat inclusion. The chemical composition of pigs at 8 weeks was substantially different from the chemical composition of initial pigs at 4 weeks. The ratio of protein to fat was 1.1 at 4 weeks but was increased to 1.8 4 weeks later. Even though the pigs in the balance-slaughter experiment were fed restrictively, these results demonstrate that pigs immediately post weaning

use body reserves (fat) while protein accretion has high priority. It is therefore important that pigs are weaned with relative large body reserves in the form of fat depots.

Table 3. Dry matter and energy intake and utilisation together with retained protein and fat in the balance-slaughter experiment.

| | Fat source | | | P- value | Lecithin | | | P- value |
|----------------------------|------------|--------|-------|-------------|----------|------|------|-------------|
| | 0 | Rapes. | Anim. | | 0 | 20 | 40 | |
| DM intake, g/d | 323 | 322 | 323 | 0.66 | 323 | 323 | 323 | 0.95 |
| ME intake, MJ/d | 5.09 | 5.47 | 5.53 | 0.0001 | 5.34 | 5.34 | 5.41 | 0.17 |
| ME % GE | 80 | 82 | 82 | 0.001 | 82 | 81 | 81 | 0.85 |
| Retained energy (RE), MJ/d | 1.20 | 1.45 | 1.47 | 0.0004 | 1.41 | 1.38 | 1.32 | 0.40 |
| RE % ME | 24 | 27 | 27 | 0.030 | 27 | 26 | 24 | 0.23 |
| Heat energy, MJ/d | 3.89 | 3.99 | 4.06 | 0.092 | 3.92 | 3.95 | 4.08 | 0.092 |
| Retained protein, g/d | 41 | 45 | 44 | 0.019 | 44 | 44 | 43 | 0.60 |
| Retained fat, g/d | 5 | 9 | 11 | 0.003 | 9 | 8 | 8 | 0.79 |

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Low protein diets reduce greenhouse gas production by sows

*S. Möhn, J.K.A. Atakora, D.J. McMillan & R.O. Ball**

Swine Research & Technology Centre, 4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5

Summary

Reducing the dietary protein intake by 20% will reduce the N excretion by sows by 20 to 30%. Additionally, the CO₂ production may be reduced by up to 5%. The effects of diet ingredients and dietary protein level on CH₄ emissions appear to be linked to the dietary content of non-starch polysaccharides. Methane emissions were greater for pigs fed barley- than corn-based diets, but could be reduced for barley-based diets if the dietary protein content was lowered. In conclusion, significant reductions can be achieved for the N₂O, CH₄ and CO₂ emissions by reducing dietary protein contents.

Keywords: sow, protein, greenhouse gas, heat production

Introduction

Low protein diets with added synthetic amino acids reduce the nitrogen (N)-excretion of pigs, thereby reducing the emissions of N₂O from manure. Reducing dietary protein contents leads to an increase in the content of carbohydrates, which contain less carbon and are used more efficiently than protein in intermediary energy metabolism. Reducing carbon intake or improving carbon utilization can be expected to reduce the production of CO₂, although it is unclear which mechanism is the predominant cause. Associated with the change of protein content is a change in fiber content and composition in the diets, which may influence hindgut fermentation and thus CH₄ production by sows. The objectives of this experiment were to assess the effect of low protein diets on N-excretion, CO₂ and CH₄ emissions of empty, gestating and lactating sows.

Materials and methods

In experiment 1, the effect of dietary protein reduction on gas exchange was determined in non-pregnant sows in a Latin square experiment. Four sows were adapted to each experimental diet for at least one week, before indirect calorimetry was performed twice per diet to measure CO₂- and CH₄ production and O₂ consumption during a 4-h period. In experiment 2, twenty-two sows were fed conventional or AA supplemented low protein diets during gestation and lactation of the second and third parities. CO₂ production and O₂ consumption were measured over 4 h during early and late pregnancy and lactation. Nutrient digestibility was estimated using Cr₂O₃ as a marker; urinary N excretion was estimated using the urinary N:creatinine ratio.

Equipment

Respiration boxes approximately 2 m³ in volume were built around commercial farrowing crates, fitted with a feeder and drinker; in addition, a removable box for suckling piglets was also designed. Air was drawn through these boxes at rates of 240 to 350 L min⁻¹. After passing through a cold water condenser, air flow was measured with commercial air meters. Part of the air was drawn off and delivered to the gas analyzers. The digitalized data were acquired at maximum rate

(4 readings per second) and the average gas concentration for each minute was recorded throughout the experiment.

Diets and feeding

In experiment 1, conventional diets based on barley-canola meal (B-HP) or corn-soybean meal (C-HP), and protein-reduced, AA supplemented diets (B-LP and C-LP) were formulated. The nutrient contents (Table 1) met or exceeded the recommendations of NRC (1998). Sows were fed twice daily to achieve equal energy intake of 10% in excess the maintenance energy requirement (NRC, 1998). In experiment 2, isoenergetic conventional (HP) or protein reduced (LP), amino acid supplemented diets were formulated to fulfill the requirements according to NRC (1998). The allowance for the single daily meal in gestation was based on body weight and back fat. During lactation, the sows were offered ad libitum access to feed. During the respiration measurements, the sows were fed at hourly intervals.

Calculation of results and statistical analysis

The CO₂ equivalent was calculated based on the potency as a greenhouse gas, the factors being, per Mol, 1 for CO₂, 21 for CH₄ and 310 for N₂O. Treatment effects were estimated using the general linear model procedure (SAS, 1999).

Results

The mean values for empty sows were 1.22 L min⁻¹ (SE 0.02) CO₂, 1.16 L min⁻¹ (SE 0.03) O₂, 0.014 L min⁻¹ (SE 0.001) CH₄ and heat production (Brouwer formula, McLean and Tobin, 1987) at 24.6 kJ min⁻¹ (SE 0.5). Base ingredients did not influence CO₂-, heat production and O₂ consumption ($P > 0.6$). The CO₂ production increased with carbon intake ($P = 0.02$) and was numerically greater for the LP diets. CH₄ production was lower for the corn- versus the barley-based diets, and lower by 60% for B-LP vs. B-HP ($P = 0.001$), but similar for both corn diets ($P > 0.1$). The CH₄ production increased with NDF intake ($P = 0.001$). CH₄ and CO₂ production were not correlated ($P = 0.57$).

Sows in gestation produced on average 1.10 L min⁻¹ CO₂ (SE 0.01), consumed 1.37 L min⁻¹ O₂ (SE 0.02) and produced 27.1 kJ min⁻¹ (SE 0.3) of heat. CO₂-, heat production and O₂ consumption during pregnancy were reduced ($P < 0.02$) in LP by 4.8%, 5.9% and 5.7%, respectively. During lactation, sows including piglets produced on average 2.39 L min⁻¹ CO₂ (SE 0.03), consumed 2.69 L min⁻¹ O₂ (SE 0.03) and produced 55.5 kJ min⁻¹ heat (SE 0.7). CO₂ production was lower by 6.6% ($P = 0.005$) for LP, while O₂ consumption ($P = 0.061$) and heat production ($P = 0.054$) tended to be lower by 9.4% and 8.8%, respectively. In gestation and lactation, the reduction in CO₂ production was mainly due to the lower carbon content and carbon digestibility of the LP diet, while the utilization of digested carbon in the two diets was similar ($P > 0.10$). The fecal excretion of C and N was similar ($P > 0.10$) for LP and HP. Overall, the urinary N excretion in LP was reduced ($P = 0.001$) by 25.7%.

The CO₂ equivalent as the sum of CO₂ and CH₄ production was lower ($P = 0.002$) for the corn diets in experiment 1, and not affected ($P = 0.22$) by protein level. In experiment 2, the greenhouse gas production as CO₂ was reduced for LP. Additionally, reduced N excretion will lead to a lower greenhouse gas production, as up to 30% of the excreted N may be converted to N₂O (Béline et al., 1999). This conversion rate would mean that in experiment 1, given quantitative excretion of N taken in at maintenance, the production of CO₂ equivalent was reduced by 16 - 17% when feeding LP diets. Given an average reduction in urinary N excretion of 25.7% when feeding LP diets to gestating and lactating sows, at least the same degree of reduction in CO₂ equivalent can be expected.

Table 1. Nutrient contents of the experimental diets and daily feed intake.

| | Maintenance diets | | | | Gestation diets | | Lactation diets | |
|---|-------------------|------|------------|------|-----------------|------|-----------------|------|
| | Barley-based | | Corn-based | | HP | LP | HP | LP |
| | HP | LP | HP | LP | | | | |
| Energy ¹ , MJ kg ⁻¹ | 11.9 | 11.9 | 13.6 | 13.6 | 14.7 | 13.8 | 15.2 | 14.6 |
| CP, % | 14.6 | 11.8 | 13.2 | 9.7 | 16.2 | 13.5 | 21.1 | 18.2 |
| Lysine, % | 0.56 | 0.55 | 0.57 | 0.51 | 0.66 | 0.68 | 0.93 | 0.92 |
| Threonine, % | 0.52 | 0.48 | 0.48 | 0.44 | 0.56 | 0.52 | 0.70 | 0.65 |
| NDF, % | 25.7 | 22.4 | 19.6 | 15.5 | 16.0 | 15.5 | 13.4 | 16.3 |
| Feed intake, kg d ⁻¹ | 2.48 | 2.53 | 2.18 | 2.20 | 2.38 | 2.34 | 6.25 | 5.64 |

¹ Maintenance diets: calculated metabolizable energy. Gestation and lactation diets: determined digestible energy.

Conclusions and implications

Feeding LP diets will reduce CO₂ production by gestating and lactating sows due to the lower C content in such diets. Reducing dietary protein, associated with lower NDF contents, reduced CH₄ production in empty sows. However, the main effect of reducing the dietary protein content is the reduction of N excretion, which has the potential to reduce greenhouse gas (N₂O) production from manure.

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The effect of different carbohydrates on the efficiency of energy utilisation in broiler chickens

V.R. Pirgozliev^{1,2}, S.P. Rose¹, A. Reppas¹ & V. Batson¹

¹ The National Institute of Poultry Husbandry, Harper Adams University College, Newport, Shropshire, UK TF10 8NB

² Queen's University, AESD, Newforge Lane, Belfast, BT9 5PX

Summary

Two experiments were conducted to quantify the efficiency of utilisation of TME for carcass energy retention in broiler chickens when different proportions of wheat and barley were fed and to examine whether changing the digesta viscosity of broiler chickens (by the addition of guar gum) affected their efficiency of energy retention. In experiment 1, five dietary treatments were prepared by blending the wheat and barley in the following ratios respectively, 4:0, 3:1, 2:2, 1:3 and 0:4. TME was determined in adult cockerels and the efficiency of energy retention was determined in broiler chickens from 7 to 21d of age. Experiment 2 compared the efficiency of energy retention between a sample of maize and the maize sample with 10g/kg added guar gum. Increasing levels of wheat, in substitution for barley, tended to increase ($P>0.05$) the efficiency of utilisation of TME for carcass energy retention. The broiler chickens fed the maize-based guar gum treatment had a lower weight gain ($P<0.001$) and their retained energy per kg of added maize was markedly reduced ($P<0.001$). The experiments have demonstrated the large effect that digesta viscosity can have on the efficiency of energy utilisation in broiler chickens.

Keywords: chicken, energy efficiency, cereals

Introduction

Cereals are composed mostly of starch that is the major energy-providing nutrient in practical poultry feeds. Cereals are the major components of practical, nutritionally-complete feeds. There is a need to accurately determine the utilisable energy content of cereals, or cereal-replacers, in order to make economic decisions about their inclusion rates in proprietary poultry feeds. Although metabolisable energy (ME) is conventionally used to determine available energy concentrations of feedstuffs for poultry, there are a number of factors that affect the efficiency of utilisation of ME. Prediction equations and computer simulations can be used to estimate the net energy of complete feeds (MacLeod, 2002) but there is evidence that there are differences between individual feedstuffs that cannot be entirely explained by these methods (Pirgozliev *et al.*, 2001). There is a need to understand the factors that cause variations in the efficiency of utilisation of available energy so that the most cost efficient diet formulations can be decided upon by the commercial poultry industry. In northern Europe, wheat and barley are primarily used as cereals for poultry feeds. Both cereals contain non-starch polysaccharides that may cause high digesta viscosity in broilers, although the high β -glucan content of barley tends to produce the greater number of high digesta viscosity problems (Svihus & Gullord, 2002).

Two experiments were completed. The objectives of the first experiment were to examine if there were consistent differences in the efficiency of utilisation of TME for carcass energy retention in broiler chickens when different proportions of wheat and barley were used in a nutritionally-complete diet. The second experiment examined whether changing the digesta viscosity of broiler chickens (by the addition of guar gum) affected the energy retention of broiler chickens.

Material and methods

Experiment 1

A sample of wheat and a sample of barley were obtained. Five dietary treatments were prepared by blending the wheat and barley in the following ratios, 4:0, 3:1, 2:2, 1:3 and 0:4. The TME of each of the five blended samples was determined in adult cockerels (four replicate birds per dietary treatment) using a rapid ME determination assay in which the previously un-fed cockerels were precision-fed 50g of the sample and their droppings collected for the following 48 h. The protocol was as described by McNab & Blair (1988).

The carcass energy retained per kg of cereal fed was determined by substitution of the cereal blend into a basal diet (40 parts cereal : 50 parts basal) (Table 1) that was fed to cages (floor area of 930 cm² with two birds per cage) of female broiler chickens from 7 to 21 d of age. Each diet was fed to eight replicate cages of birds. The cages were housed within an environmentally controlled room. The daily feed intakes of the birds were restricted (90% of *ad lib*) to equalise nutrient intakes between replicate birds within treatments and to equalise basal feed intakes for the five cereal blend treatments. Treatment differences were compared by randomised block analysis of variance and, where appropriate, quantitative treatment-group variance was partitioned by linear regression.

Table 1. Composition of the basal diet used in experiments 1 and 2.

| Feedstuff | Amount (kg/tonne) |
|---|-------------------|
| Ground wheat | 300.0 |
| Maize gluten meal (Prairie meal) | 33.4 |
| De hulled soybean meal | 83.3 |
| Full fat micronised soya | 433.4 |
| Fish meal | 83.3 |
| Lysine hydrochloride | 3.3 |
| Methionine | 5.0 |
| Dicalcium phosphate | 25.0 |
| Vitamin and trace element premix ¹ | 33.3 |

¹ The proprietary supplement was supplied by Ian Hollows Feed Supplements Ltd., Whitchurch, UK. It provided the following nutrients (mg/kg supplement) : 38.4 retinol acetate, 0.6 cholecalciferol, 2000 α-tocopherol acetate, 240 thiamin, 800 niacin, 1200 pantothenic acid, 240 pyridoxine, 1.2 cyanocobalamin, 20,000 choline chloride, 10 biotin, 120 folic acid, 1600 iron, 80 copper, 8000 manganese, 6400 zinc, 80 iodine and 16 selenium.

Experiment 2

For experiment 2, a sample of maize was obtained and a second dietary treatment was produced by adding 10g/kg of guar gum to the sample of maize. The carcass energy retained per kg of cereal fed was determined by substitution of the maize and maize plus guar gum into a basal feed as described for experiment 1. A fourteen day restricted feeding period was given to pairs of caged female broiler chickens (eight replicate cages per treatment) as described in experiment 1. Treatment differences were compared using a randomized block analysis of variance.

Results

Experiment 1

Increasing levels of substitution of barley with wheat increased ($P<0.01$) the determined TME of the cereal blend. Increasing levels of wheat tended to increase ($P>0.05$) the growth of the broiler chicks and tended to increase ($P>0.05$) the efficiency of utilisation of TME for carcass energy retention (Table 1).

Table 2. The effect of different proportions of wheat and barley on the determined TME and efficiency of its utilisation for carcass energy retention.

| Cereal blend | TME (MJ/kg) | Broiler weight gain (7 - 21 d) (kg/bird) | Efficiency of utilisation of TME for carcass energy retention |
|----------------------|-------------|---|---|
| % wheat % barley | | | |
| 100 0 | 14.041 | 0.438 | 0.527 |
| 75 25 | 13.375 | 0.443 | 0.539 |
| 50 50 | 13.016 | 0.436 | 0.510 |
| 25 75 | 13.080 | 0.422 | 0.488 |
| 0 100 | 13.216 | 0.437 | 0.499 |
| SEM | 0.2138 | 0.0063 | 0.0468 |

Experiment 2

Although the addition of the guar gum did not affect ($P>0.05$) the determined TME of the maize sample (13.59 MJ/kg), the *in vivo* digesta viscosities of the broiler chickens were markedly increased (Table 3). The broiler chickens fed the guar gum treatment had a lower weight gain ($P<0.001$) and their retained energy per kg of added maize was markedly reduced ($P<0.001$).

Table 3. Effect of addition of guar gum to maize on digesta viscosity and carcass energy retention.

| Treatment | Digesta viscosity (\log_{10} cP) | Broiler weight gain (kg/bird) | Retained energy (MJ/kg added maize) |
|------------------|--|----------------------------------|--|
| Maize | 0.637 | 0.342 | 7.08 |
| Maize + guar gum | 2.181 | 0.286 | 2.34 |
| SEM | 0.1266 | 0.0181 | 0.625 |

Discussion

The wheat and barley samples had similar proximate nutrient compositions although the crude protein and fibre content was higher in the barley. The large decrease ($P<0.001$) in determined TME with increasing barley in the mixture indicated, as expected, that the energy availability in barley was less than wheat. There was a tendency ($P>0.05$) for the efficiency of utilisation of TME

to decrease with increasing proportions of barley. Wheat and barley have a similar starch digestion coefficient and, although wheat may be retained in the anterior jejunum for longer, the overall rate of starch digestion is similar (Weurding *et al.*, 2001). Emmans (1994) proposed that the efficiency of utilisation of metabolizable energy is reduced with increasing dietary fibre but the relatively small difference in crude fibre between the diets was not large enough to explain the differences. Barley has a high B-glucan content that increases digesta viscosity in comparison to wheat-based diets and so it is possible that high viscosity conditions within the digestive tract had an effect on the efficiency of energy utilisation. The increase in digesta viscosity that was achieved by the addition of guar gum in the second experiment was much higher than would be expected in barley-based diets, but it indicated the large negative effect that high digesta viscosity has on the efficiency of energy utilisation in broiler chickens. Smits & Annison (1996) indicated that there was an interaction between digesta viscosity and intestinal bacteria populations and that high viscosity allowed bacterial proliferation in the distal ileum. Higher proportions of bacterial fermentation would increase the heat increment of digestion and so reduce the efficiency of energy utilisation in these diets.

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Energy utilization of rice straw supplemented with urea and molasses in swamp buffalo heifers

A. Purnomoadi¹, E. Rianto¹, N. Takusari², F. Terada² & M. Kurihara²

¹ Faculty of Animal Sciences, Diponegoro University, Semarang 50275, Indonesia

² National Institute of Livestock and Grassland Sciences, Tsukuba, Ibaraki 305-0901, Japan

Summary

Eight swamp buffalo (*Bubalus bubalis*) heifers (age 10 months; weight 163 kg) were used to study the energy utilization of rice straw supplemented with urea and molasses. The buffalo were kept in individual pens within a barn and allowed rice straw *ad libitum* as a basal diet. Four of the heifers received rice straw supplemented with 40 g urea (equal to 1% of estimated DMI based on a DM requirement of 2.5% liveweight) and 150 g molasses (equal to 0.1% liveweight). The balance trials were measured by total collections obtained during a 4-day period following a 2-week feed adaptation period. Methane production was measured by the facemask method for 10 minutes at 3-hour intervals for 2 days immediately after the 4-day period ended and was then converted to daily total production. The data were analyzed with the *t*-test.

The results showed that supplementation of rice straw with urea-molasses increased dry matter intake (DMI, 3133 vs 2626 g/d; $P<0.05$), nitrogen intake (NI, 41.4 vs 20.1 g/d; $P<0.001$) and gross energy intake (GEI, 48.1 vs 41.5 MJ/d; $P<0.05$). Furthermore, the supplementation reduced methane production (4.7 vs 5.2 MJ/d, equal to 9.7 vs 12.7% of GEI; $p<0.05$). However, the supplementation did not increase the intake of DE (14.2 vs 14.6 MJ/d) or of ME (8.9 vs 8.9 MJ/d). The reasons why the supplementation did not increase the heifers' energy utilization of rice straw in this experiment are that the amount of supplementation and the ratio of urea to molasses were not optimal. We should clarify both the amount and the ratio of these supplements to improve the energy utilization of rice straw by swamp buffalo heifers.

Keywords: *buffalo heifers, rice straw, methane*

Introduction

Ninety percent of the world's buffalo population is raised in the tropics under a 'backyard farming system' for draught as well as for meat, milk and offspring. In comparison to that for cattle or goats, feeding management for buffalo has been relatively less developed - even in milking operations - because the low reproduction rate of buffalo keeps buffalo's meat productivity low. One factor in the reproduction rate of the buffalo is its prolonged weaning period. On Indonesian farms, buffalo are weaned at a wide range of ages, from one to three years. During this period, the calf still suckles the mother even after it starts eating roughage. This is because the farmers typically do not know how to raise young buffalo and thus tend to leave the matter to nature.

The buffalo is known for its superiority, compared to other ruminants, in utilizing highly fibrous and poor-quality feeds of tropical forages. Rice straw, for example, is the main roughage for buffalo on small-holder farmers. Rice straw may give the animal little available energy because its digestibility is low and because more energy is lost in the form of methane than is the case with high-quality feeds (Leng, 1993). Methane is a major component in greenhouse gases. The high production of methane from animal agriculture has been pointed out to tropical countries, which have at least half of the world's cattle population and most of the world's buffalo population. Urea-molasses is a widely used supplement to improve the feed availability of rice straw in order to support productivity (Leng, 1993) as well as to reduce methane production and in turn increase

energy utilization. The lack of data on energy utilization of rice straw and its supplementation with urea-molasses in buffalo, especially buffalo heifers, led us to conduct this experiment. The experiment is also intended to provide basic information toward the reduction of methane production.

Materials and methods

Eight swamp buffalo (*Bubalus bubalis*) heifers (age 10 months; weight, 163 kg) were used in this study. They were fed rice straw *ad libitum* as a basal diet. The buffalo were split into two groups. One group (RS-UM) was given urea (40 g, equal to 1% of estimated DMI based on the DM requirement of 2.5% liveweight; LW) and molasses (150 g, equal to 0.1%LW), while the other group (RSO) was not given any supplement. The rice straw was provided unchopped, while molasses was offered in liquid form. In the RS-UM group, urea and molasses mixed with 500 g rice straw was given first, and when all of the feed had been consumed (about 30-60 minutes later), more rice straw was given, but this time without the supplement. The balance trial was done by total collection after 4 consecutive days. The dry matter intake was measured by weighing the total feed given and the orts. Feed, feces and urine were analyzed to determine the digestibilities of the dry matter, crude protein, energy and metabolizable energy. Crude protein and energy were determined by the Kjeldahl method and bomb calorimeter, respectively.

Energy loss as methane was measured during the 2 days immediately after the end of the 4-day total collection, using the facemask method as established by Kawashima *et al.* (2001). In this method, a mask is connected to a methane analyzer (infra-red gas analyzer, Horiba Ltd., Japan) to measure the methane concentration (%). The analyzer was equipped with an airflow meter for total air volume (L/min). The data on methane and air flow were averaged and recorded automatically every 3 seconds by an IBM computer. This measurement was done for 10 minutes at 3-hour intervals for 2 x 24 hours. The methane volume (L/d) was converted to kJ by multiplying by 39.54 (Brouwer, 1965). Daily methane production was averaged from the 2 days of measurements. The data were analyzed with the *t*-test.

Results and discussion

The rice straw used in this study contained 0.8% N, 29.9% CF, 40.6% NFE and gross energy of 15.81 MJ/kg. The urea and molasses contained, respectively, N 48.6 and 0.6%, and gross energy 8.74 and 7.87 MJ/kg. The balance trial results are presented in Table 1. The dry matter intake of rice straw (RSO) and of rice straw supplemented with urea-molasses (RS-UM) averaged 1.6 and 1.9% LW, respectively. These values were in the normal range (Roxas *et al.*, 1975). In comparison with RSO, the RS-UM group had an increased ($P<0.05$) intake of dry matter (DMI), crude protein (CPI), and gross energy (GEI) by 19.3, 106.0 and 15.9%, respectively. This increase in feed intake by the urea-molasses supplementation increased the lost energy in feces (26.0%; $P<0.05$), and a similar tendency was observed in lost energy in urine (24.9%). The urea-molasses supplementation also reduced methane production (4.7 vs. 5.2 MJ/d, equal to 9.7 vs. 12.7% GEI; $P<0.05$). The increases in energy excreted through feces and urine in RS-UM lowered the digestibility of dry matter, energy (DE/GE), and energy metabolizability (ME/GE) by 3.9, 5.9 and 4.0%, respectively. With the exception of small increases in crude fiber (CF) digestibility (2.7%) and in CP digestibility (31.2%; $P<0.05$), the results were unexpected, because for the most part urea-molasses supplementation has been thought to increase feed utilization and productivity. The loss of nitrogen and energy through feces rather than through urine showed that urea-molasses supplementation failed to lift the utilization of rice straw. Thus, similar intakes of DE and ME were observed. This phenomenon leads to a consideration of the low rumen activity in newly weaned buffalo. A review of each animal's history showed that weaning had been completed at an average age of 6 months (4 months before their use in this experiment), and from the age of 4

Table 1. Daily intake, fecal excretion, urinary excretion and methane production from balance trials of buffalo heifers fed rice straw with or without supplementation by urea and molasses.

| | Rice straw | Rice straw + urea molasses | Statistical significance |
|----------------------------------|------------|----------------------------|--------------------------|
| Liveweight, kg | 162 | 166 | |
| Nutrient intake per day | | | |
| Dry matter, g | 2626.0 | 3132.9 | 0.011 * |
| Crude protein, g | 125.5 | 258.7 | 0.001 *** |
| Crude fiber, g | 784.6 | 893.6 | 0.034 |
| Apparent digestibility, % | | | |
| Dry matter | 29.1 | 25.2 | 0.243 |
| Crude protein | 17.2 | 48.6 | 0.001 *** |
| Crude fiber | 40.2 | 42.9 | 0.366 |
| Energy (DE/GE) | 35.2 | 29.3 | 0.134 |
| Nitrogen loss through, g per day | | | |
| Feces | 16.6 | 21.2 | 0.010 ** |
| Urine | 9.5 | 10.8 | 0.249 |
| Energy intake, MJ per day | | | |
| Gross energy (GE) | 41.5 | 48.1 | 0.022 * |
| Digestible energy (DE) | 14.6 | 14.2 | 0.439 |
| Metabolizable energy (ME) | 8.9 | 8.9 | 0.496 |
| Energy loss through, %GEI | | | |
| Feces | 64.8 | 70.7 | 0.134 |
| Urine | 1.2 | 1.3 | 0.377 |
| Methane | 12.7 | 9.7 | 0.011 * |
| Metabolizability (ME/GE), % | 21.3 | 18.3 | 0.286 |

months they received green grass in addition to milk from the mother. Even though they were adapted to rice straw for 2 months prior to the experiment, this period may not have been long enough to develop the rumen's ability to fully utilize rice straw.

The average methane conversion rate (MCR, %GEI) of the unsupplemented and supplemented rice straw (RSO 12.7% and RS-UM 9.7% GEI) was similar to that of cattle receiving tropical grasses (Kurihara *et al.*, 1999), namely mature Angleton grass (*Dicanthium aristatum*) and immature Rhodes grass (*Chloris gayana*) (10.4 and 11.4%, respectively). This study also showed that rice straw produced more methane, by greater than 12% GEI, the upper limit of methane production in cattle (Czernawski, 1969). The effect of urea-molasses supplementation in MCR in this study, however, may stem from its contribution to GEI rather than to its effect of increasing feed utilization.

Based on all of these phenomena, we can attribute the buffalo heifers' low efficiency and energy utilization of the urea-molasses supplementation of rice straw in this study to the low digestibility of rice straw. This can be explained from three points. First, the 1:3 ratio of urea to molasses did not provide the optimal amount of energy (Chowdhury and Huque (1996) found 1:5 was the best ratio of urea to molasses), so the supplementation of urea-molasses in this study may have wasted the urea as a source of NPN because of a low readily available energy supply from the molasses. Second, the buffalo heifers used in this study were young, and the rumen's ability to degrade and utilize rice straw and urea-molasses was still poor. The third reason is that the liquid form of molasses given may have resulted in digestive difficulty, as the high viscosity may have disturbed the absorption or degradation of molasses in the buffalo rumen (Sanchez and Preston, 1980).

These results showed that urea-molasses was not utilized properly to improve the utilization of rice straw by swamp buffalo heifers. We should clarify the amount and ratio of these supplements to improve these animals' utilization of the energy provided by rice straw.

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Research on the influence of dietary macronutrients on food intake and its regulatory mechanisms in broiler chickens

Q. Swennen¹, G.P.J. Janssens², E. Decuyper¹ & J. Buyse¹

¹ *Laboratory for Physiology and Immunology of Domestic Animals, Department of Animal Production, K.U. Leuven, Kasteelpark Arenberg 30, B-3001 Leuven, Belgium*

² *Laboratory of Animal Nutrition, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium*

Summary

An experiment was conducted with two isocaloric diets (low protein (LP): 16.63 MJ/kg gross energy (GE), 126 g/kg protein, 106 g/kg fat; low fat (LF): 16.73 MJ/kg (GE), 242 g/kg protein, 43 g/kg fat) to search for the role of macronutrients in the regulation of the parameters of the energy balance and of food intake of broiler chickens. Broilers receiving the LP food had an excessive energy intake relative to their protein intake, resulting in a higher heat production and more fat accretion. Moreover, these animals had a more efficient protein metabolism. There was no clear relationship between diet induced thermogenesis (DIT) and level of food intake.

Keywords: *energy metabolism, protein metabolism, broilers*

Introduction

Research with mammals has shown that causal relationships exist between diet composition, diet induced thermogenesis, nutrient balance and the satiety power of the different macronutrients. However, for avian species, in particular domestic poultry, information is scarce. It is well documented that dietary composition has a major impact on body composition of chickens (Buyse *et al.*, 1992; MacLeod, 1992), but the relationship between metabolisable energy (ME) intake and changes in energy expenditure is still unclear in poultry. Chickens that received diets with a very high energy protein ratio, showed a reduction in heat production (Q) (MacLeod, 1992). In contrast, Buyse *et al.* (1992) found that broilers reared on a 15 % crude protein diet consumed an excessive amount of energy due to an increased food intake, which caused an increased fat deposition as well as a higher Q compared to broilers fed an isocaloric diet with 20 % crude protein. Given the discrepancies between studies, more research is warranted to explain the effect of isocaloric diets with different macronutrient contents on the components of the energy balance, including diet induced thermogenesis.

The regulation of voluntary food intake is very complex with several levels of control. Several models have been proposed to understand the mechanisms that match energy and nutrient balance with food intake and energy expenditure in mammals (Stubbs, 1999). However, far less is known about the relationship between these processes in avian species. An important question is whether the energy or nutrient metabolism is involved in the regulation of food intake.

The aim of this experiment, conducted with two isocaloric diets with isocaloric substitutions between protein and fat contents was to search for the role of macronutrients in the regulation of the components of the energy balance and the effect on food intake, as well as on some relevant plasma hormone and metabolite concentrations in broiler chickens.

Material and methods

Day-old male broiler chickens (Avibel, Zoersel, Belgium) were divided over two floor pens in a poultry house under standard housing conditions. Until 14 days of age, a commercial starter diet was provided *ad libitum* (see Buyse *et al.*, 2001 for diet composition). From 8 days of age, each group received one of two isocaloric diets, which contained the same ingredients, though some in different quantities to create pair-wise changes in protein and fat content with more or less constant ME content and carbohydrate concentrations. The LP diet contained 16.63 MJ/kg (GE), 126 g/kg protein, 106 g/kg fat and 514 g/kg non-fibre carbohydrates. The LF diet contained 16.73 MJ/kg (GE), 242 g/kg protein, 43 g/kg fat and 504 g/kg non-fibre carbohydrates. Starting at 21 days of age and repeated each week during 4 consecutive weeks, 3 LP and 3 LF chickens were placed individually in one of the six respiratory cells (as described by Buyse *et al.*, 1998). After an adaptation period (48 h), the animals were fasted for 24 h. Then they were refed a known amount of food during 5 h for measuring the diet induced thermogenesis (DIT). For the next 2 days, the animals were fed *ad libitum* to calculate a total energy balance (EB).

Gas exchanges (CO_2 and O_2) were measured continuously during fasting, DIT and EB periods. Heat production (Q) was calculated according to the short formula of Brouwer (1965): Heat production (kJ/h) = $16.18 V_{\text{O}_2} (\text{l/h}) + 5.02 V_{\text{CO}_2} (\text{l/h})$. At the start of the experimental period, after fasting, after DIT and EB measurements the individual body weights (BW) were measured and after DIT and EB, food intake per chicken was determined. Excreta were collected quantitatively after EB. Blood samples were collected in iced tubes after fasting, DIT and EB measurements from a wing vein by using a heparinised syringe. After euthanasia, weights of liver and abdominal fat pad were recorded. Gross energy and N content of excreta and food samples were determined. A total energy and protein balance and diet induced thermogenesis were calculated based on the measurements and analysis mentioned above. Respiratory quotients (RQ) were determined for all measuring periods. DIT was calculated as the Q during 5 consecutive h above fasting Q.

Plasma 3,5,3'-triiodothyronine (T_3) and thyroxine (T_4) were measured by radioimmunoassay (Darras *et al.*, 1992). Plasma triglycerides, free fatty acid and uric acid concentrations were measured spectrophotometrically with an automated apparatus (Monarch Chemistry System, Instrumentation Laboratories, B-1930, Zaventem, Belgium).

All data were analyzed by analysis of variance (ANOVA) with diet composition as classification variable (SAS Institute Inc., Cary, NC., 1998).

Results and discussion

Energy balance

From week 4 to 6, the LP animals had a significant lower BW than the LF animals (Table 1). Therefore, all parameters related to energy and protein metabolism were expressed per kg metabolic weight ($\text{kg}^{0.75}$). Food intake was significantly higher for the LP compared to the LF group. Since the gross energy (GE) content of both foods was about the same, this resulted in a significantly higher GE intake by the LP compared to the LF chickens. The $\text{GE}_{\text{excreta}}$ was not affected by the food. As a consequence, the apparent ME (AME) intake -calculated as the difference between GE intake and $\text{GE}_{\text{excreta}}$ - of the LP chickens was significantly higher and hence also the metabolisability of the LP diet. The better metabolisability of the LP food might be due to the extracaloric effect of the higher fat content of the LP diet compared to that of the LF diet (Mateos *et al.*, 1982). The higher AME intake of the LP chickens, both expressed as kJ/kg $\text{BW}^{0.75}$ and per g of protein intake, caused dramatic changes in Q and energy retention (RE). Indeed, Q and RE of LP chickens were increased with respectively 23 % ($P=0.04$) and 39 % ($P=0.0044$) compared to the values of the LF chickens. Furthermore, the partition of RE over protein (RE_p) and

Table 1. Influence of the diet macronutrient ratio on the parameters of the energy and protein balance, combined over the experimental period of 4 weeks.

| Parameter | LPfood | LFfood | % Change | |
|---|------------------------|------------------------|----------|---------|
| | Mean (\pm Stderror) | Mean (\pm Stderror) | LP vs LF | P-value |
| Body weight, g | | | | |
| Week 4 | 726.7 (\pm 33.5) | 1254.1 (\pm 20.5) | +73 | <0.0001 |
| Week 5 | 1000.6 (\pm 77.1) | 1613.1 (\pm 35.2) | +61 | <0.0001 |
| Week 6 | 1518.2 (\pm 108.8) | 2334.5 (\pm 56.9) | +54 | <0.0001 |
| Week 7 | 1854.7 (\pm 328.7) | 2550.0 (\pm 133.7) | +37 | 0.1215 |
| Food intake, g/kg ^{0.75} *d | 108.90 (\pm 5.99) | 83.70 (\pm 6.10) | -23 | 0.0073 |
| Gross energy intake ¹ | 1743 (\pm 95) | 1346 (\pm 98) | -23 | 0.0096 |
| Gross energy excreta ¹ | 412 (\pm 35) | 437 (\pm 42) | +6 | 0.66 |
| Apparent metabolisable energy intake ¹ | 1322 (\pm 71) | 909 (\pm 63) | -31 | 0.0003 |
| Heat production ¹ | 616 (\pm 57) | 477 (\pm 28) | -23 | 0.04 |
| Energy retention ¹ | 706 (\pm 72) | 432 (\pm 47) | -39 | 0.0044 |
| Energy retention as protein ¹ | 187 (\pm 8) | 230 (\pm 25) | +23 | 0.12 |
| Energy retention as fat ¹ | 519 (\pm 68) | 202 (\pm 30) | -61 | 0.0003 |
| Metabolisability | 0.766 (\pm 0.016) | 0.697 (\pm 0.015) | -9 | 0.0006 |
| Efficiency of energy retention | 0.468 (\pm 0.041) | 0.304 (\pm 0.028) | -35 | 0.0032 |
| Efficiency of protein retention | 0.580 (\pm 0.026) | 0.470 (\pm 0.041) | -19 | 0.032 |
| Respiratory quotient | 1.204 (\pm 0.010) | 1.129 (\pm 0.010) | -6 | <0.0001 |

¹Expressed as kJ/kg^{0.75} *d

fat (RE_f) was also affected by diet composition. The RE_p of LP chickens was somewhat lower (23 %, P>0.05) whereas the RE_f was significantly higher (61 %, P=0.0003) compared to that of the LF chickens.

The RQ-values were greater than 1 during energy balance, which means that fatty acid synthesis was occurring. Since the RQ value for the LP group was significantly higher, these animals seem to synthesize more fat *de novo* compared to the LF group, which is reflected in the higher value of RE_f as well as in the higher efficiency of energy retention. The efficiency of protein retention was significantly higher in the LP group, suggesting that these chickens had a more efficient protein metabolism. This factor may also contribute to the improved metabolisability of the LP diet.

The plasma concentrations of triglycerides were significantly higher in the LP compared to the LF chickens, as were the proportional liver weights, indicating a higher *de novo* lipogenesis. Free fatty acid concentrations were significantly higher in the plasma of the LP chickens. The most plausible explanation for this observation is a lower free fatty acid-uptake from the blood, since the LP animals had an already excessive energy intake. The proportional weight of the abdominal fat pad was significantly higher in the LP chickens. Together with the results of RE_f, efficiency of energy retention and RQ, this proves that the LP animals had a higher *de novo* lipogenesis and hence a higher fat accretion.

The levels of uric acid in the plasma of the LF animals were significantly higher compared to the LP animals. Since uric acid is a measure of protein oxidation, this suggests that the LP animals oxidised less proteins, corroborating the conclusion that LP chickens had a more efficient protein metabolism.

The ratio of T₃- and T₄-concentrations in the plasma of the LP animals was significantly higher compared to the LF chickens. This reflects the higher Q-values of the LP group and could be due to stimulation of hepatic ORD-I deiodase by the LP food.

Diet induced thermogenesis

Stubbs & O'Reilly (2000) formulated the hypothesis that the Q from oxidation of nutrients or the DIT shows an inverse relationship with food intake. Since Q from the oxidation of proteins is higher than that from fat, it was expected that the DIT would be influenced by the diet composition, which would have an effect on their food intake. However, the results did not show a significant food effect on DIT nor on food intake during the 5 h measuring period. Consequently, the hypothesis could not be proven or refuted. It must be kept in mind that the theory was formulated for adult mammals, whereas this experiment was done with growing chickens. It is also possible that the refeeding period used (5 h) was not long enough or the diet composition was not divergent enough to observe differences between diets.

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Energy utilization of diets with different starch content in nulliparous rabbit does fed different levels of intake

I. Toschi, L. Rapetti, L. Bava, G.M. Crovetto & C. Castrovilli

*Istituto di Zootecnia Generale, Facoltà di Agraria, Università degli Studi di Milano,
via Celoria 2, 20133 Milano, Italy*

Summary

Sixty four nulliparous rabbit does were divided into 4 groups, allocated to individual metabolic cages and fed (restricted or *ad libitum*) one of the following diets: C) 23% starch on DM; CP) 19% starch, based on Citrus Pulp; MM) 30% starch, based on Maize Meal; MF) 33% starch, based on Maize Flaked, to determine DE and ME. HP and RE were computed by indirect calorimetry on 32 animals. Diet C had a significantly lower digestibility and metabolizability, while no difference was registered between diets for HP and RE. The high intake level decreased digestibility and metabolizability significantly. From all the energy balances a regression equation was developed to predict RE from ME intake; ME_m resulted to be 417 kJ/kg^{0.75}, k_g 0.77, k_f and k_p 0.86 and 0.67, respectively.

Keywords: rabbit does, energy utilization, intake

Introduction

In the intensive breeding rithm, the simultaneous high energy requirements for pregnancy and lactation of rabbit does determine an energy deficit due to the insufficient voluntary dry matter intake (VDMI). Increasing digestible energy (DE) concentration of the diet over the typical value of 10 MJ*kg⁻¹ up to 11 MJ*kg⁻¹, considered the limit of chemostatic regulation of VDMI in rabbit does, allows higher DE intakes (Xiccato *et al.*, 1995). The use of diet with high starch content increases the energy concentration but can promote digestive disorders (Cheeke & Patton, 1979). Alternatively, it is possible to substitute part of the starchy feeds of the diet with feeds rich in non-starch polysaccharides. The scarcity of data on the energy requirements of rabbits, particularly nulliparous does, and the different methodology utilized (comparative slaughter, direct or indirect calorimetry), suggest to improve the scientific knowledge on this topic. Aim of this study was to evaluate the energy utilization of diets with different starch concentration, fed to nulliparous rabbit does at two levels of intake, in the last days before mating, when often the energy concentration of the diet is increased.

Material and methods

Sixty four hybrid growing nulliparous rabbit does (17 weeks of age) were allocated, at 20°C, to 4 dietary treatments and fed one of the following diets (table 1): C) Control, 23.3% starch on DM; CP) 19.0% starch, based on Citrus Pulp; MM) 30.4% starch, based on Maize Meal; MF) 33.2% starch, based on Maize Flaked. Each diet was offered at two levels of intake: restricted (R) (35 g*kg⁻¹ LW) or *ad libitum* (AL). Individual apparent digestibilities were determined on 4 days-period; respiratory exchanges were recorded individually on thirty two animals by indirect calorimetry for three cycles of 24 hours during each period of digestibility. The effect of the diet, the effect of the level of intake, and their interaction were studied by GLM analysis.

Table 1. Ingredients and chemical composition of the experimental diets.

| Diet | C | CP | MM | MF |
|---------------------------------------|------|------|------|------|
| <i>Ingredients (%)</i> | | | | |
| Citrus pulp | | 25.0 | | |
| Flaked maize | | | | 30.0 |
| Maize meal | 5.2 | 16.0 | 30.0 | |
| Alfalfa meal | 30.0 | 24.0 | 27.0 | 27.0 |
| Soybean meal | 21.0 | 27.0 | 24.5 | 24.5 |
| Barley | 19.8 | 3.0 | 13.2 | 13.2 |
| Wheat bran middlings | 19.0 | | | |
| Oil | 1.5 | 1.5 | 1.5 | 1.5 |
| Minerals & vitamins | 3.5 | 3.5 | 3.8 | 3.8 |
| <i>Chemical composition (% of DM)</i> | | | | |
| Crude Protein | 21.5 | 21.1 | 20.8 | 20.7 |
| Ether Extract | 3.9 | 3.3 | 3.4 | 3.3 |
| NDF | 25.9 | 23.0 | 22.4 | 22.7 |
| NFC ¹ | 39.4 | 42.8 | 44.1 | 44.3 |
| Starch | 23.3 | 19.0 | 30.4 | 33.2 |

¹ Non Fibrous Carbohydrate calculated as: DM-(ASH+CP+EE+NDF).

The thirty two energy balances determined in the trial were also analysed with simple linear regression model to determine the metabolizable energy for maintenance (ME_m) and the efficiency of utilization of ME for growth (k_g). Multiple regression analysis was applied to determine the efficiency of utilization of ME for protein (k_p) and fat (k_f) deposition, calculating the energy retained as fat (RE_f) as the difference between total RE and the energy retained as protein (RE_p).

Results and discussion

Two does were eliminated from the experiment because of adaptation problems. Statistical analysis revealed no significant interaction between intake level and diets.

Energy balance data (table 2) show that digestibility and metabolizability of diet C were significantly lower as compared to the other diets, with the exception of the metabolizability of diet CP, due its higher urinary energy excretion. This is consistent with the higher NDF content of diet C and the high level of non-fibrous carbohydrates of the other diets.

The DE and ME content (MJ/kg DM) of the four experimental diets resulted to be: 12.31 and 11.62 (C), 12.50 and 11.68 (CP), 12.50 and 11.81 (MM), 12.55 and 11.83 (MF), with no significant differences between treatments. Therefore, flaking maize grain did not improve energy utilization; moreover, citrus pulp resulted to be an excellent energy source for the rabbit. The *ad libitum* intake level decreased energy digestibility and metabolizability significantly.

The metabolizable energy for maintenance (ME_m) was computed using the equation reported in figure 1 derived from all the 30 energy balances determined in the experiment. Assuming $RE=0$, ME_m resulted to be $417 \text{ kJ/kg}^{0.75}$. This value is higher than those obtained in non-pregnant rabbit does with the comparative slaughter technique by Parigi Bini *et al.* (1990) ($378 \text{ kJ/kg}^{0.75}$ assuming $ME=0.95 \text{ DE}$) and with direct calorimetry by Partridge *et al.* (1986) ($310 \text{ kJ/kg}^{0.75}$); however, Eriksson (1952), using indirect calorimetry, determined in small Chinchilla breed a ME_m value of $385 \text{ kJ/kg}^{0.75}$.

The determination coefficient and the RSD of the linear regression (figure 1) indicate a high relationship between the two variables considered. The k_g value determined, 0.77, is higher than

Table 2. Daily energy utilization and partition (R=restricted; AL=*ad libitum*)

| | Diets | | | | | Intake level | | | |
|--------------------------|-------------------|-------------------|-------------------|-------------------|------|--------------|------|------|-----|
| | C | CP | MM | MF | SE | R | AL | SE | P |
| kg ^{0.75} | 2.50 | 2.55 | 2.55 | 2.53 | 0.02 | 2.46 | 2.60 | 0.01 | |
| DMI g/d | 136 | 132 | 134 | 134 | 5.1 | 103 | 164 | 3.5 | |
| IE kJ/kg ^{0.75} | 1002 | 934 | 947 | 950 | 34.4 | 767 | 1150 | 23.9 | *** |
| DE kJ/kg ^{0.75} | 664 | 637 | 648 | 657 | 22.6 | 537 | 767 | 15.7 | *** |
| %IE | 66.5 ^A | 68.7 ^B | 68.8 ^B | 69.5 ^B | 0.53 | 70.0 | 66.8 | 0.37 | *** |
| UE kJ/kg ^{0.75} | 36 | 41 | 36 | 37 | 1.7 | 34.2 | 40.4 | 1.21 | *** |
| %IE | 3.7 ^B | 4.5 ^{Aa} | 3.8 ^b | 4.0 | 0.19 | 4.5 | 3.5 | 0.13 | *** |
| ME kJ/kg ^{0.75} | 628 | 597 | 613 | 620 | 21.8 | 502 | 726 | 15.2 | *** |
| %IE | 62.8 ^B | 64.2 | 65.0 ^A | 65.4 ^A | 0.58 | 65.5 | 63.2 | 0.40 | *** |
| HP kJ/kg ^{0.75} | 461 | 469 | 461 | 456 | 10.5 | 431 | 492 | 7.2 | *** |
| %ME | 74.2 | 80.7 | 79.8 | 77.5 | 2.64 | 87.4 | 68.6 | 1.80 | *** |
| RE kJ/kg ^{0.75} | 188 | 136 | 127 | 145 | 24.5 | 64 | 235 | 16.7 | *** |
| %ME | 25.8 | 19.3 | 20.2 | 22.5 | 2.64 | 12.6 | 31.4 | 1.80 | *** |

Values on the same row with different letter differ significantly (A,B: P<0.01; a,b: P<0.05)

that reported by Parigi Bini & Xiccato (1998) as average value (0.54) of different experiments on growing rabbits, all conducted with the comparative slaughter technique. Partridge *et al.* (1986), in the work previously mentioned, found a k_g value of 0.67 in non pregnant does fed an high fat diet.

From multiple regression analysis between ME intake (MEI) and RE_f and RE_p the following equation was obtained:

$$\text{MEI (kJ/kg}^{0.75}) = 419 + 1.16 \text{ RE}_f + 1.49 \text{ RE}_p \quad (n=30, \text{ RSD}=28.6, R^2=0.96, P<0.001)$$

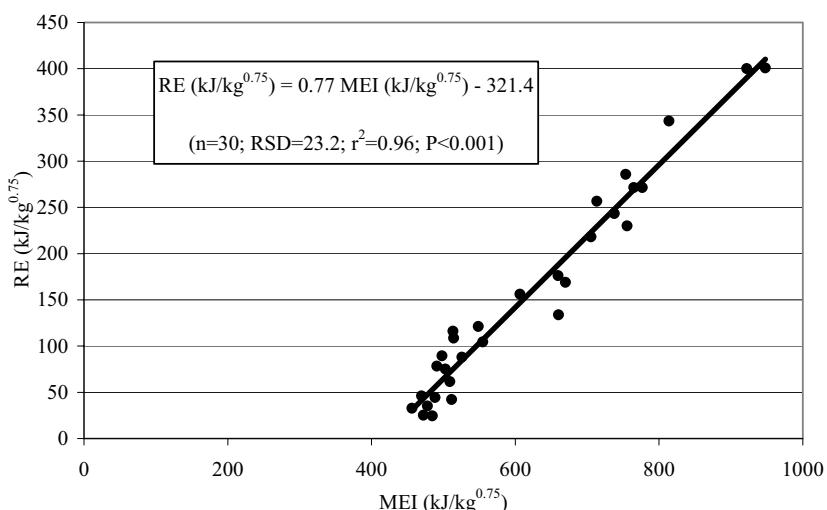


Figure 1. Relationship between the metabolizable energy intake and the retained energy.

The k_f and k_p derived were 0.86 and 0.67, respectively. These values, particularly the latter, are high in comparison with those (0.63-0.74 and 0.40-0.46, respectively) reported in literature for growing rabbits (de Blas *et al.*, 1985; Partridge *et al.*, 1989).

The high k values found in our work could be explained by the different technique applied and by the high energy concentration of the feed; the latter improves the efficiency of energy utilization (Reid *et al.*, 1980) also by means of a reduction of the fermentations in the caecum, the yield of volatile fatty acids and the heat increment involved in the process.

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Fat vs. starch as energy sources in diets for high yielding lactating dairy cows

J. Voigt¹, K. Gaafar¹, H. Hagemeister¹, W. Kanitz² & D. Precht³

¹ Research Unit Nutritional Physiology "Oskar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

² Research Unit Reproductive Biology of the Research Institute for the Biology of Farm Animals, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

³ Institute for Chemistry and Physics, Federal Dairy Research Centre, Kiel, Germany

Summary

The aim of our study was to explore whether the substitution of starch by rumen protected fatty acids in diets of dairy cows results in a transfer of carbon atoms from lipids into milk lactose. Because the carbon atoms of fat are naturally depleted in ¹³C as compared to carbon atoms of carbohydrates we hypothesized that the ¹³C content in milk lactose is lower when dietary fat carbon atoms are used for lactose synthesis. For this purpose the ¹³C/¹²C ratio in milk lactose, fat, and in blood CO₂ of samples from Holstein-Friesian cows was assessed weekly during the first 15 weeks of lactation. Treatments consisted of a corn silage-based diet supplemented with starch (control, C) or protected fatty acids (calcium salts, CS). Treatments were isoenergetic and isonitrogenous. The following results were found (C/CS, Mean±SEM): milk yield 38.8±1.7/41.5±1.0 kg/d; milk protein 3.1±0.05/2.9±0.03% (P<0.05); milk fat 4.3±0.2/4.0±0.2%; milk lactose 4.7±0.03/4.7±0.03%; milk urea 217±6/242±8 mg/l (P<0.05); blood glucose 3.5±0.08/3.4±0.05 mmol/l. Carbon atoms in the milk fat of the CS group were ¹³C-depleted in comparison to the milk fat of the C group (P<0.05). The ¹³C level in milk lactose did not differ between the groups. Surprisingly the ¹³C in blood CO₂ of the C group was depleted in comparison to the CS group. It can be concluded that the entry of ¹³C-depleted carbon atoms from fatty acids into the citric acid cycle seems to be inhibited by dietary fatty acids. Transfer of carbon atoms from fatty acids into lactose by the citric acid cycle and gluconeogenesis from oxalacetate is apparently not enhanced by feeding protected fat.

Keywords: dairy cows, dietary fat, ¹³C-transfer

Introduction

In dairy cows, glucose supply to the mammary gland is the main determinant of the milk amount (Kronfeld, 1982).

Dietary supplementation with ruminal protected fat may improve the utilization of metabolizable energy (Bergner and Hoffmann, 1996). However, in contrast to starch, fat neither supplies glucogenic components to the liver and mammary gland, nor energy to ruminal microbes. It was demonstrated that in phases of increased energy demand carbon atoms from body fat reserves will be transferred into milk lactose (Schulze et al., 1992). Weinmann et al. (1957) discussed that after β -oxidation the carbon atoms of fatty acids would be channelled as acetyl-CoA into the citric acid cycle where they substitute the carbon atoms of oxalacetate and were in this way transferred into the process of gluconeogenesis. Since the ¹³C/¹²C ratio in dietary fat is lower than in dietary carbohydrate, the ¹³C/¹²C ratio in milk lactose should tend to decreased values when carbon from lipid is transferred into glucose.

The aim of our study was to explore whether substitution of dietary starch with fatty acids results in a transfer of carbon atoms into milk lactose.

Material and methods

The experiment was carried out in two groups of high yielding dairy cows (16 Holstein-Friesian cows in each group) in the first 100 d of lactation. The groups were fed on isocaloric and isonitrogenous diets on the basis of corn silage, corn meal and soybean meal. In the experimental group, 2 kg of tapioca were replaced by one kg of protected fat (Ca soap of palm fat and soya oil, in addition to protected soybeans). The animals were given controlled diets semi-*ad libitum* which focused on maintaining a maximum energy intake. The feed supply was corrected weekly on the basis of individual performance. Milk yield was recorded for the three milkings at 0500, 1100, and 1900 h. Milk and heparinised blood samples were taken weekly from week 2 to week 15 *post partum* (p. p.) from all cows and frozen till analysis.

Feed samples were assayed for nutrients by the Weender method. Blood glucose was measured by the glucose oxidase method. All milk samples were analysed by infrared spectroscopy for fat, lactose and protein. Fat and lactose were isolated for isotope measurement by centrifugation and protein precipitation at pH 4.6. The $^{13}\text{C}/^{12}\text{C}$ ratio in feed samples, milk constituents and blood CO_2 was determined by the continuous flow technique on a Delta S instrument (Finnigan MAT, Bremen, Germany), coupled with an elemental analyser (EA 1108, FISON, Rodano, Italy) or a Gasbench II (Finnigan MAT) as interface. ^{13}C abundance was measured against a reference which had been calibrated against the international PDB standard. The results are expressed as a difference $\delta^{13}\text{C}$ between the $^{13}\text{C}/^{12}\text{C}$ ratios of the samples and the PDB-standard:

$$\delta^{13}\text{C} \text{‰} = \frac{^{13}\text{C}:^{12}\text{C}_{\text{sample}} - ^{13}\text{C}:^{12}\text{C}_{\text{standard}}}{^{13}\text{C}:^{12}\text{C}_{\text{standard}}} \times 1000 \quad (1)$$

Results and discussion

The results of dietary input and milk output are given in Table 1. The energy balance of cows in both groups did not differ. Protected fat insignificantly increased the daily milk yield. The milk protein content decreased significantly by 0.2 % but the protein output remained unchanged. Identical results were found earlier as reviewed extensively by Wu and Huber (1994).

Milk fat percentage and output (kg/d) of milk fat were similar for cows fed both diets. The lactose content was not affected, however lactose output (kg/d) in milk tended to be higher ($P<0.12$) in cows fed protected fat instead of starch. Also Casper and Schingoethe (1989) and Chouinard et al. (1997) reported higher lactose production in cows fed a high fat diet. Stimulated lactose production and unchanged lactose concentrations would suggest that glucose metabolism was probably not stressed by feeding fat instead of starch. Also the levels of blood glucose were similar in both groups (3.5 ± 0.08 vs. 3.4 ± 0.05 mmol/l, not shown).

It is generally agreed that the mammary glucose supply (and consequently the milk volume) is a function of the supply of glucose and glucogenic nutrients from the digestive tract and the rate of gluconeogenesis in liver (Kronfeld, 1982). Since lactose production was not reduced, sufficient glucose appeared to be available for lactose synthesis in cows that received fat. The question is whether carbon atoms from (dietary) fatty acids are transferred into lactose as discussed above. Due to the substitution of starch with fatty acids carbon atoms in the dietary concentrate mixture were depleted on ^{13}C by 0.85 $\delta\text{‰}$ (-20.10 vs. -20.95 $\delta\text{‰}$). In comparison to the carbon atoms of tapioca starch the carbon atoms of fatty acids were depleted by 4.1 $\delta\text{‰}$ (-24.1 vs. -28.09 $\delta\text{‰}$). As can be seen in Fig. 1, carbon in the milk fat of the fat-fed cows was ^{13}C -depleted in comparison to that of the starch-fed cows ($P<0.05$). On the other hand, the ^{13}C level in milk lactose was higher and did not differ between the two groups. Wilson et al. (1988) and Schulze et al. (1992) found that as a consequence of increased lipolysis of body fat and entry of ^{13}C -depleted carbon atoms into the Krebs cycle in fasted or restricted fed cows the $\delta^{13}\text{C}$ values of lactose approached the $\delta^{13}\text{C}$ level

Table 1. Intake and output in the first 100 d of lactation (mean; n=16)

| Item | Diet | | SEM | P |
|---|---------------------------|-------|-------|---------|
| | Starch | Fat | | |
| Intake | DM, kg/d | 20.9 | 20.2 | 1.5 |
| | NEL, MJ/d | 146.4 | 148.1 | 11.3 |
| | Starch, kg/d | 4.96 | 3.22 | 0.57 |
| | Fat, kg/d | 0.54 | 1.29 | <0.0001 |
| Milk, | kg/d | 38.8 | 41.5 | 4.12 |
| | Fat, % | 4.31 | 4.04 | 0.59 |
| | Protein, % | 3.12 | 2.91 | 0.16 |
| | Lactose, % | 4.69 | 4.71 | 0.13 |
| | Fat, kg/d | 1.67 | 1.69 | 0.28 |
| | Protein, kg/d | 1.20 | 1.20 | 0.11 |
| | Lactose, kg/d | 1.82 | 1.94 | 0.20 |
| | Energy, MJ/d ¹ | 124.2 | 126.0 | 14.1 |
| Tissue energy balance ² , MJ/d | -15.4 | -16.1 | 11.3 | 0.867 |

¹= Milk production (kg/d) x [(0.38 x %fat) + (0.21 x %protein) + 0.95]

²= Intake NEL (MJ/d) - (milk energy (MJ/d) + NEL maintenance (MJ/d))

NEL maintenance = 0.293 x BW^{0.75} (GfE, 2001)

of milk fat. Surprisingly, the ¹³C in blood-CO₂ of the control group was depleted when compared with the experimental one (Fig. 1). This means that in fat supplemented cows more carbon atoms of other sources than lipid were oxidised to generate ATP.

Conclusion

In this study, the carbon atoms of the dietary fatty acids were incorporated to a large extent into the milk fat of early lactating dairy cows. The entry of ¹³C-depleted carbon atoms from fatty acids into the citric acid cycle seems to be inhibited by high intake of dietary fatty acids. Transfer of carbon atoms from fatty acids into lactose by the citric acid cycle and gluconeogenesis from

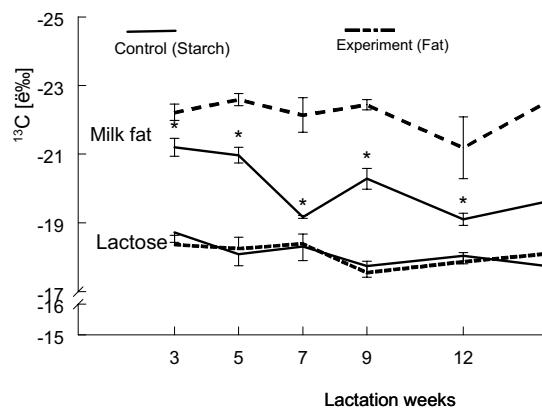


Figure 1. ¹³C in milk fat and lactose.

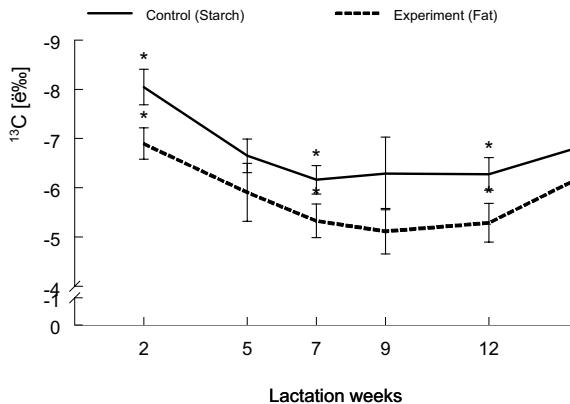


Figure 2. ^{13}C in blood CO_2

oxalacetate is apparently not enhanced by feeding protected fatty acids to dairy cows in early lactation.

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Energy session 3

Tissue, Organ and Whole Body Energy Metabolism and Methodology

Adipose tissue: an expanding role in the regulation of energy metabolism

R.G. Vernon

Hannah research Institute, Ayr, KA6 5HL, Scotland

Summary

Adipose tissue, the major energy reserve of the body, is under both acute, homeostatic control by hormones, locally-produced factors and sympathetic nervous activity, and chronic, homeorhetic control by hormones. In addition, the amount of adipose tissue is subject to autonomic control by factors produced within the tissue which act either locally (eg modulation of insulin action) or outwith the tissue (eg modulation of appetite) to adjust adiposity. Adipose tissue also secretes factors that act on other tissues including liver and muscle.

Keywords: *leptin, tumour necrosis factor α , adiponectin*

Introduction

Our ideas of adipose tissue have come a long way since the days it was considered to be just an inert storage tissue. We now know that it is a metabolically very active tissue subject to complex acute and chronic endocrine control, and also acute sympathetic control. In addition, it is subject to autocrine and paracrine control, and is a source of signals which modulate both appetite and the metabolism of other tissues (eg liver, muscle) which have key roles in energy metabolism. That the control of adipose tissue function is so complex reflects its critical role in mammalian physiology. Indeed mice engineered to lack adipose tissue mostly died before adulthood, while those that survived showed severe diabetes (McKnight, 1998).

The relatively low daily energy requirements of poikilothermic vertebrates means such creatures do not need substantial reserves of energy (Pond, 1992; Sheridan, 1994). By contrast, the high energy demands of homeothermy, coupled with in the main a terrestrial existence, means mammals need an efficient (weight-wise) store of energy. Triacylglycerols, relatively light, and hydrophobic (hence stored fat contains little stored water) provide an excellent solution. Accumulation of triacylglycerols, however, does impair the function of most cells, hence the need for a specialist storage organ - adipose tissue (Pond, 1992). The use of adipose tissue as an energy reserve pre-dated the evolution of mammals, being present in reptiles, however, in these adipose tissue stores are relatively limited and mostly abdominal (mesenteric) (Pond, 1992; Sheridan, 1994). Fatty acids released from triacylglycerols stored in these depots enter the portal blood and hence the liver before reaching the general circulation. The liver also has some capacity to store triacylglycerols, but when the amount exceeds 5-10% of liver mass, it can lead to impairment of liver function in mammals (Grummer, 1993; Drackley *et al.*, 2001). The development of mesenteric adipose tissue can thus be viewed as an 'out-sourcing' of the liver's lipid store. This intimate relationship between (mesenteric) adipose tissue and liver is still maintained in mammals; indeed adipose tissue secretes hormones which modulate liver function. Skeletal muscle can also store lipid, and is an important reserve of energy in fish. However, as with liver, accumulation of triacylglycerols in myocytes in mammals leads to impairment of muscle function (there is a strong positive correlation between the accumulation of triacylglycerols and the development of insulin resistance in muscle) (Shulman, 2000; McGary, 2002). Interestingly adipose tissue also secretes hormones which modulate muscle metabolism. Thus as we move from fish to mammals, we find a change from liver and skeletal muscle as key centres of energy storage to adipose tissue.

Mammals have a multiplicity of adipose tissue depots arranged throughout the body, some in the abdominal cavity, some under the skin and some within the musculature, plus a number of small, specialised depots (eg in the hoof and behind the eye) (Pond, 1992). This arrangement of depots evolved at a very early stage (it is found in marsupials) and has been retained, sometimes with modification to meet specialist needs of a species, ever since. Having such an array of depots spread through out the body does, however, create the potential for adipose tissue mass becoming excessive - ie obesity.

Possession of adipose tissue has allowed mammals to explore a variety of challenging habitats from the arctic to the desert, in which food availability is erratic. Arctic survival has required development of seasonal changes in adipose tissue with accumulation of considerable amounts of lipid in late-summer and autumn to help survive the winter (Mercer *et al.*, 2000). Adipose tissue also has an important role in reproduction; lipid reserves are often accumulated during pregnancy for use during the later stages of pregnancy, at parturition or during lactation (Vernon & Pond, 1997). However, while having a reserve of energy provides a buffer against starvation and provides mammals with options they would not otherwise have, there is a potential cost in that it can render an animal a succulent meal for a predator. For predators, obesity reduces the chances of catching a meal. Thus most mammals in the wild adjust their adiposity depending on whether starvation or predation is the greater threat; eg, antelope on the plains of Africa are relatively thin as predation is a greater threat than starvation. By contrast, reindeer, facing an Arctic winter, deposit large amounts of fat in the late summer and autumn, as during the winter, starvation is the greatest threat to survival (Larsen *et al.*, 1985). Foxes in northern Europe are generally fatter than those in southern Europe, reflecting the greater threat of starvation in the north (Lindström, 1983). Domestication has removed (mostly) threats of predation and starvation in Western countries and breeding (eg for leanness) may have altered some of the mechanisms regulating adiposity. Nevertheless, seasonal changes in appetite and adiposity are found in sheep for example, (Kay, 1985; Vernon *et al.*, 1986) while domestic ruminants show changes in adiposity during the cycle of pregnancy and lactation (Vernon & Pond, 1999).

It is not surprising then that adipose tissue metabolism is not only subject to complex homeostatic and homeorhetic controls, but in addition, there are further controls, operating both locally and elsewhere in the body, to keep adipose tissue mass within appropriate limits for the animal.

Pathways of lipid metabolism in adipocytes

Triacylglycerols are synthesized from fatty acids and glycerol-3 phosphate, the latter derived from the metabolism of glucose (figure 1). Fatty acids may be synthesized de novo within adipocytes, mostly from acetate or glucose depending on the species (almost all from acetate in ruminants, although the process is still glucose-dependent); key enzymes include acetyl CoA carboxylase and fatty acid synthase as well as the glucose translocase, Glut 4. In addition fatty acids are obtained from plasma lipoprotein triacylglycerols by the action of lipoprotein lipase. Saturated fatty acids may be converted to monounsaturated fatty acids by stearoyl CoA desaturase. Triacylglycerols are hydrolyzed by the action of hormone-sensitive lipase; the glycerol and some fatty acids so released pass into the blood, while some fatty acids may be re-esterified. Rates of synthesis and hydrolysis (lipolysis) of triacylglycerols vary with adipocyte size; both occur simultaneously, so whether there is net accretion or loss of lipid depends on the relative rates of these two processes (Vernon, 1992).

Acute, homeostatic control

Insulin is the most important acute anabolic regulator of adipose tissue metabolism, promoting lipogenesis by a variety of mechanisms including increased glucose uptake, activation of key lipogenic enzymes (eg acetyl CoA carboxylase), and secretion of lipoprotein lipase activity; insulin

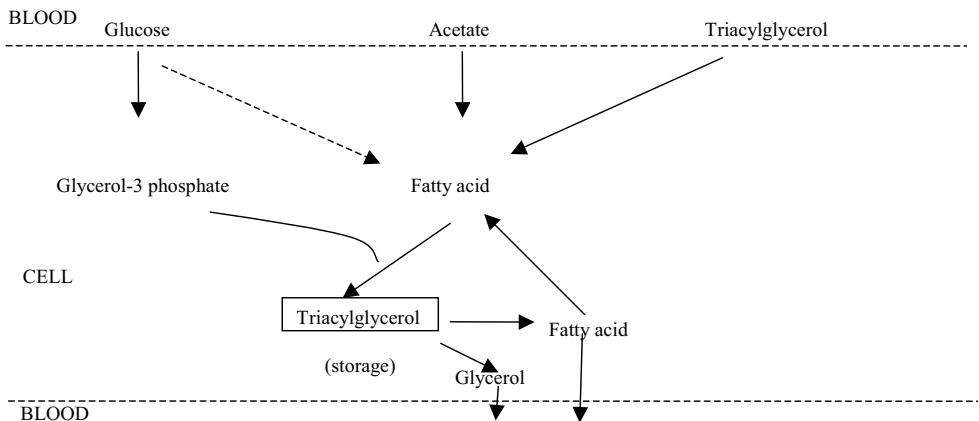


Figure 1. Pathways of lipid metabolism in ruminant adipocytes.

also inhibits lipolysis (Vernon, 1992). In addition to its acute effects, insulin also has chronic anabolic effects, promoting synthesis of key lipogenic enzymes including acetyl CoA carboxylase and lipoprotein lipase. Following binding to its receptor, insulin activates a branching cascade of signalling pathways (Vanhaesebroeck & Alessi, 2000; Saltiel & Kahn, 2001; Khan & Pessin 2002) - a much simplified version of which is shown in figure 2.

With respect to metabolism, most effort is still focussed on identifying pathways involved in the control of glucose transport by insulin, with surprisingly little work on other steps in the control of lipogenesis. However, both acute and chronic effects of insulin on the lipogenic and lipolytic pathways are thought to involve phosphoinositide-3 kinase and protein kinase B. This has been shown for sheep adipose tissue, as wortmannin, an inhibitor of phosphoinositide-3 kinase, prevented the induction of lipogenesis and an increased in acetyl CoA carboxylase activity and mRNA concentration by insulin (Barber M.C., Travers, M.T. & Vernon, R.G. unpublished observation). Protein kinase B is another a key branch point, with pathways transmitting the insulin signal to protein synthesis and glycogen synthesis as well as the anti-apoptotic effect of insulin (Vanhaesebroeck & Alessi, 2000). Further steps from protein kinase B involved in lipid metabolism are not known. Protein kinase C zeta and the related isoform iota/lamda appear to be involved in metabolic signalling by insulin, but their precise role, and downstream events are again

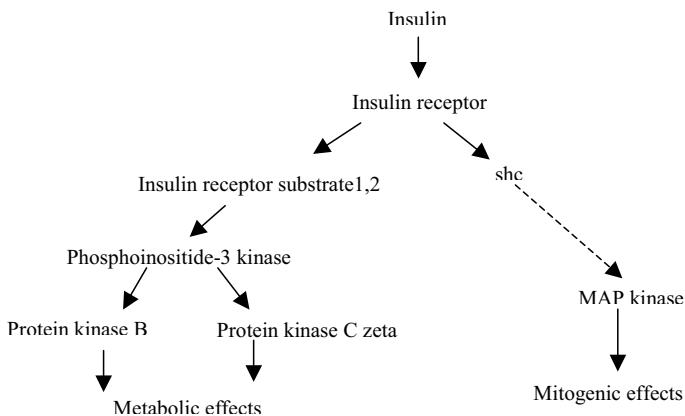


Figure 2. Insulin signalling pathways.

unknown (Khan & Pessin, 2002). Recent studies have shown that there is a second signalling pathway involved in insulin stimulation of glucose transport. This involves phosphorylation of a protein, c-Cbl by the insulin receptor; c-Cbl then binds with CAP, flotillin, (a protein associated with lipid rafts in the plasma membrane) and TC10, a protein implicated in insulin stimulation of glucose transport membrane (Litherland *et al.*, 2001; Khan & Pessin 2002).

Lipid synthesis is also subject to acute, autocrine control by acylation-stimulating protein (ASP) (Cianflone *et al.*, 2003). Adipocytes secrete several proteins (factors B, C3 and D; the latter is also known as adipasin) of the alternative pathway of complement production. Factors B and C3 bind to chylomicrons, which allows proteolytic cleavage of C3 by adipasin to produce factor C3a. Subsequently the terminal arginine residue is removed by the action of a carboxypeptidase to yield ASP. ASP binds to adipocytes to promote fatty acid esterification and also glucose uptake (Cianflone *et al.*, 2003). This system is likely to be of greatest importance for animals consuming diets rich in fat, but a recent study shows that ASP increased esterification slightly in bovine adipocytes (Jacobi & Miner, 2002).

The catecholamines, noradrenaline (released from nerve terminals in adipose tissue) and adrenaline (released from the adrenal medulla) in response to sympathetic activity, act acutely to inhibit lipogenesis and stimulate lipolysis (Vernon, 1992). Lipolysis is initiated by hormone sensitive lipase, although there is now evidence for the involvement of a second system (Saltiel 2000). The lipolytic effects of catecholamines are modulated by locally-produced factors such as adenosine and prostaglandin E (Vernon, 1996a). Signalling systems involved have been largely elucidated, at least with respect to the activation of hormone sensitive lipase by phosphorylation (Figure 3). Prostaglandin E, acting via its own receptor, inhibits lipolysis by the same mechanism as adenosine. Interestingly, microdialysis studies with sheep adipose tissue *in vivo*, showed that prolonged stimulation with noradrenaline increased release of prostaglandin E, with a concomitant decrease in lipolysis (Thompson & Vernon, 1992). In addition to activation, the lipolytic signal may cause translocation of hormone-sensitive lipase from the cytosol to the fat droplet (Londos *et al.*, 1999). This is thought to involve perilipin, a protein located on the surface of the fat droplet,

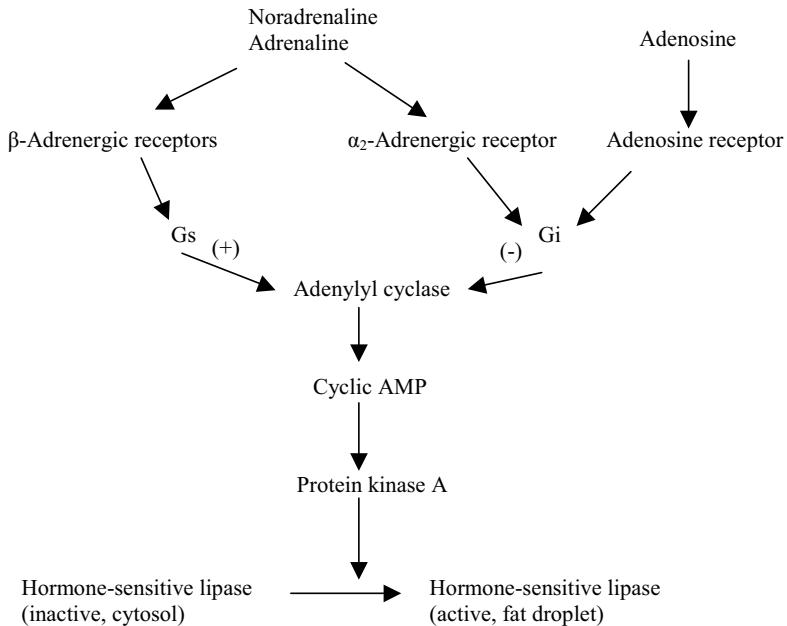


Figure 3. Regulation of hormone-sensitive lipase activity.

which is also phosphorylated in response to lipolytic signals (Londos *et al.*, 1999). Perilipin appears to act as a barrier to lipolysis (Brasaemle *et al.*, 2000), indeed, transgenic mice, lacking perilipin, fail to accumulate triacylglycerol (Martinez-Botas *et al.*, 2001).

Lipolysis is also influenced by blood flow (Vernon & Houseknecht, 2000). Fatty acids are removed from adipose tissue bound to serum albumin, which has two high-affinity binding sites for fatty acids (Vernon & Clegg, 1985). Serum albumin concentration is about 0.5mM and blood flow through adipose tissue about 20-50 µl/min per g tissue. Thus the maximum rate of lipolysis, if both binding sites are free, is only 50 nmol/min per g tissue (significantly less than found with isolated adipocytes *in vitro*) (Vernon & Clegg, 1985). Interestingly, catecholamines, adenosine and prostaglandin E, which regulate lipolysis are also vasoactive (Crandall *et al.*, 1997; Vernon & Houseknecht, 2000). Catecholamines initially cause vasoconstriction in adipose tissue, but then cause vasodilation, facilitating the removal of fatty acids from the tissue. Adenosine and prostaglandin E inhibit lipolysis and are also vasodilatory, so may be involved in the rapid suppression and removal of fatty acids from adipose tissue following a lipolytic stimulation (Vernon & Houseknecht, 2000).

Chronic, homeorhetic control

The homeostatic controls which modulate adipose tissue metabolism, are subject to chronic, homeorhetic control to meet the needs of the particular physiological state (Bauman, 2000). For example, during lactation, there are a series of adaptations in a variety of tissues, to ensure the preferential use of nutrients by the mammary gland (Bauman, 2000). Lactation usually results a fall in lipogenesis in adipose tissue around parturition when there is a rise in lipogenesis in the mammary gland (Vernon & Pond, 1997; Barber *et al.*, 1997). These adaptations involve reciprocal changes in the expression of key lipogenic enzyme genes and also reciprocal changes in the activation state of enzymes such as acetyl CoA carboxylase in the two tissues.

Homeorhetic adaptations also include changes in the intracellular signalling systems that regulate metabolism. Thus in adipocytes the ability of insulin to stimulate lipogenesis is diminished during lactation (Vernon & Pond, 1997; Bauman, 2000). The molecular basis of, and the factor responsible for, this impairment are not known. Insulin activation of protein kinase B appears to be unimpaired in adipose tissue from lactating sheep, but there is some loss of protein kinase C zeta (Vernon & Finley, 1999). Lactation causes increased lipolytic response of adipocytes to β-adrenergic agents, which should facilitate the mobilisation of adipose tissue lipid for use by the mammary gland (Vernon & Pond, 1997; Bauman, 2000). Both increased numbers of β-adrenergic receptors and an increased amount of adenylyl cyclase have been implicated in this adaptation (Vernon & Pond, 1997). Paradoxically, there is also an increased response to the antilipolytic effect of adenosine during lactation; the physiological advantage of this is still uncertain and the molecular basis unresolved.

The best described homeorhetic modulator of adipocyte metabolism is growth hormone (GH), reflecting its extensive use in the USA and elsewhere to promote milk production (Bauman & Vernon, 1993). GH also decreases adipose tissue accretion and enhances muscle deposition (Etherton & Bauman, 1998). Serum GH increases during early lactation in ruminants and facilitates the partitioning of nutrients between adipose tissue and the mammary gland (Bauman & Vernon, 1993). GH exerts its effects on adipose tissue metabolism both by altering the amount of key metabolic enzymes and by altering the signalling ability of systems which mediate the acute effects of homeostatic hormones (Bauman & Vernon, 1993; Etherton & Bauman, 1998). GH attenuates both the increase in acetyl CoA carboxylase and fatty acid synthase gene expression induced by insulin in adipose tissue and decreases the ability of insulin to activate acetyl CoA carboxylase (Bauman & Vernon, 1993; Etherton & Bauman, 1998). GH also decreases lipoprotein lipase in adipose tissue (de la Hoz & Vernon, 1996). In vitro studies suggest that GH also has a direct effect on lipogenesis independent of insulin (Vernon, 1996b). Chronic treatment of pigs

with GH did not prevent the response to an insulin challenge on lipogenesis in vivo, but it did reduce sensitivity to insulin (that is, it reduced response to a sub-maximum dose of insulin) (Etherton & Bauman, 1998). Chronic exposure of sheep adipose tissue to GH in vitro did not prevent an initial response to an insulin challenge, but it did prevent a sustained response, due to an impairment at or before, the level of protein kinase B (figure 2) (Vernon R.G. & Finley E., unpublished observation). By contrast, insulin activation of protein kinase B does not appear to be impaired by lactation (Vernon & Finley, 1999). Thus while GH is probably responsible for some changes in adipose tissue during lactation, other factors must also be involved.

GH enhances lipolysis by enhancing signalling through the adrenergic signalling cascade, primarily by decreasing signalling by antilipolytic factors such as adenosine and prostaglandin (figure 3) (Vernon, 1996a; Etherton & Bauman, 1998). In sheep adipose tissue GH impairs the interaction between Gi and adenylyl cyclase (figure 3) (Doris *et al.*, 1998) and also increases the number of β -adrenergic receptors, thus increasing sensitivity to catecholamines (Vernon 1996a). GH may thus be the factor responsible for the increase in β -adrenergic receptor number and the increased response and sensitivity of adipocytes to catecholamines during lactation. By contrast, the increase in serum GH cannot be the cause of the enhanced response to adenosine during lactation. There is at least one further mechanism, as GH decreases the release of prostaglandin E by sheep adipose tissue following a catecholamine challenge (Doris *et al.*, 1996). Prolonged high levels of GH in the blood thus modify adipose tissue metabolism to favour lipolysis rather than lipogenesis.

Glucocorticoids, sex steroids and also thyroid hormones are thought to have chronic effects on adipose tissue metabolism, but there is not much information for these hormones, especially for ruminants (Vernon, 1992). The glucocorticoid analogue, dexamethasone, for example, modulates insulin action in sheep adipose tissue in vitro, antagonising the effects of low concentrations of insulin on lipogenesis, but accentuating the lipogenic effects of high concentrations of insulin (Vernon 1992). Dexamethasone also enhanced the lipolytic response to catecholamines and enhanced antilipolytic response to adenosine (Vernon, 1996a); increased serum glucocorticoid concentrations could thus contribute to the increased lipolytic response to catecholamines, and enhanced response to adenosine during lactation.

While the obvious sex differences in overall adiposity, and depot specific differences in adipocyte size and lipogenic activity between males and females suggests a role for sex steroids, probably androgens (Robeline, 1986; Eguinoa *et al.*, 2003), attempts to demonstrate direct effects using in vitro systems have been unsuccessful. This suggests that the sex steroids may exert their effects on adipocytes indirectly. However, rodent adipocytes rodents at least have sex steroids receptors (Rebuffe-Scrive *et al.*, 1989), so direct effects are possible.

Autonomic control

The chronic, homeorhetic adaptations are designed to adjust adipose tissue metabolism to meet the needs of other systems of the body. In addition there are other mechanisms that control the amount of adipose tissue which I have called ‘autonomic’. Adipocytes can vary in volume from a few picolitres to a few nanolitres, but they cannot enlarge indefinitely (Vernon, 1992). Lipogenic capacity and the rate of lipolysis vary with adipocyte size, but the anabolic response to insulin diminishes as adipocytes enlarge which is probably why average rates of synthesis and breakdown of triacylglycerol eventually become equal and net accretion stops (Vernon, 1992). Adipocyte number is also under complex endocrine and local control (Scanes 2003), but the systems are readily subverted, hence in obese individuals adipose tissue mass can exceed 50% body mass. The ultimate determinant of adiposity of course, is the long-term balance between energy intake and expenditure.

In contrast to homeorhetic control, which is achieved via hormones from outwith the tissue, autonomic control is achieved via factors produced within adipose tissue, which may act locally

or as hormones. Indeed adipose tissue secretes a plethora of factors (Table 1) (Frübeck *et al.*, 2001; Vernon *et al.*, 2001; Guerre-Millo, 2002), the number increasing annually. For some (eg leptin, adiponectin) secretion rate varies with depot (Wajchenberg *et al.*, 2002). While roles of a few are now quite well understood, for others their function is still unclear. For some, including leptin, tumour necrosis factor (, resistin and adiponectin, secretion rate varies with adiposity, suggesting a possible autonomic role in the maintenance of adipose tissue mass (Vernon *et al.*, 2001). Leptin, arguably the long postulated lipostat, discovered by Friedman and colleagues (Zhang *et al.*, 1994), appears to be the most important of these.

Table 1. Some substances secreted by adipose tissue.

| | | |
|--|------------------------------|------------------------------------|
| Fatty acids | Leptin | Complement System |
| Prostaglandin E ₂ | Resistin | Factor B |
| Prostacyclin (Prostaglandin I ₂) | Tumor necrosis factor (| Factor C3 |
| Monobutyryl | Interleukin -1 & -6 | Factor D (adipsin) |
| Angiotensinogen/Angiotensin II | Adiponectin (AdipoQ, Acrp30) | Acylation-stimulating protein |
| Atrial natriuretic peptide | IGF-I | IGF-binding proteins |
| Plasminogen activator inhibitor-1 | Oestrone | Cholesterol ester transfer protein |
| | Oestradiol | |
| Lipoprotein lipase | Testosterone | Retinol binding protein |

Leptin in the circulation is mostly secreted by adipocytes, and acts on hypothalamic neurones to decrease appetite (Ahima & Flier, 2000; Frübeck *et al.*, 2001; Williams *et al.*, 2001; Vernon *et al.*, 2001). In the fed state, serum leptin concentration varies directly with the amount of adipose tissue in the body (Friedman & Halaas, 1998). Animals lacking leptin or its receptor overeat and become very obese, while administration of leptin can decrease appetite (Friedman & Halaas, 1998; Ahima & Flier, 2000). Such observations suggest leptin can act as an adipostat. However, leptin is only one of many factors regulating feeding, and in the fed state it probably acts as a restraint, attenuating the orexigenic effects of other agents. Thus in rats, which normally eat at night, the nocturnal feeding is paralleled by an increase in serum leptin; this increase in leptin cannot be the cause, but could be the consequence, of the increased food intake (Vernon *et al.*, 2002). Interestingly, during lactation in rats (which are hyperphagic, but continue consuming most food at night), the nocturnal increase in leptin is markedly attenuated; this is consistent with a restraining role in the fed state which is diminished during lactation, facilitating the increased intake (Vernon *et al.*, 2002).

In addition to its effects on appetite, leptin can enhance energy expenditure via sympathetic nervous stimulation of brown adipose tissue thermogenesis (Ahima & Flier, 2000; Vernon *et al.*, 2001; Williams *et al.*, 2001). Leptin secretion is decreased by fasting (Ahima, 2000) and during periods of negative energy balance (eg early lactation in ruminants) (Vernon *et al.*, 2002). Leptin increases thyroxine secretion, which should increase energy expenditure (Flier *et al.*, 2000). Indeed leptin reverses the effects of fasting on the secretion of various pituitary hormones, including GH and the gonadotropins as well as thyrotropin (Ahima, 2000; Ahima & Flier, 2000). Leptin thus appears to be an important signal of too little adipose tissue lipid and loss of such lipid. Leptin is also seen as having a key role in the adaptations to under-nutrition which are designed to increase metabolic efficiency and hence spare the use of energy and other reserves, so increasing survival time (Ahima, 2000; Flier *et al.*, 2000; Vernon *et al.*, 2001).

Leptin may also have direct effects on rodent and human adipocytes (increased lipolysis and fatty acid oxidation, and decreased glucose transport and lipogenesis) (Reidy & Weber, 2000), but others have failed to find any effects (Vernon *et al.*, 2001). However, ovine leptin had no effect on

sheep adipocytes in vitro, other than a weak, insulin- antagonistic effect on lipogenesis, but this was only achieved when using high, non-physiological concentrations of leptin (Newby *et al.*, 2001).

Obesity results in the increased production of tumour necrosis factor α (TNF α) by adipose tissue which does have direct effects on adipocyte metabolism (Sethi & Hotamisligil, 1999; Bulló-Bonet *et al.*, 1999; Vernon *et al.*, 2001). TNF α antagonizes the effects of insulin and enhances lipolysis in rodent adipocytes, which should slow net lipid accretion. In addition, it inhibits preadipocyte differentiation, but this effect can not resist the pressure of sustained overeating. Treatment of cattle with TNF α in vivo caused insulin resistance and increased blood fatty acid levels (Kushibiki *et al.*, 2001a, 2001b, 2002, 2003), and a relationship between serum TNF α , insulin resistance and fatty liver was found during lactation in cows (Ohtsuka *et al.*, 2001). We have found a weak (compared to GH), insulin-antagonistic effect of TNF α on lipogenesis, but no effect on lipolysis, in sheep adipose tissue in vitro (Melrose, S.E., Finley, E. & Vernon, R.G., unpublished observations). TNF α thus has similar effects on adipocyte metabolism as GH. The insulin antagonistic effects of TNF α in rodent adipocytes, is thought to be due to serine phosphorylation of insulin receptor substrate-1, rendering it an inhibitor of insulin receptor kinase activity (Sethi & Hotamisligil, 1999). As noted, GH also acts on an early stage in insulin signalling pathway. We recently explored the possibility that the insulin-antagonistic effects of TNF α and GH might be additive, but surprisingly TNF α attenuated the inhibitory effect of GH on both lipogenesis and also protein kinase B phosphorylation in sheep adipose tissue (Vernon, R.G. & Finley, E., unpublished observation). The molecular basis of the lipolytic effect of TNF α in rodent adipocytes is uncertain, but like GH it can attenuate the antilipolytic effect of adenosine (Vernon *et al.*, 2001). However, in rodents TNF α decreased the amount of the inhibitory, GTP-binding protein, Gi, whereas in sheep GH prevents the association of the activated alpha subunit of Gi with adenylyl cyclase (Vernon *et al.*, 2001).

Resistin, another peptide hormone secreted by adipocytes, which could be involved in the autocrine control of adiposity, as in rodents it acts directly (ie in an autocrine fashion) on adipocytes, antagonising the actions of insulin (Steppan & Lazar, 2002). Initial studies in rodents suggested that serum concentration of resistin increased with obesity providing a link between obesity and insulin resistance; furthermore, administration of exogenous resistin increased, while an antibody against resistin decreased, insulin resistance in vivo (Steppan & Lazar, 2002). However, other studies found that resistin mRNA levels in adipocytes and serum resistin decreased during obesity; the reason for these discrepancies is not clear. Studies with human adipose tissue suggest that resistin is produced primarily by stromal-vascular cells rather than adipocytes; also resistin levels do not vary with adiposity in humans (Hotamisligil, 2003; Guerre-Millo, 2002). Nothing appears to be known about resistin in domestic species.

Even less is known about the autocrine/paracrine effects of other peptides secreted by adipocytes. Adiponectin (also called AdipoQ or Acrp30) has endocrine effects (below), but mice lacking adiponectin show increased TNF α secretion by adipocytes and decreased clearance of fatty acids from the blood (Maeda *et al.*, 2002), suggesting possible direct effects on adipocytes. Plasminogen activator inhibitor type-1(PAI-1), secretion of which by adipose tissue increases with obesity, is implicated in the development of vascular problems associated with obesity (Guerre-Millo, 2002). Lack of PAI-1 decreases adiposity in mice and also reduces TNF α secretion by adipose tissue (Schäfer *et al.*, 2001), while a recent study suggests PAI-1 antagonises insulin action in a fibroblast-derived cell-line (López-Alemany *et al.*, 2003). Thus PAI-1 may also have direct effects on adipocyte function. Whatever, it is now clear that there are a number of locally produced factors which modulate adipose tissue metabolism either directly or indirectly (figure 4). Importantly, several act to diminish response to insulin, which should act to slow down the rate of fat accretion.

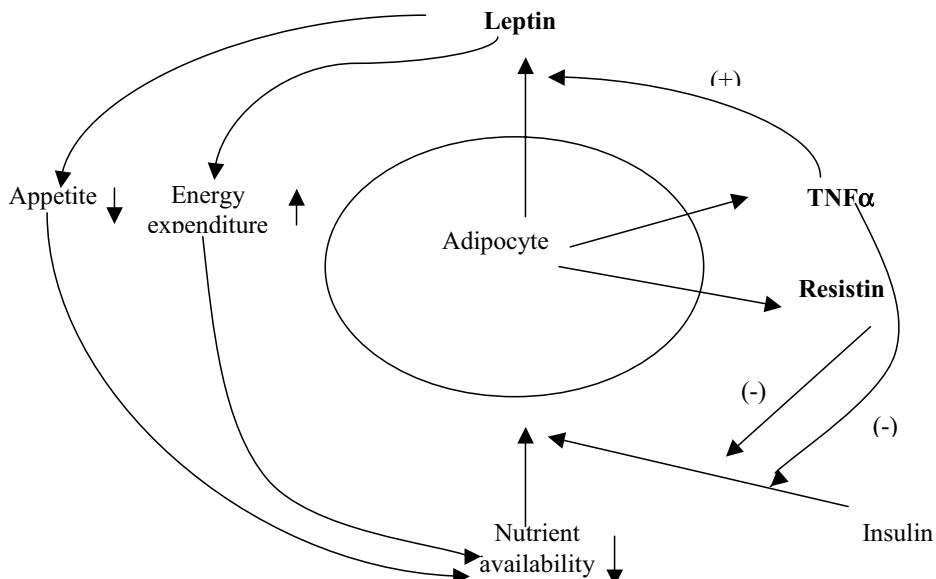


Figure 4. Factors regulating adiposity.

Adipose tissue interactions with other organs

Factors released by adipose tissue have both paracrine and endocrine effects on other organs of the body. Several tissues are in intimate contact with adipose tissue and so are susceptible to paracrine modulation, and could also benefit from an immediate source of fatty acids for oxidation. The mammary gland requires a bed of adipose tissue for early development at least; the reason for this is still not resolved, but insulin-like growth factor 1, prostaglandins and extracellular matrix components released by adipose tissue have been implicated (Vernon & Houseknecht, 2000). It has also been suggested that leptin is needed for mammary differentiation during pregnancy in the mouse (Chehab 2000), but our recent studies show that the absence of leptin delays, but does not prevent, the onset of copious milk production at parturition (Knight *et al.*, 2002). Leptin does, however, influence fertility as it enhances secretion of gonadotropins by the pituitary gland and may also have direct effects on ovarian cells (Vernon *et al.*, 2001; Chehab, 2000). Low levels of leptin may contribute to the delay in the restoration of oestrous in very thin cows (Liefers *et al.*, 2003). Reproduction imposes considerable energy demands on the mother, so a mechanism restricting fertility when body reserves are low is advantageous. Lymph nodes are located in adipose tissue depots (often smaller depots) (Pond, 2003). In guinea pigs the lipolytic responsiveness of adipocytes and also the fatty acid composition of their constituent triacylglycerols varies with distance from the lymph node; this is thought to arise from release of cytokines by the lymph nodes Pond, 2003). However, we found no evidence for such a relationship in sheep popliteal adipose tissue (Melrose, S.E. & Vernon, R.G., unpublished observations). Leptin is also required for the development and function of T cells of the immune system (Lord *et al.*, 1998; Vernon & Houseknecht 2000); mounting an immune response also requires a considerable drain on energy supply. Intriguingly, the heart has a cap of adipose tissue, which is present even in the foetus in sheep (Marchington *et al.*, 1989); the role of this depot is unknown but it could well be needed for cardiac development or function.

In addition to effects noted above, leptin has a further interesting function in energy metabolism, acting directly (ie not via the hypothalamus) to restrict triacylglycerol accumulation in muscle and

other cells (excluding adipocytes of course); this is achieved in part at least by stimulation of fatty acid oxidation (Unger, 2000). Thus it would appear that with the evolution of the adipocyte, a specialist lipid storage cell, a mechanism developed to limit triacylglycerol accumulation in other cell types. Some triacylglycerol can be stored in the liver, but when the amount exceeds 5-10% of liver mass (as can happen during early lactation in cows) it can have deleterious effects on liver function leading to ketosis (Grummer, 1993; Drackley *et al.*, 2001). Similarly muscle has retained some ability to store triacylglycerols in mammals, but increasing amounts of lipid is associated with a diminished responsiveness to insulin, and can easily lead to the onset of non-insulin dependent (type 2) diabetes (Shulman, 2000; McGary 2002). The reason why adipocytes can store such large quantities of fat is not unknown, but probably relates to the type of protein network around the lipid droplet (eg presence of perilipin). The hypolectinaemia of lactation may thus contribute to increase triacylglycerol levels found in liver and muscle, and hence to the decreased responsiveness of muscle to insulin (Vernon *et al.*, 2002). The latter probably benefits lactation, as it should favour glucose utilization by the mammary gland.

Leptin is not the only hormone released by adipocytes modulating liver and muscle metabolism. Adiponectin enhances the ability of insulin to decrease hepatic glucose production, and may also increase fatty acid oxidation in muscle (Stefan & Stumvoll, 2002). Adiponectin differs from most other peptide hormones secreted by adipocytes in that its concentration falls with obesity (Stefan & Stumvoll, 2002). Serum adiponectin also decreases during pregnancy and lactation in mice, and this may contribute to the diminished response to insulin in these states (Combs *et al.*, 2003). By contrast to adiponectin, resistin (serum concentration increased by obesity) attenuates the ability of insulin to suppress hepatic glucose production in rodents (Steppan & Lazar, 2002; Hotamisligil, 2003). Whether resistin affects muscle metabolism in rodents is not clear, but in humans it is not thought to cause insulin resistance, which implies it is not effecting insulin stimulated glucose uptake in muscle (Steppan & Lazar, 2002; Hotamisligil, 2003). TNF α is also thought to contribute to the insulin resistance associated with obesity (Bulló-Bonet *et al.*, 1999; Vernon *et al.*, 2001; Frübeck *et al.*, 2001), but while it antagonizes insulin action in adipocytes, it is not clear if it has direct action on muscle. In addition to these peptide hormones, fatty acids released by lipolysis not only provide a fuel for tissues such as muscle and liver, they also diminish glucose utilization by muscle (Shulman, 2000; McGary 2002) and may promote glucose production by liver (Lam *et al.*, 2003). Effects on muscle were initially thought to involve inhibition of pyruvate dehydrogenase activity by acetyl CoA derived from fatty acid oxidation, but more recent studies show that fatty acids also impede glucose uptake by inhibiting insulin signalling, probably at the level of protein kinase B (Shulman, 2000). Are there other signals? Probably. Selective knockout of Glut 4 in adipocytes decreased insulin sensitivity in muscle and liver; this was not due to any obvious changes in serum leptin, TNF α or fatty acids (Abel *et al.*, 2001). Surgical removal of perirenal and epididymal (but not mesenteric) adipose tissue from the abdominal cavity increased whole body and hepatic insulin sensitivity; again this does not appear to be due to changes in serum fatty acid levels, but leptin and TNF α secretion by subcutaneous adipose tissue were diminished (Barzilai *et al.*, 1999; Gabriely *et al.*, 2002).

Adipose tissue thus communicates with liver and muscle, the original sites of lipid storage in vertebrates, in several ways. Mechanisms (eg leptin) operate to direct fatty acid storage to adipocytes, which normally ensure that triacylglycerol levels are kept low in other tissues. Adipocytes are specially equipped to accumulate large amounts of triacylglycerols, serving both as an energy store and as a sink for surplus fatty acids, ensuring that blood levels are normally low. Short-term fluctuations in blood fatty acid levels are not damaging, and even more sustained periods of elevation as occur during early lactation, are not necessarily injurious; while fat will accumulate in liver and muscle, in part due to hypolectinaemia, this is normally lost as lactation progresses. Likewise the accumulation of fat prior to the winter in some species does not appear to be injurious, probably as it is again transient. On the other hand, sustained over-eating leading to obesity, can have damaging effects on liver, muscle and other tissues of the body, both due to

the sheer mass of adipose tissue resulting in sustained, elevated levels of fatty acids in the blood and changes in secretion of various hormones (TNF α , PAI-1, resistin etc). In addition, cells appear to become insensitive to leptin during obesity. Hormones such as TNF α probably have a role in preventing the development of obesity, inhibiting the lipogenic effects of insulin on adipocytes. If such signals are ignored, then useful physiological agents can have pathological effects, impairing the normal functioning of muscle and other tissues. However, while effects of some adipose tissue hormones on other tissues may occur by accident, others such as leptin and adiponectin, clearly have important beneficial, endocrine roles in the non-obese.

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On-line, continuous determination of $^{13}\text{CO}_2$ / $^{12}\text{CO}_2$ ratios by non-dispersive infrared absorption in indirect calorimetry facilities

S.J.J. Alferink, J.J.G.C. van den Borne, W.J.J. Gerrits, S.C.W. Lammers-Wienhoven & M.J.W. Heetkamp

Wageningen Institute of Animal Sciences (WIAS), Wageningen University, P.O. Box 388, 6700 AH Wageningen, The Netherlands

Summary

The objective of this study was to implement a cost effective, labour saving method for on-line measurement of $^{13}\text{CO}_2$ concentrations based on Non Dispersive InfraRed absorption (NDIR). For this purpose, two NDIR gas-analysers were used for measuring total CO_2 and $^{13}\text{CO}_2$ concentrations. The analysers were connected to an automated data-acquisition system where inflowing and outflowing air of the respiration chambers is analysed in rotation. Conversion of the signal from these analysers to $^{13}\text{CO}_2$ concentrations and ^{13}C enrichment was done as follows: (1) Calibration of the units was performed with gas mixtures having a high and low known CO_2 , $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ concentration. (2) To correct for overlapping absorption spectra of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$, six different gas mixtures containing 0% to 1.29% pure $^{12}\text{CO}_2$ were used. Preliminary data of an experiment where a single dose of 300 mg of U- ^{13}C -glucose was fed to two preruminant calves was analysed and compared with Isotope Ratio Mass Spectrometry (IRMS) data of parallel samples. These data showed a very good correlation of IRMS and NDIR $^{13}\text{CO}_2$ enrichment. Based on experiments with 150 kg preruminant calves fed diets differing in natural ^{13}C enrichment the system showed that differences of one delta (δ) can be detected. This allows for the quantification and measurement of the kinetics of labelled substrate oxidation in indirect calorimetry facilities.

Keywords: *indirect calorimetry, nutrient oxidation, NDIR*

Introduction

In metabolism studies ^{13}C enriched substrates are often used as tracers to enhance knowledge on substrate kinetics and utilisation. Depending on the substrate, isotope labelled oxidation products can appear in the exhaled air. There are many medical applications that use $^{13}\text{CO}_2$ measurement, for example ^{13}C -labelled urea is used for diagnosis of *Helicobacter pylori* infections by measurement of $^{13}\text{CO}_2$ in exhaled air (Braden et al. 1994). In breath tests usually only a small number of samples of exhaled air over a small time span is analysed by either non-dispersive infrared absorption (NDIR) or isotope ratio mass spectrometry (IRMS). In these tests, total production of labelled and unlabelled oxidation products is unknown. IRMS analysers are very expensive and/or not suited for on-line continuous measurement. A high quality vacuum and sophisticated purification system for the sample gas is needed. Long time collection and analysis of a great number of samples by IRMS is very labour intensive (Braden et al. 1994).

The NDIR analyser techniques used for measurement of the ^{13}C labelled oxidation products are widely used in medical applications and are described by Wagner et al. (2000). For medical applications, mainly synthetically enriched substrates are used. The isotope concentration is measured directly in the exhaled air that has a high concentration of the oxidation product.

The combination of open circuit climate and respiration facilities and accurate on line NDIR measurement of $^{13}\text{CO}_2$ opens the way to continuous and quantitative determination of labelled oxidation products. This may help in making the step from measurement of heat production by indirect calorimetry to quantification and measurement of kinetics of substrate oxidation.

Substrates that are naturally enriched in ^{13}C have low isotope enrichment compared to synthetically ^{13}C enriched substrates. To be able to determine labelled oxidation products of these naturally enriched substrates in the outgoing air of respiration chambers it is necessary to detect differences of one to two δ values at low CO_2 concentrations (0.2% -1.0%).

Material and methods

To study and quantify kinetics of substrate oxidation and relate them to indirect calorimetry data an Advance Optima Uras-14 NDIR analyser system (ABB automation analytical division, Frankfurt a. M. Germany) for separate analysis of total CO_2 and $^{13}\text{CO}_2$ was coupled to existing open circuit indirect calorimetry facilities. This new system was integrated in the automated data-acquisition system where incoming and outgoing air of sets of 2 respiration chambers are analysed in 6 minute intervals (Verstegen et al. 1987). The new analyser system was put in series with existing O_2 and CH_4 analysers. The flow of the sampled air was approximately 1 litre/min. The analysers were connected with short tubes to analyse the same air sample at a given time in all analysers. Besides the standard calorimetry data, self-developed computer software stored the rough percentages of CO_2 and $^{13}\text{CO}_2$ in a central database. These rough data can be used to calculate isotope enrichment and can be expressed as δ values.

The conversion from rough data to real $^{13}\text{CO}_2$ concentrations is based on a 3-step correction procedure. In **step 1**, two calibration gas mixtures (CAL1 and CAL2) are used for linear zero and span point corrections of all gases, also for $^{13}\text{CO}_2$. CAL1 contains 1.083 % CO_2 and 19.617 % O_2 in N_2 and CAL2 contains 0.000 % CO_2 , 21.008 % O_2 and 505 ppm CH_4 in N_2 (certified $\pm 0.5\%$ accuracy). In CAL1, analysed with IRMS as described by Boutton (1991), the ^{13}C atom % was 1.0776 % ($=117 \text{ ppm } ^{13}\text{CO}_2$) and $^{12}\text{CO}_2$ concentration was 1.071 %. Twice daily CAL1 and CAL2 were directed automatically through the analyser series and measured concentrations of all gases were stored. In later data correction this frequency was shown to be sufficient to correct for mechanic and electronic drift of the analysers.

Step 2 corrects $^{13}\text{CO}_2$, calculated in step 1, for overlapping infrared absorption spectra of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ in the NDIR $^{13}\text{CO}_2$ sensor. A calibration gas (CAL3) with 1.29 % $^{12}\text{CO}_2$ in O_2 and N_2 was used. This gas was free from $^{13}\text{CO}_2$. Monthly, CAL2 and CAL3 were manually blended to produce six gas mixtures gradually increasing in $^{12}\text{CO}_2$ between 0 % and 1.29 %. Based on measurements of total CO_2 and O_2 , the ratio of CAL2 to CAL3 was calculated to determine the precise concentration of $^{12}\text{CO}_2$. The relation of calculated $^{12}\text{CO}_2$ concentration and readings from the $^{13}\text{CO}_2$ analyser determined by linear regression of data from the 6 gas mixtures is given in formula 1.

$$^{12}\text{CO}_2 \text{ interference (ppm } ^{13}\text{CO}_2\text{)} = \% ^{12}\text{CO}_2 * 7.8243 + 0.0104 \quad (R^2=0.9996) \quad (1)$$

Repeated tests showed this interference to be very constant but this may be a characteristic of this analyser. All air samples were corrected for $^{13}\text{CO}_2$ concentration according to formula 2.

$$^{13}\text{CO}_2 \text{ step 2} = ^{13}\text{CO}_2 \text{ step 1} - ^{12}\text{CO}_2 \text{ interference} \quad (2)$$

The $^{12}\text{CO}_2$ concentration in formula 1 is calculated as total CO_2 % minus $^{13}\text{CO}_2$ % from step 1. The error occurring while using an interference uncorrected $^{13}\text{CO}_2$ from step 1 for calculating $^{12}\text{CO}_2$ % to be used in formula 1 is very minimal and therefore neglected.

In **step 3** a conversion of $^{13}\text{CO}_2$ % is performed because CAL1 contains $^{12}\text{CO}_2$. This means that after step 2 the $^{13}\text{CO}_2$ % is not correct because at span point (using CAL1) $^{13}\text{CO}_2$ is overestimated. This means that the CAL1 117 ppm $^{13}\text{CO}_2$ span point correction causes an underestimation. Therefore a linear correction depending on the $^{13}\text{CO}_2$ concentration compared to the concentration of $^{13}\text{CO}_2$ in CAL1 was performed according to formula 3.

$$^{13}\text{CO}_2 = ^{13}\text{CO}_2 \text{ step2} (1 + 8.39 / 117) \quad (3)$$

In **step 4**, for every 6-minute measurement of both chambers and incoming air, the $^{13}\text{CO}_2$ concentration from step 3, together with the total CO_2 concentration from step 1 is used to calculate

δ values according to formula 4. In this calculation the isotope enrichment is expressed against the PDB standard value of 0.0112372.

$$\delta = (\% \text{ } ^{13}\text{CO}_2 / (\% \text{ total CO}_2 - \% \text{ } ^{13}\text{CO}_2) - \text{PDB standard}) / \text{PDB standard} \times 1000 \quad (4)$$

Results and discussion

NDIR data of an experiment where a single dose of 300 mg of U- ^{13}C -glucose was fed to two 150 kg preruminant calves housed in separate climate respiration chambers was analysed and compared with IRMS data of 19 parallel samples. The levels of CO_2 in the sampled outflowing air of the climate respiration chambers ranged from 0.68% to 0.95% while $^{13}\text{CO}_2$ levels ranged from 0.007% to 0.010%. Figure 1 shows the ^{13}C enrichment measured in this experiment. Comparing the $^{13}\text{CO}_2$ enrichment of both calves analysed by NDIR and IRMS shows excellent agreement. With linear regression analysis a high linear correlation ($Y = 0.9831X - 0.0716$; $R^2 = 0.9962$) over a range of 26 δ was found (see figure 2).

In this experiment calves were fed a milk replacer diet with a $^{13}\text{CO}_2$ enrichment of -28.0 δ for calf A and -26.2 δ for calf B (triple IRMS analysis). The difference in ^{13}C enrichment between diets was established by choosing different protein sources and lactose originating from cows fed either a grass or a maize diet. Figure 3 shows the ^{13}C enrichment measured over one day of the outflowing air of the respiration chambers 15 days after first administration of the experimental

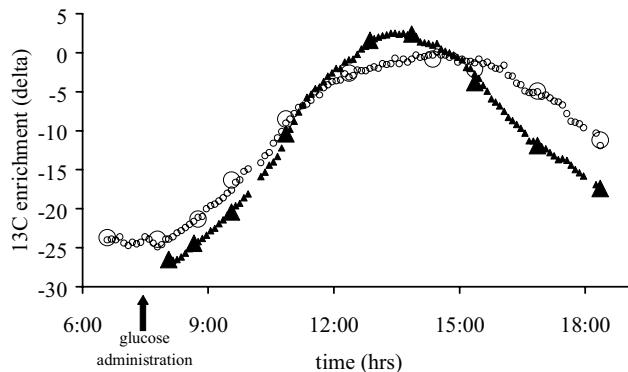


Figure 1. NDIR (calf A ○; calf B ▲) and IRMS (calf A ○; calf B ▲) ^{13}C enrichment in time after single ^{13}C -glucose administration.

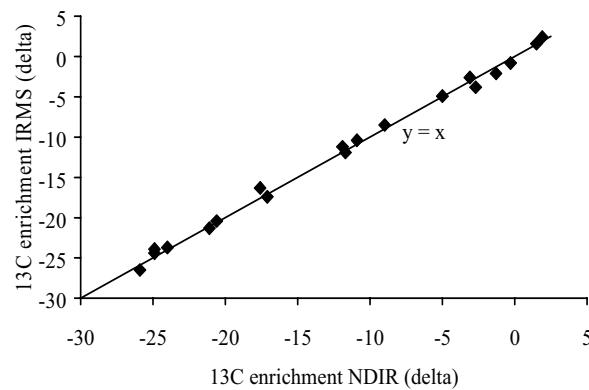


Figure 2. NDIR vs. IRMS ^{13}C enrichment after single ^{13}C -glucose administration.

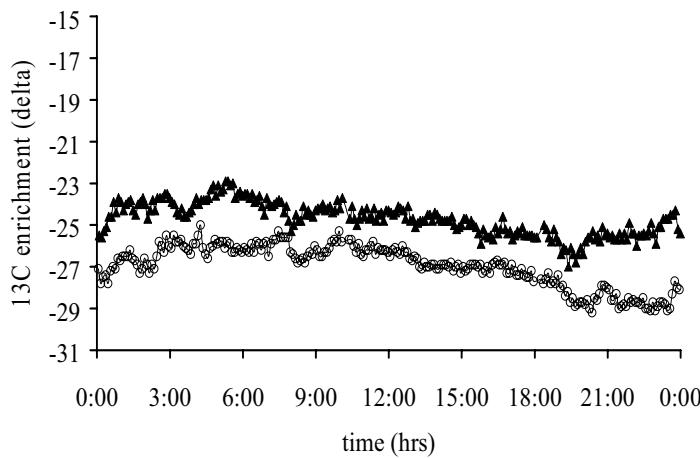


Figure 3. ^{13}C enrichment over day of outflowing respiration chamber air from calf A (○); calf (B ▲).

diets. The average (standard error) of ^{13}C enrichment over this day of calf A and B are -26.96 (0.07) and -24.68 (0.05) respectively.

Conclusions

We have found a very close relation between NDIR and IRMS techniques of determining $^{13}\text{CO}_2$ enrichment of outflowing respiration chamber air. The results presented in figure 1 and 3 show that the sensitivity of these measurements is around 1 δ . This allows for the quantification and measurement of the kinetics of labelled substrate oxidation in indirect calorimetry facilities.

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Lipogenesis in subcutaneous adipose tissue and in oxidative or glycolytic muscles from Angus, Black Japanese x Angus and Limousin steers

M. Bonnet¹, Y. Faulconnier¹, J.F. Hocquette¹, C. Leroux¹, P. Boulesteix², Y. Chilliard^{1,a} & D.W. Pethick³

¹ INRA, Unité de Recherches sur les Herbivores, Theix, 63122 Saint-Genès Champanelle, France

² UPRA France Limousin Sélection, Lanauad, France

³ Murdoch University, Division of Veterinary and Biomedical Sciences Perth, Australia 6150

^a corresponding author

Summary

The impacts of the breed and/or anatomical sites on enzyme activities linked to *de novo* lipogenesis and esterification were investigated. Samples of subcutaneous adipose tissue (SCAT), *rectus abdominis* (RA) and *semitendinosus* (ST) muscles were obtained from Limousin, Angus and Black Japanese x Angus steers to assay fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and glycerol-3-phosphate dehydrogenase (G3PDH) activities.

FAS ($P = 0.03$) and G6PDH ($P = 0.02$) activities expressed per mg of soluble protein were lower in SCAT, RA and ST from Limousin than from Angus and Black Japanese x Angus. Whatever the breed, FAS, ME, G6PDH activities were higher ($P < 0.0001$), and G3PDH activity was lower ($P < 0.0001$), in SCAT than in muscles. G3PDH activity was lower ($P < 0.05$) in the oxidative RA than in the glycolytic ST. Significant interaction between breed and anatomical sites were observed for FAS ($P = 0.01$), G6PDH ($P = 0.004$) and G3PDH ($P < 0.0001$) which indicated that (i): breed effect on FAS and G6PDH activities was only significant in SCAT, (ii): anatomical site differences for G3PDH activities were dependent on the breed, the decreasing rank order was SCAT < RA < ST in Angus and Black Japanese x Angus, and SCAT < RA = ST in Limousin steers.

Keywords: lipogenesis, adipose tissue, muscle, breeds, steers

Introduction

Quantitative and qualitative differences for fat deposition depending on anatomical sites and breeds have been reported in numerous studies of the bovine (for review see Kempster 1981; Bas & Sauvant, 2001). Differences in lipogenic and metabolic pathways between adipose tissues (AT) and skeletal muscles have been partly identified in the bovine (Howarth *et al.*, 1968; Vernon *et al.*, 1987; Belk *et al.*, 1987; Middleton *et al.*, 1998). However, the effect of breed on lipogenic pathways has mainly be addressed in AT from breeds known to deposit large quantities of intramuscular fat (Miller *et al.*, 1991; May *et al.*, 1994; Belk *et al.*, 1997). Hence, to further understand the key metabolic pathways of lipogenesis, we have measured lipogenic enzyme activities involved in *de novo* lipogenesis and fatty acid esterification in subcutaneous AT (SCAT), and in an oxidative and a glycolytic muscle, from three breeds of steers, two that accumulate AT readily (Angus and Black Japanese x Angus) and another that does not (Limousin).

Materials and methods

Twelve Limousin (23 months, 738 ± 35 kg), 10 Angus (23 months, 622 ± 40 kg) and 10 Black Japanese x Angus (28 months, 639 ± 30 kg) steers were slaughtered after a 6 month-finishing

period with a cereal-rich (70%) diet which allowed them to express their genetic potential for development of AT (see Hocquette *et al.* in the present symposium).

At slaughter, samples of SCAT, *rectus abdominis* (RA) and *semitendinosus* (ST) were placed in liquid nitrogen and stored at -80°C. Activities of enzymes involved in *de novo* lipogenesis (fatty acid synthase [FAS], glucose-6-phosphate dehydrogenase [G6PDH], malic enzyme [ME]) and in fatty acid esterification (glycerol-3-phosphate dehydrogenase [G3PDH]) were assayed as described by Chilliard *et al.* (1991). Enzyme activities were normalized by content of soluble proteins assayed in homogenates mainly according to Bradford (1976) using bovine serum albumin as standard and the Bio-Rad Protein Assay procedure (Bio-Rad, Marnes la Coquette, France).

Data were analysed using the MIXED procedure of SAS (1989). Fixed effects included breed (B), anatomical sites (S) and their interaction (B x S). Animal within breed was the random effect. When applicable, a multiple comparison of means was performed using the LSMEANS statement of MIXED procedure. Differences between breed and/or anatomical sites were considered to be significant when $P \leq 0.05$.

Results

De novo lipogenesis and esterification differed depending either on the breed, anatomical site or both (Figure 1).

Differences between breeds were observed for FAS and G6PDH activities, which were higher in tissues from Angus or Black Japanese x Angus than from Limousin steers (+ 28 %, $P = 0.03$ and + 41 %, $P = 0.02$ for FAS and G6PDH, respectively). Additionally, there was a significant interaction between breed and anatomical sites for FAS ($P = 0.01$) and G6PDH ($P = 0.004$). In SCAT only, FAS and G6PDH activities were higher ($P < 0.05$) in Angus (+13 % and +29 %, respectively), but lower ($P < 0.05$) in Limousin (-17 % and -19 %, respectively), relative to activities in Black Japanese x Angus steers.

Differences between anatomical sites were observed for the four enzymes. FAS, G6PDH and ME activities were 23-, 164-, 14-fold higher ($P < 0.0001$) in SCAT than in muscles. Conversely G3PDH activity was 39 % lower ($P < 0.0001$) in SCAT compared with muscles; and G3PDH activity was higher in the oxidative RA than in glycolytic ST (+28 %, $P < 0.05$). Furthermore, the effect of anatomical sites on G3PDH activity depended also on the breed ($P < 0.0001$ for breed by anatomical sites interaction). Compared to Limousin and Angus, G3PDH activity was lower in SCAT ($P < 0.05$) and higher ($P < 0.05$) in ST from Black Japanese x Angus. In RA, G3PDH activity was the highest ($P < 0.05$) in Limousin.

Discussion

We report here evidences that lipogenic activities involved in *de novo* lipogenesis and esterification differ between Limousin, Angus, Black Japanese x Angus and/or between AT, oxidative and glycolytic muscles.

Breed differences were observed for FAS and G6PDH activities in SCAT and for G3PDH activity both in SCAT and muscles. Such higher levels of FAS and G6PDH activities in "fat breeds" were already observed in SCAT from Angus compared either to Santa Gertrudis (Miller *et al.*, 1991) or Wagyu crossbreed (May *et al.*, 1994). To our knowledge, our results are the first highlighting a breed-related level of G3PDH which was also dependent on the anatomical sites. However, these differences for G3PDH do not seem to explain the relationship between breed and the ability to accumulate SCAT or intramuscular fat. Indeed, the rank order for G3PDH activity was Limousin = Angus < Black Japanese x Angus in ST and Limousin > Angus = Black Japanese x Angus in RA. The rank order for triglyceride contents was Limousin < Angus = Black Japanese x Angus both in RA (69 and 71 versus 15 mg/g fresh tissue) and in ST (22 and 18 versus 5 mg/g) muscles

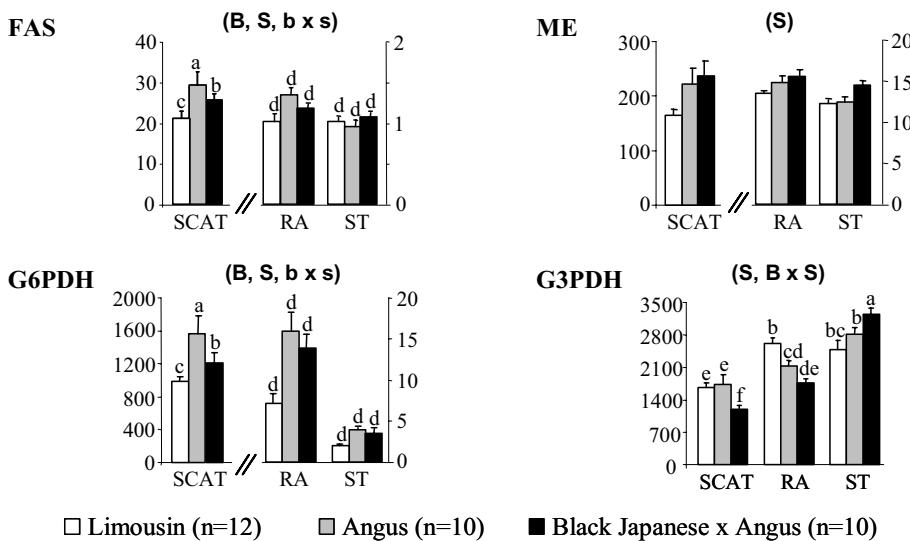


Figure 1. Activities (nmoles/min/mg protein) of fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and glycerol-3-phosphate dehydrogenase (G3PDH) in subcutaneous adipose tissue (SCAT), rectus abdominis (RA) and semitendinosus (ST) from Limousin, Angus or Black Japanese x Angus steers. Results are means \pm SEM. B, S: significant effect of breed or anatomical site ($P < 0.05$ or 0.0001 , respectively); b x s or B x S: significant interaction ($P < 0.01$ or $P < 0.0001$). Data with different superscript differ ($P < 0.05$).

(Jurie *et al.*, 2002). Although there is no clear relationship between G3PDH activity and triglyceride content of muscle, the contribution of G3PDH to marbling requires further study. Surprisingly, a negative correlation was observed in bovine skeletal muscle between lipids, triglyceride content and diacylglycerol acyltransferase activity also involved in esterification (Middleton *et al.*, 1998). Additionally to esterification, we could also consider fatty acid uptake, lipolysis and fatty acid oxidation to explain the great difference in muscle triglyceride content in the three studied breeds. Some of these metabolic pathways are reported by Hocquette *et al.* in the present symposium or remain to be studied.

Whatever the breed, we observed a great difference in lipogenic potential between SCAT and both oxidative and glycolytic muscles. The ratio of FAS, G6PDH and ME activities measured in SCAT to those found in muscles were about 23, 164 and 14 respectively. To our knowledge there is no such published work for FAS activity while published ratios for G6PDH were about 13 in steers (Belk *et al.*, 1997) and 55 for non-lactating sheep (Vernon *et al.*, 1987). Published ratio for ME was about 2 in sulking calves (Howarth *et al.*, 1968). Conversely to enzymes involved in *de novo* lipogenesis, G3PDH activity was 1.6-fold higher in muscles than SCAT, and was higher in the glycolytic than in the oxidative muscle types. The few data available in ruminants report a 2-fold higher activity in skeletal muscles than SCAT from sheep (Vernon *et al.*, 1987). All these results suggest that in muscles from steers, G3PDH is not only involved in triacylglycerol synthesis but also in the glycerol phosphate shuttle. This last metabolic pathway transfers the cytosolic NADH into the mitochondria during the ATP synthesis from glucose. This could explain the higher G3PDH activity in the glycolytic than in the oxidative muscle that we report here.

Our results highlight breed-related differences for FAS and G6PDH activities in SCAT and for G3PDH activity both in SCAT and muscles. The activities of FAS and G6PDH were higher in bovine breeds showing an increased propensity for fatness. The high G3PDH activity in glycolytic

muscle suggests that the G3PDH is not only involved in triacyglycerol synthesis but also in the glycerol phosphate shuttle in muscles of steers.

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Estimation of energy expenditure in free-living adult and growing ruminants by heart rate measurement: from cardiovascular system to whole animal

A. Brosh¹, D. Robertshaw², Y. Aharoni¹, R.E. Rawson², M. Gutman³, A. Arieli⁴, E. Shargal⁵ & I. Choshniak⁵

¹ Beef Cattle unit, A.R.O., Newe Yaar Research Centre, PO 1021 Ramat Yishay 30095, Israel

² Dept. Biomedical Sciences, Cornell University, Ithaca, NY, 14853, USA

³ Institute of Field Crops, A.R.O., PO Box 6 Bet Dagan 50250, Israel

⁴ Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

⁵ Dept of Zoology, Tel Aviv University, Ramat-Aviv 69978, Israel

Summary

Studies on the basic physiology of O₂ mobilization were performed using animals with implanted blood flow probes in confined conditions, animals in the field and in feedlots. The studies covered a variety of parameters: diet energy, production, reproduction, time of day, exercise and heat load levels. The aim of the studies was to investigate whether energy expenditure (EE) could be estimated from heart rate (HR) measurements. Daily EE was calculated by multiplying the 24h HR measurements by O₂ consumption per one heartbeat (O₂ Pulse, O₂P) that was measured over a short period of time on constrained animals. The main purpose of these studies was to investigate the stability of O₂P under the various test conditions. The findings reveal that EE can be accurately determined by this method. Extreme heat load and exercise affect O₂P, consequently, the O₂P value should be corrected to accommodate for such conditions.

Keywords: heart rate, cardiovascular, energy balance

Introduction

The development of an effective method for the measurement of energy expenditure (**EE**) is essential for management of both grazing and confined feedlot animals. The use of heart rate (**HR**) as a method of estimating EE in free-ranging large ruminants has been limited to day by the shortage of suitable field monitoring equipment and by the absence of empirical understanding of the relationship between cardiac function and metabolic rate. Recent developments in microelectronics however, now offer the possibility of using small HR devices to monitor free-ranging animals. Estimation of oxygen (**O₂**) uptake (**VO₂**) of animals from their HR should to be based upon a consistent relationship between HR and VO₂. The question as to whether, or to what extent, feeding level, environmental conditions and reproductive state affect such a relationship has not yet been answered. Consequently, our research objectives were: 1) To study the effect of diet energy density, level of exercise, thermal conditions and reproductive state on cardiovascular functioning as it relates to O₂ mobilization. 2) To validate the use of HR to predict EE of ruminants by measuring and calculating the energy balance (**EB**) components in different productive and reproductive states. 3) To study the effect of diet metabolizable energy (**ME**) and of ME intake (**MEI**) of grazing ruminants on their HR, EE and EB.

Materials and methods

Studies on the basic physiology of O₂ mobilization were carried out in the USA at Cornell University. Studies on the basis of EB measurements were carried out in several sites in Israel:

Newe-Ya'ar Research Center, using cows and growing calves in feedlots; Karei Deshe Experimental Range farm, using grazing cows; and the Faculty of Agriculture, the Hebrew University of Jerusalem, using growing lambs in metabolic cages. The relations of VO_2 to HR were studied under differing heat load conditions in growing lambs, in young Friesian calves and in high-yielding dairy cows. The studies covered a variety of conditions in order to investigate the possibility of using HR to estimate EE..

In Cornell: The effects of exercise, ambient temperature, heat load, reproductive state and diet energy on O_2 mobilization and blood flow distribution were measured. Six female sheep at any one time were used in these experiments. Animals were maintained on hay and concentrate diet. They were surgically prepared by implanting flow probes (Transonic Systems, Inc., Ithaca, New York). The probes were placed on the cardiac artery for direct cardiac output measurement, the external iliac artery for blood flow to one hind limb, and the common carotid artery to measure blood flow to one side of the head. The wires from the flow probes were brought through an opening in the skin over the back and were connected to a flow meter on the day of the experiment to monitor the blood fluxes by a system of computers. Blood samples were collected from the carotid artery, and from the jugular and saphenous veins, to determine arterial and venous oxygen concentrations, and to calculate arterial-venous differences.

Oxygen consumption was measured using a loose fitting mask through which air was ventilated at a constant rate and expired air sampled for oxygen and carbon dioxide content. The system was calibrated by application of nitrogen.

The animals were trained to run on a treadmill and exercise intensity was expressed as a percentage of maximal VO_2 . From the measurements of blood flow, whether cardiac output, carotid flow or iliac flow, heart rate could be calculated by Fourier transform analysis of the constant flow measurements. In order to sample arterial blood, a Teflon plate with a central groove was surgically placed underneath an exteriorised common carotid artery. In effect, this allowed the artery to be placed in a subcutaneous position, which was readily accessible by simple palpation for the insertion of a collecting cannula. Thus, arterial blood samples could be taken for analysis of either oxygen and carbon dioxide tension or oxygen content.

In Israel: Three experiments were carried out in the hot summer to define changes in VO_2 per HR, the O_2 pulse (O_2P , $\text{ml O}_2 * \text{kgBW}^{-0.75} * \text{heart beat}^{-1}$) attributable to changes in the time of day or in the heat load (Aharoni 2003). **In Exps. 1 and 2**, calves ($n = 8$) and lambs ($n = 7$), respectively, were measured simultaneously for HR and VO_2 five and six times, respectively, during a 24-h period. **In Exp. 3**, high-yielding dairy cows ($n = 20$) were measured simultaneously for HR and VO_2 four times during a 4-month summer season, in a wide range of heat load conditions. Two additional experiments, (**Exps. 4 and 5**) were conducted to examine the reliability of the HR method for estimating the animals' EE by calculating their EB. The null hypothesis of Exps. 4 and 5 was that EE could be estimated from the HR and the O_2P with acceptable precision, if the ratio of MEI to (EE + RE) and the ratio of the EE calculated by the difference (MEI-RE) to the EE measured by the HR, were not significantly different from 1.0 and the variation among animals (i.e. standard error, SE) was small. In Exp. 4, (Arieli et al. 2002) a comparison was made between assessments of EE by the HR method and the comparative slaughter technique. Growing lambs were kept individually in metabolic cages and were fed *ad libitum* one of three feeding regimes using different proportions of concentrate and alfalfa hay. The body energy content was determined in four animals at the start of the experiment, and in 12 animals at its termination. The entire experimental period was divided into four sub-periods. For each diet, metabolizability and MEI were determined by total faecal collection. EE was calculated from HR monitoring for three consecutive days, and short-term VO_2 measurement for each period. In Exp. 5 (Brosh et al. 2002) six mature beef cows were used, intake was measured individually and ME and MEI were calculated from *in vivo* digestibility. RE in the cows' BW was calculated on the basis of BW changes, on the assumption that all changes were caused by gain or loss of fat tissue. The energy content of the newborn calf and accompanying uterine tissues was calculated from the calf's live

weight at birth and published equations. Milk production was measured three times according to the weight increment of calves following suckling. In Exp. 6, The EE of grazing cows and the ME content of the pasture were measured in the course of three years under 29 representative treatments of grazing season, stocking rate (high and low) and two periods of confinement. Ten cows were used per treatment. Individual faecal output (**FO**) was measured during six of the grazing periods for the two stocking rates (twelve treatments), and MEI was calculated from FO and *in vitro* digestibility of herbage representative samples.

In all the studies carried out in Israel, EE was estimated from daily HR measurements for three to seven days and O₂P determinations over 10 to 15 min. An open-circuit system for measuring VO₂ was used. The N₂ recovery of this system was measured gravimetrically according to McLean and Tobin (1990).

Results and discussion

The physiological studies conducted using sheep with implanted flow probes, show that: 1) although stroke volume decreases during intense exercise, O₂P actually increases and measurement of EE by HR and constant O₂P may underestimate VO₂, unless the slope of the regression relating to heart rate and VO₂ is also determined; 2) alterations in VO₂ associated with the level of feeding and the effects of feeding itself have no effect on O₂P; 3) both pregnancy and lactation may increase blood volume, especially lactation; but they have no effect on O₂P; 4) ambient temperature in the range of 15 to 25°C in the resting animal has no effect on O₂P; and 5) severe heat stress, induced by exercise, elevates body temperature to a sufficient extent that 14% of cardiac output may be required to dissipate the heat generated by exercise rather than for O₂ transport. However, this is an unusual situation and its affect on EE estimation in a freely grazing animal, especially when heart rate is monitored over several days, is minor. In growing animals, the time of day, or the heat load (Exps. 1, 2), affected HR and VO₂, but had no effect on O₂P. On the other hand, the heat load, induced by THI higher than 75, affected the O₂P measured in high-yielding dairy cows (Exp. 3). This is similar to the finding in sheep with implanted probes under extreme heat load.

The MEI ratio to the EE+RE measured in the EB trials (Exps. 4, 5) was not significantly different from 1.0, being 1.062±0.026 for beef cows during one year of their reproductive cycle and 0.957 ± 0.024 for growing lambs.

Similar to the finding for confined cows (Brosh *et al.* 2002), the dependency of EE and RE of grazing cows on pasture on MEI (Exp. 6) were highly significant (P<0.001). The predicted value of EE at zero intake (326 kJ*kgBW^{-0.75} * day⁻¹) was similar to that estimated by NRC (1984). The EE at maintenance of the grazing cows, (EE=MEI, 523 kJ*kgBW^{-0.75} * day⁻¹) was in the range of 465 to 525 suggested by NRC (1996 pp 6-7) for beef cows. The average daily HR and EE significantly increased during lactation, being P<0.001 and P<0.02 respectively, while grazing ME significantly increased HR and EE, being P<0.001 and P<0.001 respectively. In contrast to the finding for confined ewes and cows, the O₂P of the grazing cows was significantly affected by the combined treatments (P<0.001); this effect was significantly related to the diet ME (P<0.001) and consequently to the MEI (P<0.03). Grazing significantly increased O₂P compared to confinement. Thus, if EE of grazing animals during a certain season of the year is estimated using the HR method, the O₂P must be re-measured whenever grazing ME significantly changes.

EE and RE depend significantly on diet ME; the cows' lactation state and the biomass of the grazing area also generate significant effects. Therefore, the grazing cows' energy balance variable, including the DM intake, can be estimated using a simple analysis of the herbage sample's *in vitro* digestibility and the diet ME calculation, using the coefficients presented in table 1. The estimation is limited to the range of the independent variables, i.e. ME and biomass of the grazed area used for the regression calculation.

Table 1. Statistical data for the prediction of energy expenditure (EE) and of the retained energy (RE) ($\text{kJ} \cdot \text{kg} \cdot \text{BW}^{-0.75} \cdot \text{day}^{-1}$) of free-grazing cows. The prediction is based on the concentration of metabolizable energy (ME) in grazed herbage and the effects of lactation and of biomass. These factors were previously found close to ($P < 0.1$) or to significantly affect EE.

| | EE | | | | RE | | | |
|------------------------|----------|-------|------------|--------|----------|--------|-----------|--------|
| | Estimate | SE | t (df=190) | P | Estimate | SE | t (df=65) | P |
| Intercept | -170.2 | 72.5 | -2.35 | 0.02 | -841 | 247 | -3.41 | 0.001 |
| Effect of ME (kJ/KgDM) | 106.56 | 8.59 | 12.41 | <0.001 | 103.1 | 25 | 4.12 | <0.001 |
| Lactation Effect | 75.1 | 30.2 | 2.49 | 0.014 | 254.3 | 81.1 | 3.14 | 0.003 |
| Biomass (kgDM/hectare) | -0.012 | 0.007 | -1.71 | 0.089 | 0.076 | 0.0331 | 2.3 | 0.025 |

In conclusion, the studies were conducted to investigate physiological mechanisms of cardiovascular and O_2 mobilization in a variety of domestic ruminant species, ages, reproductive states, diet ME, time of intake and time of day. These variables were compared under grazing and confinement conditions. From these combined studies we can conclude that EE can be determined from HR measurements taken over the course of several days, multiplied by O_2P measured over a short period of time (10-15 min). The study shows that RE can be determined during the growing phase without a need for slaughtering. In the near future the development of microelectronic devices will enable wider use of the HR method to determine EE and energy balance. This will open up new scope for physiological and agricultural research with minimal strain on the animals. The method is also potentially useful as a tool for herd management.

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Evaluation of dual-energy X-ray absorptiometry to determine the *in vivo* body composition of broilers

J. Buyse¹, Q. Swennen¹, G.P.J. Janssens², E. Decuyper¹ & R. Geers³

¹ Laboratory for Physiology and Immunology of Domestic Animals, Department of Animal Production, K.U. Leuven, Kasteelpark Arenberg 30, B-3001 Leuven, Belgium

² Laboratory of Animal Nutrition, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium

³ Laboratory for Quality Care in Animal Production, Department of Animal Production, K.U. Leuven, Bijzondere weg 12, B-3360 Lovenjoel, Belgium.

Summary

A number of experiments was conducted to optimize the use of dual-energy X-ray absorptiometry for measuring the *in vivo* body composition of chickens and to validate these measurements by means of a chemical carcass analysis. The results indicated that an accurate estimation of the body composition of broilers is possible for the parameters fat tissue mass, lean tissue mass and water mass when the animals are scanned in dorsal position on 4 mm polystyrene as AM and in 'detail slow' scan mode. There was a close agreement between predicted body composition based on estimating linear equations and the chemical carcass composition for body weight, lean and fat tissue mass.

Keywords: dual-energy X-ray absorptiometry, broilers, *in vivo* body composition

Introduction

Meat-type chickens have been intensively selected for body weight gain. Besides a greater growth rate and improved food efficiency, this selection strategy has entailed undesirable, indirect selection responses such as augmented depot fat deposition, leg problems and metabolic diseases (*e.g.* ascites) (Scheele, 1997). Some of these undesirable responses can be partly counteracted by genetic means as well as by management.

In order to study the fat deposition in broilers, an accurate *in vivo* measurement of the fat content of animals is necessary. Several methods are known to validate the body composition of animals. Some invasive techniques are carcass analysis and dilution techniques (Hedrick, 1981), whereas bioelectrical impedance (Brodie *et al.*, 1998) and skinfold thickness (Mirosh *et al.*, 1981) are non-invasive measurements. Still, an accurate non-invasive method for measuring the *in vivo* body composition of chickens is lacking.

Dual-energy X-ray Absorptiometry (DXA) is a non-invasive technique used to determine *in vivo* body composition that evolved from Dual-energy Photon Absorptiometry (DPA). However, DXA has a higher precision, shorter scanning times and lower radiation doses (Blake and Fogelman, 1997). Therefore, DXA is now considered by many as the method of choice to determine human body composition. For animals, use of DXA to measure body composition has been limited to pets, laboratory animals such as mice (Nagy and Clair, 2000), pigs (Geers *et al.*, 1998), etc. As far as we know, there is only one report on the use of DXA for measurements of the *in vivo* body composition of chickens (Mitchell *et al.*, 1997). The results suggest that DXA might be appropriate for measuring the body composition of chickens weighing above 2000 grams. For chickens weighing less, there was a problem with either the use of DXA or, more likely, with the algorithms used in the calculations of body composition of small animals and further research was recommended (Mitchell *et al.*, 1997).

The purpose of the present study was to optimize the use of DXA for measuring the *in vivo* body composition of chickens and to validate these measurements by means of a chemical carcass analysis.

Materials and methods

Body composition was measured with a total-body DXA scanner (LunarTM DPX-L, Lunar Corporation, Madison). The measurements are based on the differential attenuation of low (38 keV) and high energy (70 keV) X-rays by fat and other tissues. A total body scan is made and the fat and lean content is determined for each pixel of this scan that does not overlie bone. The soft tissue attenuation coefficient (R-value) is the ratio of the mass attenuation coefficients (μ) at 38 and 70 keV (Godfredsen *et al.*, 1986): $R = \mu^{38} / \mu^{70} = \ln(I_0^{38}/I^{38})/\ln(I_0^{70}/I^{70})$. Besides the whole composition values for fat and lean content, DXA measurement estimates the bone mineral content (BMC) and density (BMD).

The purpose of the first trial was to evaluate the precision of the DXA measurements, as well as the influence of the scan mode and the position of the chickens on the DXA-parameters. Two broilers out of three different body weight groups (900, 1600 or 2200 grams) were scanned *post mortem* in two scan modes ('high resolution medium' and 'detail slow') and two positions (dorsal or ventral). Each bird was scanned twice on two subsequent days for each combination of mode and position.

The effects of attenuating materials (AM) on the precision of the measurements and on DXA-parameters were tested in trial two. The birds used in trial one were scanned *post mortem* using either polyvinylchloride (PVC, 2 mm) or polystyrene (PS, 4 mm) as AM. The scan modes were 'detail slow' and 'high resolution medium'. Each chicken was scanned twice on two subsequent days in dorsal position per combination of mode and AM.

The accuracy of the DXA measurements was tested in trial three by comparison with chemical carcass analysis. Fourteen male broiler chickens (Cobb) were grown under standard conditions. At 14 days of age, the birds were randomly assigned to one of three dietary treatments (21.9 % crude protein and 10.724 MJ metabolisable energy/kg, 21.2 % crude protein and 12.040 MJ metabolisable energy/kg or a 50:50 mixture of both), to induce a wide range in body composition of the animals. Five birds per group were scanned at weights ranging from 900 g to 2100 g. The scans were made in 'detail slow' mode, in ventral position and with 4 mm PS as AM. The birds were anaesthetized with Zoletil 100 (Virbac Laboratories, France), injected into the pectoral muscle in a dose of 30 mg/kg body weight. After the scan, the animals were fasted overnight, then killed and frozen. Thereafter, the frozen carcasses were scanned in the 'detail slow' mode on 4 mm PS and after the scan, a chemical analysis (Weende analysis) was performed to determine the correlations between DXA measurements and the results of chemical analysis. Chickens were autoclaved overnight and samples were taken immediately after homogenization. Previous investigations demonstrated no significant differences in proximate analysis due to autoclavation (non-published).

Finally, a validation experiment was performed to assess the estimating linear equations that were formulated based on the results of trial three. Eleven other chickens of varying body weight (ranging from 960 to 2400 g) were scanned *post mortem* for a chemical analysis.

The whole-body scans were acquired and analyzed using small animal total body research software (LunarTM DPX-L, Lunar Corporation, Madison). The DXA measurements provided readings of R-value, total tissue mass, percentage fat, fat tissue mass, lean tissue mass and BMC and BMD.

The effects of scan mode, positioning and AM on the DXA parameters were tested using analysis of variance and t-test comparisons were performed between the *in vivo* body composition estimated on the basis of the linear equations and the chemically determined body composition.

Results and discussion

The precision of the DXA measurements is expressed as coefficient of variation: CV = (standard deviation/average) * 100. These results showed that the DXA measurements were precise for the parameters body weight and lean tissue mass (CV<1%). However, for BMC (2.4% \leq CV<4.2%) and fat tissue mass (6.2% \leq CV<8.4%), the precision was less. Furthermore, the precision of all parameters except body weight was influenced by scan mode but not by position of the chicken. The effects of scan mode and position of the animals on the obtained values of the DXA-parameters were also assessed in trial one. Scan mode had a significant influence on the values of R-value, % fat, fat tissue mass, BMC, and BMD ($P<0.0001$). The position of the broiler (dorsal or ventral) had no effect on the DXA-parameters, nor was there an interaction between scan mode and positioning. Body weight and lean tissue mass values were not dependent on these methodological factors. Covariance analysis indicated a significant influence of the body weight of the chicken on DXA measurements, besides scan mode.

The results of the second trial showed a linear relationship between R-value and % fat, but only when $R \leq 1.385$. When the R-value exceeded 1.385, the estimated % fat remained constant at approximately 4 %, corroborating the findings of Mitchell *et al.* (1997). Therefore it was attempted to increase the accuracy of the measurements by placing the chickens on top of an extra layer of AM, which lowered the R-value ($1.33 \leq R \leq 1.37$). The AM did not have a significant effect on the precision of the measurements.

The influences of scan mode and AM on the values of the DXA-parameters were assessed in trial two. The R-value was significantly influenced by scan mode as well as by AM: 'detail slow' mode and 2 mm PVC increased R-value compared to 'high resolution' mode and 4 mm PS, respectively. Percentage fat was significantly influenced by both factors whereas this was not the case for body weight and lean tissue mass. For fat tissue mass, BMC and BMD, the model was significant but r^2 was low. This indicates that the variation not explained by the model is large and that possible effects of other factors might be overlooked. However, these results confirm the significant difference between 'detail slow' and 'high resolution medium' scan mode for all parameters except body weight and lean tissue mass.

The correlations between the results of the DXA measurements and the results of chemical carcass analysis were investigated in trial three. Estimating linear equations were estimated to assess the body composition based on the DXA-parameters (table 1). The relationships between the DXA-parameters and the results of the chemical carcass analysis were highly significant. The equations concerning body weight, fat tissue mass, lean tissue mass, BMC (compared to total ash weight) and BMD (compared to total ash weight) were the most reliable (highest r^2). For % fat, the model is less reliable. There was no linear relation between the DXA-parameters BMD and BMC and the % ash, as measured via chemical carcass analysis. Total protein mass and water content are highly correlated to the lean tissue mass, as measured with the DXA, which is in agreement with Mitchell *et al.* (1997).

There was a close agreement between the predicted body composition on the basis of the linear equations and the chemical carcass composition for the parameters body weight, lean tissue weight and fat tissue mass (validation experiment). Notwithstanding the rather high r^2 of the regression models between total ash mass and BMC and BMD respectively, the validation experiment did not reveal a good agreement between predicted g ash and chemically measured g ash.

In conclusion, when the chickens are scanned in dorsal position, with 4 mm polystyrene as attenuating material and in 'detail slow' mode, an accurate estimation of the body composition of chickens in terms of fat tissue mass, lean tissue mass, water mass with the total body DXA scanner seems to be possible. The predictions of proportional tissue weights based on the DXA % tissue values are less accurate. Also, the system needs to be refined if total ash mass is to be predicted from the DXA parameters BMC and BMD.

Table 1. Estimating linear equations (\pm standard error) for assessing chemical body composition (Y) with the total body DXA scanner (X).

| X | Y | Model | r ² | P |
|-----------------|------------------|--|----------------|---------|
| Body weight | Body weight | $Y = 0.913 (\pm 0.006) X - 25.11 (\pm 10.84)$ | 0.999 | <0.0001 |
| Fat tissue (g) | Fat (g) | $Y = 0.863 (\pm 0.111) X + 17.767 (\pm 25.86)$ | 0.833 | <0.0001 |
| % fat tissue | % fat | $Y = 0.605 (\pm 0.238) X + 5.714 (\pm 3.073)$ | 0.351 | 0.0256 |
| Lean tissue (g) | Protein (g) | $Y = 0.231 (\pm 0.03) X - 48.86 (\pm 43.615)$ | 0.830 | <0.0001 |
| BMD | Ash (g) | $Y = 388 (\pm 50) X - 111 (\pm 20)$ | 0.830 | <0.0001 |
| BMD | % ash | $Y = 3.666 (\pm 2.555) X + 1.407 (\pm 1.018)$ | 0.146 | 0.177 |
| BMC | Ash (g) | $Y = 1.245 (\pm 0.108) X + 12.191 (\pm 2.911)$ | 0.918 | <0.0001 |
| BMC | % ash | $Y = 0.00936 (\pm 0.008) X + 2.635 (\pm 0.216)$ | 0.102 | 0.2646 |
| Lean tissue (g) | Moisture content | $Y = 0.735 (\pm 0.0515) X - 72.537 (\pm 74.575)$ | 0.945 | <0.0001 |

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Two different methods for measuring of gas exchange and heat production in farm animals

A. Chudy

Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Dummerstorf, Forschungsbereich Ernährungsphysiologie "Oskar Kellner", Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany

Summary

Two diverse methods are described for measuring the gas exchange - CO₂- and/or CH₄-production (excretion) and O₂-consumption - in farm animals under different keeping conditions volumetric or gravimetric.

Keywords: respiration, gas exchange measuring methods, heat production

Introduction

Different methods of respiration technique are necessary considering varying experimentally aims. New technical developments make it possible, the respiration technique to simplify and to reduce the costs as well as to adapt better to the aim of experiments. The paper informs about two diverse methods of volumetric (air mass) respectively gravimetric measuring of gas exchange.

Methods

Volumetric method

The method for measuring of gas exchange described below can be used for all kinds of animals under different keeping conditions. The special advantages of this method are: the possibility to use light constructions of chambers, the possible renunciation of absolute air tightness of the chambers and of sub atmospheric pressure and last not least this method is also to use in special

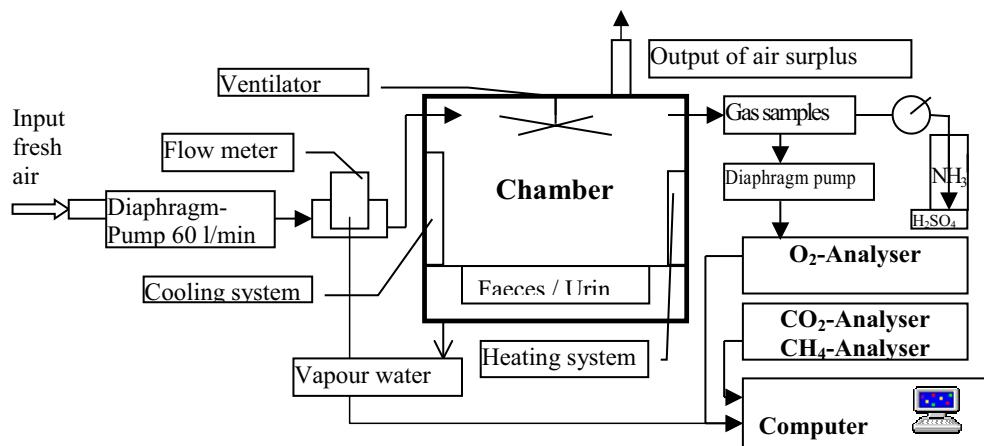


Figure 1. Diagram of respiration unit with volumetric measuring the gas exchange.

rooms under normal conditions with single animals or with animals groups. The extent of use is limited by the capacity of influx of fresh and/or conditioned fresh air, by the possibility of use and by the accuracy of flow measurements.

This method is perfectly suitable for measuring the energy metabolism during days, weeks and months. It was made perfect and applied in respiration experiments with poultry (laying hens), piglets and Chinchilla to 24 h measurements with a break of 20 minutes for feeding, treatment and weighing of animals, collection of excrements and animal products (eggs) as well as for calibration of gas analysers. The measuring principle is shown in figure 1:

The input of fresh air is taken from the environment using a diaphragm- pump or other equipments (blower). The pump does not work with lubricating oil as piston pumps in order to avoid any pollution of breath air. We used a diaphragm pump with a power of up to 60 l/min. The quantity of air input into the chamber is measured by a flow meter (HASTING or other products) before the entry in the respiration chamber or in special keeping rooms continuously. In our experience it is better to work with flow meters that with flow controllers. The scaled dates of volume measurements (mA or mV) were registered directly by the computer, converted and stored in defined intervals (every 20 seconds) for calculation in Excel data files. Because the new flow meter measures the air mass (number of molecules) instead of air volume it is not necessary also to register the air temperature, air pressure and air humidity in order to compute the standard volume (V_0). This is a great simplification in the respiration technique.

The air input is to adjust to the experimental conditions (temperature, humidity) before or in the chamber with heating, cooling and condensation systems (air condition systems). The air in the chamber/room must be mixed and ventilated very well, so that the taken air samples represent the real average of concentration of CO_2 , CH_4 and O_2 in the chamber/room. The surplus of air is diverted in the outside environment. Therefore by using this method it is not necessary to seal the chamber hermetically. The output is to regulate according to a light excess pressure (approximately 1-2 cm water column) in the chamber as safety factor against the entry of outside (room) air.

Generally, it is possible to use this method for measurements of gas exchange of single animals or animals' groups in normal or special rooms under defined keeping conditions.

The samples of the chamber air of approximately 80 l/h were taken with a small diaphragm pump and were analysed with oxygen-, carbon-dioxide- and methane-analyser (from Firm Maihak: O_2 -Analyser OXYGOR 610, CO_2/CH_4 -Analyser UNOR 610) continuously or - if more than one chamber is used - in automatically controlled intervals in each chamber. The registered results are given out to the computer, converted and stored for the different chambers and defined intervals (seconds or minutes). For recording of nitrogen losses separate aliquot samples were measured by a gas meter and the ammonia nitrogen absorbed in diluted sulphuric acid was analysed and calculated up to the quantity of N-losses per animal and day as input in the C- and N-balance.

Based on this dates one can estimate the gas exchange - carbon dioxide production in litres (l) and oxygen consumption in litres (l) - and calculate the heat production either totally or as its course over the measuring periods:

$$\text{CO}_2[\text{l}] = \Sigma(\text{Input}[\text{l}/\text{min}] * (\text{chamber air [%]} - \text{fresh air [%]} \pm \text{drift corr. [%]}) * \text{time factor}) * 24 / \text{D}[\text{h}]$$

$$\text{CO}_2[\text{g}] = \text{CO}_2[\text{l}] * 1.964 \quad \text{C}[\text{g}] = \text{CO}_2[\text{g}] * 0.2729 \text{ (necessary for C-N-balance)}$$

$$\text{O}_2[\text{l}] = \Sigma(\text{Input}[\text{l}/\text{min}] * (\text{fresh air [%]} - \text{chamber air [%]} \pm \text{drift corr. [%]}) * \text{time factor}) * 24 / \text{D}[\text{h}]$$

$$\text{RQ} = \text{CO}_2[\text{l}] / \text{O}_2[\text{l}]$$

$$\text{Heat production [MJ]} = 16.18 * \text{O}_2[\text{l}] + 5.16 * \text{CO}_2[\text{l}] + 5.90 * \text{N}[\text{g}] - 2.42 \text{ CH}_4[\text{l}]$$

Where: N = urinary nitrogen excretion in g (poultry: N = N_{total} - N_{AA}; CH₄ = 0)

The calibration of this measuring method can be carried out with alcohol oxidation (CO₂ and O₂ calibration) and easier only CO₂-calibration with dry ice (CO₂-ice), weighed) in a polystyrene box for delayed sublimation. The divergences between CO₂ input and measured CO₂ respectively O₂ consumption amounted to ± 1 - 2 %.

Gravimetric method

The gravimetric method is compared to the above-described method a special method for small animals, especially for animals, which are very strong sensitive to keeping conditions (adrenaline stressed animals as mice), or for experiments with intensive handlings of the animals.

The method based on a cycle system with a total absorption of CO₂ and H₂O. The animals are kept in a hermetically sealed chamber under defined experimental keeping conditions. The best materials for this chamber are plexiglass or other plastic materials. For the methodically necessary bigger air buffer room and especially for the handling (feeding, collection of excrements, animal treatments etc.) it is suitable to build a relatively big chamber, appointed additionally with rubber gloves for the handling from outside. In dependence on the aim of experiments the air condition in the chamber is to regulate by means of external environmental room temperature or with a heating-cooling control system additionally installed in the chamber.

The inputs in this cycle system are only oxygen (O₂), feed and water for the animals. In the course of experiments not any outputs of substances or escapes of gases are permissible!

The measuring principle shown in figure 2:

The air is sucked out of the chamber and piped through a series of absorption bottles by means of a hermetically sealed diaphragm pump. N-losses (NH₄) out of the chamber are recorded by absorption in diluted sulphuric acid (1st absorption column) and following titration. The following two washing bottles with concentrated sulphuric acid (H₂SO₄) absorb water, totally. After them the totally dried air is piped in two absorption bottles with 30 per cent caustic potash solution (KOH) for the absorption (compounding) of carbon dioxide (CO₂ + 2 KOH → K₂CO₃ + H₂O). Afterwards the air must be dried again absolutely with concentrated sulphuric acid (H₂SO₄) according to the HALDANE-principle (Schiemann et al., 1957). Finally the cycle is closed by passing the dry air over a glass wool filter recovering to the chamber.

The increase in weight of the last absorption system (two washing bottles with KOH and two washing bottles with concentrated sulphuric acid (H₂SO₄)) is directly the animal's CO₂-production in the period between the weighings (figure 2).

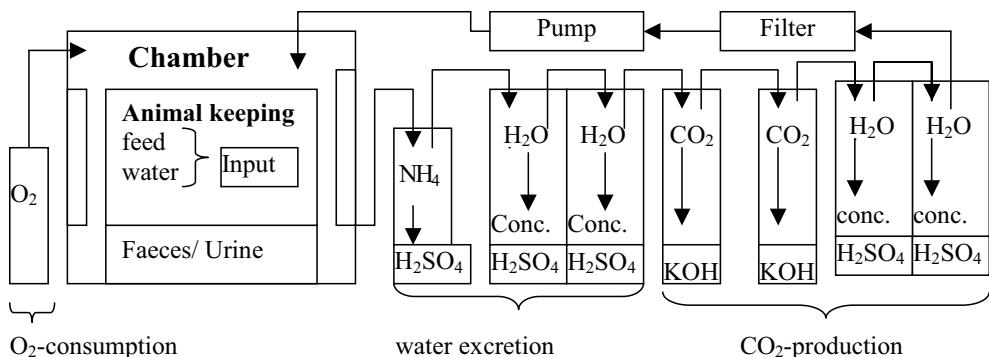


Figure 2. Diagram of a respiration unit with gravimetric measuring gas exchange for small animals.

The animals consume oxygen (O_2), produce carbon dioxide (CO_2) and lose water by intermediary oxidation of hydrogen (animal's additionally urine, faeces and feed water). The CO_2 and the water (H_2O) are absorbed and taken out of the recovered air. The volume of the air recovered to the chamber is reduced by the O_2 consumption of the animals. The CO_2 -concentration adjust to equilibrium in dependence on the extend of ventilation (pump power). Therefore the O_2 -consumption arises as a vacuum in the chamber. This pressure deficit in the chamber must be compensating continuously with a supply of O_2 out of an oxygen bottle. This is to regulate with a reduction valve or an automatically pressure regulatory (control) system. At the end of the daily experiment the pressure equilibrium is necessarily to establish. The weight difference of the oxygen bottle amount to the O_2 -consumption in the measuring period. This method is also right for experiments with stable isotopes because it is possible in quantity to record ^{15}N , ^{13}C , ^{18}O and deuterium separately.

The use of this method is at present very easy because from the industry at one's disposal are very accurate electronically scales up to 30 kg indication of net weight. With such equipments the CO_2 -production and the O_2 -consumption are to estimate very easy and accurate gravimetrically.

Conclusions

The volumetric measuring method described above has good hopes for the future because it opens new possibilities for investigations of energy metabolism under conditions of production. For example the energy requirement of laying hens in floor keeping can be estimated. This method releases the respiration measurements from investments for special buildings and reduces the expenditure for respiration chambers in extreme to an expense for a plexiglass (plastic) bonnet. The gravimetric measuring can be used method for special investigations with small animals under consideration of especially conditions with reference to the animal's characteristics and demands upon keeping and treatments. Outgoing from our calibrations the gravimetric measuring method has a very high exactness. From the point of view of employment and environment protection the work with concentrated acids and bases are disadvantages. But this method needs no great investments for buildings and equipments.

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Apomorphine-susceptible and apomorphine-unsusceptible rats as model for High-resisting and Low-resisting pigs: Metabolic differences

S.B. Degen¹, M.W.P. Hof¹, W.J.J. Gerrits², M.J.W. Heetkamp² & A.R. Cools¹

¹ Department of Psychoneuropharmacology, University of Nijmegen, P.O.Box 9101, NL-6500 HB Nijmegen, The Netherlands

² Wageningen Institute of Animal Sciences, P.O.Box 338, NL-6700 AH, Wageningen, The Netherlands

Summary

This study was undertaken to provide detailed information on the energy metabolism of apomorphine-susceptible (APO-SUS) and apomorphine-unsusceptible (APO-UNSUS) rats, a model for High-resisting (HR) and Low-Resisting (LR) pigs, respectively. It is known that HR and LR pigs differ in the partitioning of ingested energy. To further test the validity of the model, energy partitioning was measured for two weeks using indirect calorimetry.

APO-SUS rats had both a higher daily food intake and growth rate (gram/day) than APO-UNSUS rats. When scaled with metabolic body weight, however, both gross energy and metabolisable energy intake did not differ between the two lines. Activity related heat production did not differ between the lines whereas total heat production tended to be lower and non-activity related heat production was lower in APO-SUS than in APO-UNSUS rats.

Finally, APO-SUS rats appeared to deal more efficiently with their energy reserves than APO-UNSUS rats. Both ratlines retained similar quantities of protein, but mobilised body fat reserves. APO-SUS rats tended to mobilise less body fat than APO-UNSUS rats. As a consequence, APO-SUS rats still retained energy during the experimental period. In contrast to APO-UNSUS rats that mobilised energy reserves. Overall, APO-SUS rats have a more efficient metabolism than APO-UNSUS rats do.

Keywords: apomorphine, rat, indirect calorimetry

Introduction

The aim of the following study was to provide detailed information on the energy metabolism of apomorphine-susceptible (APO-SUS) and apomorphine-unsusceptible (APO-UNSUS) rats. In addition to their differential susceptibility to the dopamine agonist apomorphine, APO-SUS and APO-UNSUS rats (Cools *et al.* 1993; Cools & Gingras 1998) share several behavioural, endocrinological, physiological and immunological features with the so called High-resisting (HR) and Low-Resisting (LR) pigs (Hessing 2003), respectively. For instance, APO-SUS/HR animals are more aggressive, have a similar corticosterone response to ACTH, and a higher mean heart rate than APO-UNSUS/LR animals do, providing thereby a genetically based model for the behaviourally selected HR and LR pigs.

Previous research revealed that from birth on (Degen *et al.* submitted) APO-SUS rats had a significant higher body weight than APO-UNSUS rats at any time (Rots *et al.* 1995). This seemed contradictory because we also showed that for several somatic markers, for instance anogenital distance and digit separation, APO-SUS rats developed slower than the APO-UNSUS rats. This leads to the question whether or not these differences in body weight result either from a difference in feed intake or from a difference in partitioning of ingested energy, or a combination of both. The association between a differential apomorphine susceptibility and performance and partitioning of energy has not been studied in rodents so far. For HR and LR pigs on the other

hand it is already known that they differ both in the partitioning of ingested energy (Heetkamp *et al.* this symposium) as well as in their susceptibility to apomorphine (Bolhuis *et al.* 2000). To further test the validity of our rodent model we determined the energy and nitrogen balance of the APO-SUS and APO-UNSUB rats using the method of indirect calorimetry.

The response to environmental stressors, in this case the transfer into the climate respiration chamber, may differ between the APO-SUS and APO-UNSUB rats. Therefore, the measurements were performed during two subsequent weeks.

Materials and methods

Subjects

Eleven males of the apomorphine-susceptible rat line (APO-SUS) and 12 males of the apomorphine-unsusceptible rat line (APO-UNSUB) were used. These rats were pharmacogenetically bred and reared in the Central Animal Laboratory of the University of Nijmegen, the Netherlands, as described (Cools *et al.* 1990). Litters were weaned at postnatal day 28 and subsequently housed with 2-3 littermates per cage (42 x 26 x 15 cm) using a bedding of fine wood shavings. Standard food (RMH-B, Hope-farms) and water were available *ad libitum*.

Experimental design

The experiment consisted of 4 successive trials in which a total of 8 groups, 4 from each rat line, were tested. Upon arrival at the experimental facilities of the Wageningen University the rats were 53 days old. Each trial consisted of a pre-experimental period of two weeks, allowing animals to acclimatise after transport. During the experimental period of each trial, being two successive periods of 7 balance days, 2 groups were put in one of the two small climate respiration chambers in which heat production was determined by indirect calorimetry (Verstegen *et al.* 1987). Within each trial, energy (E) and nitrogen (N) balance measurements were performed for 2 consecutive 7-days periods. Temperature was maintained at $21 \pm 0.4^\circ\text{C}$, relative humidity at $60 \pm 3\%$ with a fixed 12-hour light/dark cycle (light on 0700).

Ad libitum food intake was measured by weighing start and end weight of feed troughs. Fresh food contained 19.529 kJ of Gross energy (GE) per gram, 4.17% of N and 91.3% of dry matter. Faeces, urine, and fine wood shavings were collected quantitatively and sampled for E and N analysis. All measurements were quantitatively corrected for the fine wood shavings. GE was determined by adiabatic bomb calorimetry and N by Kjeldahl. Metabolizable energy (ME) intake was calculated as E minus E losses in faeces and urine. Heat production (Q_{tot}) was measured in 9-min intervals by determining exchange of oxygen and carbon dioxide as described by Verstegen *et al.* (1987). During the last 6 days of both balance periods Q_{tot} was measured. Total energy retention (RE) was calculated by subtracting Q_{tot} from ME intake. The retention of N was calculated as N intake minus N losses in faeces and urine, in aerial NH_3 and in NH_4^+ of water that condensed on the heat exchanger. Energy retention as protein (RE_p) was derived from the N retention, and energy retention as fat (RE_f) was calculated from RE and RE_p . From RE_p , RE_f and ME intake. Metabolisable energy requirements for maintenance (ME_m) were calculated assuming fixed efficiencies for utilisation of ME for protein retention (0.54) and fat retention (0.74), as commonly applied for pigs (ARC, 1981).

Physical activity was recorded in the same time intervals as Q_{tot} . Physical activity per group of rats was monitored with a radar Doppler device described by Heetkamp *et al.* (this symposium). Per group and per day the 9-min data on Q_{tot} were related to activity counts. Subsequently, activity related heat production (Q_{act}) and heat production not related to physical activity ($Q_{\text{cor}} =$ heat production corrected for activity = $Q_{\text{tot}} - Q_{\text{act}}$) were calculated.

The effect of genotype and balance period (week 1/week 2) on energy and N balance traits as well as the mean values of Q_{tot} , Q_{act} and Q_{cor} per balance period were tested by ANOVA using a split-plot model, with balance periods within groups taken as repeated measurements.

Preliminary results

The results showed that both daily food intake and daily weight gain in gram per day were higher in APO-SUS rats than in APO-UNSUS rats ($p<0.01$ and $p=0.03$ respectively), consistently over the 2-week period. When scaled with metabolic body weight ($\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$), however, both the gross energy and metabolisable energy intake did not differ between the two ratlines but a decrease of both parameters ($p=0.01$ and $p<0.01$ respectively) was seen in time. In other words, the APO-SUS rats are capable of gaining significantly more weight than the APO-UNSUS rats with equal amounts of energy available.

The total heat production (Q_{tot} , $\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) tended to be lower in the APO-SUS rats than in the APO-UNSUS rats (only numerical because $p=0.14$), whereas activity related heat production (Q_{act} , $\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) did not differ between the two lines. The non-activity related heat production (Q_{cor} , $\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) was lower in APO-SUS than in APO-UNSUS rats ($p=0.04$). A decrease of heat production was seen in time, this holds for all three parameters (Q_{tot} , $p<0.01$; Q_{act} , $p=0.05$; Q_{cor} , $p<0.01$). When considering the heat production during the light and dark periods separately, heat production during the dark period was higher for the APO-UNSUS group ($p<0.05$), which was partly related with an tendency to a higher Q_{act} ($p=0.10$) (Heetkamp *et al.* this symposium). Finally, APO-SUS rats appeared to deal more efficiently with their available energy reserves than APO-UNSUS rats. Both ratlines retained similar quantities of protein, but, surprisingly, mobilised body fat reserves during both balance periods. APO-SUS rats mobilised less body fat compared with APO-UNSUS rats ($P=0.10$). As a consequence, APO-SUS rats still retained energy during week 2 whereas APO-UNSUS rats mobilised energy reserves. Estimated maintenance energy requirements were numerically lower for the APO-SUS rats than for the APO-UNSUS rats ($p=0.12$).

Discussion

The present study was designed to evaluate differences in performance and partitioning of energy in APO-SUS and APO-UNSUS rats for two successive balance periods of 7 days.

In agreement with our hypothesis, namely that APO-SUS and APO-UNSUS rats differ in their metabolism (see introduction), APO-SUS rats had a more efficient use of their available energy. Metabolizability of the dietary energy was similar between the two lines. APO-SUS rats gained more weight, due to a higher food intake. When scaled with metabolic body weight, these differences disappeared.

Heat production data showed that the non-activity related heat production, is lower in the APO-SUS rats compared to the APO-UNSUS rats. The equal ME, equal N retention and different heat production in the two ratlines have as a consequence that the APO-SUS rats retained more energy in a numerical way. The differences in efficiency became apparent at the level of fat retention. Numerically, APO-SUS rats mobilised less body fat compared to APO-UNSUS rats during both experimental weeks. As a result, APO-SUS rats still showed energy retention during the second week in contrast to APO-UNSUS rats, which mobilised their energy reserves. The differences in fat retention are in agreement with a pilot study which revealed that the APO-SUS rats indeed had higher amounts of body-fat than the APO-UNSUS rats (Degen *et al* unpublished data). It is known that higher amounts of fat results in lower maintenance energy requirements, which were numerically lower in the APO-SUS rats.

In contrast to our rats, the LR pigs show a higher average daily weight gain than the HR pigs (Heetkamp *et al.* this symposium). Therefore, it seems that from a metabolic point of view the

pigs differ from our rats. It should be noted, however, that the pigs in the study of Geverink were fed restrictedly, whereas in this experiment, food was offered *ad libitum*. In addition, other differences in experimental design might explain the differences. The rats were analysed within their home cage whereas the pigs were transferred from their home pen into the, more stress-full, climate chamber. Therefore, further research is necessary to draw firm conclusions on the rodent model for HR and LR pigs, with respect to metabolism.

Overall, when taking all measured parameters together they gain sufficient critical mass for the conclusion that the APO-SUS rats have a more efficient metabolism compared to APO-UNSUS rats. Something that is also reflected by the maintenance energy requirements, which seemed to be lower in the APO-SUS rats.

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Energy metabolism in Landrace x Duroc crossbreed pigs and their parent breeds

H. Fandrejewski, S. Raj, D. Weremko & G. Skiba

The Kielanowski Institute of Animal Physiology and Nutrition; 05-110 Jabłonna, Poland

Summary

Experiment comprised 78 pigs, 26 from each of Duroc and Landrace pure breed and Landrace x Duroc crossbreeds. Pigs were fed diet (0.63 g lysine/1 MJ EM) according to age (from 13 kg to 30 kg body weight) or at two feeding regimens (*ad libitum* and 13% lower) from 30 till 85 kg. Pigs were slaughtered at 13, 30 and 85 kg and their body was analyzed to content of protein and fat. In the first stage of growth the only insignificant differences between breeds in performance and body chemical data were found. However during period 30-85 kg crossbred pigs temporary exhibited higher appetite, and at similar rate of fat deposition retained respectively 9 and 5% more protein than pigs of Duroc and Landrace breed. Heat production associated with protein anabolism and maintenance for was lower in crossbred pigs than their parental breeds.

Keywords: *pigs, Duroc, energy metabolism, protein retention, heterosis*

Introduction

Duroc pigs carry almost twice as much intramuscular fat as most modern populations of white breeds. Therefore, used this breed is often considered as a way to rapid improvement of the eating quality of pork from the modern breeds. Duroc crossbreeds often showed also positive heterosis in the growth rate and visible fat accumulated in the carcass (Lo et al., 1992), what can be derived from altering energy metabolism in their body.

It is rather striking that energy metabolism of crossbred pigs is almost unknown. In the study reported here the sows of Landrace breeds were mated with Duroc boars, as data from literature concerning reciprocal cross differences (eq., Sellier, 1976) indicate an advantage of using Duroc as sire breed.

Materials and methods

The experiment comprised 78 pigs, 26 from each of breed combinations. Three groups of pigs: Duroc (D) and Landrace (L) breed and Landrace x Duroc crossbred (L*D) were used. In each combination the pigs were progeny of 8 mothers and 4 sires. Furthermore, in-group of L and L*D, the maternal sows were full sisters, whereas in the group of D and L*D the same paternal boars were used to production of piglets. Pigs from 8 weeks of age (13,4±1.9kg) to 30 kg live weight (±0,16) were fed according to the age, but then till 85 kg body weight two feeding regimens (restricted and free allowance to feed) were used. Diet was based on cereals and soybean meal and contained 153g CP, 7,8g lysine and 16.2 MJ GE/kg. Animals were kept individually in 2.6m² cages in termo-neutral environmental. Feed consumption and body weight were measured weekly. The pigs were slaughtered at 13 kg (n=15), 30 kg (n=15) and 85 kg (n=48) body weight after 16-h fasting period and then their carcass, hair and emptied offal were analyzed for protein, fat and ash content. Energy content in the body was estimated from protein and fat content using coefficients: 23,9 and 39,8 kJ/g, respectively (Wenk et al., 2001). Apparent digestibility of fed components was determined during a three-day collection in 4 animals of each breed pigs at approximately 20 and 50 kg body weight. Content of ME in feed was estimated with digestible

protein, fat, crude fiber and non-nitrogen extractives, as proposed Schiemann (1988). The composition of feed, faces and body was analyzed according to AOAC procedures. Corrected heat production (HP') was calculated as ME-(1.4 fat gain +1.15 protein gain), where 1.4 is the equivalent of ME needed for fat deposition in growing gilts (Fandrejewski, 1992) and 1.15 is the equivalent of protein synthesis *in vitro* (Reeds and Mersmann, 1991). Heterosis effect was estimated as difference to traits of parental breeds. Statistical analyses were performed using SAS procedure (1997): in analysis one or two factors, breed or/and dietary level, were taken into account. Differences between groups were tested at P<0.05.

Results

Digestibility of energy did not differ among breeds and digestion trial, but digestion coefficient was relatively low (80.4%). Estimated ME content in the feed was 12.35 MJ/kg.

In the first stage of growth (13-30 kg) only negligible differences between breeds in the performance data were detected (Table 1). At the mean value for daily gain equals 590g, the L piglets grew somewhat faster than those from D and D*L group

In the period of 30-85 kg body weight the crossbreeds fed *ad libitum* consumed more food, particularly over 60 kg body weigh when they significantly enhanced appetite. Daily gain of L*D pigs was 48g higher than of Landrace pigs (P=0.03) and 40g (4.8%) higher than average body gain of parental breeds.

Landrace pigs contained in the body more protein and less fat than Duroc pigs (Table 2), but such differences were statistically significant only at terminal weight, whereas crossbreeds were always intermediate. From 13 to 30 kg body weight the breed differences in daily deposition of protein (mean 88g) and fat (mean 116g) were small (P>0.05), therefore are not presenting in tables. During period from 30 to 85 kg body weight the Landrace pigs consumed the same amount of energy as Duroc pigs (Table 3), but deposited more protein (by 7 kJ/kg^{0.75}) and less fat (by 58 kJ). The F₁ pigs retained more protein than their pure-breed analogues and similar amount of fat as Duroc pigs. As a consequence, utilization of energy was similar in Duroc and crossbred pigs (36.5%) and better than in Landrace pigs (33.1%). Heterosis for energy balance parameters ranged from 5.9 to 13.6%, being the highest as heat production (corrected) related to protein deposition.

Table 1. Performance of Duroc (D) and Landrace (L) pigs and their crossbreds (L*D) fed restricted (r) or *ad libitum* (a)

| Trait | Period | D | | L | | L*D | | CV % | Statistical |
|--------|----------------------|------|------|------|------|------|------|------|-------------|
| | | R | A | r | a | r | a | | |
| FI, kg | 13-30kg ¹ | 1.35 | - | 1.38 | - | 1.35 | - | 5,8 | - |
| | 30-85kg ² | 2.42 | 2.74 | 2.43 | 2.77 | 2.39 | 2.84 | 8,3 | a>r |
| ADG, g | 13-30kg ¹ | 586 | - | 601 | - | 584 | - | 9,4 | - |
| | 30-85kg ² | 778 | 890 | 764 | 874 | 835 | 898 | 8,4 | L*D>L |

¹ 26 pigs in each breeds

² 8 pigs in each subgroups

Table 2. Chemical body composition (g/kg empty body weight) of Duroc (D) and Landrace (L) pigs and their crossbreds (L*D) fed restricted (r) or ad libitum (a)

| Trait | Body weight | D | | L | | D*L | | CV | Statistical |
|---------|-------------|------|------|------|------|------|------|------|-------------|
| | | R | A | r | a | R | a | | |
| EBW | 13 kg | 12,6 | - | 12,5 | - | 12,3 | - | 12,4 | - |
| | 30 kg | 28,0 | - | 27,7 | - | 28,8 | - | 2,4 | - |
| | 85 kg | 82,1 | 81,5 | 82,9 | 82,1 | 82,4 | 81,6 | 8,1 | - |
| Protein | 13 kg | 148 | - | 151 | - | 147 | - | 4,8 | - |
| | 30 kg | 152 | - | 156 | - | 147 | - | 3,4 | - |
| | 85 kg | 148 | 147 | 155 | 153 | 151 | 151 | 3,4 | L>D, L*D |
| Fat | 13 kg | 98 | - | 83 | - | 98 | - | 16,0 | - |
| | 30 kg | 173 | - | 150 | - | 160 | - | 10,6 | - |
| | 85 kg | 241 | 240 | 218 | 202 | 222 | 232 | 9,4 | D>L*D>L |
| | 30 kg | 32,1 | - | 32,1 | - | 30,0 | - | 6,7 | - |
| | 85 kg | 30,6 | 30,3 | 31,0 | 30,1 | 30,7 | 30,9 | 4,7 | - |

Table 3. Energy metabolism of Duroc (D) and Landrace (L) pigs and their crossbreds (L*D) fed restricted (r) or ad libitum (a) (kJ per day and kg^{0,75})

| Traits | Level of feeding | | | | | | CV % | Statistical | Heterosis % | | |
|---------------------|------------------|------|------|----------------|------|------|------|---------------|-------------|--|--|
| | restricted (r) | | | ad libitum (a) | | | | | | | |
| Genotype | | | | | | | | | | | |
| | D | L | L*D | D | L | L*D | | | | | |
| ME | 1411 | 1420 | 1398 | 1609 | 1614 | 1669 | 12,0 | a>r | 3,5 | | |
| ER _p | 124 | 132 | 141 | 140 | 145 | 151 | 8,7 | L*D>L>D, a>r | 8,6 | | |
| ER _f | 394 | 356 | 386 | 444 | 367 | 443 | 14,5 | L*D, D>L, a>r | 6,1 | | |
| ER/ME | 36,7 | 34,4 | 37,7 | 36,2 | 31,7 | 35,6 | 8,5 | L*D>D>N, r>a | 5,9 | | |
| HP' | 717 | 770 | 695 | 826 | 933 | 775 | 11,9 | L*D<D<N a>r | 10,9 | | |
| HP'/ER _p | 5,76 | 5,83 | 4,93 | 5,90 | 6,43 | 5,80 | 11,1 | L*D<D<N a>r | 13,6 | | |

Discussion

Heterosis was estimated as the data of crosses minus average data of the pure breeds. In this study heterosis estimated were not detected for content of chemical components of the body. It is in agreement with reported experimental estimates for carcasses traits, which have in general been small (Lo et al, 1991). Also heterosis was not important for performance and energy metabolism in young pigs (up to 30 kg body weight). An explanation is lack of differentiation in chemical body composition within young modern pigs. Such difference among current breeding pigs, including Duroc breed, appeared usually at later age of animal, what shown, among others, Raj (2002) in the study on pigs from various sires lines.

Crosses weighing over 30 kg showed heterosis for all energy balance parameters. In the case of rate of protein deposition, the crossbreds were better than each parent breeds, so we can say that

for this trait occurred even “true” heterosis. Crossbreds grew at least 30 g per day better and deposited at least 6.5 g more protein, what indicated that founded heterotic effect in body gain was entirely connected with enhance of protein retention. Furthermore, it also seems that such process caused change in energy metabolism. Usually deposition of protein involves an overall increase of energy metabolism, which is probably partly due to the increase in protein turnover. (Fuller et all., 1987). Therefore, in growing animals, the energy costs of maintenance and protein deposition should be considered jointly rather than separately (Kielanowski 1976). Estimates in present work suggest that enhance of protein deposition in crossbreds pigs did not demand extra energy from food. Heat production related to maintenance and protein deposition was reduced by $0.5 \text{ kJ/kg}^{0.75}$ per kJ energy additionally retained as protein. Thus, such response of crossbred pigs is similar to those found in pigs fed diet with improving the amino acid composition (Fandrejewski et al., 2001).

Conclusion

In conclusion, positive heterosis in retention of energy as protein can be expected in Landrace x Doric crossbreeds. Furthermore, such enhanced of protein retention did not demand extra energy from food.

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Influence of feeding intensity on corporeal development, body composition and sexual maturity in New Zealand White male rabbits

Kinga Fodor¹, L. Zöldág¹, S.Gy. Fekete¹, A. Bersényi¹, A. Gáspárdy¹, Emese Andrásoszky¹, Margit Kulcsár², F. Eszes¹ & M. Shanli¹

¹ Faculty of Veterinary Science Budapest, Szent István University, Institute on Animal Breeding, Nutrition and Laboratory Animal Science

² Department of Obstetrics and Reproduction, H-1400 Budapest, P.O. Box 2, Hungary

Summary

Experiment with young male New Zealand White (NZW) rabbits was carried out to establish the live body weight changes, body measurements, body composition and sexual maturity in function of feeding intensity. The animals (n=10) were fed *ad libitum* (Group 1, "AL"), while their pairs' feeding was restricted (n=10) to 70% of *ad libitum* (Group 2, "RS"). The starting live weights practically were the same: 0,907±0,146; 0,911±0,147 kg in Group AL and RS, respectively. The feeding trial lasted between 6 and 22 weeks of age. The average body weight from 7 to 22 weeks of age was significantly higher in AL-Group. At 22 week of age the body weight of Group RS rabbits was 85,64 percent compared to weight of AL animals (3,22±0,52 kg; vs. 3,76±0,33 kg). Data of average body weight shows that the 8, 9, 11, 19 and 21 weeks of age restricted males were similar to 7, 8, 10, 15 and 16 weeks of ages to those fed *ad libitum*. The development of bucks, fed restricted, tended to be allometric. The most significant difference had been experienced at the age of 16 and 18 weeks, the least differenced had been checked at the age of 6, 12, 15 and 19 weeks. Based on the present data, the restricted feeding of 70% cannot be proposed either for the future breeding bucks or slaughtering broiler males. To determine the major chemical components in the body, rabbits were euthanised. Original dry matter and crude fat content of the body had been significantly decreased using restricted feeding ($p < 0,05$); on the other hand percentage of protein within the dry matter increased (49,6%; 65,0%) and that the fat decreased (40,2%; 22,1%) significantly. The libido unambiguously decreases in consequence of feed withdrawal. The most conspicuous difference was in the level of blood testosterone. Although a few RS bucks gave sperm but only much later than rabbits fed *ad libitum*. On the other hand there was no difference in motility of spermatocytes and volume of fluid in comparison with AL animals. We could not find any relationship between the reproductive status and fat content of the body; there is no correlation between the higher body fat content and the reproductive status of bucks in the present trial.

Keywords: New Zealand White, male rabbit, body measures, chemical composition

Introduction

There had been widely spread the *ad libitum* breeding at feeding of rabbits. For breeding animals, one of the most significant purposes of prospective development is to achieve sexual maturity in an optimum age. Fodor *et al.*, (2000., 2001.) studied effect of feeding intensity of New Zealand White female rabbits. Body weight of 18-weeks-old New Zealand rabbits, that had been 70% fed restricted, was 84,4 % of the rabbits that had been fed *ad libitum*. In females New Zealand White breed the restricted feeding delayed sexual maturity. Some scientists (Zahraah, 1981.; Tacke *et al.* 1995.) dealt with connection of feeding and sexual maturity of rabbits as well as producing of spermatocytes. As a consequence of various feeding insufficiencies (for example disorder in energy supply) the number of abnormal spermatocytes increases and the motility as well as the

ability on fertility decreases. Our present experiment is covering New Zealand White bucks since we made the same experience with New Zealand White does before (Fodor *et al.* 2001).

Materials and methods

Twenty 5-week-old New Zealand White male rabbits were used. Animals were divided into two groups: the control ($n=10$) which were fed *ad libitum* (AL) while with their pair's ($n=10$) feeding was restricted exactly to 70% of *ad libitum* intake (RS). To avoid the effect of photoperiod (Adam & Robinson, 1994), controlled daily lighting periods were applied (16:8 h light to dark). The rabbits were allowed to drink tap water *ad libitum*. The rabbits were fed a commercial diet, containing 15.2% crude protein, 14.1% crude fiber and 11.5 MJ DE/kg (Bácska Ltd). The trial lasted between the ages of 6 to 22 weeks of the animals. To determine the major chemical components in the body, at the beginning 10 and at the end 20 rabbits were euthanised at 5 and 22 weeks of age, respectively. For details of methodology see Fekete and Brown (1993). The measures of the live animals were recorded weekly. The body indices were calculated from the body measurements taken during the experiments. Most of the body indices used in this investigation is widely applied in other domestic species (Gáspárdy *et al.*, 2001; Püske *et al.*, 2001) in the same or in a modified form. Some of the indices (index of head capacity, index of ear surface) have created by us in order to evaluate the body development of the rabbit. At the age of 8, 16 and 20 weeks the following hormonal treatments were used in both two groups: HCG and GnRH. Plasma testosterone levels were determined from the samples, which were collected 0., 3. and 6. hours following hormone treatment. Animals were allowed to jump on female rabbit once a week in order to examine the libido and to gather sperm. At finishing of trial the weight of testicles was measured and they were submitted to histological examination.

Results

At the beginning of the experiment the live weights of groups were practically the same: (0.91 ± 0.146 kg vs. 0.91 ± 0.147 kg). Live weight of animals fed *ad libitum* was significantly larger ($p < 0.05$) even from age of week, than weight of rabbits fed restricted (1.49 ± 0.13 kg and 1.3 ± 0.08 kg), and this fact was valid throughout the whole trial. At the end of experiment the live weight of 22-weeks-old rabbits was 85.64 % compared to that of control animals (3.76 ± 0.33 kg; 3.22 ± 0.52 kg) (Figure 1).

During the time of trial mean increase of body weight of restricted fed animals were 87.5 % in comparison with *ad libitum* fed rabbits (0.16 ± 0.08 kg; 0.14 ± 0.1 kg). When the RS bucks reached

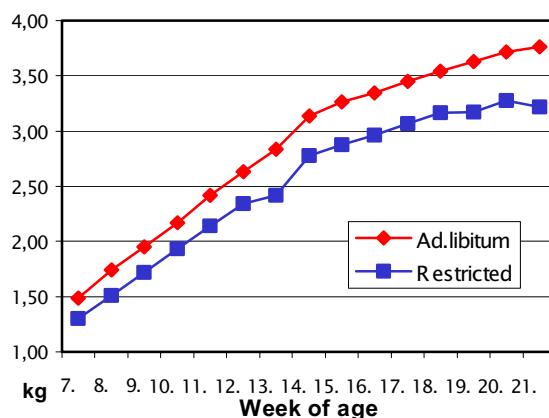


Figure 1. Change of live weight in function of ag.

the body weight of *ad libitum* fed animals at an older age, they showed significant differences in their following body measurements: rear cannon length were in the same size or shorter, capacity of head remained smaller and a retardation was found in heart girth.

The comparison of body measurements at specific ages demonstrates that the effect of restricted feeding manifests itself first in a decrease of body weight gain, length of head and trunk, rump width and head capacity, as well as in a change of the fore cannon to body weight and body weight to heart girth ratio. Finally the width of head and the trunk length to rump width ratio will change. Restricted breeding has negligible or no effect on head capacity to body capacity ratio and fore cannon to rear cannon length ratio in animals of the same age. It can be established that restricted feeding causes retarded growth of the hind legs and trunk, i.e. body parts which have considerable slaughter value.

Original total content of dry matter and crude fat (aether extract) of the body had been significantly decreased after restricted feeding ($p < 0,05$), on the other hand percentage rate of crude protein within dry matter increased as well as the crude fat and ash decreased significantly (Figure 2). The difference in ash between the two group is not significant. Calculating the parameters of chemical maturity (Moulton, 1923), i.e. ash/fat free dry matter (FFDM) and CP/FFDM, % it can be stated that 6 week old rabbits did not reach chemical maturity (Fekete *et. al* 1997), but bucks at the end of the trial did, like in the study of Coudert & Lebas (1985). Libido unambiguously decreases in consequence of feed withdrawal. Thirty percent of animals fed restricted are not willing to mate at all, while all of bucks' fed *ad libitum* covered female rabbit at latest by the age of 16 weeks. It was successful to take seminal fluid from all of animals in control group at the 15th or 16th weeks. However there were only 4 animals of group had been fed restricted that could give sperm up to end of experimental time. Although a few bucks fed restricted gave sperm but only much later than rabbits fed *ad libitum*. On the other hand there were no difference in motility of spermatocytes and amount of fluid in comparison with animals fed *ad libitum*. The weight of both left and right testicles was heavier in case of animals fed *ad libitum*, however these differences were not significant compared to each other, and values of mean relative weight were the same in case of two groups. These results are analogous which was found Gábor *et al.*, (1995). The most conspicuous differences were in level of blood testosterone.

The animals fed differently with same age had nearly the same concentrations of testosterone in blood before the hormone treatment. Following the treatments, concentration of testosterone increased suddenly in blood at the hours 3 and 6. However, much higher testosterone levels were measured in group fed *ad libitum* (2.2-3.4 times more than in the 0. hour), compared to the group fed restricted (1.7-2.0 times more than in the 0. hour). We could not find a similar correlation between the reproductive status and fat content of the body, like we did it in the New Zealand

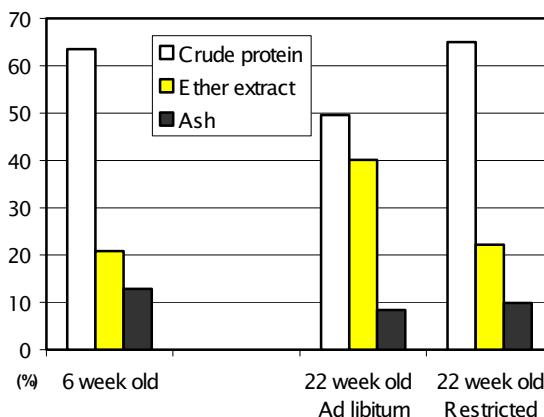


Figure 2. The body composition in the dry matter at equal live weight and different ages.

White female rabbits. (Fodor *et. al.*, 2001). The sperm production also seemed to be relatively independent from the testicles size. There is no relationship between the bucks of higher body fat content and the bucks of better reproductive status in the present trial.

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Heat production of growing heifers that differ in composition of *Bos indicus* and *Bos taurus*

H.C. Freetly, J.A. Nienaber & T.M. Brown-Brandl

*USDA-ARS, U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933-0166,
USA*

Summary

Previous studies using indirect-calorimetry have reported that heat production scaled to body weight of *Bos indicus* cross cattle is lower than that of *Bos taurus* cattle; however, in a comparative slaughter study, estimated fasting heat production of *Bos indicus* × *Bos taurus* steers was not lower than that of *Bos taurus* steers. We hypothesize that fasting heat production decreases at different rates as the proportions of *Bos indicus* and *Bos taurus* parentage changes. Calves were produced by artificially inseminating F_1 (Brahman-MARC III; $n = 55$) and MARC III ($n = 60$) cows to either Brahman ($n = 9$) or MARC III ($n = 9$) sires. Thirty-one heifers were produced 7 - 0% Brahman:100% MARC III (0B:100M), 13 - 25% Brahman:75% MARC III (25B:75M), 5 - 50% Brahman:50% MARC III (50B:50M), and 6 - 75% Brahman:25% MARC III (75B:25M). The indirect-calorimetry measurements were made when heifers were 30.2 ± 0.3 week of age and then every six weeks for a total of six measurements. Heat production (kcal/kg) decreased linearly over the 30 weeks of the development phase and heifers with differing ratio of Brahman and MARC III differed in the rate of decrease ($P < 0.001$). The heat production (kcal/kg) as a function of age (x , weeks) was $f(x) = -0.268(0.034)x + 38.7$ (1.6) for 0B:100M, $f(x) = -0.211(0.023)x + 35.9$ (1.1) for 25B:75M, $f(x) = -0.164(0.033)x + 33.6$ (1.51) for 50B:50M, and $f(x) = -0.068(0.047)x + 29.4$ (2.1) for 75B:25M heifers. The current data set supports our hypothesis that fasting heat production decreases at different rates as the proportion of *Bos indicus* and *Bos taurus* parentage changes and supports that apparent discrepancies between indirect-calorimetry studies and comparative slaughter studies is due to the age at which heat production is measured and the inference space over which it is applied.

Keywords: growth, heifer, metabolic rate

Introduction

Previous studies using indirect-calorimetry have reported that heat production scaled to body weight of *Bos indicus* cross cattle is lower than that of *Bos taurus* cattle (Vercoe, 1970; Frisch & Vercoe, 1977); however in a comparative slaughter study, estimated fasting heat production of *Bos indicus* × *Bos taurus* steers (Brahman and Boran) was not lower than that of *Bos taurus* steers Ferrell & Jenkins (1998). Brody (1945) reported that heat production scaled to body weight decreases as cattle age and the rates of decrease differ between beef and dairy types of cattle. Freetly et al. (2002) reported that the rate of decrease in fasting heat production with aging in sheep differ between breeds. Two factors may contribute to the observed differences in fasting heat production between the indirect-calorimetry studies and the comparative slaughter study. First, the proportion of *Bos indicus* and *Bos taurus* in the parentage and second, the age at which measurements were conducted. We hypothesize that fasting heat production decreases at different rates as the proportions of *Bos indicus* and *Bos taurus* parentage changes. To test our hypothesis, American Brahman (a composite of *Bos indicus* cattle primarily from India) and the MARC III (a *Bos taurus* composite consisting of Hereford, Angus, Pinzgauer, and Red Poll) were used to create heifers that had different Brahman:MARC III ratios.

Materials and methods

Calves were produced by artificially inseminating F₁ (Brahman-MARC III; n = 55) and MARC III (n = 60) cows to either Brahman (n = 9) or MARC III (n = 9) sires. Thirty-one heifers were produced 7 - 0% Brahman:100% MARC III (0B:100M), 13 - 25% Brahman:75% MARC III (25B:75M), 5 - 50% Brahman:50% MARC III (50B:50M), and 6 - 75% Brahman:25% MARC III (75B:25M). Calves were born between March 15 and April 30. Heifers and their dams grazed improved grass pastures before weaning and did not receive additional feed. Heifers were weaned at 152 ± 2 d of age and were allowed ad libitum access to a diet that contained, on a dry matter basis, 20% chopped alfalfa hay, 22% corn, 54.3% corn silage, 0.5% limestone, 3% soybean meal, and 0.2 % sodium chloride. Heifers remained on this diet for the remainder of the study except when feed was removed to determine heat production.

Five or six heifers were placed in each pen (72 m²) and heifers had access to an open fronted barn. Following weaning, heifers were periodically moved to stanchions and trained for sample collection with indirect-calorimeters. The first calorimetry measurements were taken when heifers were 30.2 ± 0.3 week of age and then every six weeks for a total of six measurements. Feed was withheld 55 h before calorimetry measurements started. Composite gas samples were collected from 55 though 71.5 hours of feed removal. Gas analysis were conducted as described by Nienaber & Maddy (1985). Following calorimetry measurements, heifers had ad libitum access to brome grass hay for two days and were returned to their normal diet over an eight day period.

Data were analyzed as a split-plot design. Age was treated as a continuous effect. The model was animal(breed type), breed type, age, and breed type×age. Breed differences were tested with animal(breed). Age and breed×age were tested with the residual error.

Following the last measurement during the heifer development phase, heifers were mated to an Angus bull and calorimetry measurements were made 23 weeks after being placed with the bull. Pregnant heifers were fed a diet that contained, on a dry matter basis, 27% chopped alfalfa hay, 67.3% corn silage 5.5 % soybean meal and 0.2% sodium chloride to provide 172 kcal ME/BW^{0.75·d⁻¹}. The same sample protocol for measuring heat production in young heifers was used in bred heifers. Data were analyzed with an ANOVA.

Results

Fasted body weight increased linearly with increased age and the relationship between fasted body weight and age differed between breed types (breed type×age; P = 0.0001; Table1). The relationship between heat production scaled for metabolic body size (BW^{0.75}) and age differed between breed types (breed type×age; P = 0.0001). Heat production scaled for metabolic body size decreased (P < 0.01) as 0B:100M (-0.50 ± 0.13 kcal/kg^{0.75·week⁻¹) and 25B:75M (-0.26 ± 0.08 kcal/kg^{0.75·week⁻¹) heifers aged; however, the change in heat production for 50B:50M (-0.12 ± 0.14 kcal/kg^{0.75·week⁻¹) and 75B:25M (0.28 ± 0.18 kcal/kg^{0.75·week⁻¹) did not differ from zero as they aged (P > 0.12). The relationship between heat production scaled for body weight and age differed between breed types (breed type×age; P = 0.0001). Heat production scaled to BW decreased as 0B:100M, 25B:75M, and 50B:50M heifers aged (P = 0.0001); however, changes in heat production with increased age did not differ from zero (P = 0.28) for 75B:25M heifers (Figure 1).}}}}

Fasted body weight of pregnant heifers tended to differ with breed type (P = 0.08). Fasted body weights were 463 ± 44 kg for 0B:100M heifers, 422 ± 31 kg for 25B:75M heifers, 429 ± 20 kg for 50B:50M heifers, and 429 ± 28 kg for 75B:25M heifers. Heat production of pregnant heifers scaled to body weight did not differ with different ratios of Brahman and MARC III (P = 0.87). Heat productions were 19.8 ± 2.2 kcal/kg·d⁻¹ for 0B:100M heifers, 19.3 ± 1.4 kcal/kg·d⁻¹ for 25B:75M heifers, 19.6 ± 2.8 kcal/kg·d⁻¹ for 50B:50M heifers, and 20.4 ± 4.4 kcal/kg·d⁻¹ for 75B:25M heifers.

Table 1. Relationship between fasted body weight (kg) and age (weeks) of heifers differing in breed composition of Brahman (B) and MARC III (M).

| Breed type | Slope | \pm SE | Intercept | \pm SE | R ² |
|------------|-------|----------|-----------|----------|----------------|
| 0B:100M | 6.64 | 0.43 | 0.72 | 20.48 | 0.86 |
| 25B:75M | 6.02 | 0.26 | 9.45 | 12.09 | 0.88 |
| 50B:50M | 5.68 | 0.18 | 24.73 | 8.26 | 0.97 |
| 75B:25M | 5.60 | 0.40 | 32.57 | 18.06 | 0.86 |

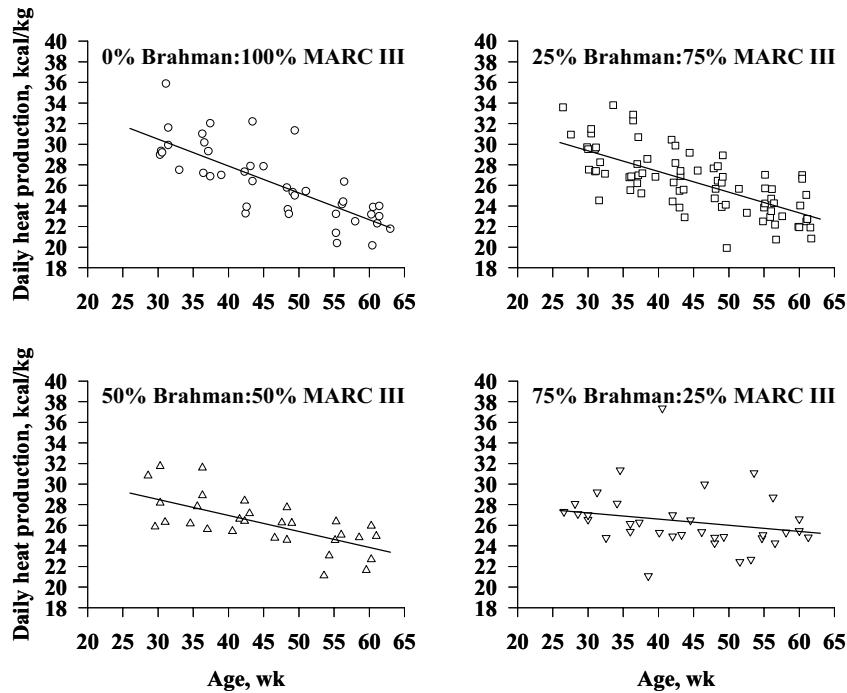


Figure 1. Heat production (kcal/kg) as a function of age (x, weeks) $f(x) = \text{slope} (\text{SE}) + \text{intercept} (\text{SE})$. $\circ f(x) = -0.268 (0.034)x + 38.7 (1.6)$, $R^2 = 0.61$; $\square f(x) = -0.211 (0.023)x + 35.9 (1.1)$, $R^2 = 0.52$; $\Delta f(x) = -0.164 (0.033)x + 33.6 (1.51)$, $R^2 = 0.47$; and $\nabla f(x) = -0.068 (0.047)x + 29.4 (2.1)$, $R^2 = 0.06$.

Discussion

The current data set supports our hypothesis that fasting heat production decreases at different rates as the proportion of *Bos indicus* and *Bos taurus* parentage changes. In our study, the relative ranking in fasting heat production in young heifers was highest for MARC III and decreased as the proportion of Brahman increased; however, the rate of decrease in heat production followed the same ranking resulting in a reversal in rank of heat production in older heifers. The comparative slaughter study of Ferrell and Jenkins (1998) was conducted on steers that were 36–56 weeks of age. This age span is centered around where the rank reversal in our study occurs, suggesting that cumulative heat production during this time span would not differ between the breed types. These findings support that the apparent discrepancy between indirect calorimetry and comparative

slaughter studies is due to the age at which heat production is measured and the inference space over which it is applied.

At 86 weeks of age, pregnant heifers did not differ in fasting heat production suggesting that, while the rate of decrease in fasting heat production differs between *Bos indicus* and *Bos taurus* cattle, eventually fasting heat production scaled to body weight becomes constant across breeds. In our sheep studies, we found that a single model of the decrease in heat production with aging could be used across breeds when the relative proportion of mature body weight of the animal was used rather than chronological age (Freetly et al., 2002). Inherent in the sheep model is that mature ewes of all breeds reach the same heat production per unit weight at maturity. Since heat production did not differ in older heifers, it may be possible to apply a similar type of model to beef cattle to describe fasting heat production.

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Utilization of metabolizable energy for protein energy and fat energy deposition of growing bulls

M. Gabel¹, K. Schmundt¹, H.-J. Papstein¹ & K. Ender²

¹ University of Rostock, Institute for Ecologically-Compatible Animal Husbandry, J.-v.-Liebig-Weg 8, 18059 Rostock, Germany

² Research Institute for the Biology of Farm Animals, Research Unit Muscle Biology & Growth, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

Summary

Nutrient and energy retention of growing bulls (age 6 to 12 months) of different breeds (German Holstein, Belgian Blue, German Angus, Galloway) were measured in individual feeding trials using serial slaughterings and whole-body analysis. All animals were fed with a diet containing grass silage and concentrate (40 resp. 60 % of ration DM). The nutrition level was similar for all breeds (10.7 - 11.0 MJ ME/kg DM). Utilization of ME for protein energy deposition, fat energy deposition and whole energy deposition were estimated by regression analysis from the results of nutrient and energy retention as well as energy intake, both with (I) and without (II) specification of maintenance requirement (MR) ((I) $k_p = 0.49$, $k_f = 0.92$, $MR = 0.694 \text{ MJ ME/kg LM}^{0.75}$, $k_{pf} = 0.80$, $MR = 0.705 \text{ MJ ME/kg LM}^{0.75}$. (II) $k_p = 0.24$, $k_f = 0.48$, $MR = 0.530 \text{ MJ ME/kg LM}^{0.75}$, $k_{pf} = 0.37$, $MR = 0.530 \text{ MJ ME/kg LM}^{0.75}$).

Keywords: protein/fat degradation, energy deposition, energy utilization

Introduction

Partial quantification of metabolizable energy (ME) utilization for protein and fat energy deposition (PED, FED) at defined energy intake implies a broad variation of PED and FED. Furthermore, the autocorrelation between these two parameters should be as small as possible (Henkel, 1976). Up to now quantification of utilization of ME for PED and FED was done from investigations on growing animals of one breed with different energy supply (Flachowsky, 1978; Rohr, 1980; Klein & Hoffmann, 1989; Kirchgeßner et al., 1994; Gabel & Papstein, 1995). Genetic influences on the utilization of ME for FED could not be verified (Hoffmann, 1996). Thus, the aim of the presented study was to determine the partial utilization of ME for PED and FED based on measurements of body mass gain on growing bulls of different breeds at similar energy supply.

Material and methods

One hundred and eighty male young cattle of the breeds German Holstein (GH), Belgian Blue (BB), German Angus (GA) and Galloway (Ga) were stalled up at the age of 4 months for an individual feeding experiment with serial slaughterings and whole-body analysis. Table 1 shows the number of animals per group and slaughtering step. The inhomogeneous number of animals per slaughtering step has to be ascribed to capacitive reasons at slaughter date.

The diet (DM) contained 40 % wilted grass silage and 60 % concentrate. Crude nutrient and energy contents of the ingested diets are listed in table 2.

Development of live weight (LW) was determined monthly. Slaughtering of animals was conducted after 24 h starvation in the experimental slaughter plant of the Research Institute for the Biology of Farm Animals, Dummerstorf/Rostock. Details of slaughtering procedure, sampling and sample processing are presented by Pieper et al. (1984) and Gabel & Papstein (1995). For

Table 1. Number and age of animals per breed and slaughtering step.

| Breed | Slaughtery step/age (months) | | | | Sum |
|-------|------------------------------|---------|----------|---------|-----|
| | I / 6 | II / 12 | III / 18 | IV / 24 | |
| GH | 10 | 10 | 11 | 12 | 43 |
| BB | 8 | 9 | 15 | 14 | 46 |
| GA | 10 | 10 | 14 | 14 | 48 |
| Ga | 11 | 10 | 12 | 10 | 43 |

Table 2. Crude nutrient and energy contents of ingested diets.

| Breed | Crude nutrient and energy content | | | |
|-------|-----------------------------------|------------|------------|-------------|
| | g XP/kg DM | g XL/kg DM | g XF/kg DM | MJ ME/kg DM |
| GH | 147 | 24 | 252 | 10.9 |
| BB | 162 | 23 | 242 | 11.1 |
| GA | 151 | 24 | 252 | 10.8 |
| Ga | 128 | 23 | 261 | 10.7 |

calculation of energy content from protein and fat content of the carcass the factors determined by Böhme & Gädken (1980) were used (22.6 MJ/g protein, 39.0 MJ/g fat).

Statistical evaluation was performed by multiple regression analysis between energy intake (EI) and PED and FED according to Kielanowski & Kotarbinska (1970):

$$EI \text{ (MJ ME/d)} = a * PED \text{ (MJ/d)} + b * FED \text{ (MJ/d)} + C \text{ (MJ ME/kg LW}^{0.75}) \quad (1)$$

$$EI \text{ (MJ ME/d)} = a * PED \text{ (MJ/d)} + b * FED \text{ (MJ/d)} + 0.530 \text{ (MJ ME/kg LW}^{0.75}) \quad (2)$$

$$EI \text{ (MJ ME/d)} = a * PED \text{ (MJ/d)} + b * FED \text{ (MJ/d)} + C \text{ (MJ ME/kg LW}^{0.75}) \quad (3)$$

$$EI \text{ (MJ ME/d)} = a * PED \text{ (MJ/d)} + b * FED \text{ (MJ/d)} + 0.530 \text{ (MJ/kg LW}^{0.75}) \quad (4)$$

In equations (2) and (4) maintenance requirement is predetermined by GfE/AfB (1985).

Statistical calculations were performed with the software package SPSS 7.5.2G.

Results

From the animal performances which are important regarding the utilization of the ME, energy intake (table 3), development of empty body weight (EBW) and the energy deposition in terms of PED and FED shall be presented.

Table 3. Intake of metabolizable energy (ME) per breed depending on the age of the animals.

| Age (months) | GH (MJ ME/d) | BB (MJ ME/d) | GA (MJ ME/d) | Ga (MJ ME/d) |
|--------------|--------------|--------------|--------------|--------------|
| 6 | 50.1 ± 8.4 | 49.9 ± 10.5 | 42.9 ± 4.1 | 39.4 ± 4.0 |
| 12 | 82.6 ± 7.4 | 79.4 ± 9.1 | 78.4 ± 8.5 | 64.7 ± 6.5 |
| 18 | 106.6 ± 12.7 | 94.2 ± 6.9 | 97.1 ± 7.2 | 79.1 ± 12.6 |
| 24 | 114.8 ± 9.8 | 100.7 ± 9.6 | 102.8 ± 7.4 | 99.2 ± 6.0 |

Based on metabolic live weight (average of experimental period) the energy intake between BB (805 MJ ME/d), GA (807 MJ ME/d) and Ga (793 MJ ME/d) was similar. Due to a higher DM-intake (880 MJ ME/d) the GH had a 10 % higher energy intake.

EBW reached at the end of month 24 differed not significantly between GH, BB and GA (table 4).

Table 4. Development of empty body weight per breed depending on the age of the animals.

| Age (months) | GH (kg) | BB (kg) | GA (kg) | Ga (kg) |
|--------------|--------------|--------------|--------------|--------------|
| 6 | 166.3 ± 22.0 | 175.0 ± 26.5 | 164.3 ± 17.5 | 143.2 ± 13.8 |
| 12 | 359.1 ± 26.2 | 159.3 ± 14.1 | 334.9 ± 23.2 | 294.3 ± 26.7 |
| 18 | 514.6 ± 57.0 | 553.9 ± 47.7 | 498.8 ± 32.1 | 404.7 ± 35.1 |
| 24 | 635.8 ± 48.3 | 641.9 ± 45.2 | 650.7 ± 30.4 | 535.0 ± 29.6 |

Differences in EBW were evident in all slaughtering steps between GH, BB and GA on one hand and Ga on the other hand. Daily gain of EBW were 869 g for GH, 865 g for BB, 901 g for GA and 726 g for Ga.

Table 5 shows PED and FED realized with this development of EBW.

Table 5. Development of protein and fat energy deposition (PED, FED) per breed depending on the age of the animals.

| Age (months) | GH | | BB | | GA | | Ga | |
|-----------------|---------------|---------------|---------------|---------------|---------------|----------------|---------------|---------------|
| | PED (MJ) | FED (MJ) | PED (MJ) | FED (MJ) | PED (MJ) | FED (MJ) | PED (MJ) | FED (MJ) |
| | 717 ± 93 | 721 ± 169 | 804 ± 125 | 218 ± 110 | 724 ± 72 | 675 ± 128 | 633 ± 46 | 594 ± 131 |
| 6 | 1539 ± 120 | 2443 ± 335 | 1711 ± 71 | 772 ± 138 | 1466 ± 119 | 1910 ± 233 | 1304 ± 114 | 1595 ± 287 |
| 12 | 2104 ± 230 | 4428 ± 899 | 2564 ± 208 | 1778 ± 394 | 2056 ± 179 | 4215 ± 562 | 1792 ± 188 | 2543 ± 263 |
| 18 | 2489 ± 200 | 6527 ± 988 | 2998 ± 212 | 2151 ± 443 | 2633 ± 176 | 5794 ± 1121 | 2179 ± 171 | 5178 ± 712 |
| 24 | | | | | | | | |

Regression analysis of means listed in table 5 led to the following regression equations:

$$\pm 0.868 \quad \pm 0.371 \quad \pm 0.039$$

$$EI (\text{MJ ME/d}) = 2.038 \text{ MJ PED/d} + 1.084 \text{ MJ FED/d} + 0.694 \text{ MJ ME/kg LW}^{0.75} (\pm 22) \quad (1)$$

$$\pm 1.108 \quad \pm 0.445$$

$$EI (\text{MJ ME/d}) = 4.215 \text{ MJ PED/d} + 2.105 \text{ MJ FED/d} + 0.530 \text{ MJ ME/kg LW}^{0.75} \quad (2)$$

$$\pm 0.330 \quad \pm 0.038$$

$$EI (\text{MJ ME/d}) = 1.250 \text{ MJ (PED + FED)/d} + 0.705 \text{ MJ ME/kg LW}^{0.75} (\pm 22) \quad (3)$$

$$\pm 0.192$$

$$EI (\text{MJ ME/d}) = 2.676 \text{ MJ (PED + FED)/d} + 0.530 \text{ MJ ME/kg LW}^{0.75} \quad (4)$$

The reciprocal values of regression coefficients a and b result in the following values for k_p , k_f und k_{pf} :

| | | | |
|---------------|-----------------|--------------|--|
| equation (1): | $k_p = 0.49$ | $k_f = 0.92$ | maintenance requirement = $0.694 \text{ MJ ME/kg LW}^{0.75}$ |
| equation (2): | $k_p = 0.24$ | $k_f = 0.48$ | maintenance requirement = $0.530 \text{ MJ ME/kg LW}^{0.75}$ |
| equation (3): | $k_{pf} = 0.80$ | | maintenance requirement = $0.705 \text{ MJ ME/kg LW}^{0.75}$ |
| equation (4): | $k_{pf} = 0.37$ | | maintenance requirement = $0.530 \text{ MJ ME/kg LW}^{0.75}$ |

Estimation of ME input for energy deposition with linear single or multiple regressive approaches (equation 1 - 4) comprises the estimation of maintenance requirement of ME, too. Thereby, interactions between ME input for energy deposition and the maintenance requirement which could not be excluded, are frequently reflected in a relatively high input for maintenance requirement (equations 1 and 3). The values of k_p , k_f and k_{pf} (according to the equations (1) and (3) with high values for maintenance requirement (0.694 and $0.705 \text{ MJ/kg LM}^{0.75}$) are similar to those found for growing cattle by Cherepanow (1977), Flachowski (1978), Menke et al. (1980) and Kirchgeßner et al. (1994).

Linear regressions for estimation of maintenance requirement are discussed controversially (Gabel & Papstein, 1995). But non-linear regressions (Gabel & Papstein, 1995) as well as whole metabolism measurements (van Es, 1978; Hoffmann et al., 1981) led to lower values. Thus, in equation (2) and (4) maintenance requirement according to GfE (1995) was predetermined with $0.530 \text{ MJ/kg LM}^{0.75}$. Resulting utilization coefficients (k_p , k_f , k_{pf}) are explicitly lower and match the level which was determined by Gabel & Papstein (1995) from non-linear regressive generalisation of deposition measurements. Values of k_p , k_f and k_{pf} presented by Kirchgeßner et al. (1994) from deposition measurements with beef cattle (German Simmental) at a maintenance requirement of $0.535 \text{ MJ/kg LM}^{0.75}$ are marginally higher. An influence of utilization coefficients of ME for energy deposition by metabolizability of energy (q) and the nutrition level (NL) could be precluded, because both factors did not vary in the presented study. The utilization of ME for PED, FED resp. whole energy deposition (determined for growing cattle of different breeds at approximately the same NL) showed the same level, as could be determined for growing cattle of the breed Black and White (Gabel & Papstein, 1995).

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Comparing two techniques to estimate energy expenditure on physical activity in group housed animals

M.J.W. Heetkamp¹, H. van den Brand¹, S.B. Degen² & W.J.J. Gerrits¹

¹ *Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O.Box 338, 6700 AH Wageningen, The Netherlands*

² *Department of Psychoneuropharmacology, University of Nijmegen, P.O.Box 9101, 6500 HB Nijmegen, The Netherlands*

Summary

Quantification of physical activity in animal energy metabolism studies is important because it may cover up effects experimentors are trying to show, or alternatively, it may be the subject of study. Two different techniques to measure activity of group-housed animals, i.e. radar Doppler and short-term weight variation were compared to evaluate effects on estimations of energy expenditure on physical activity. Compared with radar Doppler, the relationship between weight-based activity parameters and heat production was clearer, but appeared to be non-linear, implying that the energy costs per unit of activity is not constant. The use of different weight-based activity parameters, using non-linear curve fitting to assess the energy costs per unit activity, results in a large variation in estimates of activity related heat production. It may be an artefact of the weighing technique, at some points probably enlarged by the non-linear curve fitting.

Keywords: *energy expenditure, physical activity, group housing*

Introduction

Quantification of energy expenditure on physical activity is important because experimental treatments (e.g. diets, housing conditions) can affect animal behaviour. This may cover up effects experimentors are trying to show, or alternatively, it may be the subject of study. Within our calorimetry facilities, in individually as well as in group-housing settings, we use a radar-Doppler technique for continuous recording of physical activity of various animal species under various experimental conditions. Apart from the advantages (e.g. non invasive, easy to automate), some disadvantages of this technique can be mentioned: (i) Continuous standing or lying could theoretically result in the same number of activity counts, whereas the energetic implications are quite different (Nienaber et al; 1987); (ii) The radar Doppler method accounts for movements, but not for the mass of movement. (iii) Tuning the sensitivity of radar systems is difficult and involves arbitrary choices regarding movements that are considered to be physical activity (e.g. breathing, ear or tail movements).

To test the validity of the radar Doppler method with regard to the possible disadvantages mentioned above, this method was compared with activity data computed from short term weight variation recorded by a standard electronic weighing device (comparable with the force-sensor technique) using group housed rats.

Materials and methods

Energy partitioning of two genetic lines of rats (as described by Degen et al., presented at this symposium) was measured using indirect calorimetry. Eight groups of 3 rats of one of 2 different genetic lines were housed during 2 weeks in cages with fine wood shavings, inside one of two identical small climate-controlled respiration chambers. The rats were either selected for or against

apomorphine susceptibility (APO-SUS and APO-UNSUS, respectively). We continuously measured heat production and physical activity using both the radar-Doppler technique and a weighing device (Mettler-Toledo; KA32s bench scale; resolution output 0.1 gram) underneath each cage. In the radar technique, high frequency radiation (radar) is continuously emitted and, if reflected from a moving surface, the change in frequency (Doppler effect) is monitored within the same activity sensor. If the change in frequency exceeds a threshold value (=sensitivity) electrical pulses are transferred to a counter inside a computerized data-acquisition system. The radar data were stored as 1-minute counts. Throughout the experiment, every 0.7 second, weights (± 0.1 g) of both weighing devices were recorded by a computer system.

Heat production (Q) was measured in 9-minute periods. Therefore, measurements of both methods of physical activity were calculated over the same 9-minute periods as well. Short term weight variation was calculated by summing the absolute differences from successive weights over 9 minute periods. To separate possible "noise" from noticeable movements, 4 different activity parameters were computed, each with different threshold values for differences in successive weights: 0.1 (resolution of devices), 1.0, 2.0 and 3.0 gram for variables W0, W1, W2 and W3 respectively. In addition, two extra parameters were calculated by summing the difference between successive weights and a moving average of 20 and 100 successive weights (parameters Wa20 and Wa100 respectively). Because preliminary data analysis showed a non-linear relation between heat production and the above mentioned weight activity parameters, we separated the effect of the number of movements from the effect of the weight change per movement, both present in parameters W0 to W3 and Wa20 and Wa100. Therefore, new weight activity parameters were calculated, only taking into account the number of movements (as counts). A count was defined as the difference in successive weights exceeded a threshold value of 0.1, 1.0, 2.0 and 3.0 gram for variables WC0, WC1, WC2 and WC3, respectively.

The relation between radar counts and weight activity data first were visually evaluated. Subsequently, for each of the 9 minute periods, energy expenditure on physical activity (Q_{act}) was calculated as the first derivative of the relationship between total heat production (Q) and physical activity of all observations of that day (= energy cost per unit of physical activity), multiplied by the measurement of physical activity over that 9-minute period. For the radar measurements, a straight line was fitted to the data of heat production vs. activity counts. Addition of a quadratic component to this relationship did not improve the fit in most cases ($P>0.05$). For the weight measurements a quadratic component did improve the fit in most cases ($P<0.05$). Therefore, 2 models were fitted to the data of heat production vs. activity measurements: 1) a linear relationship (including a fixed effect for light/dark period); 2) an allometric relationship ($Y = a + bX^c$) estimated using the data of the active (=dark) period only. Q and Q_{act} were calculated per day but also for light and dark periods separately, and were averaged over a week. These variables were analyzed for each of the two weeks by analysis of variance for effects of genetic lines using a repeated measurement design. Different estimates of Q_{act} were compared.

Results and discussion

With increasing threshold weights above 1g, the number of observations with activity=zero increased dramatically. Therefore, parameters W2, W3, WC2 and WC3 were omitted from further calculations. Parameters Wa20 and Wa100 showed similar data as W0 and are therefore not presented separately.

Figure 1a presents a typical example of the relation between radar and weight based activity. Parameter W1 showed similar results. There was a remarkable absence of a clear relationship, with at low levels of weight activity, the range of radar activities still being considerable. Apparently, the sensitivity of the radar-based system is far higher, because movements, picked up by radar, were not detected with the 0.1-g sensitive weighing device. Figure 1b shows a typical example of the relation between Q and radar activity. In figure 1c and 1d this relation is presented

for Q and the weight activity parameter W0 and WC0, respectively. Figure 1c and 1d with parameters W1 and WC1 on the X-axis showed similar graphs but with much more zero activity points. The radar technique caused a much wider range of activity data at different levels of Q compared with the weight technique. The clear non-linear relation between Q and weight activity (Fig.1c & 1d) is remarkable, and requires non-linear curve fitting for determining the energy cost per unit of activity.

The activity related heat production (Q_{act}) calculated with different activity parameters and models are presented in Table 1. To summarise the main points from table 1: (i) The level of Q_{act} estimated based on radar corresponded with all weight-based estimates except WC0 based measurements. (ii) Regardless calculation methods, estimates of Q_{act} based on W1 were lower than those based on W0 measurements. (iii) During the dark -but not during the light- period, WC based estimates generally led to increased estimates of Q_{act} compared with W based estimates. This may reflect a difference in sensitivity of the calculation of weight counts, and could be corrected for by choosing an appropriate threshold level. (iv) non-linear curve fitting reduced the dark-light difference for all W-based measurements, but increased those for WC-based measurements. This is related to the shape of the fitted curve, which showed a decreasing slope for W-based measurements (Fig 1c) and an increasing slope for WC based measurements (Fig 1d) (v) Standard errors (relative to the mean) of all W based estimates were lower compared with the radar method.

Choice of threshold level: These results show a clear dependency of estimates of Q_{act} on the sensitivity of the measuring system. For example, Q_{act} estimated based on W1 were lower

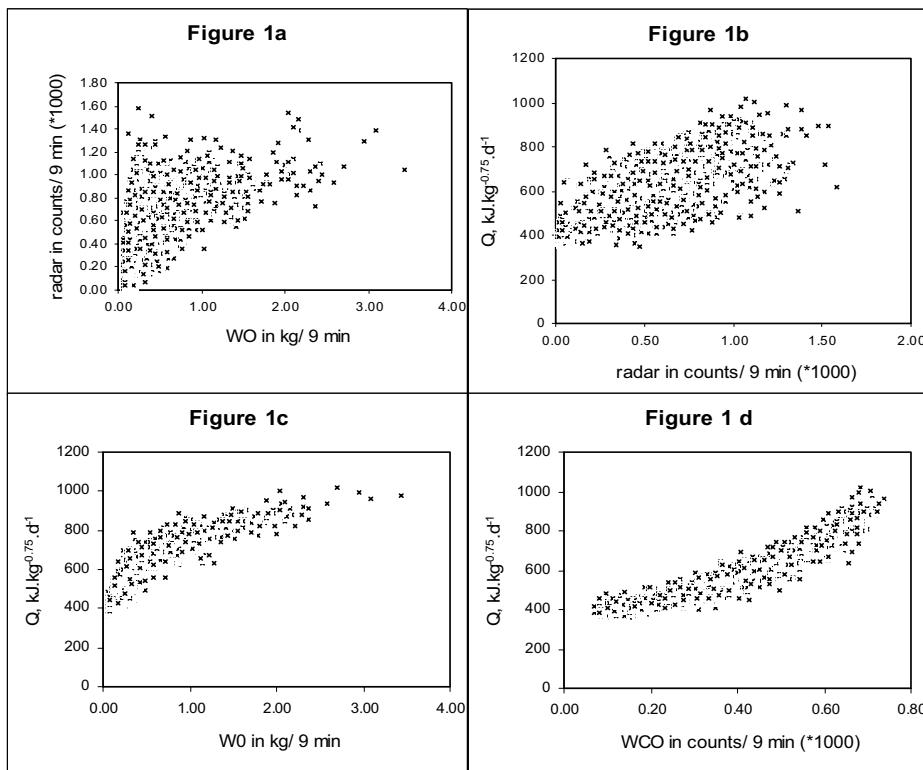


Figure 1. Typical examples of relations of: 1a: Radar and W0 activity; 1b: Q and radar activity; 1c: Q and W0 activity; 1d: Q and WC0 activity.

Table 1. LSmeans of Q and Q_{act} calculated from different activity parameters with different models.

| Parameter | APO- | | SEM | P- value |
|--|-------|-----|------|----------|
| | Model | SUS | | |
| Daily heat production, $\text{kJ} \cdot \text{kg}^{-.75} \cdot \text{d}^{-1}$ | | | | |
| Q | 549 | 583 | 8.3 | .07 |
| Q _{act-radar} 1 | 94 | 105 | 6.0 | .28 |
| Q _{act-W0} 1 | 104 | 98 | 4.0 | .39 |
| Q _{act-W1} 1 | 77 | 71 | 2.9 | .26 |
| Q _{act-WC0} 1 | 197 | 218 | 9.9 | .23 |
| Q _{act-WC1} 1 | 109 | 105 | 2.5 | .41 |
| Q _{act-W0} 2 | 125 | 122 | 4.3 | .65 |
| Q _{act-W1} 2 | 91 | 84 | 2.4 | .15 |
| Q _{act-WC0} 2 | 187 | 231 | 8.4 | .03 |
| Q _{act-WC1} 2 | 91 | 92 | 5.0 | .90 |
| Heat prod. 12 h light period, $\text{kJ} \cdot \text{kg}^{-.75} \cdot \text{d}^{-1}$ | | | | |
| Q | 440 | 455 | 8.1 | .28 |
| Q _{act-radar} 1 | 58 | 58 | 5.9 | .97 |
| Q _{act-W0} 1 | 42 | 37 | 1.9 | .15 |
| Q _{act-W1} 1 | 22 | 19 | 1.1 | .10 |
| Q _{act-WC0} 1 | 112 | 116 | 9.7 | .79 |
| Q _{act-WC1} 1 | 40 | 34 | 1.2 | .03 |
| Q _{act-W0} 2 | 89 | 99 | 6.2 | .35 |
| Q _{act-W1} 2 | 55 | 58 | 2.2 | .37 |
| Q _{act-WC0} 2 | 51 | 54 | 6.3 | .73 |
| Q _{act-WC1} 2 | 9 | 7 | 0.5 | .06 |
| Heat prod. 12 h dark period, $\text{kJ} \cdot \text{kg}^{-.75} \cdot \text{d}^{-1}$ | | | | |
| Q | 659 | 710 | 8.5 | .02 |
| Q _{act-radar} 1 | 130 | 152 | 6.3 | .09 |
| Q _{act-W0} 1 | 166 | 160 | 6.1 | .53 |
| Q _{act-W1} 1 | 131 | 123 | 5.0 | .34 |
| Q _{act-WC0} 1 | 282 | 319 | 10.2 | .08 |
| Q _{act-WC1} 1 | 177 | 177 | 4.2 | .93 |
| Q _{act-W0} 2 | 161 | 145 | 4.0 | .07 |
| Q _{act-W1} 2 | 126 | 110 | 3.7 | .05 |
| Q _{act-WC0} 2 | 323 | 409 | 12.0 | .01 |
| Q _{act-WC1} 2 | 172 | 177 | 9.6 | .77 |

compared with W0. Generally, a reduced sensitivity reduces the x-values in the activity vs. heat production relationship, whereas the y-values remain the same. Consequently, the estimated Q_{act} is decreased as well. Also, the extreme high estimates using WC may illustrate this point.

Counts vs. weights based measurement: Based on these data, it is impossible to conclude if accounting for the mass of movement improves the quality of the Q_{act} estimates. Improvement of the relationship between Q and activity, however, illustrates that the precision of short-term measurement in heat production is not likely a limiting factor (compare Figure 1b vs. 1c and 1d). *Are the energy costs per unit of activity constant?* As illustrated by Figure 1c and 1d the non-linear relationship between Q and all weight based activity parameters was surprising. It suggests

that the energy cost per unit activity depends on the quantity of activity, exhibited during a 9-minute period. It may be explained by the heat increment of digestion occurring while activity levels are lowered again (Figure 1c) because rats may rest after eating. Evidence of this kind of behaviour was not found in literature (Clifton, 2000), and can only be demonstrated when eating patterns are known. Alternatively it may be an artefact of the measurement, enlarged by sophisticated curve-fitting to assess the energy costs per unit activity accurately. The observations were not equally distributed over the X-axis: the number of observations with activity=zero increases sharply already with the less sensitive parameter W1. Probably a linear plateau model, with data as in figure 1d, would allow estimation of the inflection point of a horizontal and an increasing line. This point then reflects the point at which activity=0, and an estimate of the desired threshold level of the system. Energy costs per unit activity then can be calculated as the slope of the increasing line.

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The relationship between muscle metabolic pathways and marbling of beef

J.F. Hocquette¹, C. Jurie¹, Y. Ueda¹, P. Boulesteix², D. Bauchart¹ & D.W. Pethick³

¹ INRA, Herbivore Research Unit, Theix, 63122 Saint-Genès Champanelle, France

² UPRA France Limousin Sélection, France

³ Murdoch University, Division of Veterinary and Biomedical Sciences, Perth, Australia

Summary

Three muscle types were taken from 4 groups of steers of different genotypes which express either high (Angus, Japanese Black x Angus) or low (Limousin) levels of marbled meat. Expression or activities of different proteins or enzymes controlling energy metabolism were determined. Angus and Crossbred animals were characterised by a more oxidative muscle metabolism (especially a higher cytochrome-c oxidase activity) and a higher protein content of adipocyte fatty acid binding protein. The latter was considered as a good predictor of marbling.

Keywords: muscle, fat, metabolism, adipocyte, meat quality

Introduction

Intramuscular fat deposition (marbling) influences many quality attributes of beef. Marbling refers to the visually discernible depots of fat within muscles. It is the primary factor determining the quality grade of beef meat in North American and Asian markets. By contrast, beef produced from the French cattle breeds contains a low amount of fat.

Triacylglycerol (TAG) is the major component of fat in muscles. It is stored to a minor extent within the myofibers and to a major extent within the intramuscular adipocytes. Therefore, the number and the diameter of intramuscular adipocytes may be a good predictor of marbling (Cianzo *et al.*, 1985). The content of fat within muscle also results from the balance between uptake, synthesis and degradation of TAG. Therefore, many metabolic pathways in both adipocytes and myofibers could contribute to the variability of intramuscular fat content. They involve the ability of muscle to utilise circulating fats via the activity of lipoprotein lipase (LPL), the intracellular trafficking of fatty acids (FA) that is facilitated by fatty acid binding proteins (FABP), the muscle capacity for *de novo* FA synthesis from carbon precursors, and the activities of key enzymes that direct FA towards oxidation within mitochondria or storage within intramuscular adipocytes (reviewed by Hocquette *et al.*, 1998).

Our hypothesis is that the metabolic activity of muscles may differ between bovine breeds with different ability to develop marbling. Our purpose is to take advantage of this natural genetic variability to identify precisely these metabolic differences.

Material and methods

Animals and samples

Four bovine groups of steers from three different genotypes were used: Angus and Crossbred Angus × Black Japanese which produce a marbled meat and Limousin which produce a weakly marbled meat. All animals had a long finishing period with a cereal-rich diet which allowed them to express their genetic potential for intramuscular fat deposition.

In experiment 1, one group of 12 Limousin steers and two groups of 10 Angus and 10 crossbred Angus x Black Japanese were fattened for 6 months with a similar diet [rolled wheat (47-50%), triticale (17-18%), hay (14-18%), lupins (9%), Metabolizable energy: 12 KJ/kg DM, Crude Protein: 15%]. Animals were slaughtered between 23 and 28 months of age. In experiment 2, one group of 8 Angus steers was reared in the experimental farm of Murdoch University in Australia. Animals were fattened during 10 months and slaughtered at 28 months of age. These extremely fat animals were compared to the extremely lean Limousin of experiment 1.

At the time of slaughter, samples of three muscle types were taken from Limousin steers: *Rectus abdominis* [RA] (oxidative), *Longissimus thoracis* [LT] (rather oxidative) and *Semitendinosus* [ST] (glycolytic). RA and ST samples were taken from the two groups of ten Angus and crossbred Angus x Black Japanese steers (experiment 1). LT samples were taken from the eight 28-month-old Angus steers (experiment 2).

Measurements

In all muscle samples, activities of lipoprotein lipase (LPL), oxidative enzymes (isocitrate dehydrogenase [ICDH], citrate synthase [CS], cytochrome-*c* oxydase [COX], hydroxyacyl-CoA dehydrogenase [HAD]), glycolytic enzymes (phosphofructokinase [PFK], lactate dehydrogenase [LDH]) as well as protein and DNA contents were measured according to the methods cited by Hocquette *et al.* (1998) and Piot *et al.* (2000). The muscle contents of two isoforms of fatty acid-binding proteins specific of muscle fibers (H-FABP) or intramuscular adipocytes (A-FABP) were determined by ELISA (Piot *et al.*, 2000). All results were expressed per g tissue wet weight or per mg DNA or protein muscle content.

Differences between genotypes and muscles of experiment 1 were analysed by variance analysis using SAS. Fixed effects included genotype, animal nested within genotype, muscle type and interaction between muscle and genotype. The effect of genotype was tested against animals within genotypes. Differences between Limousin and Angus steers for LT muscle of experiment 2 was tested by the Student *t* test. Covariance analyses were made using SAS.

Results

Differences in TAG content and in metabolic activity between muscles and genotypes

In the first 3 groups of animals (experiment 1), TAG contents were on average 3.4-fold higher in RA than in ST muscle. TAG contents were also 4.4-fold higher in Angus and in crossbred steers than in Limousin steers, but the difference was much higher in RA (69 and 71 vs 15 mg/g of fresh muscle, $P < 0,001$) than in ST (22 and 17.5 vs 5.4 ; $P < 0,06$). Whichever the genotype, glycolytic enzyme activities (PFK, LDH) were on average 1.3-fold higher in ST than in RA muscle. Conversely, some enzyme activities of oxidative metabolism (ICDH, COX) were on average 2-fold higher in RA muscle. These results confirmed the expected differences between the muscles. For RA and ST muscles together, enzyme activities of glycolytic metabolism were 1.4-fold higher in Limousin steers. Conversely, enzyme activities of oxidative metabolism and H-FABP contents were at least 1.5-fold higher in the 2 two other genotypes ($P < 0,005$). Muscles from Angus and crossbred Angus Black Japanese were clearly more oxidative and less glycolytic than those of Limousin steers. Thus, samples could be classified from the more oxidative to the more glycolytic: (i) RA Angus, (ii) RA Limousin and ST Angus (iii) ST Limousin.

Protein content in A-FABP (which is expressed in adipocytes only) were 2.3 and 3.6-fold higher in RA muscles from Angus and crossbred steers respectively compared to Limousin steers ($P < 0,002$), but no significant differences were observed in ST muscle. Protein content in A-FABP were also higher (x 1.1 to 7.5) in RA muscle than in ST muscle but the difference was significant in Angus and crossbred steers only ($P < 0,001$).

The above differences between genotypes were confirmed in LT muscle in experiment 2 when extremely fat Angus steers were compared to Limousin steers. Intramuscular TAG content was 9.4-fold higher in Angus steers. Among the studied metabolic parameters, the biggest differences were observed for A-FABP protein content, COX activity and ICDH activity which were 3.8, 2.1 and 1.6-fold higher in Angus compared to Limousin steers. The differences in other metabolic enzymes were lower (x 1.5 for activities of HAD and CS and x 0.60 to 0.83 for LPL, PFK and LDH activities in Angus compared to Limousin steers).

Since DNA and proteins contents were 1.02 to 1.19 higher in muscles in Limousin steers than in other genotypes, the differences between genotypes were slightly amplified when results were expressed per mg of DNA or per mg of proteins.

A-FABP protein content in relation to TAG muscle content

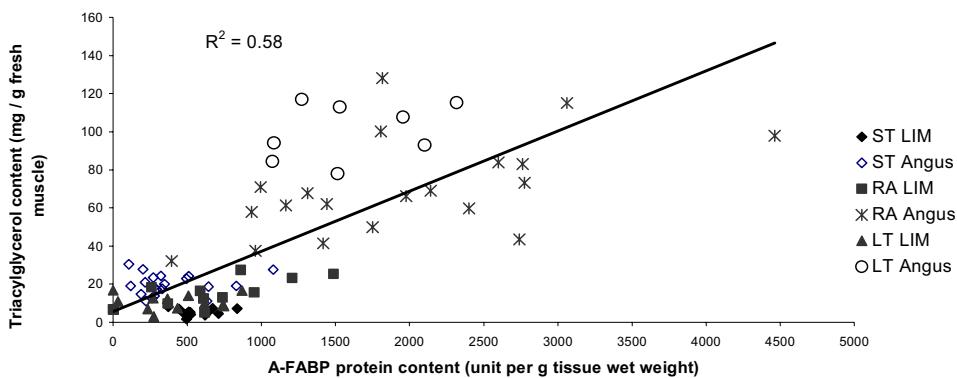


Figure 1. Triacylglycerol content in relation to A-FABP protein content in 3 muscles [Rectus abdominis (RA), Longissimus thoracis (LT) and Semitendinosus (ST)] from 3 genotypes of steers [Limousin (LIM), Pure Angus and Crossbred Angus (both labelled as Angus)].

Among the various parameters studied in the two experiments, three of them (A-FABP protein content, COX and ICDH activities) were characterised by high coefficients of correlation (> 0.70) with TAG muscle content when data of the three muscles (RA, ST and LT) from the four groups of steers were taken into account (84 values). The other parameters exhibited coefficients of correlation which ranged between 0.18 and 0.59. LPL activity, which is involved in uptake of circulating TAG, was not positively related to intramuscular fat.

Covariance analysis indicated that A-FABP protein content alone explained 58% of total variability in TAG content (Figure 1). The combination of COX activity and A-FABP protein content allowed us to explain 67% of total variability with these two parameters. The unexplained part of the rest of variability may be due to genotype effect (17%) and muscle effect (3%) independently of A-FABP content and COX activity.

Discussion

Our study was conducted with extreme muscle types and genotypes to display a wide range of intramuscular fat content. This allowed us to identify the major metabolic indicators which may contribute to the variability of fat deposition within muscle.

Comparison of muscle types clearly indicate that fat deposition was higher in oxidative muscles than in glycolytic muscles. Furthermore, the potential of intramuscular fat content was higher for genotypes characterised by a high oxidative muscle metabolism (Angus and Crossbred animals) than for Limousin (with a high glycolytic muscle metabolism). This confirms previous

observations in rabbits (Gondret *et al.*, 2001). It is, however, surprising because the more FA are catabolised, the less they should be deposited. It has been speculated that a high FA turnover (which is characteristic of oxidative muscles) would favour fat deposition (Gondret *et al.*, 2001). Among the oxidative metabolic markers, COX activity (an enzyme of the respiratory chain) was better correlated to TAG content than enzymes within the mitochondrial matrix (HAD, CS, ICDH).

Another important indicator of TAG deposition is A-FABP protein content. Given this protein is expressed within adipocytes exclusively (and not within muscle fibers), it is probable that the accumulation of A-FABP protein is an indicator of adipocyte number within the muscle tissue. By itself, this indicator explained a high proportion of the variability in TAG (58%). This indicates that intramuscular adipocyte number is the major biological mechanism which contributes to TAG accumulation. This result confirms those of Cianzo *et al.* (1985). The originality of our work is that A-FABP protein content is a parameter which can be easily measured routinely in frozen samples compared to adipocyte number.

The unexplained part of the variability (33%) may be linked to other metabolic pathways such as *de novo* lipogenesis which indeed differed between genotypes (not shown).

Acknowledgement

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Genotype differences in the long-term response to different levels of energy supply of dairy cows kept under tropical conditions

A. Jenet^{1,2}, A. Yimegnuhal¹, S. Fernandez-Rivera¹, A. Tegegne¹, P.O. Osuji¹, G. McCrabb¹, H.-R. Wettstein² & M. Kreuzer²

¹ International Livestock Research Institute (ILRI), P.O. Box 5689, Addis Ababa, Ethiopia

² Institute of Animal Science, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

Summary

Forty-eight *Bos indicus* cows of Boran type and Boran × Holstein crosses were fed low, medium and high levels of energy for approximate 5 years starting 1.5 years before first calving. Body weight change, milk production and reproductive performance were evaluated in the first three lactations. While crossbreds responded to higher feeding levels with increased milk production, Boran cows did not react. However, *post partum* anoestrus and calving intervals were reduced by the high energy supply in both genotypes. Receiving a low feeding level, Boran cows seem to cope better in reproductive performance. Changes in energy supply in lactation 3 did not clearly affect performance, but prolonged or reduced calving intervals of crossbreds switched to low or high feeding level regimes, respectively.

Keywords: *Bos indicus*, reproductive performance, under-nutrition

Introduction

Long-term or life-time under-nutrition and poor production and reproduction performance of dairy cattle in tropical small-holder systems are often a result of low feed availability and poor feed quality. Genotype options for small holders in the tropics include either indigenous, mostly *Bos indicus* genotypes, or their crossbreds with *Bos taurus* dairy breeds. *Bos indicus* and *Bos taurus* genotypes have been selected with different breeding purpose for long. While indigenous breeds evolved under seasonal and day-to-day fluctuations and shortages in feed availability, *Bos taurus* dairy breeds were selected for high milk yield under conditions of unlimited feed supply. In this study the response to feeding level during the entire first three lactations was investigated in Boran (*Bos indicus*) and Boran × Holstein cows, with treatment feeding already starting long before first conception. The study also tested the effect of changing feeding levels in the third lactation.

Materials and methods

Twenty-four Boran and 24 Boran × Holstein (F1 and F2 with 75% Holstein blood) cows were fed either low, medium or high feeding levels corresponding to an energy supply of approximately 1.0 (low), 1.2 (medium) and 1.4 times (high) of maintenance energy requirements. Allocation to feeding level remained unchanged from 1.5 years before first parturition until cows calved for the third time. In the third lactation, the cows previously fed on low, medium or high level were split into two groups each, which from then on were exposed either to low or to high level of energy supply, simulating an annual feed fluctuation versus a productive life-time plane of nutrition. The diet always consisted of grass hay, mainly Bermuda (*Cynodon dactylon*), and wheat bran (2:1). This type of diet also ensured that limitations were given clearly by energy supply.

Results and discussion

Differences in body weight (lower with low and medium feeding level) developed mostly before first calving (data not shown). Boran cows fed the high level gained weight in the first reproduction cycle and body condition scores were higher than in the corresponding crossbred group. The time period required for the occurrence of the first oestrus after calving was shorter with high feeding level in both genotypes while the effects of low versus medium level differed between lactations (Table 1). *Post partum* anoestrus does significantly limit productivity of dairy cattle in tropical regions and is directly correlated with the recovery of body weight after under-nutrition. In a study at ILRI crossbred cows resumed cycling after a period of severe depletion when 0.21 of the body weight loss was replenished and conceived when 0.51 of the loss was regained (Zerbini et al., 1996). Differences in non-return rates of successfully inseminated cows were not significant and ranking of feeding levels varied between lactations. However, two Boran cows receiving the high feeding level and four crossbred cows from the low treatment did not come into heat and did not get pregnant. This suggests that Boran tolerated the low feeding level and reproductive performance suffered with the high feeding level, in that case because cows got obese, whereas the opposite was observed for the crossbreds. Gestation length differed between genotypes (277 vs. 281); however, the difference was only 4 days on average.

Table 1. Effect of feeding level (L = low, M = medium, H = high), genotype and lactation (1 & 2) on the occurrence of the first oestrus after calving^{2,3,4}, non-return rate⁵, gestation^{3,5} and lactation length^{2,3,5}, calving interval³ and milk yield^{1,2,4} in Boran and crossbred cows.

| Genotype | Boran | | | Crossbreds | | | SEM |
|-----------------------------|----------|-----|-----|------------|-----|-----|------|
| | L | M | H | L | M | H | |
| Feeding level | | | | | | | |
| <i>Lactation 1</i> | <i>n</i> | 5 | 7 | 5 | 7 | 7 | 8 |
| 1 st oestrus (d) | 251 | 168 | 114 | 255 | 246 | 92 | 20.7 |
| Non-return rate | 1.4 | 2.0 | 4.7 | 1.8 | 1.7 | 2.4 | 0.29 |
| Gestation (d) | 279 | 276 | 277 | 282 | 276 | 278 | 0.9 |
| Lactation (d) | 264 | 282 | 229 | 403 | 459 | 377 | 27.4 |
| Calving interval (d) | 525 | 475 | 538 | 578 | 587 | 479 | 16.4 |
| Milk (kg/13 weeks) | 268 | 316 | 233 | 449 | 553 | 637 | 19.7 |
| <i>Lactation 2</i> | <i>n</i> | 5 | 8 | 3 | 7 | 7 | 8 |
| 1 st oestrus (d) | 92 | 105 | 53 | 186 | 188 | 88 | 13.2 |
| Non-return rate | 1.0 | 2.4 | 1.3 | 3.6 | 1.8 | 2.4 | 0.26 |
| Gestation (d) | 280 | 285 | 287 | 279 | 280 | 278 | 0.9 |
| Lactation (d) | 156 | 185 | 164 | 455 | 386 | 374 | 29.6 |
| Calving interval (d) | 264 | 282 | 229 | 403 | 459 | 377 | 10.1 |
| Milk (kg/13 weeks) | 271 | 299 | 247 | 454 | 525 | 721 | 21.3 |

¹ Effect of feeding level, $P < 0.05$.

² Effect of genotype, $P < 0.05$.

³ Effect of lactation, $P < 0.05$.

⁴ Interaction of feeding level \times lactation, $P < 0.05$.

⁵ Interaction of genotype \times lactation, $P < 0.05$.

Lactation length was nearly half in the Boran cows compared to the crossbreds but similarly long as described in the review of Mukasa-Mugerwa (1989). The difference is explained by the strong tendency of Boran cows to terminate milk production after weaning which is far less obvious in the crossbreds. Calving intervals only differed between lactations. Milk yield, calculated for lactations 1 and 2 over the first 13 weeks, was about two times higher in the crossbreds compared with the Boran, respectively. Calculated over the whole lactations, differences were even higher due to the shorter lactation lengths found in the Boran cows. Crossbreds responded to feeding level in milk yield (on average of the first 13 weeks of lactations 1 & 2 (5.4, 6.5 and 8.7 kg/d with low, medium and high level), while Boran cows showed no significant response in milk yield to any feeding level (overall average of 3.2 kg milk/d). Before first parturition, the same Boran animals responded in growth rate to the high feeding level, but also less clear than the crossbreds (Jenet et al., 2002).

In lactation 3, when part of the cows were shifted from high to low level and vice versa, the response in milk yield was considerably smaller than expected from the changes in energy supply in the magnitude of $\pm 40\%$ (Figure 1). By contrast, milk production of the cows in the third lactation was still largely influenced by the previous feeding level, i.e. their nutritional history, and previous genotype differences persisted. This suggests that under-nutrition of dairy cows of any genotype for a great part of their whole productive life-time results in irreversible losses in productivity while cows well-fed for the same time seem to tolerate a period of under-nutrition without major drop in performance. Also in reproductive performance there were no clear responses to the changed energy supply. There are some indications that both, low and high plane of nutrition, might have slightly impaired reproductive performance of the crossbred cows.

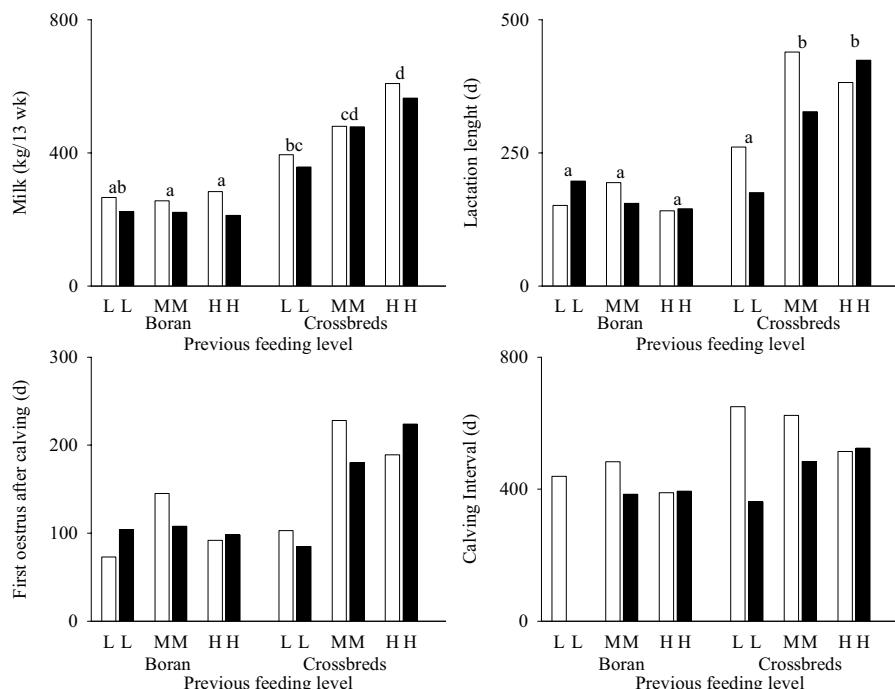


Figure 1. Effect of previous feeding level in lactations 1 & 2 and actual feeding level in lactation 3 (white columns = low; black columns = high) on milk yield and reproductive performance in Boran and crossbred cows in their third lactation.

Accordingly, there was a tendency towards prolonged or reduced calving intervals of cows switched to low or high feeding level regimes, respectively.

Carry-over effects of previous periods of under-nutrition of a kind that no clear response in milk yield will take place when feed supply is improved, are known from indigenous *Bos indicus* breeds (Olaloku and Oyenuga, 1974), but crossbreds were mostly found to respond at least moderately in milk yield to additional feed or supplements (Gemedo et al., 1995; Sandoval-Castro et al., 2000). The same holds true for the restoration of an impaired fertility, such as anoestrus caused by under-nutrition and work stress, by re-alimentation (Zerbini et al., 1996). The almost completely absent response of any cow group to changes in energy supply in lactation 3 is, therefore, unexpected.

Conclusions

When exposed to different energy supply for their whole productive life, milk yield of crossbred cows, but not of indigenous cows, is affected by the feeding level. Results from lactation 3 show carry-over effects of long-term exposure to another level of energy supply in such a way that the response was weak, both, to improvements and to impairments of feeding. These results have implications for market-oriented production systems and for rural areas with poor natural resources not strongly linked to markets and in which increasing milk yield is often not a priority. Opportunities for effectively using additional feed resources are of high priority in peri-urban areas, where an increase in marketable milk production is feasible with improved feeds for crossbred cows. Indigenous breeds, better adapted to low water and feed supply, would be the choice for remote tropical regions with unfavorable conditions.

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Utilization of α -ketoglutarate (AKG) in young growing pigs after intra-venous and intra-duodenal administration

Peter Junghans¹, Michael Derno¹, Stefan Pierzynowski², Ulf Hennig¹, Wolfgang-Bernhard Souffrant¹

¹ Research Institute for the Biology of Farm Animals, Research Unit Nutritional Physiology “Oskar Kellner”, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany

² Lund University, Department of Animal Physiology, Helgonavägen 3B, 22362 Lund, Sweden

Summary

AKG is a key intermediate substance in oxidative metabolism and nitrogen metabolism. Some studies have reported beneficial metabolic effects of dietary supplementation of AKG, but the role of AKG in the gut is still obscure. We aimed to quantify how the route of administration (intraduodenal vs. intravenous) affects AKG utilization rate and heat production. Castrated male pigs (15 kg) were supplied with a complete nutrient solution (Kabiven™, Fresenius Kabi, Uppsala, Sweden) via catheters. To explore metabolic effects of AKG, 1 g AKG * kgBW⁻¹*d⁻¹ was infused simultaneously with the nutrient solution (NS). After 5 days of adaptation [¹⁻¹³C]AKG (15 mg * kg BW⁻¹, 3 h-infusion) was provided to estimate the AKG utilization (AKG UTIL), which was approximated as: AKG UTIL = 100 - ¹³C recovery (in % of ¹³C dose). ¹³C recovery was calculated from the ¹³C enrichment in breath CO₂ and the corresponding whole-body CO₂ production. Results show that the route of administration of AKG (intraduodenal vs. intravenous) has significant influence on the utilization of AKG (% of ¹³C dose): 59.8 ± 4.9 vs. 68.5 ± 1.7 and whole body heat production (kJ*d⁻¹*kgBW^{-0.62}): 745 ± 68 vs. 948 ± 45, respectively. Results indicate that intravenous infusion of AKG may improve utilization of nutrients whereas utilization of AKG is smaller as compared to intraduodenal administration route.

Keywords: α -ketoglutarate, animal nutrition, pig model

Introduction

AKG is a key intermediate substance in oxidative metabolism (conversion of AKG to succinate with loss of CO₂ and formation of NADH) and nitrogen metabolism (formation of glutamate by transamination or scavenging free ammonia). Furthermore, AKG participates in the non-enzymatic oxidative decarboxylation during hydrogen peroxide decomposition, and enhances of the proper metabolism of fats that could suppress oxygen radical generation and thus prevent lipid peroxidative damages (Velvizhi et al., 2002). Some studies have reported beneficial metabolic effects of AKG in human and animal nutrition. Thus, enteral feeding of AKG in the early postoperative phase may improve gut integrity and reduce infectious complications after trauma and surgery in man (Wiren et al., 2002). In animal nutrition, it has been proposed to include AKG, because AKG could replace dietary dispensable amino acids by shunting ammonium back into the dispensable amino acid pool (Pierzynowski and Sjodin, 1998). Nevertheless, the role of AKG in the gut is still obscure (Lambert et al., 2002). Therefore, we aimed to quantify how the route of administration (intraduodenal vs. intravenous) affects AKG utilization rate and energy metabolism.

Materials and methods

Animals

For the investigations 4 castrated male pigs (German Landrace) per group with initial body weight (BW) of about 15 kg, aged nearly 2 month, were used. About four weeks after weaning the animals were taken from our own pig experimental station in Dummerstorf and housed in individual metabolic cages. During an one week adaptation period the animals were fed with the diet "Start 2" (Treder und von Pein, Itzehoe, Germany) and kept under controlled ambient temperature (22°C). Because the animals should be supplied continuously with nutrients and AKG animals were surgically fitted with three catheters (into jugular vein and duodenum for infusion of nutrients and AKG, into carotid artery for blood sampling).

Diets

Animals obtained an infusion of nutrient solution (NS) consisting of Kabiven™ emulsion supplemented with Soluvit' vitamin solution (both from Fresenius Kabi, Uppsala, Sweden). About 1800 ml per day of the nutrient solution was infused to an animal of 15 kg BW. This amount covered the 2.5 fold of the requirement for maintenance (about 5850 kJ*d⁻¹).

Experimental procedures

The NS infusion was started immediately after operation (1st day) whereas the infusion of AKG (1 g AKG * kgBW⁻¹*d⁻¹) was started on the 3rd day. In the intermediate time catheters for AKG infusion and blood sampling were kept free by means of heparinised isotonic saline solution (Serum-Werk Bernburg AG, Germany) with a flow rate of 2ml per hour. Depending on the experimental group both NS and AKG were given intraduodenally and/or intravenously to the animals 24 hours a day via catheters by means of peristaltic pumps (VEB MLW Labortechnik, Ilmenau, Germany). On the 3rd day the pigs were placed with the metabolic cages into the respiration chambers. After 3 days of adaptation to these conditions CO₂ production and O₂ consumption were measured over 3 days. Over the same periods urine was quantitatively collected every day. On the 7th day a dose of 15 mg [1-¹³C]AKG (Chemotrade, Leipzig, Germany) per kg BW solved in 50 ml isotonic saline solution was infused according to the experimental group via catheter by means of syringe pumps (Perfusor compact®, B/Braun, Melsungen AG, Germany) over a period of three hours. For calculating of AKG utilization gas samples were taken from the respiration chambers and transferred directly into breath bags (Tesseraux, Bürstadt, Germany) for subsequent measurement of the enrichment of ¹³C in breath CO₂ by means of the IRIS infrared ¹³C isotope analyser (WATV GmbH, Bremen, Germany (Junghans et al., 1997)). Sample time points were 24 and 1 hours before and 0.5; 1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; 5; 6; 9; 12 and 24 hours after start of [1-¹³C]AKG infusion.

Calculations

The AKG utilization (AKG UTIL) was approximated as:

$$\text{AKG UTIL} = 100 - \frac{\text{recovery}}{\text{dose}} \times 100 \quad (\text{in \% of } ^{13}\text{C dose}).$$

The heat production (kJ*d⁻¹) was calculated from CO₂ production, O₂ consumption (L*d⁻¹) and urinary N excretion (gN*d⁻¹)

$$\text{Heat production} = 5.16 \cdot \text{CO}_2 \text{ production} + 16.18 \cdot \text{O}_2 \text{ consumption} - 5.90 \cdot \text{N excretion}$$

as reported by Brower (1965).

Results

Results show that the route of administration of AKG has significant influence on the utilization of AKG and whole body heat production (Table.1).

Table 1. Dietary metabolic effect of AKG.

| Experimental No. | NS | AKG | AKG utilization % of ^{13}C dose | Heat production |
|--|----|-----|--|---|
| | | | | $\text{kJ} \cdot \text{d}^{-1} \cdot \text{kg} \cdot \text{BW}^{-0.62}$ |
| <i>Route of administration[*]</i> | | | | |
| 1 | id | id | 59.8 ± 4.9 a) | 745 ± 68 a),A) |
| 2 | iv | id | 62.7 ± 6.3 | 849 ± 113 |
| 3 | iv | iv | 60.7 ± 10.3 | 936 ± 92 B) |
| 4 | id | iv | 68.5 ± 1.7 b) | 948 ± 45 b) |

^{*}id: intraduodenal, iv: intravenous; Values are expressed as mean \pm SD. Different letters indicate significant differences ($p < 0.05$) within rows.

Discussion

The utilization of AKG corresponds with values reported for carboxyl- ^{13}C labelled amino acids (e.g. Junghans et al., 1998). The utilization of AKG was determined indirectly from the recovery of ^{13}C after decarboxylation of the C-1 in AKG. Therefore, the differentiation between the direct decarboxylation of AKG to form succinate and the formation of glutamate with the subsequent reverse reaction to AKG and succinate is not possible. Also, the incorporation of ^{13}C into metabolites or body constituent was not determined. Losses of ^{13}C via urine and faeces were found to be negligible.

Results indicate that intravenous infusion of AKG may improve utilization of nutrients whereas utilization of AKG is smaller as compared to intraduodenal administration route.

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Relationship between body composition, net feed intake and gross feed conversion efficiency in composite sire line sheep

S.A. Knott^{1,2}, B.J. Leury², L.J. Cummins¹, F.D. Brien¹ & F.R. Dunshea^{2,3}

¹ *Pastoral & Veterinary Institute, Department of Primary Industries, Private Bag 105, Hamilton, VIC. 3300, Australia*

² *Institute of Land & Food Resources, The University of Melbourne, Parkville, VIC. 3010, Australia*

³ *Victorian Institute of Animal Science, Department of Primary Industries, 600 Sneydes Road, Werribee. VIC. 3030, Australia*

Summary

Net feed intake (NFI) and gross feed conversion ratio (FCR) are commonly used to identify animals that are more efficient at converting feed into gain, however little work has evaluated the potential to use NFI or FCR in the sheep industry. Variation of NFI and FCR between different stages of maturity, and the influence of body composition on NFI and FCR were studied in a group of composite sire ram lambs. The results suggest that careful consideration of the stage of maturity of animals when measuring NFI and FCR is necessary when using these parameters for selection purposes. NFI and FCR were also found to be highly correlated to carcass lean tissue mass, which supports previous work undertaken in cattle.

Keywords: *feed conversion efficiency, body composition, sheep*

Introduction

Net feed intake (NFI) and gross feed conversion ratio (FCR) are commonly used to identify animals that are more efficient at converting feed into gain. NFI has been shown to be moderately heritable and negatively correlated with carcass lean in cattle (Herd and Bishop, 2000). However, little work has evaluated the potential to use NFI or FCR in the sheep industry. Improved feed conversion efficiency could have substantial benefits for the industry, through the reduction of feed costs, whilst maintaining or increasing level of production. This study examined the variation of NFI and FCR between sheep at different stages of maturity, and investigated the influence carcass composition and organ weight has on these parameters.

Material and methods

Composite sire ram lambs ($n=34$; 4 months age; $\bar{x} = 36$ kg), sourced from a pedigreed research flock, were individually fed a concentrate-based diet *ad libitum* (metabolisable energy 12 MJ/kg DM; crude protein 16% DM) for 28 d. Live weight and total dry matter intake (TDMI) were determined weekly and NFI was calculated as the difference between actual feed intake and estimated feed intake given the animal's requirements for maintenance and growth (Arthur *et al.* 1996). Animals were ranked on NFI and FCR, with a subset of animals ($n=12$) selected and allocated to either a high (H) group representing the highly efficient animals, or to a low (L) group, representing the least efficient. Lambs were then returned to pasture.

At 11 months of age ($\bar{x} = 41.8$ kg) H and L lambs were individually fed a similar concentrate-based diet. Twice weekly body weight and TDMI were measured for 41 d. After 41 d, animals were slaughtered and organ weights recorded. Total organ mass was calculated as the sum of the empty GIT, liver, pancreas, spleen, heart and lungs. Carcasses were scanned using dual energy x-

ray absorptiometry (DXA) to determine lean tissue mass (LTM), fat tissue mass (FTM) and inorganic matter (IOM). Following scanning, carcasses were boned out into lean, bone and fat. Statistical analyses were conducted using GenStat (GenStat, 2000) Correlations were considered significant when $P<0.05$.

Results

At 4 months of age there were differences in average daily gain (ADG), NFI and FCR (Table 1) and therefore it was possible to identify extreme groups of animals. Across all sheep, NFI was correlated with FCR and ADG but not with TDMI (Table 3). FCR was not correlated with TDMI. At 11 months of age, the previously identified differences between H and L sheep were no longer evident, however TDMI was significant between the groups (Table 2). There was no correlation between NFI measured at 4 and 11 months of age, and similarly with FCR at the two different

Table 1. Mean (\pm SD) ADG, TDMI, NFI and FCR of sheep at 4 months of age.

| Group | n | ADG (g/day) | TDMI (kg) | NFI | FCR |
|-------|----|-------------------------|-------------------------|----------------------------|--------------------------|
| All | 34 | 364 (57) | 35.9 (4.4) | -0.286 (0.20) | 4.78 (0.78) |
| H | 6 | 422 (43) ^a | 32.4 (3.7) ^a | -0.570 (0.10) ^a | 3.67 (0.43) ^a |
| L | 6 | 305 (31.8) ^b | 35.7 (1.7) ^a | -0.012 (0.17) ^b | 5.62 (0.6) ^b |

^{a, b} means with different letters are significantly different ($P<0.05$, H & L groups only)

Table 2. Mean (\pm SD) ADG, TDMI, NFI and FCR of sheep at 11 months of age.

| Group | n | ADG (g/day) | TDMI (kg) | NFI | FCR |
|----------------|----|--------------------------|---------------------------|----------------------------|---------------------------|
| All | 11 | 484 (81.34) | 80.3 (9.978) | -0.42 (0.179) | 4.09 (0.462) |
| H | 6 | 468 (95.35) ^a | 77.0 (11.78) ^a | -0.45 (0.182) ^a | 4.07 (0.563) ^a |
| L ¹ | 5 | 504 (65.66) ^a | 84.2 (6.397) ^b | -0.38 (0.189) ^a | 4.11 (0.370) ^a |

¹ Numbers of animals were reduced for the second experiment ($n=12$, H & L groups only)

Table 3. Selected correlations for efficiency measures for sheep at 4 months and 11 months of age with extreme FCR and NFI values ($n=12$, $n=11$, 4 months and 11 months of age respectively; values in bold are significant, $P<0.05$).

| | 4 months | ADG | 1.00 | | | | | |
|--|-----------|----------|---------------|--------------|--------------|-----------|---------------|--------|
| | | TDMI | -0.304 | 1.00 | | | | |
| | | FCR | -0.915 | 0.637 | 1.00 | | | |
| | | NFI | -0.902 | 0.562 | 0.968 | 1.00 | | |
| | 11 months | ADG | 0.038 | 0.399 | 0.137 | 0.085 | 1.00 | |
| | | TDMI | -0.282 | 0.168 | 0.311 | 0.294 | 0.821 | 1.00 |
| | | FCR | -0.380 | -0.489 | 0.117 | 0.193 | -0.668 | -0.128 |
| | | NFI | -0.483 | -0.521 | 0.215 | 0.323 | -0.560 | -0.078 |
| | | ADG | TDMI | FCR | NFI | ADG | TDMI | FCR |
| | | 4 months | | | | 11 months | | |

stages of maturity (Table 3). As a group, mean NFI and FCR at 11 months of age were lower in comparison to 4 months of age, however the range was less.

A reasonable range was observed between animals in body composition parameters and visceral organ weights (Table 4), however there were no significant correlations between total organ mass, or individual organ weight with NFI or FCR at 11 months. There was a significant difference between H and L animals for liver weight adjusted to empty body weight (H 17.59 g/kg, L 20.27 g/kg, $P<0.05$). DXA predicted lean tissue was correlated with boned out lean tissue (Table 5) and both measures of lean tissue mass were significantly correlated to NFI.

Table 4. Mean ((SD) DXA body composition values, bone out data and selected organ weights for sheep at 11 months of age (n=11, n=6, n=5 for all, H & L groups respectively)

| | DXA LTM (kg) | DXA FTM (kg) | DXA IOM (kg) | Total Lean (kg) | Total Fat (kg) | Total Bone (kg) | Total Organ mass (g) | Liver (g) | Total GIT (g) ² |
|-----|--------------------|--------------------|--------------------|-----------------------|----------------------|-----------------------|----------------------------|------------------|----------------------------------|
| All | 25.3 (2.571) | 6.0 (1.28) | 1.1 (0.07) | 18.2 (1.760) | 5.0 (0.75) | 6.7 (0.49) | 6794 (604.53) | 1085 (142.88) | 4123 (449.64) |
| H | 25.8 (1.419) | 5.9 (1.70) | 1.1 (0.06) | 18.7 (0.987) | 5.0 (0.87) | 6.6 (0.30) | 6715 (548.40) | 1077 (143.75) | 4025 (307.58) |
| L | 24.8 (3.648) | 6.1 (0.72) | 1.1 (0.01) | 17.5 (2.366) | 5.0 (0.66) | 6.8 (0.68) | 6890 (718.96) | 1096 (157.96) | 4241 (596.10) |

² GIT - gastrointestinal tract

Table 5. Selected body composition (DXA and bone out) correlations for sheep at 11 months of age with extreme FCR and NFI values. (Values in bold are significant, P<0.05).

| | | | | | | | | |
|---------|--------------|-------|---------------|---------------|--------------|------------|--------------|------|
| DXA LTM | 0.402 | 0.040 | -0.619 | -0.890 | 1.00 | | | |
| DXA FTM | 0.250 | 0.391 | 0.106 | -0.180 | 0.230 | 1.00 | | |
| Lean | 0.309 | 0.141 | -0.316 | -0.614 | 0.784 | 0.350 | 1.00 | |
| Fat | 0.612 | 0.424 | -0.465 | -0.729 | 0.624 | 0.570 | 0.628 | 1.00 |
| | ADG | TDMI | FCR | NFI | DXA LTM | DXA FTM | Lean | Fat |

Discussion

Measuring feed intake and weight gain over a defined period enables the calculation of both FCR and NFI at various stages of growth. This study explored the hypotheses that i) stage of maturity may have a significant impact on NFI and FCR values obtained for sheep; and ii) that body composition may also impact on NFI and FCR. These data suggest that the stage of maturity at which NFI and FCR are measured may alter the rank of these parameters and would need to be considered when using NFI and FCR for selection purposes. Variation in stage of maturity at 4 months of age and its potential influence on efficiency may have contributed to altering the order of FCR and NFI between the two age groups, but this is difficult to determine as composition was not measured at 4 months. It is also possible that length of time (21 d versus 41 d) needed for accurate measurement of intake and weight gain, may have also contributed to this variation, however this is unlikely as the animals were young, rapidly growing and at *ad libitum* intake

(Archer *et al* 1999). Further work needs to be undertaken to confirm the results found in this small study.

Body composition is often thought to have a significant role in influencing whether one animal is more efficient than another (Perry *et al*, 1997). The negative correlation between lean tissue mass and NFI and FCR found in this study indicates that animals with a higher proportion of lean tissue mass were more efficient in terms of NFI and FCR. This is supported by previous work undertaken in cattle (Herd *et al*, 2000). However care needs to be taken in interpreting this result due to the low numbers of animals used.

Total organ mass, in particular the liver and gut tissues, are key components of energy utilisation in animals. The liver, although it represents only 1-2% of body weight, contributes approximately 17-31% to whole animal energy expenditure, which is similar to that of the digestive tract (Ortigues, 1991). Small changes in mass of these tissues may cause significant changes in energetic efficiency between animals. Provided that level of nutrition and physiological state are similar between animals, this should be a valid comparison (Ortigues & Doreau, 1995), although there still may be variation in energetic efficiency per unit mass of tissue. These data suggest that there was no relationship between individual organ weights and FCR and NFI at 11 months. However it is interesting to note that there was a significant difference in liver weight adjusted for empty body weight at 11 months of age between H and L animals identified at 4 months of age. We conclude that body composition and organ weight is likely to have an influence on NFI and FCR in these animals, however further work needs to be undertaken in larger numbers of animals.

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High intake of metabolizable energy alters regulation of bovine cardiac mitochondria

B. Löhrke, M. Derno, G. Dietl, A. Tuchscherer, T. Viergutz & W. Jentsch

Research Institute for the Biology of Farm Animals, Department of Nutritional Physiology, „Oskar Kellner“ and Department of Genetics and Biometry, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

Summary

Increasing intake of metabolizable energy (ME) correlates with an increase in heat production. Here we show that an excessive intake of ME induces a change in energy dissipating mechanisms of bovine cardiac mitochondria.

Keywords: ATP/ADP-sensitive potassium channels, redox state, cardiomyocytes

Introduction

Whole body metabolic rate (MR) increases with an increase in an intake of metabolizable energy (ME) as shown by measurements of the oxygen consumption and CO₂ production using respiration chambers (Löhrke et al. 1997). Mitochondrial respiration accounts for about 80 % of the total oxygen consumption and control over respiration has been reported to be shared between the reactions that produce mitochondrial membrane potential (ψ_M) and those that dissipate it (Ainscow & Brand 1999). Oxygen consumption and ψ_M are correlated, however, at a high respiratory level in a nonlinear manner (oxygen consumption exceeds the increase in ψ_M *in vitro*). An intact mitochondrial inner membrane is required for coupling the ATP synthesis to oxygen consumption through a protonmotive force that drives the ATP synthase and other coupled reactions. Proton flux is generated by proton pumps of the electron transport chain, transferring protons from NADH at complex I and succinate (complex III) into the space between inner and outer membranes. ATP synthesis can be uncoupled from oxygen consumption by proton leak across the inner membrane. This is one of the most important energy-dissipating cycles regulated by uncoupling proteins, nitric oxide (Nisoli et al. 2003), and cationic channels (Murata et al. 2001) especially ATP/ADP-sensitive potassium channels (KATP/ADP) with tissue-specific differences in the impact on mitochondrial activity. The corresponding regulation of ψ_M in bovine cardiac mitochondria is unknown. In this study we focused on the responsiveness of cardiac ψ_M to differential intake of ME and on the response of ψ_M to drugs that open (activate) KATP/ADP or inhibit complex III to obtain some information about diet-induced changes in the regulation of ψ_M .

Methods

Twelve bulls (Deutsches Holstein) 289 ± 15 kg in weight and 300 ± 15 days old were allocated to 3 groups (4 in each) fed 1.0-, 1.5-, and 1.9-times the requirement of ME for maintenance (MEm) for 4 weeks. Heat production (HP) and heart rate (HR) were recorded in week 4 using four climatized (18° ± 2° C) respiration chambers. After slaughtering, specimens from the right ventricle were sampled and mitochondria were prepared using a kit from Sigma. Protein concentration of the mitochondrial suspension (10 mM Hepes pH 7.4, 250 mM sucrose, 1 mM ATP, 0.08 mM ADP, 5 mM sodium succinate, 2 mM K₂HPO₄, 1 mM DTT) was measured by the Bradford technique.

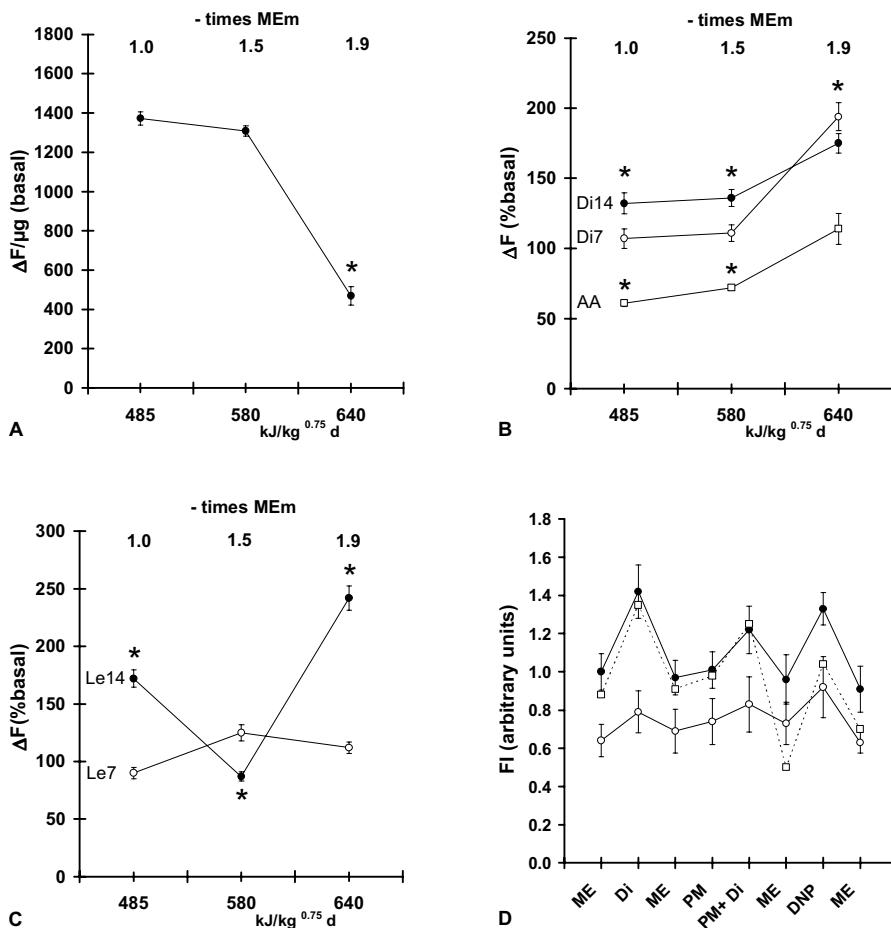


Figure 1. A-C: Ψ_M changes induced by differential MEm, complex III inhibition, and K^+ ATP/ADP openers.

Mitochondria from right ventricles of animals fed 1.0-, 1.5-, and 1.9-times MEm were incubated without additives (A), with antimycin A, AA, or diazoxide, Di, (B) and levromakalim, Le, (C) in concentrations (μM) indicated. Ψ_M was measured by JC-1 fluorescence as described in methods. The symbols stand for means, the bars for SEM, asterisks for significant difference (paired t-test vs basal, $P < 0.05$).

Figure 1. D: Redox state of FAD/FMN in response to uncoupling the respiration chain in cardiomyocytes.

Cells were successively incubated in the medium without additives (ME), diazoxide (Di, 100 μM), phorbolmyristate acetate (PM, 100 nM), PM + Di, and dinitrophenol (DNP, 0.5 mM) washing off the drugs in intervals of 1 min. FAD/FMN fluorescence intensity (FI) was measured in single cells by flow cytometry. FI increase indicates oxidation of nonfluorescent $\text{FADH}_2/\text{FMNH}_2$. Impact of PM-inducible protein kinase-C activity on FI was recorded to obtain some information about the susceptibility to inflammatory responses. The closed and open circles stand for means of the 1.0- and 1.9-MEm group, the rectangles for 1.5-MEm, the bars for SD. For clarity, the bars of 1.5-MEm means are omitted.

ψ_M was assayed by JC-1 (to 30 μM) in 20 mM MOPS pH 7.5, 110 mM KCl, 10 mM ATP, 10 mM MgCl₂, 10 mM sodium succinate, 1 mM EGTA (150-fold the volume of mitochondrial suspension). JC-1 fluorescence maximum shifts from 530 to 590 nm after ψ_M -dependent accumulation in the space between outer and inner membrane (excitation and emission, 480 \pm 20 and 620 \pm 40 nm). Fluorescence (F) accumulation (ΔF) per mg (respective μg) protein was calculated by $(F_{\text{sample}} - F_{\text{blank}})(\text{JC-1 dilution}/V(C)$, where C = protein (mg/ml) and V = volume of the suspension added (total volume, 300 μl). FAD/FMN fluorescence in intact single cardiomyocytes (obtained by digestion of small-cut ventricle specimen with 0.15 % collagenase) was measured by flow cytometry (Coulter) with an excitation and emission at 488 and 520 \pm 10 nm.

Results and discussion

Basal ψ_M of the 1.0- and 1.5-MEm groups was found to exceed (roughly 280 %) the value of the 1.9-MEm group (Figure 1A). Complex III inhibition by antimycin A (2.5 μM) decreased ψ_M only at 1.0- and 1.5-MEm (Figure 1B). In contrast, diazoxide (7 μM), an opener of mitochondrial KATP/ADP, strongly induced ψ_M only in the 1.9-MEm group (Figure 1B). This indicates that the ψ_M differences do not occur due to lacking mitochondrial integrity but due to a change in the regulation of ψ_M . The dose-dependent ψ_M responses to levcromakalim (Figure 1C), a KATP/ADP opener that activates the channels by a mechanism differing from diazoxide, support this conclusion and indicate diet-induced structural KATP/ADP changes. In contrast to 1.0- and 1.5-MEm, the major portion of intracellular FAD/FMN was reduced (nonfluorescent) at 1.9-MEm and little influenced by uncoupling the respiratory chain through dinitrophenol or by opening KATP/ADP through diazoxide (Figure 1D). In summary, the results indicate a diet-inducible change in mitochondrial energy-dissipating mechanism.

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Oral selenate improves the diabetic status in dbdb mice and modifies the activity of glycolytic and gluconeogenic marker enzymes

A.S. Mueller¹, J. Pallauf¹ & J. Rafael²

¹ Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Heinrich-Buff-Ring 26 - 32, D-35392 Giessen

² Center of Biochemistry, Ruprecht Karl University Heidelberg, Im Neuenheimer Feld 328, D-69210 Heidelberg

Introduction

Beside antioxidative functions of the trace element selenium as an integral part of glutathione peroxidases and thioredoxin reductases, an interesting physiological aspect for selenate was detected with regard to diabetes. In type I diabetic rats and in tissue cultures insulinomimetic properties have been shown to evolve from selenate (selenium VI). 10 weeks of oral selenate treatment led to a 50 - 80% reduction of the elevated blood glucose levels in rats with streptozotocin induced diabetes I (IDDM). Especially during oral glucose challenge tests the insulinomimetic properties of selenate became vitally important. Insulinomimetic properties of selenate were also achieved after intraperitoneal selenate injections for 8 weeks (McNeill et al., 1991; Battell et al., 1998). In the type I diabetic rat model not only a higher disappearance rate of glucose and an enhanced glucose tolerance was the outcome of selenate treatment but also a correction of the abnormally expressed glycolytic and gluconeogenic marker enzymes glucokinase, pyruvate kinase, phosphoenolpyruvate carboxykinase was observed as a consequence of selenate administration, indicating the involvement of selenate in major insulin dependent signalling pathways (Becker et al., 1996). An influence of selenate administration on the expression and the activity of glucose-6-phosphate dehydrogenase and fatty acid synthetase confirmed this hypothesis (Berg et al., 1995). Treatment of rat adipocytes and rat hepatocytes with 500 µM selenate affected an increased phosphorylation of several proteins including the β-subunit of the insulin receptor, IRS1, the p42 and the p44 subunits of MAP kinase, cAMP phosphodiesterase, S6 kinase and 210-, 170-, 120-, 95-, and 60 kDa proteins. In summary the enhanced phosphorylation of diverse cellular proteins is believed to be responsible for an elevated translocation of glucose transporters, an increased glucose uptake and a modified gene expression of metabolic enzymes (Hei et al., 1998; Stapleton et al., 1997; Stapleton, 2000). In the literature no information is available so far on a possible insulinomimetic role of selenate in animals with type II diabetes (NIDDM). A distinct differentiation of the insulinomimetic properties of selenate in comparison to other selenium derivatives on glucose metabolism in diabetic animal models could not be achieved so far. The present study consequently investigates possible insulinomimetic properties of selenate in C57BL/KsOlaHsd-Leprdb mice with a defective leptin receptor, featuring severe symptoms of NIDDM such as hyperglycaemia, hyperinsulinaemia and high resistance to insulin (Kodama et al., 1994; Chua et al., 2002). The study also examines whether insulinomimetic properties are only derived from selenate or if other selenium compounds like selenite which are often used as selenium supplements in laboratory animal diets display insulinomimetic effects as well.

Materials and methods

21 adult male C57BL/KsOlaHsd-Leprdb mice, weighing 46.7 ± 1.31 g, kept at 22°C and fed a common lab chow (Altromin 1310, containing 0.25 mg selenium/kg diet as sodium selenite) were put on a selenium deficient diet (<0.03 mg Se/kg) based on torula yeast. The mice were randomly

assigned to three groups of 7 animals (group 0Se = Se deficient mice, group SeIV = selenite treated mice and group SeVI = selenate treated mice). Group 0Se was kept on selenium deficiency for 10 weeks, whereas the mice of the groups Se IV and SeVI were supplemented with a daily dose of sodium selenite and sodium selenate equivalent to 15% of the individual LD50 of these selenium compounds (LD50 of selenite and selenate for mice = 3500 µg/kg body weight). Development of body selenium stores during the experiment was controlled by measuring plasma glutathione peroxidase activity (GPx3). Activity of cellular glutathione peroxidase (GPx1) in the liver and in the skeletal muscle was measured as an indicator of the final selenium status (Tappel et al., 1982). During the experimental period the actual diabetic status of the mice was monitored by testing their glucose tolerance and insulin resistance. These tests were performed before subjecting the mice to specified diets (initial status) and after 4, 6, 8 and 10 weeks of special feeding. Activities of the glycolytic marker enzymes hexokinase, phosphofructokinase, pyruvate kinase and of the gluconeogenic marker enzymes glucose-6-phosphatase, fructose-1,6-diphosphatase, pyruvate carboxylase in the liver, the skeletal muscle and in adipose tissue served as parameters of the diabetic status at the end of the experiment (Ureta et al., 1975; Uyeda, 1970; Blair et al., 1976; Allegre et al., 1988; Pontremoli & Melloni, 1975; Warren & Tipton, 1976).

Results

A differential development of plasma glutathione peroxidase activity (GPx3) was measured as a consequence of the diverse dietary conditions. Starting from a mean activity of 8.65 ± 1.39 U/mL GPx3 activity persistently decreased in the selenium deficient mice of group 0Se to a final value of 2.68 ± 0.38 U/ml at week 10, whereas a continuous increase of GPx3 activity to final values of 14.6 ± 0.59 and 12.7 ± 0.66 was measured in the selenium treated mice of the groups SeIV and SeVI, respectively. Selenium deficiency and treatment with selenite or selenate were also reflected by the activity of cellular glutathione peroxidase (GPx1) in the liver and in the hind limb muscle of the mice. After 10 weeks of selenium deficiency GPx1 activity in the liver of Se deficient mice (396 ± 139 mU/mg protein) was reduced to 23% and 25% as compared to the values measured in mice treated with selenite (1741 ± 205) and selenate (1599 ± 129) for 10 weeks. Likewise in the hind limb muscle of selenium deficient mice of group 0Se (26.8 ± 3.87 mU/mg protein) GPx1 activity decreased to about 54% and 48% in comparison to selenium supplemented mice of groups SeIV (49.7 ± 10.2) and SeVI (56.2 ± 12.4).

After 10 weeks a distinct impairment of glucose tolerance in selenium deficient and selenite treated mice could be observed in comparison to the initial status and to selenate treated mice. In contrast selenate administration led to a reduction of blood glucose peak values obtained 20 and 40 minutes after a glucose challenge. Recovery from the glucose challenge was highly delayed in selenium deficient mice and in mice treated with selenite for 10 weeks. In mice with selenate administration the return of blood glucose concentration to the initial value was comparably fast as in the mice of the initial status. 10 weeks of selenium deficiency (0Se) clearly diminished the properties of insulin. Unexpectedly the administration of selenite (Se+IV) for 10 weeks caused the most distinct impairment of insulin action associated with a markedly increased insulin resistance. 30, 60 and 90 minutes after the insulin challenge the reduction of the fasting blood glucose concentration was only 10%, 28% and 32%, respectively. Thus the acute reducing activity of insulin on blood glucose concentration in mice treated with selenite was only half of that obtained for mice at the initial status and in selenium deficient mice. Dbdb mice treated with the insulinomimetic selenium compound selenate for 10 weeks showed the highest response to an insulin challenge. The reduction of the fasting blood glucose level in selenate treated mice was most distinct (30 min: 33% reduction of the fasting blood glucose concentration, 60 min: 62%, 90 min: 75%). Thus in the present study the altered sensitivity to insulin was also reflected by the activity of some glycolytic and gluconeogenic marker enzymes in various tissues of the dbdb mice (Table 1). 10 weeks of selenate treatment led to a 3-fold increase in liver hexokinase activity as compared to selenium

deficient and selenite treated mice. The activity of phosphofructokinase in liver and adipose tissue was elevated 2-fold and 1.7-fold in selenate treated mice in comparison to their selenium deficient and selenite treated companions. Within the glycolytic marker enzymes a 2-fold higher activity of pyruvate kinase was also measured in the adipose tissue of selenate treated mice as compared to selenium deficient and selenite treated mice. In contrast to glycolytic enzymes, 10 weeks of selenate treatment repressed the activity of liver pyruvate carboxylase, the first enzyme in gluconeogenesis, by the factor 1.5 in comparison to selenium deficient and selenite treated dbdb mice.

Table 1. Activity of glycolytic and gluconeogenic marker enzymes (U/mg protein) in various tissues of dbdb mice kept on selenium deficiency for 10 weeks or treated with selenite (Se IV) or selenate (Se VI) for 10 weeks.

| Glycolytic/gluconeogenic marker enzyme | Organ | 0 Se | Se IV | Se VI |
|--|------------------|------------------------|------------------------|------------------------|
| Hexokinase | • Liver | 0.08±0.05 ^a | 0.07±0.05 ^a | 0.26±0.06 ^b |
| Phosphofructokinase | • Liver | 9.25±2.74 ^a | 12.2±0.86 ^a | 18.5±1.22 ^b |
| | • Adipose tissue | 0.22±0.07 ^a | 0.19±0.08 ^a | 0.38±0.07 ^b |
| Pyruvate kinase | • Liver | 5.20±1.58 ^a | 5.21±0.69 ^a | 6.25±0.84 ^a |
| | • Adipose tissue | 0.12±0.05 ^a | 0.09±0.03 ^a | 0.21±0.03 ^b |
| Glucose-6-phosphatase | • Liver | 2.62±0.92 ^a | 3.60±0.72 ^a | 3.89±0.51 ^a |
| Fructose-1,6-bisphosphatase | • Liver | 0.65±0.24 ^a | 1.00±0.36 ^a | 0.99±0.38 ^a |
| Pyruvate carboxylase | • Liver | 90.9±16.8 ^b | 103±14.9 ^b | 66.3±11.6 ^a |

Significant differences ($p<0.05$, Tukey test / Dunnett-T3 test) between groups are indicated by different superscripts within a line

Discussion

Hitherto investigations on in vivo insulinomimetic properties of selenate were made exclusively in streptozotocin treated type I diabetic rats and in tissue cultures of hepatocytes and adipocytes. Due to altered glucose tolerance the present study could show a distinct insulinomimetic effect of selenate also in type II diabetic animals. In type I diabetic animals the diabetic metabolism of the animals is the result of a restricted insulin secretion (Nishigaki et al., 1989). In contrast type II diabetic dbdb mice present a completely different metabolic situation (Chan et al., 1975). The diabetic metabolism in these animals is the consequence of an increased insulin production and a high resistance to insulin. The current study gives evidence for an additional role of selenate which meliorates insulin resistance of the dbdb mice in contrast to selenium deficient and selenite treated controls, indicated by a more intensive and prolonged effect of a single insulin dose. Thus selenate could be shown as acting as an insulin sensitising agent in type II diabetic animals. Both, the results of previous investigations in type I diabetic rats and the findings of the present study confirm the hypothesis that selenate influences the activation of major components of the insulin signalling pathway and amplifies insulin signalling. Downstream the insulin receptor substrates 1 and 2 insulin signalling spreads into three pathways. The RAS-RAF-MEK-MAPK pathway triggers the expression of glycolytic and gluconeogenic marker enzymes and of GLUT 3. The activation of the protein kinase B pathway is involved in the regulation of GLUT 1 synthesis and GLUT 4 translocation, whereas the activation of PI3 kinase is the second main stimulus for GLUT 4 activation (Hall & Granner, 1999; Newsholme & Dimitriadis, 2001). Altogether the observed augmentation of the glycolytic pathway by selenate treatment, indicated by enhanced activities of hexokinase in the liver, phosphofructokinase in liver, skeletal muscle and adipose tissue and of

pyruvate kinase in adipose tissue and the suppression of gluconeogenesis indicated by the decreased activity of liver pyruvate carboxylase in comparison to selenium deficient and selenite treated mice confirms the hypothesis that selenate modifies the activities of glycolytic and gluconeogenic marker enzymes by an involvement in insulin signalling transduction.

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Nursing behaviour, milk intake and growth rate in piglets

M. Neil

Swedish University of Agricultural Sciences, Dept. of Animal Nutrition and Management, Funbo-Lövsta Research Centre, S-755 97 Uppsala, Sweden.

Summary

Milk production in 5 single-housed Yorkshire sows was measured using the isotope dilution technique with D₂O as a marker. Sows were in their third parity and litter size was 7-13 piglets. Piglets were enriched twice, at 4-5 (period 1) and at 16-18 (period 2) days of age. During the measurement periods nursing behaviour of sows with litters were recorded using a time lapse video. Piglet teat order was recorded by direct observation. The individual milk intake was significantly correlated with piglet LW (+), piglet growth (+) and teat pair number (-). Teat pair number was also correlated with piglet LW and growth (both negatively), but not with piglet birth weight. No significant correlations between sow milk yield and nursing frequency or length of nursing bouts were found. Nursing frequency decreased from period 1 to period 2 (30.5 and 26.3 per day, P<0.01), as did the median length of nursing bouts (7.7 and 4.7 minutes, NS). Milk production per sow and day increased from 9.6 kg in period 1 to 14.0 kg in period 2 (P<0.05). The individual milk intake increased from 0.93 kg per piglet and day in period 1 to 1.40 kg in period 2 (P<0.05). Daily milk intake decreased by 0.059 kg (P<0.01) and piglet growth rate by 0.016 kg per day (P<0.01) with teat pair number. The conversion of milk into growth increased from 3.8 kg per kg in period 1 to 4.8 in period 2 (P<0.05). Thus, the data confirm the superiority of anterior teats over posterior teats regarding milk yield and piglet growth rate. In spite of a 14% decrease in nursing frequency from period 1 to period 2 (mean piglet age 6 and 19 days), milk yield in sows increased by 46%.

Introduction

Suckling in pigs is a phenomenon with an intricate interaction between the sow and her litter, and between littermates. The piglet suckles frequently and is utterly dependent on sow milk since consumption of other feed is insignificant before 3 weeks of age (Cole, 1990). The variation in piglet live weight at birth is large and this variation is rather increased than decreased throughout lactation. Hence, the variation in piglet growth and milk intake must be considerable. It is therefore of interest to study the individual milk intake in piglets and the total sow milk yield in relation to piglet growth and nursing behaviour. In the present study, milk intake was measured using isotope dilution. With this technique, in contrast to the weigh-suckle-weigh technique, milk intake can be measured over periods of days during which sows and piglets are undisturbed, thus allowing simultaneous studies of nursing behaviour.

Material and methods

The experimental plan and the procedures involving use of animals were reviewed and approved by the Ethical Committee for Experimentation with Animals, Sweden.

The animals used in this study were part of a larger experiment, comprising 48 Swedish Yorkshire sows and their first 3 litters. For this study 5 sows with their 3rd litters were used. Litter size was 7-13 piglets with a mean of 10.6. Sows were housed in individual farrowing pens (8.5 m²) with partly drained floor and were supplied with straw bedding. Water was available in 2 nipples, one at sow height, the other at piglet height. The latter was plugged during the periods when milk

intake was measured. Sows were fed twice daily. No creep food was fed to the piglets and the entire litters were shut into the creep corners when sows were fed. The animals were weighed at birth and at 3 weeks of piglet age.

Nursing behaviour was recorded during both experimental periods using a time-lapse video. One camera was used per pen and placed to cover the whole pen, which meant that details such as piglet identity not could be seen on the recordings. Neither was the occurrence of milk let-down always obvious. Teat order was assessed at least twice during each period by direct observation during suckling after marking the piglets with large numerals, and was expressed as teat pair number, where number 1 is the most anterior pair. For piglets changing teat pair during or between sucklings, the mean teat pair number was used. A nursing bout was defined as at least half the litter suckling simultaneously for at least 1 min. Nursing time was calculated as the sum of the durations of nursing bouts per day for each litter and period. The duration of nursing bouts was not normally distributed, and therefore median values for each litter and period were used in the analyses.

The isotope dilution technique was used to measure piglet milk intake. Deuterium oxide was used as marker. Piglets were enriched twice, at 4-5 days (period 1) and at 16-18 days of age (period 2). Piglets were taken from the sow and weighed. After 1 h, an initial blood sample for background was drawn and piglets were given an intramuscular injection in the neck. The injection dose was 1 g per kg live weight of a 10% deuterium oxide solution in saline. An equilibration sample was drawn after 1 h and then piglets were returned to the sow. After a dilution period of 3 days, piglets were taken from the sow for 1 h and weighed, after which a dilution sample was drawn and piglets were returned to the sow. To keep track of any recycling of the tracer within litter, 2-3 piglets in each litter were not enriched, only blood sampled. Blood samples were drawn from the jugular vein in evacuated serum tubes. Serum was collected and stored in tightly sealed tubes at -18°C. In each of the experimental periods, milk samples were taken from at least two teats from each sow after administration of oxytocin. Milk samples were analysed for dry matter, ash, nitrogen, fat and lactose, and protein content was calculated as Kjeldahl nitrogen \times 6.38. The atomic fraction of deuterium in water hydrogen in the serum samples was measured using an isotope ratio mass spectrometer. The analyse was performed at the Danish Institute of Agricultural Sciences according to the procedure described by Theil *et al.* (2002). Milk intake in piglets was calculated as the water turnover corrected for isotope fractionation and for the potential metabolic water stored in body fat and protein, divided by the potential water fraction in milk (for details see Theil *et al.*, 2002). In short, the amount of piglet body water was calculated from the initial dilution of deuterium at enrichment. The fractional turnover rate of body water was calculated from the dilution of deuterium, and water turnover as body water \times fractional turnover rate. Water turnover was corrected for isotope fractionation, and for the potential metabolic water stored in fat and protein. Finally the piglet milk intake was calculated as the corrected water turnover divided by the potential water fraction in milk (approximately 0.94 in sow milk). Sow milk production was calculated as the sum of intakes by her piglets. Since 2-3 piglets per litter were never enriched, their milk intakes were estimated using an imputation procedure according to Theil *et al.* (2002). The predictive model included in the imputation procedure was based on measurements available from the larger experiment.

SAS software (SAS, 1999) was used for the statistical analysis. Correlations were analysed using the CORR procedure, regarding either the sow or the piglet as the experimental unit. In the latter step the periods were also analysed separately. Nursing frequency and duration, sow milk yield and litter growth rate were analysed using the GLM procedure with sow as the experimental unit. This model comprised effect of period, of sow, and of litter size. Milk intake, piglet growth rate and live weight was analysed using the GLM procedure with the individual piglet as the experimental unit. This model comprised effect of period, of sow and of teat pair number, with the effect of random sow as an error term.

Results

Nursing occurred more frequently during the first than during the second period, ranging from 26 to 32 nursings daily in the first period and 25 to 28 in the second. Simultaneously, there was a reduction in the duration of nursing bouts from 8 to 5 minutes (ranges 4-11 and 3-8, respectively). Teat fidelity was complete in one of the five litters only. In the first period one sow was standing occasionally while nursing. In the second period two of the sows stood up during more than 50% of the nursings, and one sow stood up occasionally.

From the correlation analysis with the sow as the experimental unit it was evident that the nursing characteristics were positively intercorrelated ($P<0.05$). The correlation between nursing frequency and sow milk yield, $r=-0.61$, tended to be significant ($p=0.06$). This was probably a coincidence, since in the separate analyses of periods 1 and 2 the correlation coefficients for the two periods had opposite signs. No other significant correlations between sow milk yield and nursing frequency or length of nursing bouts were found.

The individual milk intake was positively correlated with duration of nursing bouts, piglet liveweight and growth rate, and negatively correlated with teat pair number (Table 1). Teat pair number was also correlated with piglet growth rate and tended to be correlated with liveweight in the second period (both negatively), but not with piglet birth weight. Piglet liveweight and growth rate showed a strong positive correlation.

The effect of period, but not of sow nor of litter size, was significant on nursing frequency and sow milk yield (Table 2). Nursing frequency decreased, whereas daily milk yield and litter growth rate increased from period 1 to period 2, although the increase in growth rate failed to be significant. The individual milk intake, piglet growth rate and liveweight increased from period 1 to period 2 (Table 3), and decreased with teat pair number by 0.059 kg, 0.016 kg and 0.11 kg, respectively.

Table 1. Correlation coefficients for nursing characteristics, milk intake, piglet live weight (LW) and growth rate (experimental unit piglet, N=53 in period 1, N=52 in period 2.)

| Period | Bout duration | | Teat pair | | Milk intake | | Piglet LW | | Piglet growth | |
|-------------------|---------------|-------|-----------|-------|-------------|--------|-----------|--------|---------------|--------|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Nursing frequency | 0.50* | -0.01 | -0.01 | -0.11 | 0.01 | -0.09 | -0.38* | -0.09 | 0.07 | -0.17 |
| Bout duration | | | -0.23 | 0.002 | 0.34* | 0.43* | -0.13 | 0.03 | 0.40* | 0.38* |
| Teat pair | | | | | -0.44* | -0.28* | -0.20 | -0.24+ | -0.44* | -0.25+ |
| Milk intake | | | | | | | 0.79* | 0.74* | 0.97* | 0.96* |
| Piglet LW | | | | | | | | | 0.69* | 0.71* |

+ = $P<0.10$, * = $P<0.05$, for $|r|>0$.

Table 2. Nursing frequency and duration, litter growth rate and sow milk yield (N=5).

| | Period 1 | Period 2 | P-value, effect of | | Litter size |
|-----------------------------|----------|----------|--------------------|------|-------------|
| | | | Period | Sow | |
| Daily nursing frequency | 30.5 | 26.3 | 0.01 | 0.07 | 0.11 |
| Duration of bouts, min | 7.7 | 4.7 | 0.11 | 0.21 | 0.39 |
| Total nursing time, min/day | 239 | 123 | 0.06 | 0.15 | 0.27 |
| Litter growth rate, kg/day | 2.46 | 2.96 | 0.29 | 0.76 | 0.70 |
| Sow milk yield, kg/day | 9.6 | 14.0 | 0.04 | 0.76 | 0.97 |

The effect of sow was significant, both regarding individual milk intake, piglet growth rate and liveweight. The average milk intake per live weight increased from 3.8 in period 1 to 4.8 in period 2, but was not affected by sow nor by teat pair number.

Table 3. Milk intake, piglet live weights and growth rate (N=53 and 52 in periods 1 and 2).

| | Period 1 | Period 2 | Teat pair regression | P-value, effect of | | Teat pair |
|--------------------------|----------|----------|----------------------|--------------------|-------|-----------|
| | | | | Period | Sow | |
| Milk intake, kg | 0.93 | 1.40 | -0.059 | 0.02 | 0.001 | 0.001 |
| Piglet growth rate, kg | 0.24 | 0.30 | -0.016 | 0.17 | 0.001 | 0.001 |
| Piglet LW, kg | 2.74 | 6.61 | -0.11 | 0.001 | 0.001 | 0.03 |
| Milk intake /growth rate | 3.85 | 4.75 | -0.048 | 0.006 | 0.56 | 0.82 |

Discussion

Milk production in sows is considered to be superior in the anterior glands. Although teat fidelity in the present investigation was complete in one litter only, the individual milk intake and growth rate was found to be larger in piglets suckling anterior than those suckling posterior teats, which is in agreement with the results of Kovács & Váradi (1996). The interval between nursings is a major determinant for sow milk yield according to Etienne *et al.* (1998). In spite of this, the nursing frequency in the present study showed no influence on sow milk yield. This could be due to a relatively low range in nursing frequency and a small sample size. Also, the nursing frequency was measured in two separate intervals between which the nursing frequency decreased, while milk yield of the sows increased towards peak lactation. The estimate of milk conversion into piglet growth during the first period (3.8 kg/kg) was lower than in the second period (4.8). This is logical, since piglets became heavier and thus need more of their milk intake for maintenance. Also, they grew faster and the retention of fat, which - on a live weight basis - requires more energy than lean growth does, increases with age in piglets (Pond & Houpt, 1978).

Thus, the data confirm the superiority of anterior teats over posterior teats regarding milk yield and piglet growth rate. In spite of a 14% decrease in nursing frequency from period 1 to period 2 (mean piglet age 6 and 19 days), milk yield in sows increased by 46%.

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Protein, fat, and bone mineral deposition rates in growing pigs (birth to 90 kg) of different *RyR1* genotypes studied by dual energy x-ray absorptiometry

A.M. Scholz¹ & A.D. Mitchell²

¹ University Munich, Experimental Farm, 85764 Oberschleißheim, Germany

² United States Departm. Agriculture, Growth Biology Laboratory, Beltsville, MD 20705, USA

Summary

A growth study was performed from birth to 90 kg live body weight (BW) using dual energy x-ray absorptiometry (DXA) as a non-invasive tool to measure the deposition rates of main body components protein (lean), fat, and bone mineral in different ryanodine receptor 1 (*RyR1*) “model” genotypes (*NN* → “fat”, *Nn* → intermediate, *nn* → lean). The pigs were scanned at 10, 30, 60, and 90 kg BW. Fat and bone mineral measurements were used directly, while the lean mass was used to calculate protein mass (protein deposition rate -- Pd) using a device specific regression equation based on chemical reference data. Pd increases steadily up to about 50 kg body weight ($Pd_{max} \sim 140$ g/d for *nn*) and then gradually declines. The amount of daily protein and fat deposition depends on the body composition/ genetic background (metabolic situation) while observing nearly identical total tissue deposition rates for the three genotypes over time. At the end of the growth period studied (60–90 kg BW), the leanest genotype (*nn*) has a faster decreasing Pd than the less lean (“intermediate” - *Nn*, “fat” - *NN*) pigs due to a slower total tissue growth caused by a comparatively lower (appetite) feed intake (*nn*=2.14, *Nn*=2.5, *NN*=2.46 kg/d). The linear plateau model for Pd related to energy intake holds in tendency only for the “intermediate” genotype.

Keywords: protein deposition, tissue growth efficiency, dual energy x-ray absorptiometry

Introduction

Improvement of lean or protein growth and efficiency are two of the main objectives in pig breeding (Landgraf et al., 2002, Roux, 2002, Chen et al., 2003). Selection strategies, growth models and/or feeding policies consider in many cases a wide body weight range (e.g. 25 - 100 kg) for predicting the deposition of protein (protein + water = lean), fat, and ash using constants applied for the whole growth period (Hermesch et al., 2003). However, the application of constant maintenance requirements, efficiency ratios for protein and fat deposition, and growth rates may lead to model assumptions/feeding strategies, which would not exactly meet the changing requirements of pigs of different genotypes during growth. Therefore, this experiment was aimed at the study of protein, fat, and bone mineral deposition and efficiency in pigs of different “model” genotypes (*RyR1*: *nn*=“lean”, *Nn*=“intermediate”, *NN*=“fat”) covering growth and body composition changes from birth to 90 kg live body weight using dual energy x-ray absorptiometry as a non-invasive measuring tool.

Material and methods

In the first part of the study, a total of 45 group-housed (6-8 animals/group), ad-libitum-fed (CP = 176.16 g·kg⁻¹, DE = 14.78 MJ·kg⁻¹) pigs (13 *NN* - 14 *Nn* - 18 *nn*) were analyzed by DXA (GE LUNAR DPX software version 3.8e; pediatric mode → Mitchell et al, 1998) at approximately 10 kg body weight (Table 1). By applying a three-component model, DXA measures total body lean, fat, and bone mineral masses based on the different relative tissue absorbance of x-radiation at two energy levels (38 and 70 keV).

Table 1. Number of animals within gender and live body weight (BW) group (10 - 90 kg).

| Gender | BW Group | | | | | <i>Total</i> |
|------------------|----------|---------|---------|---------|---------|--------------|
| | 10 | 30 | 60 | 90 | | |
| Male (intact) | 18* (1) | - | - | - | 18 | (1) |
| Male (castrates) | 3 (2) | 17 (7) | 10 (3) | 7 (7) | 20 (19) | |
| Female | 24 (12) | 30 (8) | 22 (12) | 10 (10) | 54 (42) | |
| Total | 45 (15) | 47 (15) | 32 (15) | 17 (17) | 92 (62) | |

*(animals for reference study)

In order to calculate the tissue growth rates between birth and 10 kg body weight, it was assumed that the individual body composition (BC%) at birth (~1.2 kg BW) was linearly related to that measured by DXA applying previously calculated regression coefficients between the body components and BW for the range 5-90 kg:

$$\text{Lean Tissue (LT in %)} = 93.6386 - 0.1697 \cdot \text{BW} \quad (1)$$

$$\text{Bone Mineral (BM in %)} = 2.5674 - 0.0041 \cdot \text{BW} \quad (2)$$

$$\text{The corresponding Fat (\%)} \text{ results from: } \text{Fat \%} = 100 (\%) - \text{LT (\%)} - \text{BM (\%)} \quad (3)$$

Since lean tissue mainly consists of the two components protein and water (+ minimal portions of carbohydrates [glycogen] and minerals), the protein deposition rate (Pd) can be calculated from the lean tissue growth rate. An equation modified after Mitchell and Conway (1994) for the protein content (g) derived from chemical reference data results in the protein deposition rates for the growth periods shown in Table 2:

$$\text{Protein deposition (Pd in g}\cdot\text{d}^{-1}\text{)} = -1.062 + 0.22 \cdot \text{DXA lean tissue growth rate (g}\cdot\text{d}^{-1}\text{)} \quad (4)$$

Fat and Bone mineral growth rates were calculated accordingly:

$$\text{Tissue growth rate (g/d)} = \Delta \text{ Tissue Mass (g)} / \Delta \text{ Age (d)} --$$

$$\text{with } \Delta = \text{Difference between [Start of growth period] and [End of growth period]} \quad (5)$$

The second part of the study using the DPX adult mode(s) started with a live body weight of ~30 kg. Then, all pigs ($n=47$; 16 NN - 22 Nn - 9 nn, Table 1) were kept in single pens. After sedation, the pigs were positioned on the DXA table with back legs extended and front legs stretched parallel to the thorax using a cushion (triangular foam) to keep a constant distance/angle between thorax and front legs (Mitchell et al., 1998). The number of animals was reduced to 32 (14-11-7) for the 30-60 kg period and to 17 (6-6-5) for the 60-90 kg period due to a corresponding reference slaughter trial (Table 1). Up to the end of the experiment, the pigs were fed the same diet mentioned above. However, they received the food semi ad libitum at 95 % of the estimated voluntary feed intake in relation to the weekly measured body weight applying the "old" NRC (1988) equation for growing finishing pigs (4.5-117 kg):

$$DE_{\text{Intake}} (\text{MJ}\cdot\text{d}^{-1}) = 55 \cdot [1 - e^{-0.0176 \cdot \text{BW}^1}] \quad (6)$$

Ellis and Augspurger (2001), and Whittemore and Green (2001) summarize more and "later" equations for growing finishing and weaned pigs. All equations have in common the fact that they cannot consider all requirements of practical feeding (like different genetics, housing, climatic conditions...), though latest feeding/growth software includes a large number of possible adjustment factors. The daily individual feed intake was recorded manually for calculating the average daily feed intake (DFI) within the growth periods 30→60 kg and 60→90 kg. On the average, the three pig genotypes consumed less food than the amount predicted by the above equation.

In order to calculate the tissue growth rates between 10 kg and 30 kg, it was also assumed that the individual BC% for the pigs scanned at 30 kg was linearly related to that at 10 kg BW -- following

the same procedure as for the period birth-10 kg BW. The age and BW differences were known, since these pigs had been studied in a previous magnetic resonance study at 10 kg BW, **but not by DXA**.

Results and discussion

Table 2. Deposition rates for protein, fat and bone mineral ($g \cdot d^{-1}$) in pigs with different RyR1-Genotypes (LSM \pm SEE: for a SAS 8.1 GLM model with fixed effects: RyR1, Sex, Breed/Line).

| Deposition ($g \cdot d^{-1}$) | | | | | | | | | |
|---------------------------------|--------------|--------------|------------|---------------------|--------------|------------|--------------|--------------|------------|
| RyR1: NN ("fat") | | | | Nn ("intermediate") | | | nn ("lean") | | |
| | Protein | Fat | Bone Min. | Protein | Fat | Bone Min. | Protein | Fat | Bone Min. |
| I*) | | 6.56 kg | | | 6.27 kg | | | 4.81 kg | |
| | 42 \pm 3 | 13 \pm 2 | 6 \pm 1 | 39 \pm 4 | 10 \pm 2 | 5 \pm 1 | 30 \pm 4 | 8 \pm 3 | 4 \pm 1 |
| II | | 20.79 kg | | | 21.50 kg | | | 21.50 kg | |
| | 104 \pm 4 | 57 \pm 4 | 13 \pm 1 | 107 \pm 3 | 59 \pm 3 | 13 \pm 0 | 115 \pm 5 | 58 \pm 5 | 14 \pm 1 |
| III | | 48.82 kg | | | 48.91 kg | | | 49.38 kg | |
| | 120 \pm 6 | 199 \pm 13 | 19 \pm 1 | 123 \pm 5 | 172 \pm 11 | 19 \pm 1 | 132 \pm 7 | 168 \pm 16 | 19 \pm 1 |
| IV | | 77.16 kg | | | 77.37 kg | | | 74.33 kg | |
| | 104 \pm 14 | 225 \pm 45 | 9 \pm 1 | 116 \pm 14 | 183 \pm 45 | 9 \pm 1 | 112 \pm 19 | 125 \pm 58 | 12 \pm 1 |

*) Mean live weight within growth periods; I: birth (1.2)-10 kg, II: 10-30 kg, III: 30-60 kg, IV: 60-90 kg

At minimally different total tissue growth rates up to ~65 kg BW (Figure 1), the protein (lean) deposition ($g \cdot d^{-1}$) increases with age until an approximate live weight range of 40-50 kg (Figure 2, 3). The protein deposition rate of the "lean" genotype (nn) exceeds that of the two other genotypes (Nn and NN) starting at a BW of ~20 kg until ~65 kg. At the end of the study (>65 kg), the protein (lean) deposition rates of the two leaner genotypes (Nn and nn) became more equal at a slightly lower level, while the fat deposition rates -- unexpectedly -- advanced in different directions with a decrease for nn and a further increase for NN and Nn. The bone mineral deposition follows in tendency the pattern of the protein deposition (Table 2).

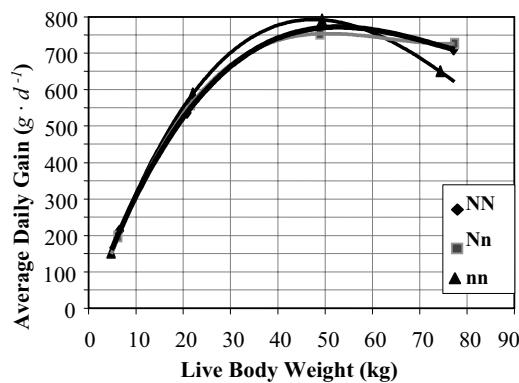


Figure 1. Average daily gain ($g \cdot d^{-1}$) in pigs of different RyR1 genotypes from birth to 90 kg BW.

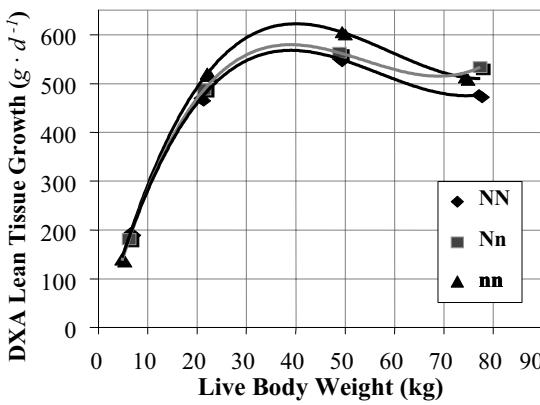


Figure 2. DXA lean tissue growth rate ($\text{g}\cdot\text{d}^{-1}$) in pigs of different *RyR1* genotypes from birth to 90 kg BW.

A linear-plateau model for the protein deposition ($\text{g}\cdot\text{d}^{-1}$) in relation to feed (energy) intake cannot be confirmed — except for the intermediate genotype *Nn* (Figure 3 - see dashed line). In addition, this study proves that the efficiency of protein deposition relative to energy (feed or protein) intake decreases as the pigs develop from birth to ~90 kg body weight (Figure 4). The (maximum) rate of protein deposition depends on the body composition/ genetic background of the pig — in this study on the *RyR1* genotype. As known from earlier studies summarized by De Lange et al. (2001), the protein deposition rate increases steadily from birth until reaching a maximum between 40 and 65 kg BW followed by a more or less significant decline. However, in some genotypes/lines, the protein deposition rate may increase up to higher body weights (Landgraf et al., 2002). Only at the end of the study (60-90 kg), the leanest genotype (*nn*) shows a reduced appetite (feed intake) compared to the two other groups (*NN* and *Nn*) confirming the results of other authors for high lean pig lines (see Ellis and Augspurger, 2001). Specifically designed selection and feeding programs may optimize the lean (protein) deposition for pigs of different genetically determined body composition and deposition rates during the various stages of growth (Landgraf et al., 2002).

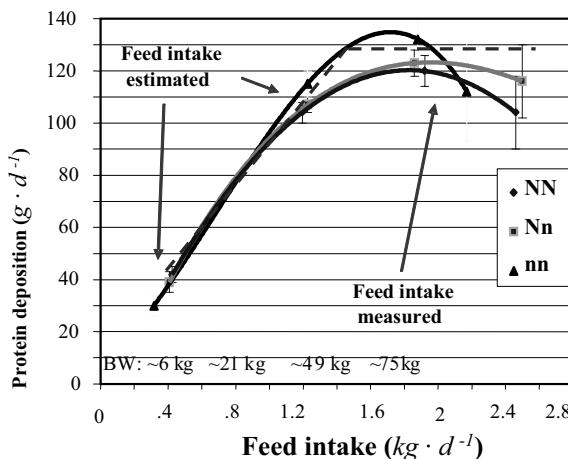


Figure 3. Average feed intake ($\text{kg}\cdot\text{d}^{-1}$) and protein deposition ($\text{g}\cdot\text{d}^{-1}$) in pigs of different *RyR1* genotypes from birth to 90 kg BW.

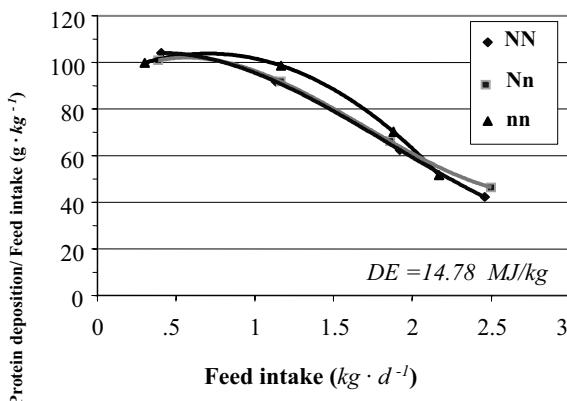


Figure 4. Efficiency of protein deposition in relation to feed intake in pigs of different *RyR1* genotypes from birth to 90 kg BW.

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Effect of bilaterally caecal ligation on utilization of dietary energy in growing chickens

Kunio Sugahara, Mako Matsumoto, Motoko Yamada & Fumiaki Yoshizawa

Department of Animal Science, Faculty of Agriculture, Utsunomiya University, Utsunomiya, 321-8505, Japan

Summary

In order to determine a role of caeca on energy utilization in growing chickens, metabolizability of the dietary energy and energy expenditure was examined within a week after caeca were bilaterally ligated and washed out. Apparent ME values were 14.30 and 13.69 kJ/g air-dry matter for the sham-operated and ligation groups respectively, and these are significantly different. Although apparent ME intake and fasting heat production were decreased by caecal ligation, partition of AME available for growth was not affected. These results suggest that caeca were responsible for metabolizing the dietary energy and that they contribute to basal metabolism with a limited extent.

Keywords: metabolizable energy, heat production, caeca

Introduction

Fibre content or concentration in the diet is critical on the dietary energy utilization in poultry. The utilization of this component is associated with the caecal microorganisms. Carre & Gomez (1994) found that caeca partly contributed to digestion of the water-soluble non-starch polysaccharide and have ability to absorb most organic produced from fermentation. In addition, the fermented non-starch polysaccharide provides the birds with metabolizable energy (Jamroz *et al.*, 2001). Therefore, it is interesting to examine how much the caeca contribute to absorb and/or to metabolize the dietary energy.

Caecectomy or caecal ligation have been used to study the role of caeca on nitrogen and energy utilization (Isshiki, 1980; Carre & Gomez, 1994; Karasawa *et al.*, 1997). However, keeping mature birds for several weeks after surgical remove or ligation of caeca in the previous studies. This period would be enough for the birds to get acclimatized to a novel environment and subsequently it is difficult to examine the early response to the reduced caecal function.

In order to examine the effect of the caeca on the energy utilization in growing period, balance and respiration tests were carried out to determine the metabolizable energy value and heat production with 3-week-old chickens within a week after operation of caecal ligation.

Materials and methods

One-day-old male layer-type chickens were housed in electrically heated battery cage through 21-day-old. On 21-day-old, they were fasted for two hours, and then were subjected to the caecal ligation operation. They were housed individually in metabolism cages in the air-conditioned room throughout the experiment period. Commercial starter diet (CP 200 g/kg, ME 2.95Mcal/kg) and water were freely given unless otherwise described. The light was provided with a fluorescent lamp from 900 to 2100 and environmental temperature was kept in the thermoneutral zone of chickens.

Caecal ligation was bilaterally done as described by Son *et al.* (1996). After anaesthetized by intraperitoneal injection of pentobarbital (40 mg/kg body weight), chicken was tied in the spine

position on a board. The abdomen skin was cut in about 5 cm. The ileo-caecal-colonic junction was confirmed by inserting a glass rod from the cloaca. The end of caeca was pull out of the cavity and the proximal caeca was ligated with a nylon thread as near their origin as possible. The distal end of caeca was cut off as small as to be inserted by a syringe tip. The caecal content was washed out using about 10ml saline. Abdominal muscle layers and skin were sutured. Antibiotics were injected into thigh muscle. Caecally ligated chickens were carefully observed and body weight and food intake were recorded. Their body weight and food intake increased day by day after ligation operation. Sham-operated chickens were treated as same as the ligated chickens except for ligation and washing out of caecal content. Ligation was confirmed by observation of excreta and by postmortem inspection of the cavity.

Automated open-circuit respirometry (Sugahara & Kubo, 1991) was used to measure the heat production of chickens. Daily heat production ($\text{kJ}/\text{kg}^{0.75}\text{body weight}$) was calculated from the oxygen consumption and carbon dioxide measured for 22 hours from 1400 to 1200 daily using a formula of Romijn & Lokhorst (1961). A pair of sham- and caecal ligated-chickens was individually housed in the respirometry chamber 48 hours after operation. Respiration test was done from 3rd to 6th day after operation and the feeding and fasting heat production were determined on the fourth and sixth days respectively. ME value was determined with a total excretion collection method on the 3rd and 4th days. Energy in excreta samples and feed was determined using an adiabatic bomb calorimeter.

The replication numbers for the experimental and sham-operated groups were 7 chickens respectively in the respiratory test. Four chicks of each group were used to determine ME value. Paired t-test was done to determine the significant difference between the treatment.

Results and discussion

Effect of caecal ligation on the energy balance in growing chickens is shown in Table 1.

Table 1. Effect of caecal ligation on energy balance during two test-days in growing chickens (air-dry base).

| Bird type | Food Intake (g) | GE ingested (kJ) | Excreta (g) | GE of excreta (kJ/g) | GE excreted (kJ) | Metabolized energy (kJ) | ME value (kJ/g) | m(E) |
|-----------|-----------------|------------------|-------------|----------------------|------------------|-------------------------|-----------------|-------|
| Sham | 54.3 | 966 | 13.0 | 14.79 | 191.9 | 776.1 | 14.30 | 0.80 |
| SD | 6.8 | 122 | 2.5 | 0.24 | 37.6 | 90.0 | 3.19 | 0.018 |
| Ligation | 56.3 | 1004 | 15.5* | 14.96 | 233.0* | 771.5 | 13.69* | 0.77* |
| SD | 11.7 | 209 | 2.9 | 0.38 | 48.9 | 161.2 | 1.69 | 0.006 |

* P<0.05, Mean values and SD of 4 replications.

No significant difference in food intake between the bird type was observed. Air-dried weight of excreta and GE excreted were significantly larger in the caecal ligation than in the sham groups, though GE per unit weight of excreta was not different. Subsequently, ME value and m (E) were significantly less in the caecal ligation than in the sham groups. Thompson & Boag (1975) observed that caecectomized Japanese quail increased food intake as well as GE of excreta, and consequently metabolized as much energy as the intact one. AME values recalculated from their results were smaller in the caecectomized (2.98 kcal/g) than intact groups (3.09 kcal/g). This is similar to the present result, but the response of excreta weight and GE of excreta was different.

The difference in AME values may be partly attributed to VFA resulted from fermentation in the caeca with the content (Jamroz *et al.*, 2001).

Effect of caecal ligation or caecectomy on AME value varies with dietary composition in poultry (Ragland *et al.*, 1999). It was expected that AME value of the commercial diet consisting of soybean and corn was hardly affected by caecal ligation. The decreased AME value of the corn-soybean diet in the caecal ligation group suggests that early responses occurred before chickens were acclimatized to novel environment. If it were true, period effect would be critical in examination of the effect of caecal ligation. In connection with this, Yamauchi *et al.* (1996) found that the response of the height and surface of duodenal villi to re-feeding after fasting occurred in 24 hours in laying hens.

Effect of caecal ligation on partition of ME in growing chickens is summarized in Table 2. AME intake was calculated by multiplying average AME values obtained in the balance test (Table 1) by food intake of the respiration test. Chickens of the ligation group ate significantly less AME than those of the sham group. The deceased AME intake was due to the decreased food intake and AME values in the ligation group. Fasting heat production of the sham group was similar to the previous studies (Sugahara & Kubo, 1988). This heat production was significantly decreased by the caecal ligation. Feeding heat production also decreased by 30 kJ, which is not statistically significance.

Table 2. Effect of caecal ligation on partition of ME in growing chickens (kJ/MBS/day).

| Bird type | AME intake | Fasting heat production | Heat increment | Retained energy | kg |
|-----------|------------|-------------------------|----------------|-----------------|-------|
| Sham | 1103 | 390 | 215 | 498 | 0.692 |
| SD | 31 | 3.1 | 8.9 | 28 | 0.045 |
| Ligation | 979* | 370* | 205 | 404 | 0.653 |
| SD | 41 | 7.0 | 7.6 | 36 | 0.064 |

Mean values and SD of 7 replications. *: P<0.05.

The decreased heat production of fasting and feeding suggests that the caecal ligation affected the energy expenditure via not only digestion but also unidentified metabolic processes. The difference in the fasting heat production between the bird type was about 5%. Spratt *et al.* (1990) estimated that chicken gut contributed to total energy expenditure as much as 6% with measuring tissue oxygen consumption. Caeca were just ligated and not removed in the present experiment. Relative weight of caeca to total gut weight was less than 20% (Isshiki, 1980). These findings suggest that the extent of contribution of caeca to total energy expenditure would not reach 5%.

Neither heat increment (feeding heat production - fasting heat production) nor retained energy (AME intake - feeding heat production) was affected by caecal ligation, though both of them were decreased. Energetic efficiency of growth (kg: retained energy/(AME intake - fasting heat production)) was not affected by caecal ligation. These results show that partitioning AME available for growth was not affected by caecal ligation.

In summary, caeca have a little effect on energy utilization, if any, in growing chickens fed a corn-soybean diet.

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Energy requirement for standing in cattle

A. Susenbeth¹, T. Dickel¹, K.-H. Südekum¹, W. Drochner² & H. Steingaß²

¹ Institute of Animal Nutrition, Physiology and Metabolism, Christian-Albrechts-University, 24098 Kiel, Germany

² Institute of Animal Nutrition, Hohenheim University, 70593 Stuttgart, Germany

Summary

Energy requirement for standing was determined with four ruminally cannulated German Red Pied steers weighing 617 kg of body weight (BW) in open-circuit respiration chambers. Animals were not forced to change their positions, so that the spontaneous behaviour in this environment was observed. To avoid any interference with eating or ruminating activity during the periods of measurement, the rumens were emptied before and no feed offered. The requirement for standing over lying was derived by regressing heat production on time spent standing within 2-h periods. Energy requirement for standing over lying amounted to $14 \text{ kJ} \cdot \text{d}^{-1} \text{ kg BW}^{-1}$.

Keywords: cattle, energy requirement, physical activity

Introduction

Energy requirement for standing over lying represents a small, however not negligible proportion of the total energy metabolism in animals. The aim of this study was to determine the energy requirement for standing in cattle under conditions where animals were not forced to change their positions and their rumens were emptied to avoid any interference by eating or ruminating activity.

Materials and methods

Animals and housing

The experiments were carried out from April to July 1999 at the Institute of Animal Nutrition, Hohenheim University, Stuttgart, Germany. Four ruminally cannulated German Red Pied steers weighing $617 \pm 53 \text{ kg BW}$ (mean \pm SD) and ranging in age from 27 to 31 months were used. Animals were kept in straw-bedded pens with free access to water. They were given 1.4 kg/d of a commercial concentrate and roughages for ad libitum intake to meet their maintenance requirement.

Experimental procedure

On the day before the gas exchange measurements were performed, animals were transferred to a tie stall, where they received 0.7 kg concentrate and about 1 kg DM of roughage at 1600. On the next morning between 0700 and 0800, the rumens were manually emptied, washed with warm water, and filled with 30 L of a buffer solution (300 mosmol/L), which contained (M) 75 NaCl, 45 NaHCO₃, 2 Na₂HPO₄, 5 KCl, 20 KHCO₃, and 2 CaCl₂. Immediately afterwards the animals were put into the open-circuit respiration chambers. The first hour in the chamber served as an adaptation period. During the hours two and three gas exchange was measured without any feed and time spent standing and lying and the number of changes of the positions were measured by a photoelectric barrier. Animals were not forced to change their positions, so that the spontaneous behaviour in this environment was observed. Animals were transferred to the pen after

measurements, where the normal rumen contents, which had been kept at body temperature after emptying, was returned to the rumen, and fresh rumen liquid from donor animals was added. Steers were used for further measurements in the respiration chambers at the earliest after two days. Air temperature, air humidity, and air exchange rate in the chambers were 20°C, 60 to 70%, and 15 m³ · h⁻¹, respectively. Representative gas samples were taken and analysed for O₂ (paramagnetic principle, Magnos 2T), and for CO₂ and CH₄ (infrared absorption principle, Uras 10E; both apparatus from Hartmann und Braun, Frankfurt/Main, Germany). Heat production was calculated from oxygen consumption using the oxienergetic equivalent of 20.5 kJ · L⁻¹ O₂ (McLean, 1972). 18 measurements per animal were carried out, resulting in a total of 72 observations. The requirement for standing was estimated by regressing heat production on time spent standing. The experiments could be carried out without any problems; the animals were well adapted to handling and the respiration chambers.

Statistical analysis

Energy requirement for standing was estimated by multiple linear regression analysis using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). As both an absolute term (intercept) and the independent variable “number of positional changes” did not show significance ($P > 0.10$), when included in the equation, the following model was used:

$H = b_1 \cdot T_l + b_2 \cdot T_s \cdot BW$, where H is heat production (kJ), T_l time spent lying (min), T_s time spent standing (min), BW expressed as kg or kg^{0.75}, and b_1 and b_2 regression coefficients, which represent heat production per kilogram of BW during lying and standing, respectively. To facilitate the comparison to other energy metabolism variables, data were converted into 24-h values.

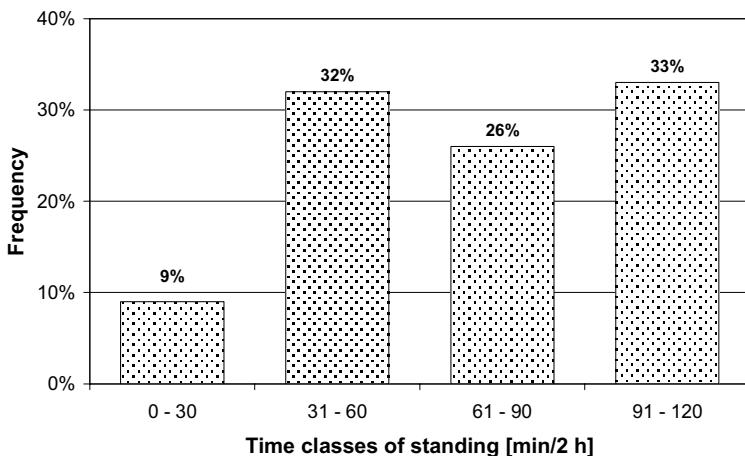


Figure 1. Relative frequency of the spontaneous standing time of the four steers within the 2-h periods of gas exchange measurements ($n = 72$).

Results and discussion

Spontaneous standing time of the animals within the 2-h periods is shown in Figure 1. The relative frequency of less than 30 min standing was 9% of the total of 72 observations, whereas the proportion within the other three time classes was of similar magnitude between 26 and 33%. The mean heat production during lying and standing is presented in Table 1. The differences between standing and lying of 14 kJ · d⁻¹ · kg⁻¹ BW and 54 kJ · d⁻¹ · kg^{-0.75} BW can be taken as estimates for the energy requirement for standing, which corresponds to a relative increase of heat

production of standing over lying by 19 and 14%, respectively. The correlation coefficient between heat production per kilogram of BW and standing time was 0.53 ($P < 0.001$). The results of the present study agree well with the mean of literature data (Table 2). However, the variation between studies is considerably high, which might be partly due to differences between experimental techniques (respiration chamber, mask technique, or trachea fistulation; different time spans of and between the respective respiration periods) and between animals. Using the above value, an animal with 600 kg of BW standing 14 h per day needs 4.9 MJ ME for standing, which corresponds to a requirement for 1 kg of milk containing 4% fat.

Table 1. Heat production during lying and standing in cattle derived from the 2-h periods of gas exchange measurement (n = 72; values are converted to a 24-h basis).

| Heat production | Lying | | Standing | | Difference | | Increase ¹ % |
|---|-------|-----|----------|-----|------------|-----|----------------------------|
| | Mean | SEM | Mean | SEM | Mean | SEM | |
| $\text{kJ} \cdot \text{kg}^{-1} \text{BW}$ | 73.3 | 1.9 | 87.4 | 1.4 | 14.1 | 2.9 | 19.2 |
| $\text{kJ} \cdot \text{kg}^{-0.75} \text{BW}$ | 376 | 10 | 430 | 7 | 54 | 14 | 14.4 |

¹Percent increase of standing over lying.

Table 2. Compilation of literature data on energy requirement for standing in cattle¹

| Source | Body weight (kg) | No. of Animals | No. of Observations | Energy requirement for standing, ($\text{kJ} \cdot \text{d}^{-1} \cdot \text{kg}^{-1} \text{BW}$) | Increase ² (%) |
|--------------------------------|------------------|----------------|---------------------|---|---------------------------|
| Dahm, 1910 | 220 | 1 | - | 11.2 | 8 |
| Von der Heide et al., 1913 | 530 | 1 | 1 | 18.7 | 20.7 |
| Fries and Kriss, 1924 | 400 | 1 | 1 | 6.6 | 9.8 |
| Forbes et al., 1927 | 468 | 1 | 1 | 15.2 | 24.9 |
| Hall & Brody, 1933 | 144 - 875 | 32 | 1938 | 9.0 | 9.0 |
| Blaxter & Wainman ³ | - | - | - | 5.9 | - |
| McLean ³ | - | - | - | 10.0 | - |
| Colovos et al., 1970 | 540 | 3 | 18 | 14.1 ⁴ | 16.1 |
| Schiemann et al., 1971 | 620 - 954 | 10 | 54 | 25.1 | - |
| Clark et al., 1972 | 460 | 4 | 32 | 11.6 | 12.2 |
| Vercoe, 1973 | 194 - 334 | 11 | 24 | 13.5 | 18.7 |
| Ku-Vera et al., 1989 | 300 | - | - | 6.6 | - |
| Neumann et al., 1994 | 567 | 3 | 30 | 24.6 | 24.3 |
| Present study | 550 - 640 | 4 | 72 | 14.1 | 19.2 |
| Mean ⁵ | | | | 13.3 | |

¹Not included here are the data of Armsby & Fries (1913, 1915), because the increment of heat production includes also eating activity, and those of Schrama et al. (1993) and of Roefs et al. (1996), because the animals were only 6 days old.

²Percent increase of standing over lying.

³Cited according to Agricultural Research Council (1965).

⁴Value after 10 min of change in position.

⁵Unweighted mean.

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Energy expenditure for chewing during eating and rumination in sheep fed *ad libitum* Timothy hay or Sudangrass hay

T. Suzuki, N. Takusari, F. Terada & M. Kurihara*

National Institute of Livestock and Grassland Science, Tsukuba 305-0901, Japan

** Present address: Japan International Research Center for Agricultural Sciences, Ohwashi 1-1, Tsukuba 305-8686, Japan*

Summary

Energy expenditure for chewing during eating and rumination were evaluated in sheep fed Timothy hay or Sudangrass hay freely, using the arterial-venous difference technique at the head. Dry matter intake and duration of eating were higher for Timothy hay than for Sudangrass hay. However, there was no difference between the two hays in either energy expenditure for chewing during eating+rumination on the one hand and per-day energy expenditure by the whole body on the other; approximately 7% of the whole-body energy expenditure was devoted to chewing activity. The energy required for chewing per unit of feed was considered to reflect the hardness of the feed, and was higher for Sudangrass than for Timothy. This suggested that feed hardness affected the amount of energy expended on chewing.

Keywords: *sheep, energy expenditure, chewing*

Introduction

In ruminants, energy is expended in fasting metabolism, the digestion and metabolism of nutrients, the maintenance of homeothermy, muscle activity, and so on. Because ruminants spend much of the day chewing, their energy expenditure for chewing during eating and rumination should not be ignored in whole-body energy expenditure. Additionally, because chewing time is affected by feed intake as well as the species and physical form of the feed, these factors may affect the amount of energy expended on chewing.

Many reports on the relationship between heat production and eating behavior in ruminants are available, but few studies have focused on heat production in relation to rumination (Sensenbrenner *et al.*, 1998). However, most of the data in these few studies are not categorized according to the origins of heat increment, such as chewing activity, digestive tract motility, or the metabolism and digestion of nutrients. Energy expenditure at the head was measured by Higuchi *et al.* (2003) using the arterial-venous difference method. Energy expended on chewing activity might be measured using this method.

Our objective is to evaluate the effect of feed species and intake on energy expenditure for chewing during eating and rumination using the arterial-venous difference method at the head.

Materials and methods

Four Suffolk sheep (mean live weight, $27.0 \pm 3.6\text{kg}$), each with a cannula attached at the jugular vein and an ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA) at the carotid artery, were treated in a crossover design. The animals were separated into two groups, one of which was fed Timothy hay and the other Sudangrass hay; both groups were allowed to eat *ad libitum* during twice-daily feedings. The chemical components of Timothy hay and Sudangrass hay on a dry matter (DM) basis were as follows, respectively: organic matter, 91.8 vs. 90.7%; crud protein, 14.5 vs. 12.7%; neutral detergent fiber, 68.9 vs. 60.7%; acid detergent fiber, 38.9 vs. 34.4%; acid

detergent lignin, 4.9 vs. 3.6%. Each sheep was assigned its own metabolic cage in a temperature- and humidity-controlled room (20°C and 60%, respectively). The experimental period consisted of a 14-day adaptation period and a 6-day measuring period for each type of hay. In the measuring period, the number of chews per animal was counted by using a tape switch attached under the jaw, and the blood flow rate was measured continuously using an ultrasonic flow probe. During each 3-day measuring period, heat production of the whole body was measured by an open-circuit indirect respiration apparatus (Iwasaki *et al.*, 1982). On the last of the 6 days, blood samples were collected from the jugular vein during eating, rumination and resting. There were at least three collections for each of these activities on that day. Hemoglobin concentration, partial pressure of the oxygen and oxygen saturation of the blood samples were analyzed using a fully automated blood gas analyzer (CHIRON 840, Bayer Medical Ltd., Tokyo, Japan). During the subsequent calculation of oxygen concentration, the arterial and venous hemoglobin concentrations were assumed to be the same, and the partial pressure of the oxygen and the oxygen saturation were assumed to be 100.7mmHg and 97.2%, respectively (Takusari; unpublished data). Oxygen consumption at the head was calculated by the blood flow rate and the difference between the arterial and venous oxygen concentrations (Higuchi *et al.*, 2003). Energy expenditure at the head was calculated as follows.

Energy expenditure at the head =

$$\frac{\text{Whole-body energy expenditure per day}}{\text{Whole-body oxygen expenditure per day}} \times \text{Oxygen expenditure at the head}$$

Energy expenditure for chewing per min was estimated from the difference between energy expenditure at the head per min during chewing and that during resting.

Statistical analysis was conducted using the GLM procedure of SAS (1992).

Results and discussion

The duration of chewing during eating was higher for Timothy hay than for Sudangrass hay, as a result of the higher intake of the former (Table 1). On the other hand, there was no significant difference between the hays in the daily energy expended on chewing during eating+rumination or in the daily whole-body expenditure. For both hays, per-day energy expenditure at the head accounted for 15% of the whole-body total, while that expended on chewing accounted for 7% of the total. Energy expenditure for chewing during eating per DM intake was higher for Sudangrass than for Timothy. This result suggests that the feeds differed in physical characteristics, such as hardness (Lee & Pearce, 1984), and that such differences affect energy expenditure for chewing during eating.

For energy expended at the head per unit of time, the activities ranked in the following order, regardless of the hay: eating > rumination > resting (Table 2). Energy expenditure for both per minute of chewing and per chew was higher during eating than during rumination. Evans *et al.* (1974) showed that the tensile strength of forage particle was lower in incubated particles in the rumen liquor than in non-incubated particles. Accordingly, the difference in per-chew energy expenditure between eating and rumination might result from the difference in hardness between the hay particles and the regurgitated particles. There was no difference between the hays in per-chew energy expenditure during eating. Because the amount of feed DM per chew during eating differed between roughages (Ulyatt *et al.*, 1986), per-chew energy expenditure during eating may be affected not only by the physical characteristics of the feed, but also by the amount of feed prehension in the mouth during eating.

Susenbeth *et al.* (1998) referred the literature data, and determined that the mean energy requirements for eating and rumination in sheep were 40 (34-55) and 11 (3-17) J/min/kgLW, respectively. The published data on per-minute energy expenditure for chewing during eating were

Table 1. Per-day feed intake, chewing activity and energy expenditure.

| | | <i>Timothy</i> | <i>Sudangrass</i> | SEM |
|--|--------------|----------------|-------------------|-------|
| DM intake | (g/kgLW) | 25.4** | 23.2 | 0.4 |
| Metabolic energy intake | (kJ/kgLW) | 233** | 227 | 4 |
| Duration of eating | (min) | 348* | 319 | 14 |
| rumination | (min) | 511 | 488 | 19 |
| No. of chews during eating | (chews) | 25,493 | 25,690 | 1,922 |
| during rumination | (chews) | 34,834 | 33,357 | 1,867 |
| No. of chews during eating per DM intake | (chews/g) | 40.8 | 43.8* | 0.3 |
| Energy expenditure by whole body ¹ | (kJ/kgLW) | 191.4 | 190.1 | 1.2 |
| at head ² | (kJ/kgLW) | 25.5 | 27.7* | 0.4 |
| for chewing during eating ² | (kJ/kgLW) | 6.6 | 6.6 | 0.4 |
| for chewing during rumination ² | (kJ/kgLW) | 6.8 | 6.6 | 0.4 |
| for chewing during eating+rumination ² | (kJ/kgLW) | 14.0 | 13.7 | 0.4 |
| for chewing during eating per DM intake ² | (J/gDM/kgLW) | 9.8 | 11.9* | 0.4 |

¹ Measured by respiration trial

² Calculated from oxygen expenditure at head

*P<0.05, **P<0.01

Table 2. Energy expenditure per unit of time or per chew.

| | | <i>Timothy</i> | <i>Sudangrass</i> | SEM |
|--|----------------|----------------|-------------------|------|
| Energy expenditure at head during eating | (J/min/kgLW) | 27.3 | 29.1* | 0.7 |
| rumination | (J/min/kgLW) | 21.5 | 22.9 | 0.5 |
| resting | (J/min/kgLW) | 8.4 | 9.6** | 0.4 |
| Energy expenditure for chewing during eating | (J/min/kgLW) | 19.0 | 19.5 | 0.7 |
| rumination | (J/min/kgLW) | 13.4** | 13.3 | 0.4 |
| Per-chew energy expenditure during eating | (J/chews/kgLW) | 0.29 | 0.27 | 0.03 |
| rumination | (J/chews/kgLW) | 0.23* | 0.20 | 0.01 |

*P<0.05, **P<0.01

higher than the values in this study. The values in the literature were calculated from the difference in whole-body heat production between eating and resting. Moreover, most of these values included energy expenditure for digestive tract motility. Meanwhile, the values in this study did not include heat production from the neck down. Therefore, the higher values found in the literature would result from the inclusion in those studies of energy expenditure for other activities, such as motility of the digestive tract.

The NDF and ADL contents, which indicate fibrousness, were higher for Timothy than for Sudangrass. Yet Sudangrass was higher in both the number of chews during eating per feed DM and energy expenditure for eating per feed DM (Table 1); furthermore, voluntary feed intake was

lower for Sudangrass than for Timothy. These results show that physical characteristics of feed are related not only to the plant's fiber components but also to its morphological structure (Perez-Barberia and Gordon, 1998). A more suitable index of the physical characteristics of feeds is necessary in order to estimate energy expenditure or feed intake.

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Validation of measuring CO₂ production and milk intake by the doubly labelled water technique in piglets

P.K. Theil, H. Jørgensen, K. Jakobsen, N.B. Kristensen & C.-T. Zheng

Danish Institute of Agricultural Sciences, Research Centre Foulum, Department of Animal Nutrition and Physiology, P.O. Box 50, DK-8830 Tjele, Denmark

Summary

This study was performed to investigate the validity of determining CO₂ production of piglets by means of the doubly labelled water (DLW) technique and concomitantly assess the milk intake by dilution of deuterated water. A total of 10 piglets were removed from the sow during the first week of lactation and fed with milk replacer by an artificial sow until weaned at 28 days of age. The piglets were enriched by intraperitoneal injection of DLW at approximately 10 days post partum. Piglets were kept in metabolic cages, and housed either pairwise (three pairs) or in a group of 4 in open-air circuit respiration chambers throughout the study, and the CO₂ production was measured 22 h daily by indirect calorimetry and for 3 d by the DLW technique. The milk replacer contained 12.7 % of DM, and 22.9 % protein and 25.7 % fat expressed on DM basis. On average, the piglets had a growth rate of 138 ± 17 g/d, the milk intake determined by D₂O dilution was 891 ± 63 g/d of milk replacer and the daily CO₂ production measured by the DLW method was 64.7 L/d. Overall, there was a high agreement between CO₂ production assessed by indirect calorimetry and by the DLW method and also between milk intake assessed by weighing the allotted milk compared to that determined by the D₂O dilution technique.

Keywords: Deuterium oxide dilution technique, doubly labelled water method, single pool model

Introduction

To be able to quantify the energy metabolism of suckling piglets, it is necessary to measure their heat production and their milk intake. Both parameters can elegantly be quantified by labelling the piglets with DLW. Further, the doubly labelled water (DLW) technique is a valuable method in combination with respiration trials for measuring the heat production of sow and suckling piglets, whereby the contribution of sow and piglets can be obtained, and the piglet milk intake and consequently the sow milk yield can be estimated. Sow milk yield is hard to quantify due to their anatomy (many glands), but also because sows respond to stressful stimuli by shutting off milk letdown. Often sow milk yield has been measured by the weigh suckle weigh method, but that is claimed to underestimate the milk yield due to interruptions of the udder massage performed by the piglets, due to loss of faeces and urine between the two weighings etc. (Klaver *et al.*, 1981). The aim of the present study was to validate the CO₂ production of piglets measured by the DLW technique by comparing CO₂ production measured by indirect calorimetry, and to validate the estimation of milk intake of piglets by comparing with weighed amounts of allotted milk.

Materials and methods

Animals and experimental procedures

Ten crossbred piglets (7 females and 3 males) from 3 litters (Y x LY, L x LY and D x LY) were used. At one week of age piglets were removed from the sows and kept individually in cages in a respiration chamber. A group of piglets (one group had 4 piglets, three groups consisted of 2

piglets) was housed in respiration chambers, and to minimise stress, piglets within a group were separated only by wire mesh so they could hear and see each other. The temperature was kept at 27 ± 0.4 °C, and the relative humidity was maintained at $64 \pm 1.3\%$. The CO₂ production was measured on a group basis for 22 h daily in the respiration unit. The piglets were enriched with doubly labelled water (DLW) approximately at 10 d of age to determine the rate of CO₂ production and milk intake. An intraperitoneal injection of a single dose of 0.5 g DLW per kg LW enriched the piglets. The DLW injectate was a mix of two thirds of 97 % H₂¹⁸O (Isotech, Netherlands) and one third of 99.9 % D₂O (Bie & Berntsen, Rødovre, Denmark). Three blood samples were drawn from each piglet, one just prior to enrichment to measure the background level of D₂O and H₂¹⁸O, the second one hour after enrichment to determine the water dilution space, and the third 3 d later to determine the CO₂ production and milk intake during that period. The piglets were fed a milk replacer every hour from 03:00 to 23:00 with an artificial sow (PigOline, Boss produkter a/s, Ulstrup, Denmark), capable of feeding 1, 2, 3, or 4 piglets individually. The milk replacer used was a commercial mixture (Milky-farm, Nukamel Olen, Belgium), and was mixed twice daily (150 g Milky-farm : 1000 g of water). The daily amount of milk allotted to the group of piglets was recorded, and control weighings of milk output to each cage was performed before and after each respiration trial to estimate the individual allotment. The individual milk intake was also measured by the D₂O dilution technique. By this technique the water intake of piglets is measured by dilution of D₂O (the hydrogen isotope of DLW), and to convert from water intake to milk intake the DM content of the milk was analysed. For that purpose a representative sample was collected throughout 24 hours (milk was lead into a bucket in a refrigerator), simulating the milk ingested by the piglets. As the D₂O dilution technique also requires account for the metabolically produced water of the piglets (Theil *et al.*, 2002), the milk replacer was also analysed for protein (N x 6.38), HCl-fat, lactose, glucose, and ash. Content of D₂O was analysed in ultrafiltrated serum water by isotope ratio mass spectrometry (Theil *et al.*, 2002), whereas ¹⁸O abundance was measured in serum water as 46/44 (m/z) ratio, calibrated against the injection solution (unpublished).

Calculations

The rate of CO₂ production ($r\text{CO}_2$) was calculated by the single pool model (equation 1) proposed by Lifson and McClintock (1966). Determination of the body water pool was done by dilution of D₂O due to the higher excess enrichment. The mean body water pool was calculated by assuming that the body water fraction was constant throughout the study. The $r\text{CO}_2$ was converted from mol/d to L/d of CO₂ by multiplying with the constants 0.08205 (atm x L x mol⁻¹ x K⁻¹) and 273.15 K.

$$\text{CO}_2 \text{ production, (L/d)} = Q_{\text{mean}} \times (0.480769 \times k_O - 0.495769 \times k_D) \times 0.08205 \times 273.15 \quad (1)$$

The milk intake was calculated as the sum of the water turnover and the potential metabolic water stored (PMWS), divided by the potential fraction of the milk (Equation 2). For a detailed description of calculations of milk intake, see Theil *et al.* (2002).

$$\text{Milk intake in piglet (g/d)} = \frac{\text{water turnover} + \text{potential metabolic water stored}}{\text{potential water fraction of milk}} \quad (2)$$

Results and discussion

The milk replacer differed chemically from sow milk in several aspects: The DM content was only 12.7%, and expressed per kg DM, the milk replacer contained 22.9% protein and 25.7% fat, whereas the ash content was as high as 10.6%. The lactose content (31.0%) was comparable to sow milk (DM, 17.9%; protein, 28.4%; HCl-fat, 37.8%, lactose, 29.3%; ash, 4.6%, V. Danielsen, unpublished), but in contrast to sow milk, carbohydrates other than lactose accounted for the

remaining 9.8 % of DM in the milk replacer. As a consequence of the lower DM content, the potential water fraction of milk was 0.948, which was higher ($P < 0.05$) than found in sow milk (Theil *et al.*, 2002). The chemical composition of the milk affected the piglet performance measured by balance and respiration trials (H. Jørgensen, unpublished). The live weight (\pm SE) of the piglets at enrichment was 3.02 ± 0.16 kg, the water dilution space was 2267 ± 119 g, while the body water fraction on average was 0.777 ± 0.008 . The latter was considerably higher than found for suckling piglets (reported as 0.706) at the same age (Theil *et al.*, 2002), which indicated that these piglets had a much lower retention of fat. This makes sense, because the dietary fat level in the milk replacer was only two third of that found in sow milk. In line with this, the piglet growth rate was only 138 ± 17 g/d, although the main part of live weight gain is accounted for by retention of protein and water (Noblet & Etienne, 1987). The feeding level (D_2O dilution technique) was 891 ± 63 g/d of milk replacer, which was lower than planned, did also contribute to the low growth rate. The artificial sow did only allow adjustment of the feeding level on a group basis, and it was necessary to slightly restrict the piglets in order to avoid milk leftover from feeding bout to feeding bout. The feed conversion ratio was as high as 6.44 g milk per g of live weight gain, which was explained by the low content of DM, protein and fat in the milk replacer.

There was a high agreement between milk intake measured by the D_2O dilution technique (891 ± 63 g/d) and weighed milk allotted by the artificial sow (910 ± 58 g/d) and the difference between the two methods amounted to 2 %. The fitted linear regression between measured (Y) and weighed (X) milk replacer was $Y = 0.98 X$ (with no intercept), or $Y = 1.065 X - 78.1$ (with intercept, Figure 1A). The latter regression showed that the discrepancy between the two estimates was 4.7 % and 2.2 % when daily milk intake was 700 and 900 g/d, respectively, while the two methods agreed fully when daily milk intake was 1200 g/d. On an individual basis, the difference between weighed amount of milk and milk intake measured by D_2O dilution was in the range of -24 g/d to 90 g/d. The discrepancy between the two methods might be explained by 1) Milk dry matter remained in the tubes leading milk replacer from the artificial sow to the drinking cup in the chamber, 2) some piglets spilt minor amounts of milk, 3) the assumption that the water fraction was constant throughout the experiment may bias the estimation and 4) the amount of potential metabolic water stored was calculated, for simplicity, by assuming that the dry matter gain of the piglets contained equal amounts of fat and protein, which does not hold for piglets with a live weight gain of considerably less than 200 g/d (Noblet & Etienne, 1987). Our findings were in line with Prawirodigdo *et al.* (1990) who reported deviations between measured and estimated milk intake of less than 3 %. The present study showed, that the D_2O dilution technique was fairly accurate when estimating the milk intake of piglets.

The average CO_2 production was found to be 64.7 L/d for both methods. This is in line with Noblet and Etienne (1987), who reported the CO_2 production to be 28.9 L/d per $kg^{0.68}$, which for the piglets in the present experiment would correspond to a daily production of 64.1 L/d of CO_2 . Determination of mean CO_2 production by the DLW technique proved to be accurate on a group basis, as the fitted regression without intercept between CO_2 production measured by (Y) DLW and CO_2 production measured by indirect calorimetry (X) yielded the equation $Y = 1.00 X$ (Figure 1B). However, the fitted model including an intercept was not very precise, due to the low variability of the initial live weight of the piglets and hence no variability of their CO_2 production. For individual groups, the CO_2 production measured by DLW ranged between 92.8 and 105.8 % of the CO_2 production measured by indirect calorimetry. Obviously, a higher initial enrichment level would reduce the lack of precision for individual groups. According to Speakman (2001), the single pool model should be used for animals weighing up to 2 kg, and the two pool model should be used for animals weighing > 10 kg. For animals in the gap between these two limits, additional data are required to evaluate which model is most appropriate. The CO_2 production was also calculated according to the two-pool model proposed by Speakman (1997). However, the CO_2 production measured by the two-pool model overestimated the CO_2 production measured by indirect calorimetry by on average 13.2 %.

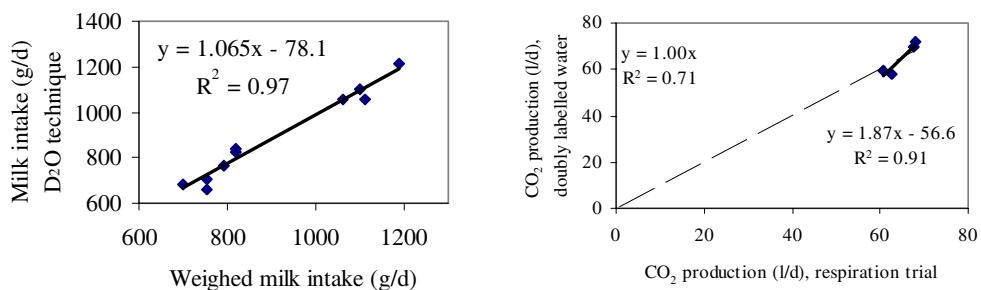


Figure 1. Validation of milk intake (1A) and CO_2 production rate (1B) in piglets, determined by dilution of D_2O and doubly labelled water techniques, respectively.

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Assessment of heat production in lactating sows by different approaches

P.K. Theil, H. Jørgensen & K. Jakobsen

Danish Institute of Agricultural Sciences, Research Centre Foulum, Department of Animal Nutrition and Physiology, P.O. Box 50, DK-8830 Tjele, Denmark

Summary

The energy metabolism of eight lactating sows was studied in week 2, 3, and 4 of lactation. The total heat production (HE) of sow and piglets was measured by indirect calorimetry (IC), while the contribution of the sow was derived by 1) the RQ method, 2) the CN method, 3) by sow heart rate (HR), and 4) the difference between IC and litter HE measured by doubly labelled water method (DLW). Equations to predict HE by means of HR were found for each sow from parallel recordings of HE and HR in four balance and respiration trials during the preceding pregnancy. Three suckling piglets in each litter were enriched with DLW to determine the CO₂ production and milk intake, which in turn was used to estimate the litter HE and sow milk yield, respectively. The HE of lactating sows remained constant throughout lactation. Overall, there was a high agreement of mean sow HE determined by the RQ method (38.05 MJ/d), by HR (36.81 MJ/d) and HE measured as the difference between IC and litter HE found by the DLW method (37.21 MJ/d), whereas the CN method probably underestimated sow HE (mean 35.15 MJ/d). The milk yield, found by dilution of deuterium in DLW enriched piglets, increased from 7.69 kg/d in week 2 to 10.64 kg/d in week 4 of lactation ($P<0.001$).

Keywords: *doubly labelled water, heart rate, indirect calorimetry*

Introduction

There is a lack in the literature of studies on energy metabolism of lactating sows, obviously due to methodological difficulties of deriving an estimate of their heat production (HE) and milk yield. The comparative slaughter technique is not an attractive way of obtaining the HE due to ethical reasons. Traditionally, indirect calorimetry (IC) has been used to measure HE of farm animals. However, two main problems arise in studies of energy metabolism of lactating sows. The first problem is, that the suckling piglets must join the sow in the respiration chamber to maintain a high milk yield and consequently contribute to the measured gas exchange. Therefore, an additional method is required to measure either the HE of the sow or HE of the litter. The second problem is to obtain a reliable estimate of the milk yield. Traditionally, milk yield has been determined by the weigh suckle weigh (WSW) method, but WSW is claimed for underestimating the milk yield because of stress, loss of faeces, urine and saliva between weighings, etc. (Pettigrew *et al.*, 1985). An elegant way of overcoming these problems is to measure piglet HE by the doubly labelled water (DLW) method, because the piglet milk intake (and thereby sow milk yield) can be measured concomitantly by evaluating the dilution of D₂O. The aim of the present study was to quantify the heat production of lactating sows by the RQ and the CN methods (corrected for piglet gas exchange), by mean daily heart rate (HR), or by subtracting the contribution of piglet HE measured by DLW in three piglets per litter from the total HE of sow + litter. The sow milk yield was assessed by determination of D₂O dilution in DLW enriched piglets.

Materials and methods

Animals and experimental procedures

Eight Danish Landrace x Yorkshire sows of 2nd parity were included when diagnosed as pregnant and subjected to, in total, 64 respiration trials during pregnancy and 48 trials during lactation. Concomitantly the sow HR was recorded. For that purpose, a biopotential transmitter (TA10CTA-D70, St. Paul, Minnesota, USA) was implanted under general anaesthesia subcutaneously, ventrally close to the sternum. The Dataquest LabPRO Telemetry System (Data Science International) was used for measurement of electrocardiogram. By means of the electrocardiograms, mean daily HR was calculated. Heat production and mean heart rate (HR) was measured once per month during pregnancy (lasting 2x24 h each) to calibrate the relation between HE and HR on an individual basis (Theil & Jørgensen, 2002). Heat production and milk intake was measured in three piglets per litter enriched with DLW, in order to estimate the litter HE and the sow milk yield. The total HE (sow + litter) was measured in three periods, approx. on d 10, 17 and 24 of lactation for 2x24 h, while sow and piglets were placed in metabolic cages for three days. An adjacent metabolic cage offered a place for piglets to rest and to urinate/defaecate, which allowed separate collection. Every 4th minute the O₂ consumption was measured by the paramagnetic principle (Magnos 4G) and CO₂ and CH₄ production was measured by the infrared principle (Uras 3) in respiration chambers. Sows were fed restrictedly twice daily with standard diets. Feed residues were collected and accounted for. The temperature was 20.7(0.9 °C during respiration trials and heat was supplied for the piglets.

Methods for assessing heat production of lactating sow

The contribution of the sow to the total HE was determined by 4 different strategies.

1. *RQ method:* The O₂ consumption and CO₂ production was separated into sow and piglet contributions according to their respective metabolic live weights (kg^{0.75}), and HE was calculated according to Brouwer (1965).
2. *CN method:* Sow HE was assessed by the CN method (CO₂ production from piglets found by the DLW method was subtracted) and it was assumed that CH₄ production, collected faeces and urine all originated from the sow. The HE was calculated by difference between ME and RE.
3. *Heart rate (HR) method:* Sow HE was predicted by means of mean daily HR, feed intake and live weight as: HE (MJ/d) = HR (beats/minute) x γ + 2.741 x kg DM intake + 0.0247 x kg LW + 4.561 MJ/d. The relation between HE and HR (γ) was calibrated individually during pregnancy (Theil & Jørgensen, 2002) and was on average 0.128 MJ per beat/minute.
4. *DLW method:* Litter CO₂ production was measured in three piglets per litter and based on these measurements, CO₂ production was calculated for the remaining littermates by means of their LW (See Theil *et al.* (2003; this volume) for validation of DLW in piglets). The piglet HE was calculated according to Brouwer (1965) by assuming that piglet RQ was equal to 0.84 (Noblet & Etienne, 1987b). Finally, the sow HE was found by the difference between total HE and litter HE.

For comparison, sow and piglet HE was calculated factorially. The piglet HE was used to subtract from the total HE (sow + litter), to estimate the sow HE.

5. *Piglet factorial method (pigFM):* According to Verstegen *et al.* (1985), piglet HE could be predicted factorially as: HE(piglets, MJ/d) = 0.406 MJ/kg^{0.75}/d x kg^{0.75} + 0.195 x LE, where LE was the energy intake from milk (MJ/d).
6. *Sow factorial method (sowFM) :* According to Noblet & Etienne (1987), lactating sow HE could be predicted as: HE(sow, MJ/d) = 0.460 MJ/ kg^{0.75}/d x kg^{0.75} + LE/0.72 - LE , where 0.72 was the overall efficiency of utilising ME for milk production and LE was the energy output in milk (MJ/d).

Results and discussion

The litter size of the sows was on average 9.25 piglets in week 2 through 4 of lactation. The DM intake and the milk yield increased throughout lactation (Table 1). From the milk yield, the DM content of milk (on average 18 %) and the energy content of the milk (averaging 25.54 MJ/kg DM), it was calculated that the total energy excreted in the milk amounted to 35.31, 42.54 and 48.47 MJ/d in week 2, 3, and 4, which corresponded to 50.1, 58.9 and 60.2 % of ME.

The total HE (sow + litter) increased considerably from week to week, but the sow HE remained rather constant irrespective of the four approaches (Table 2). The simplest and cheapest way to estimate the contribution of the sow and the piglets is to divide the gas exchange according to their metabolic live weight (RQ method). The exponent of 0.75 is used for comparison between species, whereas a lower exponent often is found when comparing within a species. In spite of that, it makes sense to apply the exponent of 0.75 in the present study, because suckling piglets and lactating sows in this context can be regarded as different species; Piglets weigh 3-7 kg, they are suckling and grow very fast, whereas sows weigh approx. 200 kg, they are lactating and they mobilise energy from their fat tissue and probably also protein pool. Relative to the RQ method, the HR and DLW approaches agreed well with the RQ method (along with the method where piglet HE was derived factorially). In contrast, the CN method estimated sow HE 7.6 % lower than the RQ method, probably because the N balance was overestimated. We did not use urine catheters in order to avoid the risk of infection. Conversely, the sow factorial approach overestimated the sow HE, especially in late lactation.

The constant HE throughout lactation seemed remarkably, because of a higher DM intake and a higher milk yield with progress of lactation. However, the sow HE found by subtracting piglet HE estimated factorially (method 5) supported our findings. The lower LW observed in the study reduced the energy requirement for maintenance (MEm), but that could only partially account for the effect. If the sow HE remains constant throughout lactation, this could either be due to a lower MEm or due to a higher efficiency of utilisation of ME for milk production (according to method 6). The latter seems more likely than the former, because a reduced MEm coinciding with peak lactation seems to be contradictory. The utilisation of ME for milk production in the present study was found to be 0.78, 0.79 and 0.81 ($P<0.05$) for week 2, 3, and 4. The factorial approach did not take into account the ME requirement for udder development. In late lactation the udder is fully developed, which may explain the apparent increase of utilisation of ME for milk production.

The DLW and the HR methods seemed to be reliable as the sum of sow HE (found by HR) and litter HE (found by DLW, data not shown) on average yielded 99 % of total HE for LF and HF sows, respectively, when total HE was measured by IC. However, the sum was slightly biased with an underestimation in early lactation (97 %) and a slight overestimation in late lactation (102 %). The sow HE (DLW approach) amounted to 53.2, 52.9 and 46.6 % of ME, which left the sow in a state of negative energy balance by an average of 4.8 MJ/d. This indicates a high priority of energy for milk, and it explains the observed sow weight loss.

Table 1. Performance of lactating sows.

| | Week of lactation | | | Mean | RR ¹ | P-value |
|-------------------|-------------------|------|------|------|-----------------|---------|
| | 2 | 3 | 4 | | | |
| Live weight (kg) | 208 | 206 | 200 | 205 | 4.0 | <0.01 |
| DM intake (kg/d) | 4.52 | 4.70 | 5.22 | 4.81 | 0.45 | 0.02 |
| Milk yield (kg/d) | 7.69 | 9.50 | 10.6 | 9.38 | 1.08 | <0.001 |

¹ RR is the root of residuals, and was calculated by PROC MIXED. RR corresponds to RMSE in the GLM procedure.

Table 2. Total heat production (HE) of sow and litter and sow HE assessed by different approaches.

| | Week of lactation | | | Mean | Deviation ¹ (%) | RR ² | P-value |
|---|-------------------|-------|-------|-------|-------------------------------|-----------------|---------|
| | 2 | 3 | 4 | | | | |
| Total HE ³ of sow & litter (MJ/d) | 53.89 | 59.39 | 63.25 | 58.84 | | 1.36 | <0.001 |
| <i>Heat production of lactating sows (MJ/d)</i> | | | | | | | |
| RQ ⁴ | 38.36 | 38.35 | 37.45 | 38.05 | . | 1.25 | 0.28 |
| CN ⁴ | 35.12 | 36.42 | 33.89 | 35.15 | -7.6 | 2.96 | 0.26 |
| Heart rate ⁴ | 35.94 | 36.37 | 38.13 | 36.81 | -3.3 | 1.80 | 0.36 |
| Doubly labelled water ⁴ | 37.27 | 37.73 | 36.64 | 37.21 | -2.2 | 1.19 | 0.22 |
| Piglet factorial ⁴ | 38.16 | 39.19 | 39.19 | 38.79 | 1.9 | 1.50 | 0.37 |
| Sow factorial ⁴ | 38.88 | 41.45 | 43.36 | 41.23 | 8.4 | 2.68 | 0.29 |

¹ Percentage deviation was calculated relative to the RQ-kg^{0.75} method

² RR is the root of residuals, and was calculated by PROC MIXED. RR corresponds to RMSE in the GLM procedure.

³ Measured by indirect calorimetry and calculated according to Brouwer (1965)

⁴ Different approaches are described in the text

In conclusion, the DLW method gave reliable results both concerning estimation of litter HE and sow milk yield. The HR method gave similar results of HE, but also the simplest method (separation of gas exchange according to kg^{0.75}) gave reasonable data.

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Prediction of rumen volatile fatty acids based on milk fatty acid profiles

B. Vlaeminck¹, V. Fievez¹, M.S. Dhanoa², A.M. van Vuuren³ & R. Dewhurst²

¹ Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium

² Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK

³ ID TNO Animal Nutrition, Edelhertweg 15, 8200 AB Lelystad, The Netherlands

Summary

The objectives of this study were to develop and validate models for predicting rumen volatile fatty acid proportions of dairy cows based on milk fatty acids. Model development, using data from two experiments, showed predictive equations explained 30 to 87 % of the variation in volatile fatty acids. Validation of the equations, using an independent experiment, showed predictions of acetate and propionate proportions were relatively accurate, with a prediction error lower than 10 % of the observed mean.

Keywords: odd and branched chain fatty acids, milk fatty acids, rumen fermentation

Introduction

Recent *in vitro* research showed the possibility to predict rumen volatile fatty acid (VFA) proportions based on rumen concentrations of odd and branched chain fatty acids (OBCFA, i.e. iso C15:0, anteiso C15:0, C15:0, iso C17:0, anteiso C17:0, C17:0, C17:1) (Vlaeminck *et al.*, 2003). If these relations persist *in vivo*, there could be scope for the use of milk OBCFA (MOBCFA) in milk-based tests predicting the supply of specific nutrients from the rumen. Indeed, MOBCFA make up around 5 % of milk fatty acids (MFA). Bivariate correlations of individual MOBCFA or their ratios with rumen VFA proportions were reported earlier (Vlaeminck *et al.*, 2002). Here, we describe the development and validation of principal component (PC) regressions aiming at predicting rumen VFA proportions based on a combination of MOBCFA. Moreover, we evaluated whether addition of even chain MFA could improve these predictions.

Material and methods

Experimental design, sampling and analysis

The current study combined data from 3 experiments, all conducted according to a 4-period incomplete or complete changeover design, with each experimental period lasting for 28 days. Diets, based on grass or legume silage and concentrate, were offered to rumen fistulated Holstein-Friesian cows. Experiment 1 (Exp. 1) was according to an incomplete changeover design, with 6 cows in the beginning of the lactation. Each cow was offered 4 of the 6 different diets, consisting of 8 kg/d of a standard dairy concentrate and one of the 6 silages *ad libitum*: grass (G), red clover (RC), white clover (WC), alfalfa, and 50/50 (DM basis) mixtures of G and RC and G and WC (Dewhurst *et al.*, 2003). Experiment 2 (Exp. 2) was designed as a 4 x 4 Latin square experiment (Hindle *et al.*, 2003), with cows in early lactation offered grass silage/beet pulp/concentrate in ratios of 43/27/30 on a DM basis. Diets were distributed once daily as a TMR and were iso-energetic and iso-nitrogenous and based on the animals requirements for energy and protein. The 4 concentrates differed in amount of (protected) starch. Experiment 3 (Exp. 3) was a 4 x 4 Latin square, with cows in mid-lactation, offered diets varying in forage to concentrate ratio (F/C).

Dietary treatments were based on *ad libitum* access to ryegrass silage and a standard dairy concentrate with F/C of 80/20, 65/35, 50/50, 35/65 on a DM basis (Dewhurst *et al.*, 2002). Samples of rumen fluid were taken every 2 h or 4 h over a 24-h period and analysed for VFA in Exp. 1 and 3 (Dewhurst *et al.*, 2003) and Exp. 2 (Hindle *et al.*, 2003) respectively. Milk samples were taken during 4 or 8 consecutive milkings and analysed for milk fatty acids (MFA) in Exp. 1 and 3 (Dewhurst *et al.*, 2003) and Exp. 2 (Fievez *et al.*, 2003) respectively.

Data analysis

Calculations

Mean molar proportions of acetate (HAC), propionate (HPR) and butyrate (BUT) [mmol/mol (HAC+HPR+BUT)] were calculated. MFA were expressed as proportion of the total fatty acid methyl esters (FAME) determined in all 3 experiments. Means of MFA were calculated per cow and diet. Since rumen bacteria differ both in OBCFA content (% of FAME) and pattern (% of OBCFA) (Viviani, 1970), individual MOBCFA were calculated relative to total MFA (% of FAME) as well as relative to the sum of MOBCFA (% of MOBCFA). The sum of C17:0 and C17:1 was introduced in the regression equation as a single independent since C17:1 has been suggested to be produced endogenously from C17:0 (Fievez *et al.*, 2003). All statistical tests were performed with SPSS statistical software, version 11.0 (Chicago, IL, USA).

Model development and validation

Multiple regressions (MR) were based on molar proportions of VFA and MFA from Exp. 1 and 2 ($n = 40$). However, as variables were correlated, PC analysis was used to derive orthogonal contrasts. Only significant MFA were included in PC analysis. Molar proportions of VFA were then regressed on these PC scores and non significant component scores were omitted from the regression using the backward procedure. Standardised coefficients were transformed to the original variables. Coefficients on a standardized scale were calculated as the product of PC coefficients and the principal score coefficients (Vlaeminck *et al.*, 2003).

Data from Exp. 3 were used for regression validation ($n = 16$). The accuracy of each of the prediction equations was evaluated based on the mean square prediction error (MSPE). The MSPE can be regarded as the sum of three components: 1/ errors from the inability of models to predict the mean response (central tendency), 2/ errors in prediction that can be accounted for by a linear correction factor (regression) and 3/ a random error of prediction (disturbance). The mean prediction error (MPE) is calculated from the root of the MSPE, and the relative prediction error (RPE) represents the MPE relative to the observed mean (Bibby & Toutenburg, 1977).

Results and discussion

Model development

Table 1 shows molar proportions of VFA used for the model development and validation. In this study, equations based on OBCFA, developed to predict molar proportions of VFA, explained 30 to 75 % of the variation in VFA (Table 2). The standardised regression coefficients (data not shown) indicated that the molar proportion of HPR was mainly determined by iso C15:0, whereas changes in C15:0 had the greatest influence on molar proportions of HAC and BUT. The high and negative coefficient of C15:0 in the HAC-predicting equation was not surprising as cellulolytic bacteria show low levels of C15:0 and C17:0 (Viviani, 1970).

In order to improve the prediction of rumen VFA proportions, additional MFA were evaluated, as they can be related to the rumen fermentation pattern. Short and medium chain MFA are (partially)

Table 1. Range of VFA proportions (mmol/mol) for the model development and validation.

| | Model development | | | | Model validation | | | |
|------------|-------------------|------|------|------|------------------|------|------|------|
| | Mean | SEM | Min. | Max. | Mean | SEM | Min. | Max. |
| Acetate | 674 | 3.17 | 627 | 715 | 664 | 3.36 | 637 | 692 |
| Propionate | 203 | 2.59 | 172 | 243 | 220 | 2.64 | 207 | 244 |
| Butyrate | 123 | 1.96 | 99 | 153 | 116 | 2.28 | 101 | 137 |

de novo synthesised from lipogenic VFA. Rumen lipolysis and hydrogenation determines concentrations of poly-unsaturated MFA (Bauman & Grinari, 2003) and depends, among others, on rumen pH which, in turn, is related to the rumen fermentation pattern (Van Nevel & Demeyer, 1996). Some measure of the former can be given by C18:0 (C18:0p) and sum of C18:2 and C18:3 [(C18:2+C18:3)p] relative to the total amount of C18-FA (Σ C18). Moreover, increased supplementation of dietary (unsaturated) long chain fatty acids, indicated by Σ C18, might increase rumen HPR proportions (Demeyer & Fievez, 2000). Prediction equations explained 56 to 87 % of the variation in VFA and were based on iso C17:0, C10:0 and C18:0p for HAC, iso C15:0, anteiso C17:0, C10:0, C12:0 and Σ C18 for HPR and iso C15:0, C15:0, (C17:0+C17:1), C6:0, C12:0, (C14:0+C14:1), (C16:0+C16:1) and C18:0p for BUT. Compared to predictions based on MOBCFA only, predictions of HPR and BUT based on MFA showed higher coefficients of determination ($p < 0.001$, $p < 0.01$ for HPR and BUT, respectively) (Table 2).

Table 2. Coefficient of determination (R^2), adjusted R^2 (R^2_{adj}) and standard error of estimate (SEE, mmol/mol) of predictive VFA model based on milk odd and branched chain fatty acids (MOBCFA) and milk fatty acids (MFA).

| | Model based on MOBCFA | | | Model based on MFA | | |
|------------|-----------------------|-------------|------|--------------------|-------------|------|
| | R^2 | R^2_{adj} | SEE | R^2 | R^2_{adj} | SEE |
| Acetate | 0.602 | 0.557 | 12.5 | 0.636 | 0.594 | 12.2 |
| Propionate | 0.303 | 0.223 | 13.6 | 0.563 | 0.484 | 10.9 |
| Butyrate | 0.748 | 0.702 | 6.34 | 0.866 | 0.813 | 4.85 |

Model validation

MSPE values suggest wide variation in the accuracy of the prediction equations for rumen VFA (Table 3). Prediction of BUT was the least accurate, with RPE higher than 30 % of the observed mean. RPE lower than 10 % of the observed mean suggests prediction equations for HAC and HPR were relatively accurate. However, a large part of the MSPE for HPR was caused by linear bias, which indicates underlying inadequacies in the structure of the model. Lower values for MSPE suggest better HAC and HPR predictions by regressions based on MOBCFA than on MFA. This might be related to high amounts of concentrates in some diets of Exp. 3, which might induce accumulation of conjugated isomers of linoleic acid. Recently, *de novo* synthesis of short and medium chain MFA has been suggested to be more sensitive to these fatty acids than to the rumen HAC supply (Bauman & Grinari, 2003).

Table 3. Accuracy and mean square prediction error components of the predictive VFA model based on milk odd and branched chain fatty acids (MOBCFA) and milk fatty acids.

| | Model based on MOBCFA | | | | | | Model based on milk fatty acids | | | | | |
|------------------|-----------------------|------|------------------|-------|---------------------------------|------|---------------------------------|-------|------------------|-------|---------------------------------|--|
| | MSPE ¹ | | RPE ¹ | | Proportion of MSPE ¹ | | MSPE ¹ | | RPE ¹ | | Proportion of MSPE ¹ | |
| | | | Mean | Line | Rand. | | | | Mean | Line | Rand. | |
| HAC ² | 218 | 2.22 | 0.388 | 0.045 | 0.567 | 362 | 2.87 | 0.331 | 0.191 | 0.478 | | |
| HPR ² | 171 | 5.93 | 0.005 | 0.411 | 0.584 | 474 | 9.88 | 0.287 | 0.497 | 0.216 | | |
| BUT ² | 1502 | 33.4 | 0.644 | 0.327 | 0.030 | 1264 | 30.6 | 0.602 | 0.370 | 0.029 | | |

¹ MSPE, mean square prediction error [(mmol/mol)²]; RPE, relative prediction error (%); mean, mean bias about the regression; line, line bias about the regression; random, random variation about the regression

² HAC, acetate; HPR, propionate; BUT, butyrate

Conclusion

The current study confirmed the potential of MFA, particularly MOBCFA, to predict rumen VFA proportions. However, in order to fully exploit the potential of these milk parameters, more experiments are needed to cover a wider range of rumen fermentation patterns.

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Effect of starch infusion into the abomasum on energy metabolism in beef cattle

J. Zhang¹ & Y. Feng²

¹ College of Life Sciences, Peking University, Beijing, China

² College of Animal Science and Technology, China Agricultural University, Beijing, China

Summary

Four mature steers fitted with permanent rumen and abomasum cannulas were used to study the effect of infusion of starch into the abomasum at three different levels (130, 330, 530 g starch/d) on energy metabolism compared to a basal diet consisting of bay-leafs, which supplied energy for 1.3-times maintenance according to a 4 x 4 latin square design in respiratory chambers. Each experimental period lasted 10 d including a 7-d adaptation period and 3-d balance period. The nutritive value of the bay-leafs was 17.3 MJ/Kg, 93.2%, 7.1%, 67.2% and 41.2% for GE, OM, CP, NDF and ADF in dry matter, respectively. Starch was infused six times per day into the abomasums via fistula every 4 h. The infusion of starch significantly decreased HP/ME ($P<0.05$) and increased K_{pf} ($P<0.05$), but there was no difference between the effect of 330 g and 530 g starch infused. The results illustrated that the efficiency of energy conversion increased by infusing starch into the abomasum, but the efficiency was not clearly increased when the starch was infused to a certain level.

Keywords: starch infusion, abomasum, energy metabolism, cattle

Introduction

The digestibility of starch in small intestine of ruminants was diversified according to literature studies. Orskov et al. (1986) could show that the digestive capacity of the small intestine of sheep was 100 g to 200 g/d, however, if the starch was processed, the digestive capacity increased to 200 - 300 g/d. Some experiments showed that the digestibility of starch in small intestine of ruminants amounted to 50 - 80% (Owens et al., 1989). However, in other studies a high digestibility in the small intestine of starch, which passed the rumen, was measured: e.g. Tuckery et al. (1984) observed a digestibility of 99.1% and Degregorio et al. (1982) of 99.3% using diets containing 40% maize.

The following factors are discussed, which may affect the digestion of starch in the small intestine in ruminants: The amount of amylase secreted was not sufficient (Karr et al. 1966); the pH is not optimal in the small intestine for amylase (Wheeler et al., 1977); the absorption of glucose in small intestine is limited (Orskov et al. 1971); the structure of starch prevented digestion (Harber et al. 1975); the time available for starch digestion in the small intestine is not long enough (Sutton et al., 1971).

Many studies stressed that infusing nutrients into the abomasum could improve the energy efficiency for ruminants. Verite et al. (1994) found that the efficiency of forage for energy conversion was improved and milk production increased by infusing protein into the abomasum in dairy cows. Some research showed that fatty acid infused into the abomasum could increase energy efficiency, because the animals could directly deposit the fatty acid into their products or tissues. This was also true for fatty acids hydrolyzed in the rumen. Lobley et al. (1982) discovered that the absorption of glucose was strengthened in small intestine by infusing CHO into abomasums, and both protein and energy retention efficiencies were improved. In general, most

studies supported the hypothesis that digestion of starch in the small intestine could increase energy efficiency compared to the fermentation in the rumen.

In order to explore the energy efficiency of post rumen starch in ruminants and regulate the diet of ruminants in practice, we designed this experiment to get some useful information for further study in the future.

Materials and methods

Four mature steers weighing 350 to 410 kg fitted with permanent rumen and abomasum cannulas were fed a basal diet consisting of bay-leafs at a feeding level of 1.3-times maintenance. The nutrition value of the bay was 17.33 MJ/Kg, 93.15%, 7.14%, 67.24% and 41.21% in dry matter for GE, OM, CP, NDF and ADF, respectively. The experiment was designed according to a 4 X 4 latin square in respiratory chambers, where the four experimental treatments were 0, 130, 330, 530 g starch infused into abomasums via cannulas six times a day.

Each experimental period lasted 10 days, including 7 days for adaptation and 3 days, in which N-balance and gas exchange was measured in respiration chambers. Samples were collected by abomasum cannulas 8 times (3 hours interval) each day in the formal period. Faecal energy, urinary energy and CH₄ were determined according to the conventional methods. HP was calculated by RQ-method.

Results

Some main experimental data are presented in the table 1.

The infusion of starch obviously increased DE and ME intake, but there were no clear differences between the groups, which received 130, 330 and 530 g starch/d. The infusion of different levels of starch significantly decreased HP/ME ($P<0.05$), and HP/ME was non-linear negatively related to the amount of starch infused. The regression equation is: HP/ME (%) = 92.9 - 3.46 lnX ($r=-0.93$, $n=4$), where X is the amount of starch infused (g/d).

Table 1. Effect of starch infusion on some parameters.

| | Starch infused (g/d) | | | |
|-------------------------------|----------------------|---------------------|---------------------|---------------------|
| | 130g | 330g | 530g | 0g |
| GE (Bay-leafs, MJ/d) | 116.21 ^a | 115.86 ^a | 113.72 ^a | 111.69 ^a |
| Starch energy infused (MJ/d) | 2.29 ^c | 5.78 ^b | 9.27 ^a | --- |
| Faecal energy (MJ/d) | 53.78 ^a | 54.24 ^a | 55.81 ^a | 53.48 ^a |
| Urinary energy (MJ/d) | 2.92 ^{ab} | 3.07 ^a | 3.21 ^a | 2.61 ^{bc} |
| CH ₄ energy (MJ/d) | 6.43 ^a | 6.41 ^a | 6.29 ^a | 5.87 ^b |
| DE (MJ/d) | 64.72 ^{ab} | 67.40 ^a | 67.18 ^a | 58.21 ^b |
| ME (MJ/d) | 55.37 ^a | 57.92 ^a | 57.68 ^a | 49.73 ^b |
| HP (MJ/d) | 42.35 ^a | 41.45 ^a | 41.55 ^a | 43.56 ^a |
| HP/ME (%) | 76.49 ^b | 71.56 ^b | 72.04 ^b | 87.59 ^a |
| RE (MJ/d) | 13.02 ^b | 16.47 ^a | 16.13 ^a | 6.17 ^c |
| K _{pf} (%) | 42.89 ^b | 50.05 ^a | 49.37 ^a | 33.96 ^c |
| Body fat deposited (d/g) | 270.97 ^b | 337.81 ^a | 312.86 ^a | 107.83 ^c |
| Body protein deposited (g/d) | 94.81 ^c | 128.25 ^b | 155.56 ^a | 79.25 ^d |

In average K_{pf} of the three infusion groups was 13.5% ($P<0.05$) higher than of the control group. Infusing 330 and 530 g/d starch increased k_{pf} by 7.2% ($P<0.05$) and 6.5% ($P<0.05$), respectively, compared with the K_{pf} -value of the group, where 130 g starch /d were given. The infusion of starch strongly increased K_{pf} ($P<0.05$) in a non-linear matter, which can be described by the regression equation: $K_{pf} (\%) = 19.0 + 5.038 \ln X$ ($r= 0.93$ $n=4$), where X is the amount of starch infused (g/d). When infusing starch in abomasums above 330 g/d, the decrease of HP/ME and K_{pf} was less pronounced. Although 130 and 330 g starch/d accounted only for 4.1% and 9.2 % of the total ME-intake, the K_{pf} increased by 8.9% and 16.1% respectively.

The amount of fat deposited in the treatment groups increased in average by 199 g/d ($P<0.05$) compared with no infusion. Protein deposition was enhanced when starch was infused into abomasums, the average increase of protein deposition in all groups was 50 g/d ($P<0.05$), the average increase of the groups with 530 and 330 g starch infusion/d was 60.8 g/d ($P<0.05$) and 33.4 g/d ($P<0.05$) higher than that of the group with 130 g starch/d.

Discussion

Based on the above research results, the efficiency of energy conversion increased by infusing starch into the abomasums, it probably proved that the starch was digested into glucose in small intestine, and the glucose provided some NADPH to improve the efficiency of conversion from acetic acid to fat in ruminants. Owens et al. (1986b) found a similar result with our experiment, and their research showed that the gain in weight for beef cattle was improved significantly by increasing starch in small intestine. Because starch was a fundamental material to increase the productivity of ruminants, the appropriate utilization of starch was a basic step to improve the producing efficiency of animal products. It will be a valuable work to regulate the proportion of digested starch in small intestine of ruminants.

Further study is necessary to learn the efficiency of energy conversion for starch digested in intestine and fermented in rumen. It is meaningful to improve animal productive efficiency by regulating the ruminant diets in practice.

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Protein session 1

Protein Metabolism and Microbiology in the Gastro-Intestinal-Tract

Intestinal microbial amino acid synthesis and its importance for the amino acid homeostasis of the monogastric host

Cornelia C. Metges & G. Loh

Research Institute for the Biology of Farm Animals, Research Unit Nutritional Physiology 'Oskar Kellner', Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

Summary

There is the possibility that the metabolic requirement of indispensable amino acids in monogastric mammals is met not only by the diet but also by amino acids synthesized de novo by the gastrointestinal microflora, which are then absorbed. It is therefore crucial to better understand and quantitate the microbial biosynthesis of amino acids in the gastrointestinal tract and its potential role in providing amino acids to meet amino acid requirement. This paper summarizes the available evidence on a contribution of microbial lysine to the host's lysine homeostasis, applying isotope tracers in humans, pigs, and rats. Between 2 and 20 % of circulating plasma lysine, urinary lysine and body protein lysine of the host, respectively, is derived from intestinal microbial sources. Factors affecting estimates of net microbial IAA contribution are discussed. It was estimated that the porcine small intestine is responsible for more than 90% of microbial lysine uptake. Microbial amino acid synthesis in the gastrointestinal tract utilizes a mixture of various nitrogen sources, i.e. endogenous amino acids, urea and ammonia. Acetate and CO₂ and to a lesser degree propionate derived from microbial carbohydrate fermentation form an active precursor pool of carbon for amino acid synthesis. Certain dietary non-starch polysaccharides and oligosaccharides, poorly digestible by mammalian enzymes can affect the composition and metabolic activity of the intestinal microflora, and are demonstrated to serve as carbon precursors for de novo amino acid synthesis in the intestinal microflora. This opens the possibility of manipulation of the microbial composition, and thus its fermentation products potentially available to the host. The intestine is a highly dynamic tissue of continuous replacement. Due to its direct vicinity to the intestinal flora it controls the effect of intestinal microbes on whole-body physiology. There is evidence that at low dietary protein intakes, or lysine concentrations splanchnic tissues benefit more from microbial amino acid sources than peripheral tissues.

In conclusion, using the ¹⁵N labeling paradigm a significant contribution of microbial lysine to the host lysine homeostasis is found. However, to assess net contribution of microbial amino is complicated by the nitrogen and amino acid recycling in the gut and the uncertainty of the precursor pool of absorption. Evidence based on ¹⁴C data and digesta exchange experiments supports the view that the de novo indispensable amino acid (i.e. isoleucine, leucine, valine, phenylalanine, lysine) synthesis by the small intestinal microflora represents a net addition to dietary amino acids absorbed from the gut.

Keywords: monogastric mammals, small intestine, intestinal microflora, adherent microbial organisms, amino acid metabolism, pig, rat

Introduction

Lysine is the first limiting amino acid in human and monogastric animal nutrition. Cereal based diets are low in lysine which is why practical pig diets are often supplemented by crystalline lysine. This aids to increase feed efficiency, lowers N excretions and efficiently utilizes the growth potential of modern breeds. An efficient lysine supply is thus of great economic importance for pig production, and it is not surprising that there are considerations regarding transgenic animals with

microbial biosynthetic pathways for these amino acids (Rees *et al.*, 1990; Saqib *et al.*, 1994). This suggests that besides dietary lysine there is a second source of lysine which might help to meet the metabolic lysine requirements of monogastric animals: The intestinal microflora.

Although it has been long known that the energy and amino acid supply of ruminants eating poorly digestible low protein diets depends on the microbial activities in their forestomachs (Clark *et al.*, 1992) it is to date insufficiently understood whether and to which extent microbially synthesized indispensable amino acids might be used to support growth and protein homeostasis of the monogastric host.

In the 1990ies new microbiological techniques using phylogenetic analysis of 16S ribosomal DNA (rDNA) sequences and other molecular based techniques became available enhancing our knowledge on the dynamics and complexity of intestinal ecosystems. This helps to elucidate the relationship between the microflora and the host, its importance in host nutrition, and in particular the identification of lysine synthesizing microbial species and its site of action within the gastrointestinal tract (GIT).

The aim of this review is to provide an overview on the microbial population in the upper GIT, intestinal microbial lysine synthesis in monogastric mammals, with emphasis on the pig, the evidence for its contribution to the host's lysine and protein homeostasis, the possibilities of dietary manipulation of the microflora, and thus possibly its amino acid synthesizing features.

Gastrointestinal microflora and lysine synthesis in monogastric mammals

Traditionally, the intestinal microflora has been analyzed by means of bacteriological culture. Enumeration of particular microbial genera or species relies on the use of selective media. A major portion of the microflora detected by microscopy, however is currently uncultivable (50% and more). More recently, molecular methods have been developed which do not rely on cultivation techniques. These include fluorescent *in situ* hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE) targeting ribosomal 16S rRNA and 16S rDNA, respectively (Kleessen *et al.*, 1999; Simpson *et al.*, 1999; Tannock 2001; McCracken *et al.*, 2001). Species can be enumerated directly by means of oligonucleotide probes based on 16S rDNA sequences. The probe molecules are labeled with a fluorescent dye and the procedure is termed *in situ* hybridization (Jansen *et al.*, 1999). DGGE on the other hand allows to screen shifts and diversity in microbial populations without the need for oligonucleotide probes of specific previously identified species (McCracken *et al.*, 2001).

The gastrointestinal tract (GIT) of monogastric animals and humans is colonized with a vast community of indigenous microorganisms. Culture-based studies indicate that the intestinal microflora may comprise up to 400 bacteria species. Such bacterial genera are: *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*, and *Ruminococcus*. Using 16S rDNA sequence analysis a total of 375 phylotypes was identified from 52 different pig samples of ileum, cecum, and colon, of which only 17% likely represented known (previously cultured) bacterial species (Leser *et al.*, 2002). Because this molecular technique does not provide abundance information it is likely that the majority of phylotypes identified represent minor constituents of the total bacterial population. It seems that most of the microbial population comprises only 30-40 species (Draser 1985).

A growing number of studies are now becoming available providing considerable insight into the complexity and dynamics of microbial communities along the GIT as affected by age, various diets, the administration of pre- and probiotics and antibiotics, and revealing so far unknown molecular microbial species (Sghir *et al.*, 1998; Simpson *et al.*, 1999; McCracken *et al.*, 2001; Suau *et al.*, 1999; Swords *et al.*, 1993; Leser *et al.*, 2002). Although within an individual some stability of microbial composition might be attained at a certain age, interindividual variability of

stable microflora between individual hosts support the hypothesis that host-related factors are involved in the determination of the GIT microbial community (Zoetendal *et al.*, 2002).

Every species of animal appears to have its unique microflora. Among monogastric animals (human, pig, rat, mouse) there are distinct differences in the anatomical and physiological parameters of the intestine, which is important in the consideration of intestinal flora. Rats have a higher gastric pH than humans and a gastric flora (Savage 1987) and are coprophagic which also occurs in the young pig. It was shown that piglets consume between 6 and 80 g of maternal feces per day (Conway 1994). As compared to humans mice and rats have large ceca relative to the overall size of their GIT (Carman *et al.*, 1993). One of the major differences between the pig gut microbiota and other omnivores such as man is the high number of bacteria in the small intestine. As demonstrated by culture-dependent and molecular approaches, there are high numbers of adherent and luminal anaerobes *Lactobacillus* and *Eubacterium* in the stomach (pars oesophagea) and small intestine of pigs which show alterations dependent on age and intestinal location (McGillivray & Cranwell, 1992; Sghir *et al.*, 1998; Henriksson & Conway 1996). *Lactobacillus* populations are one of the best-described genera inhabiting the GIT of pigs with higher prevalence in the proximal than in the lower intestine, and in weaning than adult pigs (Sghir *et al.*, 1998; Bateup *et al.*, 1998; Pryde *et al.*, 1999). Adhesion and subsequent association with the epithelium of the pig stomach mediated by bacterial saccharides or glycoproteins are considered to be prerequisites for *in vivo* colonization by *Lactobacillus* (Henriksson & Conway, 1996; Roos *et al.*, 2000).

Four microhabitats can be defined and these include the surface of the epithelial cells, the mucus gel overlying the villi, the mucus gel in the crypts and the luminal content (Conway 1994). Recent DGGE results revealed diverse bacterial populations among individual gut compartments in the porcine GIT (Simpson *et al.*, 1999).

The mean number of bacteria attached to or associated with cecal epithelial tissues of pigs was 2.7×10^7 CFU/cm² of tissue as compared to the pars oesophagea which was colonized by 10^5 - 10^6 CFU/cm² (Allison *et al.*, 1979; McGillivray & Cranwell, 1992). Stomach luminal contents were inhabited by between 10^6 to 10^9 /g contents (Bateup *et al.*, 1998). In contrast, total bacterial counts (CFU/g) were between 5.7×10^7 CFU/cm² and 8.8×10^8 CFU/g for the colonic wall, and 2.3×10^{10} for the colonic lumen (Robinson *et al.*, 1984; Pryde *et al.*, 1999).

There is evidence that microbial composition associated to the mucosa is different than in luminal contents (Allison *et al.*, 1979; Robinson *et al.*, 1994; Pryde *et al.*, 1999; Zoetendal *et al.*, 2002; Simpson *et al.*, 1999). A large fraction of the adherent population of the intestinal wall was only distantly related to known cultured bacterial types (Pryde *et al.*, 1999). The bacterial communities associated with the intestinal wall are potentially of great significance because this is the main site of nutritional and metabolic interaction with the host. Recently, in a series of elegant studies with gnotobiotic mice models microbial nutrient utilization and host nutrient production has been shown, as well as bacterial modulation of host ileal gene expression, such as Na⁺/glucose transporter, glutathione-S-transferase, and colipase (Hooper *et al.*, 2001; Hooper *et al.*, 1999). It is therefore reasonable to hypothesize that microbial inhabitants of epithelia surfaces also produce end-products of metabolism, such as amino acids, which could be absorbed by the host in competition with other microbes in the vicinity. So far this issue has received little direct experimental study.

The microflora has marked influences on the animal host which is evident from the comparison of germ-free and conventional (presence of microflora) animals (Table 1).

Besides production of metabolic end-products microorganisms alter the GIT mucosal architecture and mucus composition (Sharma *et al.*, 1995; Deplancke & Gaskins, 2001).

Amino acid synthesis in bacteria is a necessity for cell propagation and survival. This includes the synthesis of the eight indispensable amino acids usually required by monogastric mammals, such as e.g. lysine, threonine, leucine or phenylalanine, which need to be included in the diet.

Table 1. Comparison of selected metabolic and functional properties between germ-free and conventional animals (after Tannock 2001).

| Parameter | Conventional | Germ-free |
|---------------------------------------|--------------------------------|----------------------------|
| Short-chain fatty acids | large amounts of several acids | small amounts of few acids |
| Mucin content of intestinal mucus | high | low |
| Degree of mucin degradation | high | low |
| Tryptic activity | little | high |
| Rate of enterocyte replacement | fast | slow |
| Small bowel movements | fast | slow |
| Duodenal enterocyte enzyme activities | low | high |
| Intestinal mucosal surface area | great | small |
| Intestinal wall | thick | thin |

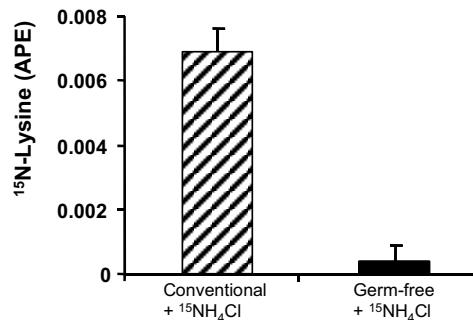


Figure 1. ^{15}N lysine enrichments in lysine of whole-body protein of conventional and germ-free rats fed a protein-free diet supplemented with $^{15}\text{NH}_4\text{Cl}$ (after Torrallardona *et al.*, 1996).

Based on the assumption that higher animals are not able to incorporate ^{15}N from non-specific nitrogen sources (e.g. urea, dispensable amino acids, ammonia) into lysine and threonine by transamination the appearance of ^{15}N labeled lysine and threonine in body fluids or proteins presumably indicates their absorption and utilization from microbial sources. Per definition germ-free animals do not have an intestinal microflora, and thus should have lost the ability to synthesize indispensable amino acids, i.e. lysine, in their intestine. This has been demonstrated in germ-free rats fed a protein-free diet containing non-specific ^{15}N , i.e. ammonium chloride, and fermentable carbohydrates (Torrallardona *et al.*, 1996) (Figure 1).

The contribution of microbial lysine to lysine homeostasis in human subjects has been estimated based on the product/precursor-ratio of ^{15}N lysine enrichment in plasma or urine : the presumable microbial protein/amino acid absorptive pool (Metges *et al.*, 1999). In an attempt to quantify the microbial amino acid contribution to the host amino acid homeostasis this ratio was multiplied by the plasma amino acid turnover, respectively, measured via intravenous ^{13}C amino acid infusion in the same subjects, or taken from the literature.

Contribution of microbial lysine to lysine homeostasis =

$$\frac{\text{Plasma free (or body protein)} \ ^{15}\text{N} \text{ lysine enrichment}}{\text{Fecal or ileal microbial} \ ^{15}\text{N} \text{ lysine enrichment}} \times \text{plasma lysine flux} \quad (1)$$

Various human and animal studies show that the administration of non-specific ^{15}N can be used to label microbial lysine and threonine, an approach which has been termed the “ ^{15}N labeling paradigm” (Metges 2000). It was observed earlier in uremic patients and in human subjects consuming low protein diets that microbial lysine can be incorporated into host body protein (ref. Metges 2000). In rats, however, the absorption of microbial lysine depends on coprophagy (Torrallardona *et al.*, 1996b).

For comparative purposes Table 2 presents a summary of the available data on microbial lysine appearance in the host body protein or amino acid pools in rats, pigs, and man. Applying the ^{15}N labeling paradigm, between 2 and 20 % of circulating plasma lysine, urinary lysine and body protein lysine of the host, respectively, was derived from intestinal microbial sources (Table 2).

Energy and nitrogen sources of the intestinal microflora

The microflora utilizes food components and endogenous compounds not easily degradable by mammalian enzymes. The monogastric host introduces into its gastrointestinal tract a variety of endogenous substances such as intestinal mucus, digestive enzymes, secreted immunoglobulins and sloughing epithelial cells, ammonia and urea. The microbial metabolic activity leads to the bacterial generation of amino acids and short chain fatty acids, important energy sources of enterocytes (Savage 1986). It has been estimated that bacterial fermentation products in the large bowel of pigs may contribute up to 25% of the maintenance energy requirement (Yen *et al.*, 1991). Also glutamate and glutamine are important metabolic fuels of enterocytes which has been shown in the classical rat studies of Windmueller and his group in the 70ies.

Via production of urease, not occurring in mammalian cells, intestinal bacteria play an important role in whole-body nitrogen metabolism. About 30% of newly synthesized urea is degraded by microbial urease. Both gram-negative and gram-positive organisms are urease producers.

Table 2. Contribution of intestinal microbial lysine to host lysine in several monogastric mammals.

| Species | Tracer | Microbial precursor lysine pool | Host lysine in | % microbial lysine label recovered in host lysine | Reference |
|----------------------------|---|---------------------------------|--------------------|---|-------------------------------------|
| Adult minipigs | $^{15}\text{NH}_4\text{Cl}$ $^{15}\text{N}_2$ urea | ileal | plasma albumin | 15.3 | Metges <i>et al.</i> , 1996 |
| Adult minipigs | $^{15}\text{NH}_4\text{Cl}$ | ileal | plasma | 12 ¹ | Backes <i>et al.</i> , 2002 |
| Grower pigs | $^{15}\text{NH}_4\text{Cl}$ ^{14}C -polyglucose | ileal cecal protein | whole-body 2 | 3.1 2003a | Torrallardona <i>et al.</i> , |
| Young rats | $^{15}\text{NH}_4\text{Cl}$ | fecal | whole-body protein | 2.8 | Torrallardona <i>et al.</i> , 1996a |
| Adult humans | $^{15}\text{NH}_4\text{Cl}$ $^{15}\text{N}_2$ urea | fecal | plasma | 7.5 ² 4.6 ^{2,3} | Metges <i>et al.</i> , 1999 |
| Adult humans / ileostomy | $^{15}\text{NH}_4\text{Cl}$ $^{15}\text{N}_2$ urea | ileal | plasma | 21 | Metges <i>et al.</i> , 1999 |
| Malnourished human infants | $^{15}\text{N}_2$ urea | urinary urea | urine | 2 | Millward <i>et al.</i> , 2000 |

¹ mean value of low and adequate dietary lysine intake

² mean value of fasted and fed states

³ not significantly different between $^{15}\text{NH}_4\text{Cl}$ and $^{15}\text{N}_2$ urea tracers

Urease, the enzyme responsible for urea breakdown generates the preferred nitrogen source, ammonia, for many bacteria. In rats urease activity in small intestinal contents (units/g collected content) was 15 % compared to that found in the large intestine (Kim *et al.* 1998). As shown for the human colon and the bovine rumen ureolytic bacteria seem to be mainly located close to the intestinal walls (Hume 1996) which might explain why there was only low enrichment in cecal luminal ammonia when ^{15}N urea was infused intravenously in human subjects (Wrong *et al.* 1985). When urea was intravenously infused in pigs urea concentration in the jejunal and in the colonic perfusate increases significantly. In contrast, ammonia concentration measured in the same animals was not changed significantly (Malmlof & Simoes Nunes 1992). At least in the pig the upper digestive tract (stomach and small intestine) represents the main site of urea secretion (Mosenthin *et al.* 1992; Malmlof & Simoes Nunes 1992).

Since mucus glycoproteins and some other digestive secretions are resistant to mammalian digestive enzymes microbial proteolytic activity is involved (Macfarlane *et al.* 1988; Quigley & Kelly 1995). Mucolytic potential is widespread among intestinal bacteria and bacterial colonization of mucus might represent a growth advantage (Deplancke & Gaskins, 2001). It has been shown that swine mucus supports the growth of numerous microbes (Allison *et al.*, 1979). Further information comes from studies in lactic acid bacteria indicating that bacteria are capable of degrading proteins and peptides, possess oligopeptide and amino acid binding proteins and transport systems (Kunji *et al.*, 1996).

Nitrogen is returned to the intestinal tract as proteins, peptides, amino acids and as urea in endogenous secretions (pancreatic, biliary, mucosal) (Fuller & Reeds 1998). Intravenous infusion of ^{15}N labeled amino acids is followed by labeled plasma amino acids appearing in the gastro-jejunal fluids within about three hours after onset of the infusion (Gaudichon *et al.*, 1994). In pigs 50 min within consumption of a ^{15}N labeled diet the tracer appeared in pancreatic secretions (Leterme *et al.*, 1996). This finding is further substantiated by our observations in the pig showing ^{15}N enrichment of amino acids (i.e. lysine, alanine, glycine, leucine, isoleucine, glutamic acid) in duodenal and jejunal proteins after a 10 days administration of $^{15}\text{NH}_4\text{Cl}$ (Metges *et al.*, 1996). The quantity of endogenous protein that is recycled in the intestine makes it a potentially significant source of nitrogen for microbial growth.

The metabolic processes by which the intestinal microflora gains carbon, nitrogen and energy from the components of the dietary and endogenous polymers is principally not different from microbial organisms in ruminants. Ammonia is the preferred source of nitrogen for the growth of enteric bacteria (Reitzer & Magasanik 1996) but only if there is sufficient ATP to drive microbial protein synthesis. As known from rumen bacteria acetate and CO_2 and to a lesser degree propionate derived from microbial carbohydrate fermentation form an active precursor pool of carbon for amino acid synthesis (Sauer *et al.* 1975). Ammonia is assimilated to form glutamate and glutamine (Reitzer & Magasanik 1996), and glutamate provides nitrogen for the synthesis of most of the other amino acids. Lysine biosynthesis has been characterized in bacteria such as lactobacilli, bifidobacteria, *E.coli* and corynebacteria as well as yeasts (Matteuzzi *et al.*, 1978, Cremer *et al.*, 1988; Saqib *et al.*, 1994; Lee *et al.*, 2001; Odunfa *et al.*, 2001). In prokaryotes lysine is derived from aspartic acid which is used to produce aspartic- β -semialdehyde which is then converted through a series of steps to the direct lysine precursor, diaminopimelic acid, required by many bacteria for the biosynthesis of cell wall (Morrison & Mackie 1996; Saqib *et al.*, 1994).

Because of its capability to bring about beneficial alterations of intestinal microbial composition the use of probiotics or prebiotics has received increasing scientific attention in recent years (MacFarlane & Cummings, 1999). Thus there are efforts to manipulate microbial metabolism, to the nutritional advantage and health of the animal tissues. However, not only addition of certain fermentable substrates alter the microbial population. Starvation for 5 days caused a dramatic reduction of luminal and mucosal *Lactobacilli* in rats (Mikelsaar *et al.*, 1987). Also, the ingestion of purified diets with a low fiber content led to a greater homogeneity of fecal microbial composition in mice (McCracken *et al.*, 2001).

Although classified non-digestible it has been shown that some oligosaccharides and non-starch polysaccharides are being partly hydrolyzed in the small intestine. Inulin was shown to be degraded by up to 50% at the level of the terminal ileum in piglets (Mikkelsen 2001). Studies in human subjects with ileostomy indicate that about 12% of inulin is degraded in the small intestine (Andersson *et al.*, 1999). This suggests that the small intestinal microflora is capable of utilizing inulin as an energy source with the potential of an increased microbial amino acid production, albeit to which extent may be species-dependent. Both *Bacteroides* and *Bifidobacterium* can hydrolyze and grow on end-products of the hydrolysis of exogenous polymers of several chemical classes found in the diet (see Table 1 in Savage 1986). Especially inulin and oligofructose support the growth of probiotic microorganisms such as *Lactobacillus* and *Bifidobacteria* followed by increased bacterial protein synthesis (Jenkins *et al.*, 1999; Gibson 1999; Schneeman 1999). Studies in rats demonstrated that inulin feeding leads to a decreased serum urea concentration, a reduced cecal ammonia level, and increased fecal nitrogen, indicating increased microbial protein synthesis (Levrat *et al.* 1993). The group of Fuller very recently demonstrated that through microbial synthesis in the gut, pigs can derive indispensable amino acids from dietary carbohydrates poorly digestible by the mammalian host (Torrallardona *et al.*, 2003a).

Absorption of microbial amino acids

Intestinal absorption of amino acids has been shown to be maximal in the mid-lower jejunum and human studies with ileal tubes show that at the ileum level dietary nitrogen is still recovered suggesting a role of the ileum for complete uptake of dietary amino acids. This conclusion is supported by the observation that peptide transporters were upregulated in the distal regions of the small intestine by a high protein diet (Erickson *et al.* 1995). The peptide transporter PEPT1 has been localized to the apical microvillous plasma membrane of the absorptive epithelial cells of the rat small intestine and shown to be responsive to nutritional condition (Erickson *et al.* 1995; Ogihara *et al.* 1999). PEPT1 appears to be exclusively expressed in small intestinal tissues but no PEPT1 mRNA could be detected in large intestinal tissues of various species (Chen *et al.* 1999; Doring *et al.* 1998). However, weak signals of PEPT2 specific fragments have been identified in rabbit colon (Doring *et al.* 1998) although the importance of this finding for peptide absorption in general as well as for other species remains to be seen. Absorption of microbial protein-derived amino acids would require that microbial protein breakdown occurs at the ileum. There is evidence for high proteolytic activity in ileal effluents due to small intestinal peptidases but also due to bacterial proteases (Macfarlane *et al.* 1988, 1989; Kunji *et al.*, 1996).

¹⁵N and ¹⁴C lysine enrichment of microbial protein after ingestion of ¹⁵NH₄Cl and ¹⁴C polyglucose changes throughout the gastrointestinal tract of pigs (Torrallardona *et al.* 2003a, 2003b). No enrichment has been found in the small intestine with the exception of the ileum while a substantial enrichment in the cecum followed by an increase towards the distal colon, comparable to the enrichments in feces, has been observed (Torrallardona *et al.* 2003a, 2003b). Based on the comparison of enrichments of both isotopes in the digesta and the carcass it was suggested that microbial lysine has been absorbed in the small intestine. The same authors studied the site of microbial lysine absorption by returning ¹⁵N labeled digesta into the small intestine of unlabeled pigs. They estimated that at least 90 % of total microbial lysine absorption occurred in the small intestine (Torrallardona *et al.* 2003b).

Our investigations in pigs with end-to-end ileo-rectal anastomosis and patients with ileostomy indicate that microbial amino acids can be synthesized in the small intestine and their appearance in the free plasma pool indicate their absorption from that site (Metges *et al.* 1996, 1999a, 1999b). However, ileostomy as well as ileo-rectal anastomosis is prone to secondary colonization and possibly alterations of digesta transit rate in the gut. Hence, while it might be somewhat different from the microbial situation in an intact gastrointestinal tract, it demonstrates the principal possibility of microbial lysine and threonine absorption from the small intestine.

There is some, albeit weak, evidence that amino acid absorption can occur in the large intestine. Between 30 and 100% of free lysine, threonine, serine, histidine and arginine from an enzymatic casein hydrolysate solution disappeared from the cecum of pigs (Olszewski & Buraczewski, 1978). This has to be viewed with caution because the surgical isolation of the cecum and the continued antibiotica lavages generate rather artificial conditions which may not be comparable to normal conditions. Using ^{15}N labeled rectum content appearance of ^{15}N lysine and other ^{15}N amino acids were detected in the colic branch of the ileocolic vein three hours after infusion into the cecum (ref. Metges 2000). Other reports indicate that amino acid absorption from the large intestine is negligible in non-ruminant animals (ref. Metges 2000). However, Fuller and Reeds (1998) summarized data on N balance measurements in pigs when protein or amino acids were infused into the large intestine and found that the whole-body N balance was always slightly improved. However, the identity of the substances absorbed may still be questioned because no nutritional benefit was seen when lysine was infused into the cecum. This latter finding does not exclude, however, the possibility of peptide absorption as mentioned above.

In conclusion, it appears that the small intestine is responsible for the major part of microbial lysine uptake although some absorption from the large intestine cannot be excluded. However, that the large intestinal amino acid absorption is of nutritional significance remains to be shown.

The gut regulates whole-body amino acid homeostasis

The intestine is a highly dynamic tissue of continuous replacement. Proliferation in the small intestine is confined to the crypts of Lieberkühn. Each of the approximately 1 million crypts in the small intestine produces about 300 new cells per day (Gordon *et al.* 1997). Due to its direct vicinity to the intestinal flora the GIT controls the effect of intestinal microbes on whole-body physiology. Gastrointestinal epithelium performs not only a barrier function but is also an active sensor of the microflora and an important intermediary in regulating and integrating cross-talk between itself and cells of the innate and adaptive immune systems (Bullard & Weaver, 2002).

A growing body of evidence suggests that the gut modulates amino acid flux and inter-organ relationships. Knowing that enterocytes have a 3-4 day life span, this may be particularly true during the absorptive period, when the intestine modulates the metabolic fate of absorbed amino acids, and consequently influences the availability of amino acids for peripheral tissues. For example, duodenal mucosa in human subjects has a fractional protein synthetic rate of about 50 % per d (Bouteloup-Demange, 1998). Proteins synthesized by intestinal tissues, are either digested and reabsorbed, or they are passed to the colon where they are degraded, and lost to the body

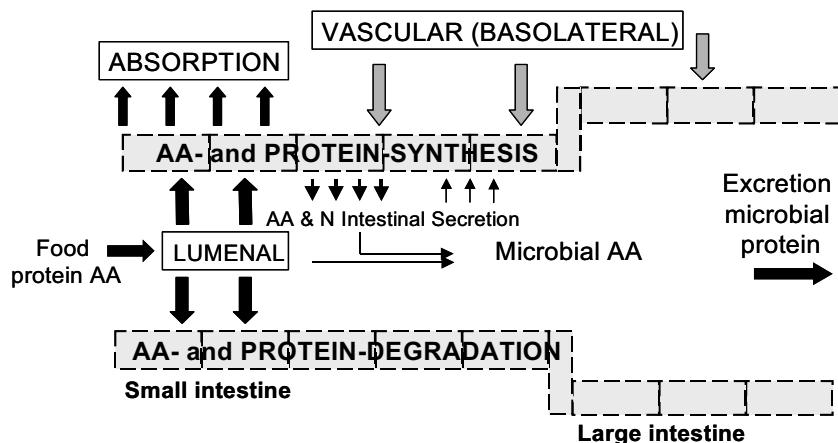
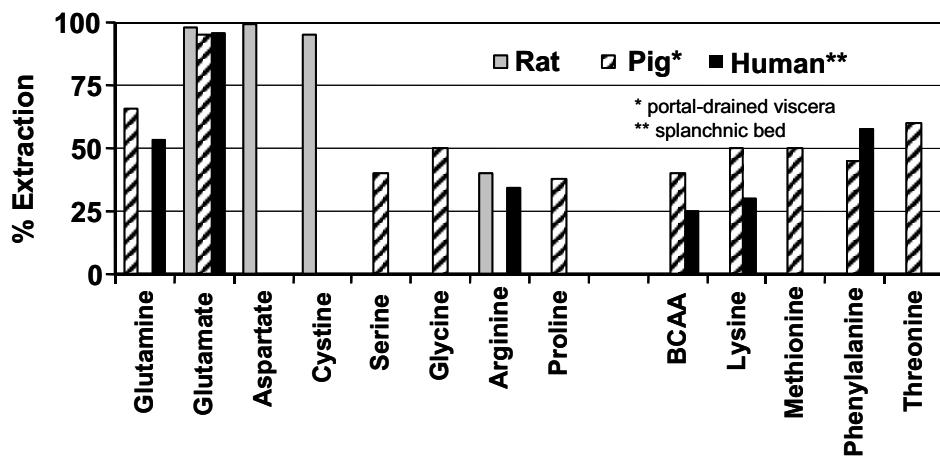


Figure 2. Amino acid metabolism in the gut.

(Figure 2). Van der Schoor *et al.*, 2002, showed that in piglets over 24 hours, 52 % of the dietary protein intake appeared in the circulation, indicating that half of the dietary protein intake is extracted by the gut. In earlier studies it has been found that 50 and 60 % of dietary lysine and threonine, respectively, is removed by the portal-drained viscera (PDV) (Figure 3).

Furthermore, it has been shown in piglets that the dietary protein intake level influences the lysine metabolism in the PDV (Van Goudoever *et al.*, 2000). In low protein-fed pigs, the use of lysine by the PDV accounted for more than 75% of its intake. In contrast to high protein feeding, both dietary and arterial lysine were used by the PDV of low protein-fed pigs in nearly equal amounts. Intestinal lysine oxidation was suppressed completely (Van Goudoever *et al.*, 2000). Thus the PDV is a key organ with respect to amino acid metabolism and the intestines use a disproportionately large amount of the dietary supply of amino acids during protein restriction. We have recently demonstrated in adult minipigs that the contribution of microbially synthesized lysine to lysine homeostasis was lower with a low lysine feeding while microbial lysine incorporation in splanchnic tissue was increased (Backes *et al.*, 2002). Therefore we concluded that minipigs do not adapt to a low lysine diet by an enhanced absorption of microbial lysine to extra-splanchnic tissues, presumably because the microbial lysine continues to be used for splanchnic protein synthesis. This implies that microbial lysine supports gut protein synthesis, particularly when the dietary lysine intake is low (Backes *et al.*, 2002). In conclusion, the intestine's enormous capacity for absorptive, synthetic and secretory functions influences the whole-body requirements and the systemic availability for specific amino acids.



*Figure 3. Extraction (%) of dietary amino acids by the small intestine (after data from Windmueller & Spaeth, 1975, 1976; Battezzatti *et al.*, 1995; Prior 1993; Reeds *et al.*, 1996, 1997; Stoll *et al.*, 1997, 1998; Metges *et al.*, 1999a; Castillo *et al.*, 1993; Biolo *et al.*, 1992; Hankard *et al.*, 1995). References are available from the authors.*

Host-bacterial relationships in the gut

In recent years we have gained a much greater insight into how the indigenous gastrointestinal microbial population interacts with the host's enterocytes (Hooper *et al.*, 2001). Enterocytes and microbial organisms inhabiting the GIT live in a subtle balance. Microflora competes directly with the host tissues for dietary nutrients and bacteria can shape the mucosal architecture (Kleesen *et al.*, 2003). In turn, intestinal enterocytes secrete a variety of molecules, called defensins, to protect themselves against microbes (Pütsep *et al.*, 2000). That commensals do have a role in the regulation of the ecological balance in the gut and that they are involved in innate immunity was

recently observed (Hooper *et al.*, 2003). It was shown that a previously uncharacterized angiogenin, Ang4, produced by mouse Paneth cells, and secreted into the gut lumen, has bactericidal activity against intestinal microbes. Ang4 expression is induced by *Bacteroides thetaiotaomicron*, a predominant member of the gut microflora. This suggests that intestinal commensal bacteria influence gut microbial ecology and shape innate immunity (Hooper *et al.*, 2003).

This example shows that the use of molecular tools in exploring gut microbiology and host responses will give us exciting new insights in a so far largely unknown ecological system and its potential benefits for the monogastric host.

Relative importance of the microbial amino acids to meet amino acid requirement

To quantify the net contribution of microbial amino acids to meet the metabolic demand for IAA would require that the material utilized to produce microbial IAA be otherwise lost or of no further value for the body. Hence, to the extent that the growth of the microbes that give rise to the labeled lysine is supported by the degradation of endogenous protein, the appearance of microbially derived lysine in plasma can be seen as part of the normal mechanism by which endogenous nitrogen and amino acids are recycled rather than microbial amino acids serving as a net source of amino acids, which are supplemental to those supplied in the diet (Metges *et al.* 1999a). Further, it is possible that microbial organisms adherent to the gut epithelium or resident indigenous bacteria in the crypts of the intestine utilize intestinal secretions (whether plasma-derived amino acids, endogenous proteins or urea) and generate and release amino acids in the surrounding mucus gel. Absorption from this pool of amino acids by the host tissue would occur without ever mixing with the intestinal luminal amino acids. Thus, by sampling GIT luminal contents this source of microbial amino acids will escape measurement because the ¹⁵N enrichment of lysine being absorbed from the gastrointestinal tract would not be accurately reflected by either the ileal or fecal microbial protein-bound lysine. Thus, to derive net microbial lysine contribution to host metabolic lysine requirement is complicated by the very intense nitrogen and amino acid recycling of the intestine. However, the use of a carbon labeled (¹⁴C) poorly digestible dietary carbohydrate and the ¹⁴C found in indispensable amino acids (i.e. isoleucine, leucine, valine, phenylalanine, lysine) in the host carcass supports the view that microbial amino acids represent a net contribution to meeting metabolic amino acid requirements. Finally, as Fuller and his group recently put it, the issue of the microbial amino acids does not invalidate the recommended amino acid requirement values. It shows, however, that the amino acid requirement has to be considered as metabolic rather than dietary requirement.

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Withdrawal of anti microbial growth promoters from pig diets increases the amino acid requirements for growth performance

P. Bikker & A. Dirkzwager

Institute for Animal Nutrition "De Schothorst", PO Box 533, 8200 AM Lelystad, The Netherlands

Summary

Two experiments were conducted to determine possible interactions between the use of an anti microbial growth promoter (AMGP) and amino acid level in the diet of growing pigs from 40-110 kg body weight. The first experiment indicated that an increase in amino acid content had more effect in a diet without AMGP. The second experiment indicated a quadratic response of daily gain and FCR to incremental levels of amino acids in diets with and without growth promoter. Estimated amino acid requirements were approximately 5% higher in diets without AMGP.

Keywords: *pigs, anti microbial growth promoter, amino acids*

Introduction

During the last decades diets for growing pigs have been fortified with antibiotics in prophylactic doses to reduce the risk of gastro intestinal disorders and improve growth performance of the animals. In a few years time this prophylactic use of AMGP in pig diets will be banned in Europe and probably in other areas thereafter. Several authors (e.g. Dierick *et al.*, 1986a,b) reported that the inclusion of AMGP in the diet of pigs improved the ileal digestibility and decreased the microbial degradation of amino acids in the digestive tract. This implies that withdrawal of AMGP may reduce the availability of dietary amino acids for protein deposition. Therefore we hypothesized that amino acid requirements are higher in pigs fed diets without AMGP. Two experiments were conducted to test the hypothesis that the withdrawal of AMGP reduces amino acid utilization for body gain and increases the amino acid requirements of growing pigs.

Material and methods

Two experiments were conducted, each with 288 group housed pigs. Pens with six pigs, castrates and females mixed, were the experimental unit. The pigs were grouped on the basis of sex and body weight and entered the pens at a body weight of 25 kg. All pigs received the same grower diet during a three week period. The pigs had free access to feed and water in a wet and dry feeder. The experimental diets were fed from 40 to 110 kg body weight. Pigs were then slaughtered and carcass lean percentage was determined by an optical probe (HGP).

The first experiment was conducted to determine a possible interaction between amino acid level and the inclusion of AMGP in the diet. This experiment comprised four treatments in a 2x2 factorial design with AMGP (0 and 30 ppm salinomycine) and dietary amino acid level (90 and 100% of the estimated requirements) as respective factors. The ileal digestible lysine content was 6.3 and 7.0 g/kg of diet respectively, the other essential amino acids were included in a constant ratio to lysine. The amino acid content was increased by an exchange of soybean meal versus manioc.

In the second experiment the effect of AMGP on the amino acid requirements was determined. The experiment comprised four treatments in a 2x4 factorial design with AMGP (0 or 30 ppm salinomycine) and amino acid level as respective factors. The amino acid content was increased

in four equidistant steps, 4.5, 5.5, 6.5 and 7.5 g of ileal digestible lysine/kg of diet. Other essential amino acids were increased according to a constant ratio to lysine. The amino acid content was increased by an exchange of soybean meal and maize gluten meal versus manioc.

Results

Experiment 1

The higher amino acid level and the inclusion of salinomycine as AMGP increased daily gain and reduced the feed conversion ratio as presented in Table 1. In the diets with and without AMGP the higher amino acid content increased daily gain by approximately 10 and 40 g/d respectively and improved the FCR by 0.06 and 0.11 respectively. Consequently, an increase in dietary amino acid content had more benefit in diets without AMGP. For daily gain, this interaction was significant. The effect of salinomycine on daily gain was bigger in the diet with the low amino acid level (44 versus 16 g/d, interaction, P<0.05). These results may indicate a positive effect of the AMGP on amino acid utilization and increased amino acid requirements in diets without AMGP. Faecal consistency was improved by inclusion of AMGP in the diet, whereas dietary amino acid content did not have any significant effect faecal consistency.

Table 1. Influence of amino acid level and AMGP in the diet on growth performance from 40-110 kg body weight in Experiment 1.

| Salinomycine, ppm | 0 | | 30 | | Effects ¹ | | | |
|---------------------------------|-------------------|------|------|------|----------------------|----|------|---------|
| | Dig. lysine, g/kg | 6.3 | 7.0 | 6.3 | 7.0 | AA | AMGP | AAxAMGP |
| Feed intake, g/d | | 2.59 | 2.60 | 2.60 | 2.60 | ns | ns | ns |
| Body gain, g/d | | 887 | 924 | 931 | 940 | ** | ** | * |
| FCR | | 2.96 | 2.85 | 2.83 | 2.77 | ** | ** | ns |
| Faecal consistency ² | | 5.4 | 5.4 | 5.8 | 5.8 | ns | ** | ns |

¹ Statistical significance of effects, * P<0.05, ** P<0.01.

² Registered on a scale from 1 (liquid diarrhoea) to 10 (hard and dry faeces) in the first five weeks of the experiment.

Experiment 2

The results of the main effects of AMGP and amino acid content in the diet are presented in Table 2. The daily gain increased quadratically and the feed conversion decreased quadratically with incremental levels of amino acids in the diet. Maximum performance was reached at the highest lysine level in the weight range from 40-70 kg and at a level of 6.5 g/kg in the weight range from 70-110 and 40-110 kg. The exclusion of AMGP from the diet significantly reduced the daily gain and increased FCR. This effect was mainly present in the weight range from 40-70 kg and smaller in the period from 70-110 kg. Lean percentage linearly increased with incremental levels of lysine without significant effect of AMGP in the diet.

In Figure 1 the quadratic response curve for daily gain and FCR is presented for diets with and without AMGP. In diets with AMGP daily gain increased quadratically and FCR decreased quadratically with increasing amino acid content, to a level of 6.5 g lysine/kg diet. No effect was found of a further increase in lysine content above 6.5 g/kg. In diets without AMGP however, a further increase of digestible lysine to 7.5 g/kg of diet improved both daily gain and FCR. At the highest level growth performance was similar for the treatments with and without AMGP.

Table 2. Main effects of amino acid level and AMGP in the diet on growth performance from 40-110 kg body weight in Experiment 2.

| | Digestible lysine, g/kg | | | | AMGP | | Effects ¹ | |
|------------------|-------------------------|------|------|------|------|------|----------------------|------|
| | 4.5 | 5.5 | 6.5 | 7.5 | yes | no | AA | AMGP |
| 40-70 kg | | | | | | | | |
| Body gain, g/d | 743 | 818 | 870 | 892 | 844 | 818 | L**Q** | ** |
| FCR | 2.97 | 2.72 | 2.53 | 2.44 | 2.61 | 2.72 | L**Q** | ** |
| 70-110 kg | | | | | | | | |
| Body gain, g/d | 885 | 942 | 970 | 960 | 944 | 935 | L**Q** | ns |
| FCR | 3.17 | 2.95 | 2.88 | 2.89 | 2.96 | 2.99 | L**Q** | ns |
| 40-110 kg | | | | | | | | |
| Feed intake, g/d | 2.51 | 2.49 | 2.50 | 2.49 | 2.49 | 2.50 | ns | ns |
| Body gain, g/d | 827 | 889 | 929 | 930 | 901 | 887 | L**Q** | ** |
| FCR | 3.09 | 2.85 | 2.72 | 2.70 | 2.81 | 2.87 | L**Q** | ** |
| HGP Lean% | 54.1 | 55.1 | 56.2 | 56.1 | 55.2 | 55.6 | L** | ns |

¹ Statistical significance of effects, L linear and Q quadratic effect, * P<0.05, ** P<0.01.

Exclusion of AMGP from the diet reduced the mean daily gain by 16 g/d and increased the FCR by 0.06 (P<0.01), but at the highest amino acid level, the AMGP effect had disappeared. These results indicate that an increase in dietary amino acid content can (partly) compensate for the loss in performance when the AMGP is excluded from the diet.

The results confirm that amino acid requirements of growing pigs are higher on diets free of AMGP. In order to quantify this effect a linear plateau-model and a quadratic model have been used to determine lysine requirements for maximum daily gain and minimum FCR. The results of the linear plateau model indicated a digestible lysine requirement of 6.0 g/kg with AMGP in the diet and a requirement of 6.3 g/kg in diets without AMGP. Using a quadratic model, requirements of ileal digestible lysine were estimated as 7.0 and 7.3 g/kg with and without AMGP. Both models indicated an increase of approximately 0.3 g/kg.

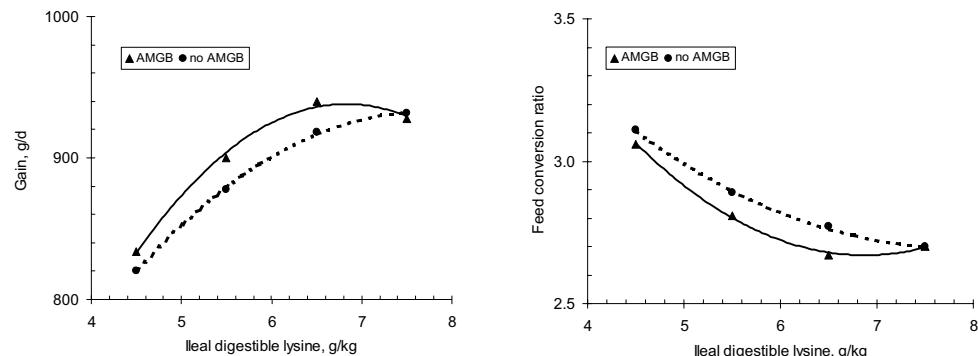


Figure 1. Interrelationships between AMGP and amino acids in Experiment 2.

Discussion

The results of this study indicate a less efficient utilisation of dietary amino acids when diets without AMGP are used. The exclusion of AMGP increased the estimated amino acid requirements by approximately 5%. Several reasons may explain this difference caused by the use of AMGP. Dierick et al. (1986a,b) reported an increase in ileal digestibility of most amino acids of 0-5 percentage units with inclusion of virginiamycin as AMGP in the diet. Furthermore these authors found a higher absorption of amino acids from an isolated loop of the small intestine in conscious pigs, when virginiamycin was added to the perfusion solution. In vitro studies with ileal digesta revealed that the inclusion of virginiamycin or spiramycin in the digesta not only reduced the incorporation of amino acids in bacterial protein but also reduced bacterial degradation of amino acids to ammonia and biogenic amines. These results indicate that with AMGP in the diet a larger proportion of amino acids is absorbed from the small intestine and available for the pig. Visek (1978) reported that the mucosal wall of the digestive tract is thinner in animals fed diets with anti microbials, probably related to a lower stress of the immune system. This may allow better absorption of nutrients from the digestive tract. In addition this may indicate a lower utilisation of amino acids by the digestive tract itself and a higher net portal flux of amino acids. This would increase the amount of amino acids available for lean tissue deposition. This hypothesis is supported by results of Roth et al. (1999) who measured protein turnover in pigs fed diets with and without avilamycin as AMGP. They reported a decrease in both protein synthesis and degradation with avilamycin in the diet and suggested that this might have been due to reduced metabolic activity of intestinal tissues. Protein retention and protein retention/protein synthesis increased with avilamycin, probably because less amino acids were used by the GI tract and more amino acids were available for whole-body protein deposition.

Conclusions

This study indicates that the exclusion of AMGP from the diet increases the dietary amino acid level required for maximum growth performance. Present amino acid recommendations for growing pigs are generally based on experiments in which AMGP were included in the diet. Therefore they may underestimate the optimal amino acid levels in diets free of AMGP.

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Effect of ensiling on the ruminal degradability and intestinal digestibility of the corn forage*

J. González¹, C.A. Rodríguez¹, J. Faría-Mármol², M.R. Alvir¹ & A. Martínez³

¹ Universidad Politécnica de Madrid, Dpto. Producción Animal, 28040 Madrid, España

² Dpto. de Zootecnia. Facultad Agronomía. Universidad de Zulia. Maracaibo, A.C. 15205, Venezuela

³ Centro de Investigación Aplicada y Tecnología Agroalimentaria. 33300 Villaviciosa, España

Summary

The effective degradability (ED) and the intestinal digestibility (ID) of dry matter (DM) and crude protein (CP) of a green corn crop (GC) and its silage (CS) were determined using *in situ* and particle transit techniques on three weaners cannulated in rumen and duodenum. Two rumen incubations with duplicates of bags at times of 0, 2, 4, 8, 16, 24, 48 and 72 h were performed for each feed, under conditions of continuous infusion in the rumen of a salt of ^{15}N . For each incubation, one bag was oven dried (80°C , 48h) to determine rumen degradation and the other bag was freeze-dried. These last samples were pooled for each rumen incubation time and 2 sub-samples were incubated into mobile nylon bags through the intestine of each animal. To determine the effective ID (EID), the above-pooled residues were composited in accordance with the rumen outflow and the kinetic of DM degradation to simulate the rumen flow. Then 4 sub-samples per animal were essayed by the same procedure. The ED values (%) obtained for GC and CS were respectively 53.1 and 57.7 (M.S.E.=0.63; P<0.05) for DM and 71.7 and 68.1 (M.S.E.=0.89; P=0.1) for CP. The evolution in the time of the colonization and the contamination fitted well to exponential equations, with higher asymptotic values for CS. The ID values for GC decreased with the time of ruminal pre-incubation for DM (P<0.001) as well as for CP (P=0.057). For CS this effect was only observed on the DM (P<0.001), although an increase was detected at 72h. The evolution of ID for CP was irregular. The silage reduced the EID of DM (17.8 vs. 14.2%; M.S.E.=0.56; P<0.05) as well as of CP (66.7 vs. 52.4%; M.S.E.=1.07; P<0.05).

Keywords: *effect of ensiling, corn forage, digestive availability*

Introduction

Usually, it is supposed that silage increases the ruminal degradability of forage crude protein (CP). However, previous works of this team also showed a slower degradation of the insoluble protein, which was imputed to a denaturalisation of proteins as a consequence of the acid accumulation in the silage. The modifications of the degradation can also affect the digestion site and, therefore, the intestinal digestibility (ID). The aims of this work were to establish the effects of silage on the effective degradability (ED) and the ID of a corn forage.

Material and methods

A green corn crop (GC) and its corresponding silage (CS) were studied in this work. These samples were liofilized after its obtention and grounded through sieves of 2 or 1 mm respectively for *in situ* studies and chemical analysis (Table 1).

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The ED and the ID of the dry matter (DM) and CP were determined on three wethers cannulated in rumen and duodenum, fed, at 40g DM/kg W^{0.75}, with a diet composed by the same quantities (DM basis) of ray-grass silage, corn silage and concentrate. Feed samples (3g) were incubated in nylon bags (46µm of pore size) in the rumen of each animal during 0, 2, 4, 8, 16, 24, 48 and 72h. Two incubations with duplicates of bags were performed for each feed. These studies were carried out under conditions of continuous infusion in the rumen of a salt of ¹⁵N to determine the microbial contamination of incubated residues, as indicated by Rodriguez et al. (2000), using bacteria isolated from the rumen particulate phase as reference sample. After the incubation, bags were stored frozen and then machine washed (3 times for 5 min). For each incubation, one bag was oven dried (80°C, 48h) to determine rumen degradation and the other bag was freeze-dried. Ruminal disappearance of DM or CP were fitted for each animal to the exponential model of Ørskov and McDonald (1979), except the CP disappearance from CS, which showed a sigmoid trend and was fitted to the model described by Van Milgen and Baumont (1995). The ED was established using the rumen outflow rate (k_p) determined in this experiment for the CS labelled with Yb by immersion as described by González et al (2003). Then, a pulse dose (40 g) was fed to each animal immediately before the first daily meal and a total of 20 samples were obtained through the duodenal cannulae, between 1 and 82h afterwards. The pattern of Yb concentrations in the duodenal digesta over time was described for each animal by fitting to the model of Dhanoa et al. [8] and rate constants derived from the decreasing phase of concentrations were used as k_p values.

The freeze-dried residues were pooled for each rumen incubation time and 2 sub-samples (0.2g) of these samples were incubated into mobile nylon bags of the same material through the intestine of each animal. To determine the effective ID (EID) the above-pooled residues were composited in accordance with the rumen outflow and the kinetic of DM degradation to simulate the rumen flow as indicated by González et al (2003). These samples were essayed by the same procedure using 4 sub-samples per animal.

Results and discussion

As GC was ensiled with a high DM content (39.3%), the reduction of the nutritive quality by ensiling was low, as showed by the small increase of minerals and fibre fractions (Table 1). However, there was a reduction of the CP content (from 96.0 to 68.7 g/kg DM), probably caused by ammonia volatilisation after the opening of the silage and/or with the dry-freezing.

Table 1. Chemical composition (g /kg dry matter) of forage samples.

| Sample | DM (%) | OM | CP | NDF | ADF | ADL |
|--------|--------|-----|------|-----|-----|------|
| GC | 39.3 | 963 | 95.0 | 436 | 215 | 14.5 |
| CS | 36.5 | 943 | 68.7 | 441 | 237 | 28.8 |

GC: green corn; CS: corn silage; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

Ensiling increased the ruminal availability of DM as a consequence of the enlargement of the soluble fraction (*a*) and a reduction of the insoluble but potentially degradable (*b*) fraction (Table 2). However, a decrease of the degradation rate was also observed as tendency, which suggests a higher resistance of insoluble materials to the microbial actions. The net result of these changes was an increase of the ED from 53.1 to 57.7%. Effects for these same fractions in CP degradation showed the same trend, although a tendency to a higher ($P = 0.082$) undegradable fraction (*u*) was also observed (Table 2), mainly as a consequence of the higher microbial N contamination (Fig.

1b). The sigmoid evolution of CP degradation in CS also suggests a slower degradation, as a consequence of the protein resistance. Therefore, the fermentative process that take place in the silo and lead to an increase of the soluble CP are balanced by a lower degradation of the insoluble protein. Estimates of ED were calculated for a fractional rumen outflow rate of 5.60 ± 1.20 (mean \pm standard deviation).

The evolution in the time of the colonization and contamination fitted well to exponential equations (fig. 1a and 1b). These values were similar in both feeds, although a trend to higher asymptotic values was showed for CS. The high values recorded for microbial nitrogen contamination showed the interest to correct this contamination to obtain reliable estimates of ED of CP in forages.

The values of ID for GC decreased with the ruminal pre-incubation time (RPT) for DM ($P < 0.001$) as well as for CP ($P = 0.057$). For CS this effect was only observed on the DM ($P < 0.001$), although an increase was detected at 72h. The evolution of ID for CP was irregular with an apparent decrease until 8h and later increases. The decrease of ID with RPT showed the progressive enrichment of forage particles in fibre or nitrogenous compounds which are unavailable in the gut with the extent of rumen degradation. The irregular behaviour observed for CP in CS may be due to the higher microbial colonization and nitrogen contamination of the CS particles (fig. 1a and 1b).

Table 2. Effect of silage on rumen degradation.

| | a (%) | b (%) | u (%) | k _d (%/h) | DE (%) |
|----------------------|-------|-------|-------|----------------------|--------|
| Dry matter | | | | | |
| GC | 29.5 | 55.2 | 15.3 | 4.08 | 53.1 |
| CS | 41.1 | 47.1 | 11.8 | 3.08 | 57.7 |
| M.S.E. | 0.47 | 1.14 | 1.02 | 0.23 | 0.62 |
| P | 0.003 | 0.038 | 0.133 | 0.089 | 0.035 |
| Crude protein | | | | | |
| CG | 57.7 | 28.4 | 13.9 | 5.77 | 71.7 |
| CS ¹ | 62.8 | 16.6 | 20.6 | - | 68.1 |
| M.S.E. | 0.19 | 1.60 | 1.46 | | 0.89 |
| P | 0.003 | 0.034 | 0.082 | | 0.100 |

a, b and r represent soluble, non-soluble degradable and undegradable fractions, respectively; k_d: fractional degradation rate of fraction b. For other abbreviations see Table 1.

¹Mean values of k₀ and k_∞ from the model of Van Milgen and Baumont (1995) were 0.99 and 10.7%/h, respectively.

Table 3. Effect of rumen pre-incubation time (RPT) on intestinal digestibility of forage samples.

| RPT (h): | 0 | 2 | 4 | 8 | 16 | 24 | 48 | 72 | M.S.E. | P |
|----------------------|-------------------|-------------------|-------------------|--------------------|--------------------|-------------------|-------------------|-------------------|--------|--------|
| Dry matter | | | | | | | | | | |
| GC | 36.5 ^a | 33.3 ^a | 24.2 ^b | 20.5 ^{bc} | 21.3 ^{bc} | 17.5 ^c | 11.1 ^d | 11.6 ^d | 1.33 | <0.001 |
| CS | 31.7 ^a | 16.1 ^b | 16.9 ^b | 14.5 ^{bc} | 11.1 ^{cd} | 9.9 ^d | 5.9 ^e | 9.9 ^d | 1.16 | <0.001 |
| Crude protein | | | | | | | | | | |
| GC | 58.7 | 60.0 | 54.1 | 49.4 | 51.7 | 49.8 | 48.3 | 44.5 | 3.24 | 0.057 |
| CS | 56.1 | 52.9 | 51.2 | 43.4 | 47.6 | 57.8 | 42.2 | 50.5 | 4.46 | 0.224 |

a, b, c, d, eValues in the same line with different superscript are different at $P < 0.05$.

For abbreviations see Table 1.

Results from samples composited based in the rumen flow showed that silage reduced the EID as for DM (17.8 vs. 14.2%; M.S.E.=0.56; P<0.05) as for CP (66.7 vs. 52.4%; M.S.E.=1.07; P<0.05). However, both last values were higher than those observed for the different incubation times. For DM, the reduction produced by ensiling may be logically associated with the increase in the concentration of fibre and lignin derived from silage fermentation. The global results recorded for CP presented some uncertainty on these values.

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Efficiency of utilization of ruminal ammonia N for milk protein synthesis in dairy cows

A.N. Hristov¹, J.K. Ropp¹, K.L. Grandeen¹, R.P. Etter¹, A. Foley¹, A. Melgar¹ & W. Price²

¹ Department of Animal and Veterinary Scienc, University of Idaho, Moscow, ID 83844, U.S.A.

² Statistical Programs, College of Agricultural and Life Sciences, University of Idaho, Moscow, ID 83844, U.S.A.

Summary

Nitrogen-15 was used to trace ruminal ammonia-N transfer into milk protein. Data from five metabolism experiments were summarized to determine the factors most responsible for the variation in the efficiency of utilization of ammonia N for milk protein synthesis. Structural analysis of the data indicated milk protein yield and the proportion of milk protein N derived from ruminal bacterial N were important variables determining the recovery of ¹⁵N-ammonia tracer in milk protein in dairy cows.

Keywords: *milk protein, ruminal ammonia, dairy cows*

Introduction

In a meta-analysis involving 256 feeding trials with Holstein dairy cows and 846 diets, average efficiency of utilization of dietary N for milk protein synthesis (NE) was $24.7 \pm 0.14\%$ (A. N. Hristov, W. J. Price, and B. Shafii, unpublished) and varied significantly between trials (from 13.7 to 40.8%). High NE was found more often with diets in which corn silage rather than alfalfa silage was the main forage ingredient, CP concentration was low, and cows were producing higher milk yields. Ammonia N losses in the rumen are a major factor determining NE (Tammenga, 1992). If energy is limiting in the rumen, ammonia uptake by ruminal microorganisms is inhibited (Nocek and Russell, 1988). Microbial protein synthesized in the rumen supplies the majority of absorbable amino acids to the small intestine (NRC, 2001). Because rumen microbes form a large proportion of their cell protein from ammonia N (Hristov and Broderick, 1996), enhanced microbial protein synthesis in the rumen may result in a more efficient transfer of ruminal ammonia N into body and milk proteins.

The objective of this study was to summarize data from five metabolism experiments and investigate the relationships between experimentally measured variables and the efficiency of use of ruminal ammonia N for milk protein synthesis.

Materials and methods

Five metabolism experiments utilizing ruminally and duodenally cannulated lactating Holstein dairy cows (four to six per trial) were conducted to investigate the effects of various dietary treatments arranged in cross-over or Latin square designs. In Exp. 1, diets differed in carbohydrate (CHO) composition: diet RFSS (barley and molasses) contained a larger proportion of ruminally available CHO in the non-structural carbohydrate fractions and diet RFNDF (corn, beet pulp and brewers grains) contained a larger proportion of CHO in ruminally available fiber. Experiment 2 investigated the effect of sodium laurate (240 g/cow per day) on ruminal fermentation, nutrient digestibility, and milk yield and composition. The objective of Exp. 3 was to evaluate the effect of dietary CP level (18.3 vs 15.8%) and degradability on utilization of dietary N in lactating dairy cows. Experiment 4 studied the effect of pure CHO sources on utilization of ruminal ammonia N

in dairy cows. Cows were fed an all alfalfa hay basal diet supplemented with either corn dextrose (**GLU**), corn starch (**STA**), fiber (**NDF**, white oat fiber), or a CHO mix (25% of each: apple pectin, GLU, STA, and NDF) at 20% of dietary DMI. In Exp. 5, cows were fed diets, in which corn, normal barley, or high-amylopectin (waxy) barley were included at 40% of dietary DM. Sampling and experimental procedures were similar between experiments (Hristov and Ropp, 2003). In Exp. 1 and 2, ruminal ammonia N was labeled through continuous 3-day intraruminal infusion of 20 atom percent excess (**APE**) ($^{15}\text{NH}_4\text{}_2\text{SO}_4$). In Exp. 3, 4, and 5, ($^{15}\text{NH}_4\text{}_2\text{SO}_4$) was pulse-dosed as contents were taken out of the rumen and mixed with the tracer. Nitrogen-15 enrichments of ruminal ammonia and bacterial N and milk protein N were studied for up to 114 or 155 h, respectively. Cows were milked either 28 times in 155 hours (Exp. 1 and 2) or 22 times in 120 hours (Exp. 3, 4, and 5).

In all experiments, milk, ruminal ammonia, and bacterial ^{15}N -enrichment (APE) curves were plotted *vs* time and fitted to various non-linear models (SigmaPlot 5.0, SPSS Inc., Chicago, IL). In Exp. 1 and 2, ^{15}N -enrichment of ammonia N varied greatly during sampling and, as a result, regression models were not fitted to these data; consequently, proportions of bacterial and milk protein N originating from ammonia N were not calculated for Exp. 1 and 2. Areas under the predicted milk protein, ruminal ammonia (Exp. 3, 4, and 5 only) and bacterial ^{15}N curves (**AUC**, ^{15}N atom % excess \times h) were computed using the trapezoidal rule (SigmaPlot 5.0). Proportions of milk protein N originating from ruminal ammonia (Exp. 3, 4, and 5 only) or bacterial N and bacterial N originating from ruminal ammonia N (Exp. 3, 4, and 5 only) were derived based on the respective AUC (Nolan and Leng, 1974). In all five experiments, the cumulative amounts of ^{15}N excreted in milk protein (as percentage of the ^{15}N infused in the rumen of each individual cow, **Cum15N**) were fitted to a logistic model (Hristov and Ropp, 2003).

The relationships between the variables measured in Exp. 3, 4, and 5 (for which a complete dataset was available) and the response, Cum15N, were investigated using multiple linear regression. In some cases, the correlation among regressor variables was high (> 0.80). In these situations, only one of the affected variables was selected for modeling. In order to avoid further multicollinearity, Principle Component Analysis (**PCA**) was used to identify several subsets of independent regressors. Each of these models was fitted according to the mixed model approach of St. Pierre (2001). The best model was selected based on the size of the Bayesian Information Criterion (**BIC**) and the magnitude, trend, and pattern of the underlying residuals. Regression analyses were weighted by the inverse of the squared Cum15N standard error. All data were analyzed using SAS (SAS Inst. Inc., Cary, NC).

Results and discussion

The average amount of ^{15}N dosed into the rumen in Exp. 1 through 5 was: 5066, 4443, 429, 429, and 429 mg/cow, respectively. The differences between Exp. 1 and 2 and 3, 4, and 5, resulted in significantly different ^{15}N -enrichment of the N pools and the respective AUC (Table 1).

As proportion of the ^{15}N dosed intraruminally, the average cumulative recovery of the tracer in milk protein (Cum15N) varied from 6 (Exp. 4) to 11% (Exp. 1). With the exception of Exp. 1 (Hristov and Ropp, 2003), dietary treatments did not result in significant differences ($P > 0.05$) in Cum15N. Average proportions of milk protein originating from bacterial N ranged from 36 to 60%. The proportion of milk protein originating from ammonia N estimated based on the AUC was considerably larger than the proportion of tracer recovered in milk gravimetrically. Recalculation of the data of Petri and Pfeffer (1987) and Petri et al. (1988) showed that from 10.3 to 12.9-14.0% of the irreversible loss of ruminal ammonia N was recovered in milk protein via microbial protein in the lactating goat. In these latter studies, the proportions of milk protein derived from ruminal bacterial N were similar (32 to 49%) to the values estimated for lactating dairy cows in Exp. 1 through 5.

Table 1. Variables used in the study (mean(SE).

| Variable | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 | Exp. 5 |
|----------------------------------|------------------|------------|------------|------------|------------|
| DMI, kg/d | 22.8±1.07 | 23.8±0.53 | 23.6±0.47 | 22.1±0.27 | 20.6±0.66 |
| N intake, g/d | 706±34.4 | 749±16.6 | 645±23.0 | 811±10.9 | 547±18.9 |
| Rumen NH ₃ , mM | 11.0±0.50 | 11.9±0.37 | 9.8±0.53 | 11.7±0.87 | 7.0±0.39 |
| PH | 6.02±0.060 | 6.08±0.041 | 6.43±0.059 | 6.15±0.049 | 6.18±0.045 |
| VFA, mM | 127±3.2 | 125±2.2 | 80±1.5 | 136±2.7 | 132±1.9 |
| CumMY ¹ , kg | 198±18.3 | 206±13.4 | 124±5.1 | 91±5.7 | 130±12.4 |
| Milk protein, % | 3.29±0.051 | 2.87±0.060 | 3.09±0.051 | 3.15±0.071 | 3.20±0.077 |
| MNY ¹ , g/d | 145±8.0 | 131±7.1 | 112±5.6 | 92±5.2 | 102±8.0 |
| NE ¹ , % | 20.7±1.14 | 17.4±0.77 | 17.6±0.75 | 11.2±0.52 | 18.3±1.14 |
| UN ¹ , % | 36±1.1 | 40±1.8 | 44±2.8 | 40±1.9 | 33±2.6 |
| MUN ¹ , mg/dl | 16.8±1.34 | 22.9±1.50 | 14.5±1.10 | 19.5±0.86 | 12.7±0.59 |
| Cum15N ² , % | 11.1±1.15 | 7.7±0.52 | 10.9±0.49 | 6.0±0.43 | 8.8±0.59 |
| AUC MP ² | 8.7±0.33 | 6.5±0.18 | 1.0±0.02 | 0.7±0.82 | 1.1±0.02 |
| AUC Bac ² | 24.4±1.45 | 18.1±1.12 | 1.7±0.06 | 1.7±0.05 | 2.3±0.09 |
| AUC NH ₃ ² | N/A ³ | N/A | 4.0±0.20 | 3.7±0.23 | 4.0±0.24 |
| MPNB ² , % | 36.7±3.05 | 36.6±1.77 | 60.7±2.31 | 45.2±1.20 | 48.0±1.19 |
| BNAN ² , % | N/A | N/A | 42.5±1.04 | 48.5±2.69 | 58.3±2.16 |
| MPNA ² , % | N/A | N/A | 25.8±1.11 | 22.0±1.42 | 28.0±1.20 |

¹ CumMY - cumulative milk yield during milk sampling (see Materials and Methods); MNY - milk protein N yield; NE - nitrogen efficiency: milk N yield ÷ N intake; UN - urinary N excretion as % of N intake; MUN - milk urea N.

² ¹⁵N from (¹⁵NH₄)₂SO₄ excreted in milk protein as proportion of ¹⁵N dosed into the rumen; AUC MP - area under the milk protein ¹⁵N curve (APE × h); AUC Bac - area under the bacterial protein ¹⁵N curve (APE × h); AUC NH₃ - area under the ruminal ammonia ¹⁵N curve (APE × h); MPNB - milk protein N derived from ruminal bacterial N; BNAN - bacterial N derived from ruminal ammonia N; MPNA - milk protein N derived from ruminal ammonia N.

³ Not analyzed.

Overall (Exp. 1 through 5), Cum15N was highly correlated to milk and milk protein yields ($r = 0.74$ and 0.75 , respectively), nitrogen efficiency ($r = 0.93$), and N intake and MUN concentration ($r = -0.64$, and -0.80 , respectively). Correlations between Cum15N and ruminal ammonia concentration and urinary N excretion were comparatively low and negative ($r = -0.50$ and -0.42 , respectively). Cum15N correlated positively to DMI ($r = 0.62$). Nitrogen efficiency was positively correlated to milk and milk protein yields ($r = 0.82$ and 0.82). All correlations were significant at $P < 0.05$. Similarly, Wilkerson et al. (1997) reported proportionally lower NE with low-producing cows (<20 kg/d) than with high-producing cows (>20 kg/d milk): 22.0 vs 29.7%, respectively. The regression analysis of the data (Exp. 3, 4, and 5 only; $n = 39$) indicated milk protein yield ($P < 0.001$) and proportion of milk protein originating from bacterial N ($P < 0.05$) were important in predicting the proportion of ammonia-¹⁵N tracer recovery in milk protein. No random effects were indicated. Mean ((SE) predicted and observed Cum15N were: 8.0(0.31 and 8.3(0.42%, respectively. The coefficient of correlation between predicted and observed Cum15N was 0.74 ($P < 0.05$).

This analysis showed that milk yield, protein content of milk, milk protein yield, N intake, the efficiency of utilization of dietary N for milk protein synthesis, and the rate of transfer of ruminal

ammonia N into bacterial N are significant factors influencing the efficiency of utilization of ruminal ammonia N for milk protein synthesis in dairy cows.

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Estimates of ileal recovery of endogenous protein and amino acids in protein supplements for pigs by means of the homoarginine method

G.S. Huang¹, W.C. Sauer¹, G. Diebold² & R. Mosenthin²

¹ Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

² Institute of Animal Nutrition, University of Hohenheim, D-70593 Stuttgart, Germany

Summary

Six barrows, average initial body weight 18 kg, were fitted with a simple T-cannula at the distal ileum, and fed the three experimental diets for 10-d periods according to a repeated 3 x 3 Latin square design. The three diets were formulated to include 18% crude protein (CP). On d 9 of each 10-d period, the experimental diets containing 50% of the protein from the original soybean meal, rapeseed meal or peas and 50% from the respective guanidated feedstuffs with homoarginine were fed, and ileal digesta were collected continuously for 24 h. The ileal recoveries of endogenous CP and most AA from rapeseed meal and peas were higher ($P < 0.05$) than from soybean meal, but there were no differences ($P > 0.05$) between rapeseed meal and peas. The real ileal digestibility values of CP and most AA were higher ($P < 0.05$) in soybean meal and peas than in rapeseed meal, but there were no differences ($P > 0.05$) between soybean meal and peas. The factors responsible for the differences may be the components of fiber, especially lignin. The lignin contents in the three diets with soybean meal, rapeseed meal and peas were approximately 0.78, 11.92 and 0.93 %. Therefore, it can be concluded that lignin rather than cellulose or other cell wall components is responsible for the differences in real ileal digestibility values of crude protein and amino acids.

Keywords: pig, endogenous protein, ileal digestibility

Introduction

It is generally accepted that apparent ileal amino acid (AA) digestibility values provide a more accurate estimate for AA availability values in feedstuffs for pigs than fecal digestibilities. However, apparent digestibility values are biased by the output of endogenous crude protein (CP) and AA in ileal digesta. The endogenous recoveries can be divided into a basal and a specific fraction. The basal recovery of CP and AA is related to the dry matter intake only but independent of the type of feedstuff, whereas the specific recovery is related to the composition of the feedstuff or diet (e. g. presence of inherent factors such as lectins, trypsin inhibitors and tannins). There is evidence that total endogenous CP and AA losses in ileal digesta largely exceed basal endogenous losses (Nyachoti et al., 1997). Corrections of apparent ileal CP and AA digestibilities for both basal and specific losses would allow for the calculation of the real digestibilities according to Low (1982). Since the production of these losses requires additional energy and AA costs to the pig, it is important to distinguish between total endogenous and dietary nitrogen losses in ileal digesta of pigs fed different feedstuffs. The objective of this study was to compare total ileal recoveries of endogenous CP and AA in soybean meal, rapeseed meal and peas by means of the homoarginine method.

Materials and methods

Six PIC barrows, average initial body weight 18 kg, were surgically fitted with a simple T-cannula at the distal ileum.

Seven corn starch-based diets were formulated to contain 18 % CP (as fed) according to NRC (1998) standards. Three normal diets contained non-guanidinated soybean meal, rapeseed meal or peas as sole protein source. Another three diets were prepared by replacing 50 % of the protein source in each normal diet with the corresponding guanidinated feedstuffs. The experiment was carried out according to a 3 x 3 Latin square design. The pigs were fed the experimental diets (Table 1) twice daily, equal amounts each meal, at 0800 and 2000 h.

Table 1. Chemical composition (%) of the experimental diets¹.

| Items | Diets ² | | | | | |
|---------------------------|--------------------|-------|------|-------|------|------|
| | SBM | G-SBM | RSM | G-RSM | PS | G-PS |
| Crude protein | 20.2 | 20.4 | 20.0 | 20.0 | 19.2 | 19.5 |
| Crude fat | 3.9 | 3.9 | 6.5 | 6.0 | 4.2 | 4.2 |
| Neutral detergent fiber | 9.7 | 10.6 | 20.0 | 19.6 | 13.4 | 13.9 |
| Acid detergent fiber | 7.1 | 5.5 | 13.8 | 16.7 | 8.4 | 8.0 |
| Indispensable amino acids | | | | | | |
| Isoleucine | 0.94 | 0.81 | 0.76 | 0.71 | 0.79 | 0.76 |
| Lysine | 1.39 | 0.70 | 1.17 | 0.64 | 1.46 | 0.77 |
| Methionine/Cystine | 0.72 | 0.65 | 0.87 | 0.81 | 0.74 | 0.69 |
| Threonine | 0.93 | 0.81 | 0.97 | 0.91 | 0.79 | 0.75 |
| Valine | 0.98 | 0.85 | 0.98 | 0.92 | 0.86 | 0.84 |

¹ Dry matter basis

² SBM: soybean meal, RSM: rapeseed meal, PS: peas, G-SBM: guanidinated SBM, G-RSM: guanidinated RSM, and G-PS: guanidinated PS

Each experimental period lasted 10 d. From d 1 to 7 the barrows were fed the non-guanidinated diets. On d 8 and 10 of each experimental period, a diet containing hydrolysed casein was fed to separate digesta from the adapting diets with guanidinated feedstuffs (containing homoarginine). On d 9 of each experimental period, the diets containing the guanidinated feedstuffs were fed. Ileal digesta were collected continuously for 24 h starting immediately after the first diets containing guanidinated feed ingredients were offered.

Results and discussion

The recoveries of endogenous CP and selected AA in ileal digesta from the diets containing guanidinated soybean meal, rapeseed meal and peas are shown in Table 2. The endogenous CP and AA recoveries in ileal digesta were higher ($P<0.05$) in rapeseed meal and peas than in soybean meal, and there was no difference ($P>0.05$) between rapeseed meal and peas. There are many factors that determine the amounts of endogenous CP and AA recoveries such as the type and level of fiber and anti-nutritional factors. The levels of NDF and ADF were higher in the diets with guanidinated rapeseed meal and peas than in the diet with guanidinated soybean meal, namely 19.57 and 16.74, 13.87 and 7.96, and 10.57 and 5.45 %, respectively. The NDF comprises a water-insoluble heterogeneous mixture of structural materials, including cellulose, hemicellulose, and lignin. Lignin is especially higher in rapeseed meal than soybean meal and peas (Bach Knudsen, 2001). Meanwhile, the water-soluble fiber, pectin and β -glycoside-linked oligosaccharides are higher in peas (e.g. Savage and Deo, 1989). This possibly explains, in part, the higher endogenous recoveries for rapeseed meal and peas compared to soybean meal.

Table 2. Recoveries of endogenous protein and amino acids in ileal digesta¹.

| Diets | Soybean meal | Rapeseed meal | Peas | SEM |
|---------------|--------------------|--------------------|--------------------|------|
| Crude protein | 27.99 ^b | 46.46 ^a | 40.18 ^a | 3.14 |
| Lysine | 0.84 ^b | 1.40 ^a | 1.20 ^a | 0.09 |
| Threonine | 1.26 ^b | 2.09 ^a | 1.81 ^a | 0.14 |
| Isoleucine | 0.70 ^b | 1.16 ^a | 1.00 ^a | 0.08 |
| Valine | 0.98 ^b | 1.63 ^a | 1.41 ^a | 0.11 |

¹ g per kg dry matter intake.

a, b Means in the same row with different superscript letters differ ($P<0.05$).

The level and type of anti-nutritional factors in feedstuffs may also affect the recoveries of endogenous CP and AA. As was reviewed by Huisman and Jansman (1991), the Bowman-Birk protease inhibitors could irreversibly bind to trypsin and chymotrypsin in the intestine. The inactivation of trypsin and chymotrypsin stimulates further the secretion of these two enzymes in pancreatic juice mediated through CCK, thereby increasing endogenous protein and amino acid losses and decreasing protein digestion (Barth et al., 1993). Another type of anti-nutritional factors includes tannins in peas and rapeseed meal. Dietary tannins are polyphenolic substances and are able to increase endogenous CP and AA by several mechanisms, including easy formation of hydrogen bonds and hydrophobic interaction of their hydroxyl groups with the carbonyl groups of protein, such as secreted enzymes, dietary protein and protein of gastrointestinal mucosa. Most of the complexes with tannins are water-insoluble, decreasing the activity of enzymes, changing the morphological structure of the mucosa, decreasing trans-membrane AA and peptide uptake, and therefore contributing to higher endogenous protein secretions (Jansman, 1993). The combined effects of fiber and anti-nutritional factors, such as trypsin inhibitors (peas) and tannins (peas and rapeseed meal) would partially explain the higher endogenous CP and AA levels for rapeseed meal and peas compared to soybean meal, in which there were lower contents of fiber and anti-nutritional factors.

As shown in Table 3, the true ileal digestibility values of CP and AA were higher ($P<0.05$) in the diets containing soybean meal and peas than in the diet with rapeseed meal, but there was no significant difference ($P>0.05$) between the soybean meal and pea diet. These relationships were consistent with the fiber contents in the three diets, especially with respect to lignin contents. As reviewed by Bach Knudsen (2001), lignin contents in non-starch polysaccharides of soybean meal, rapeseed meal and peas were 7.4, 60.9 and 6.7%, respectively. Following this ratio of lignin in polysaccharides, the lignin contents in the three diets with soybean meal, rapeseed meal and peas were approximately 0.78, 11.92 and 0.93% (DM basis). Therefore, it seems likely that lignin, not cellulose, was responsible for the differences in true ileal digestibility values of CP and AA. As a major component of the cell wall it cements and anchors the cellulose microfibrils and other matrix polysaccharides and, it also stiffens the wall thus preventing biochemical degradation and physical damage of nutrients in the cell wall (Liyama et al., 1994).

In conclusion, this study shows that recoveries of endogenous CP and AA in ileal digesta differ from feedstuff to feedstuff, the recoveries were higher from rapeseed meal and peas than soybean meal, but there was no difference between rapeseed meal and peas. The reasons for the differences among the feedstuffs possibly were type and amounts of fibers and antinutritional factors, like trypsin inhibitors and tannins. However, the real ileal digestibility values of CP and AA were higher in soybean meal and peas than rapeseed meal, with no difference between soybean meal and peas. The factors responsible for the differences among the three feedstuffs may be lignin which is a component of the NDF fraction.

Table 3. Real ileal crude protein and amino acid digestibilities (%).

| Diet | Soybean meal | Rapeseed meal | Peas | SEM |
|---------------|-------------------|-------------------|-------------------|------|
| Crude protein | 97.0 ^a | 89.4 ^b | 96.4 ^a | 0.85 |
| Lysine | 96.0 ^a | 83.3 ^b | 96.4 ^a | 0.80 |
| Threonine | 99.6 ^a | 86.3 ^b | 99.5 ^a | 0.89 |
| Isoleucine | 98.7 ^a | 87.1 ^b | 97.1 ^a | 0.70 |
| Valine | 98.8 ^a | 85.3 ^b | 97.2 ^a | 0.74 |

a, b Means in the same row with different superscript letters differ ($P < 0.05$).

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Investigations on the suitability of the synchronisation index calculated from *in sacco* results for optimising microbial protein synthesis

T. Kaswari, P. Lebzien & G. Flachowsky

Institute for Animal Nutrition, Federal Agricultural Research Centre (FAL), Bundesallee 50, D-38116 Braunschweig, Germany

Summary

Eight lactating dairy cows fitted with rumen and duodenal cannulae were used in two *in vivo* trials. Energy and protein sources differing in rumen degradability were fed to the cows four times daily in three different sequences. In feeding sequence (FS)-A equal amounts of protein and energy sources were offered at each feeding time; FS-B: 1st and 3rd feeding time were offered only the energy sources, 2nd and 4th feeding time were offered only the protein sources; FS-C: Separate feeding of energy and protein sources as in "FS-B", but in different sequence. Degradation characteristics for each feedstuff were estimated by *in sacco* incubations and used for calculating the synchronization index for each treatment (ration and sequence) based on the optimal ratio between nitrogen (N) and organic matter (OM) release in the rumen for microbial protein synthesis. None of the parameters measured for both trials was significantly correlated to the synchronization indices. The highest synchronization index was found for FS-C in the 1st trial and for FS-B in the 2nd trial. However, the efficiency of microbial protein (MP) synthesis was higher for FS-B in both trials. The investigations demonstrate that synchronization index calculated on the basis of *in sacco* experiments was not a suitable tool for getting information about the efficiency of rumen fermentation.

Keywords: synchronisation index, *in sacco* degradation, microbial protein synthesis

Introduction

Synchronising the hourly supply of energy and N in the rumen has been suggested to improve the efficiency of microbial growth and to reduce N-losses. Different experiments (e.g. Herrera-Saldana et al. 1990; Newbold & Rust 1992; Henning et al. 1993; Kolver et al. 1998; Keller et al. 2002) have been performed to test this hypothesis with varying results. Degree of synchrony is mostly given by a synchronisation index (Sinclair et al. 1993), calculated from data on the extent and rate of ruminal nutrient degradation derived from *in sacco* measurements. The aim of the present experiment was therefore to study whether such an index shows a correlation to rumen fermentation parameters, nutrient degradation or duodenal flow of MP.

Material and methods

In sacco

Ruminal OM and protein degradability of feedstuffs were estimated on the basis of the disappearance of OM and N using the nylon bag technique as described by Ørskov et al. (1980). The feedstuffs chosen for incubation consisted of corn silage, grass silage, grass hay, wheat, pea, corn and soybean meal from the same batches as those used for the *in vivo* trials. Synchronization index for the diets and sequences used in the *in vivo* experiment was calculated based on the degradation characteristics of OM and protein of each feed component in the diets from the *in sacco* experiments using the following formula modified from Sinclair et al. (1993):

$$\text{Index} = \frac{25 - \sum_{1-6}^6 \sqrt{(25 - \text{released of N/OM per four hours})}}{25} \quad (1)$$

Where 25 was assumed as 25 g N/ kg OM truly digested in the rumen which is the optimal ratio recommended by Czerkawski (1986) for the optimal MP synthesis.

In vivo

Eight lactating dairy cows fitted with rumen and duodenal cannulae, were used in each of two trials (T). Diets containing (DM basis) 7 kg corn silage, 1.3 kg grass hay, 4.2 kg wheat and 2.5 kg pea (+ 150 g urea) in T1 ; 10.3 kg grass silage, 5.2 kg corn and 1.5 kg soybean meal (SBM) in T2 were given at four feeding times. Roughages in both trials were offered in equal parts twice daily. Concentrates were offered in 4 hours intervals according to the following design : FS-A: Equal amounts of protein and energy sources were offered at each feeding time; FS-B: 1st and 3rd feeding time were offered only the energy sources (wheat or corn), 2nd and 4th feeding time were offered only the protein sources (pea or SBM); FS-C: Separate feeding of energy and protein sources as in "FS-B", but in different sequence (Table 1.).

Table 1. Feeding regimen.

| Trial | Feeding time | | | | |
|-------|--------------|--------------|-------------|--------------|-------------|
| | | 05:30 | 09:30 | 13:30 | 17:30 |
| 1 | Roughage | Corn silage | Grass hay | Corn silage | Grass hay |
| | FS- A | Wheat + Pea | Wheat + Pea | Wheat + Pea | Wheat + Pea |
| | FS- B | Wheat | Pea | Wheat | Pea |
| 2 | FS- C | Pea | Wheat | Pea | Wheat |
| | Roughage | Grass silage | - | Grass silage | - |
| | FS- A | Corn + SBM | Corn + SBM | Corn + SBM | Corn + SBM |
| FS- B | Corn | SBM | Corn | SBM | |
| | FS- C | SBM | Corn | SBM | Corn |

Following 16 days of adaptation, duodenal fluid was sampled in 2 h intervals for 5 days. Rumen pH, ammonia, and short chain fatty acids (SCFA) were sampled and determined on d 9 and d 11 during adaptation period. Milk was sampled and analyzed during duodenal collection period. Chrome oxide mixed with flour was used as a marker for determination of nutrients flow in the duodenum. Dried duodenal fluids from each cow and period were pooled together for analyzing microbial protein in the duodenal fluid by NIRS according to Lebzien & Paul (1997).

Results and discussion

The ratio of N/OM release differed between feeding times and feeding sequences for both trials. The variation between ratios for the different time and sequences were higher in the first compared to the second trial. As a consequence the calculated synchronization indices were quite differed among feeding sequences in the first than in the second trial (Figure 1).

There was inconsistency of index among feeding sequences between T1 and T2 where the highest index in the 1st T was found on FS-C but the highest index in the 2nd T was found on FS-B. Interestingly, there was consistency among feeding sequences for both trials related to the MP

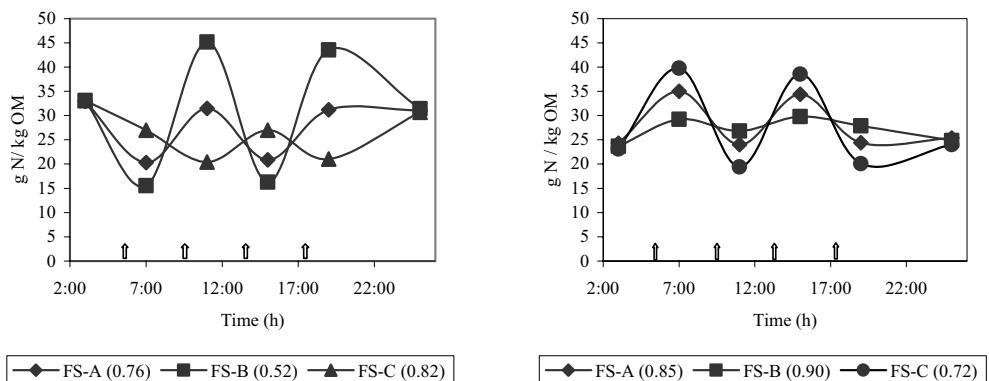


Figure 1. Relation between N and OM released in the rumen measured by in sacco at the different times of the three feeding sequences in trial 1 (left) and trial 2 (right). Arrows on the axis show feeding time and values in the brackets of legends show the synchronisation index.

synthesis and milk urea nitrogen (MUN) where the highest MP synthesis and the lowest MUN found on FS-B for both trials. The lowest MP synthesis and the highest MUN were also consistent for both trials and that found on FS-A (Table 2).

Synchronisation index of 1.0 means the perfect synchronisation of energy and protein in the rumen for MP synthesis. In the present experiments, only on FS-B of the 2nd T which had the highest synchronisation index (0.90) referred to the highest MP synthesis (2037 g/d ; 10.6 g/MJ ME). These results suggest that synchronisation index which counted only the balance between the energy and N in the rumen in period of time was not the sole factor affected MP synthesis in the rumen. Additionally, feed processing prior to incubation of nylon bags altered the characteristics of feed itself. Therefore the rate supply of energy and N calculated by the in sacco and used to derive the index could be different and did not reflect on the in vivo results. Results of Henning et al. (1993); Kolver et al. (1998); Casper et al. (1999) also suggest that synchronisation of availability of energy and N in the rumen produced minimal benefit to the ruminants.

Table 2. Effect of different feeding sequences and synchronisation indices on the rumen parameters, microbial protein synthesis and milk urea concentration .

| Parameters | Trial 1 | | | Trial 2 | | |
|--------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | FS-A (n=4) | FS-B (n=7) | FS-C (n=4) | FS-A (n=4) | FS-B (n=4) | FS-C (n=4) |
| Synchronisation index | 0.76 | 0.52 | 0.82 | 0.85 | 0.90 | 0.72 |
| Rumen Parameters | | | | | | |
| - SCFA (mmol/l) | 69 - 122 | 81 - 119 | 81 - 149 | 89 - 111 | 87 - 115 | 87 - 114 |
| - NH ₃ -N(mg/100ml) | 2 - 21 | 1 - 13 | 1 - 25 | 3 - 24 | 5 - 24 | 4 - 26 |
| Microbial Protein | | | | | | |
| - g/ day | 1515 | 1672 | 1576 | 1723 | 2037 | 1777 |
| - g/ MJ ME | 9.0 | 10.2 | 9.5 | 8.9 | 10.6 | 9.2 |
| Milk | | | | | | |
| - Urea (mg/100 ml) | 27.4 | 21.6 | 23.4 | 28.0 | 27.5 | 27.7 |

Conclusion

From this experiment it is concluded that synchronisation index calculated from the in sacco results was an inappropriate tool to estimate the efficiency of MP synthesis. Feeding sequence in which energy concentrates were given before protein sources resulted in the highest MP synthesis.

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The microbial community and its metabolic activities in the small intestine of weaning piglets

J. Kluess¹, A. Akkermans³, S. Konstantinov³, S. Kuhla¹, M. Kwella¹, V. Guiard² & W.B. Souffrant¹

¹ Research Institute for the Biology of Farm Animals, Department of Nutritional Physiology "Oskar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

² Research Institute for the Biology of Farm Animals, Department of Genetics and Biometry, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

³ Wageningen University, Laboratory of Microbiology, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands

Summary

To characterize ileal microbial flora in weaning piglets a slaughter trial was conducted. 384 German Landrace piglets of both genders were allocated to four different feeding regimes (with/without antibiotic; 3% or 8% crude fibre content). Pre- and postweaning piglets were sacrificed at defined times, the whole intestinal tract was removed and its content collected, for each section separately.

The microbial community was examined applying classical plate counting (selective agar plates) and molecular techniques (DGGE, 16S rDNA-sequencing, FISH). Furthermore a range of microbial metabolites was determined. Pre- and postweaning alterations in the microbial community of the small intestine - rather due to age than to diet - were observed.

Keywords: weaning piglets, intestinal microflora, microbial metabolites

Introduction

In commercial pig production weaning is probably one of the most dramatic incidents in a piglets life. To warrant a high operating efficiency in pig production, piglets have been weaned gradually earlier in the recent decades, so the common weaning age in Europe nowadays is between 21 and 28 days of life. As a consequence higher incidents of gastrointestinal (diarrhea etc.) and respiratory (pneumonia etc.) diseases occurred and are accompanied by stagnation or loss of body weight - a complex widely referred to as "post-weaning growth-check". To overcome these problems, antimicrobial feed additives have been commonly used in starter diets. As cross-resistances to therapeutically used antibiotics were discovered, these feed additives are now prohibited by an EU-ban. For that reason it became necessary to look for acceptable alternative strategies to alleviate weaning in commercial pig production. To evaluate potential alternatives and its properties, a profound knowledge of the porcine gastrointestinal tract (GIT) and its changes during this time is crucial.

Our investigations intend to characterize the resident microflora in the small intestine of weaning piglets as an important part of the piglet's GIT.

Material and methods

Animals and feeding regime

The experiment was conducted under constant conditions (26-28 °C ambient temperature, natural light system) in our experimental station with German Landrace piglets of both genders. Piglets were raised conventionally from the sow with sow milk (SM) as sole diet and no access to creep feed. Piglets were weaned at 28 days of age and allocated to group pens (free water access), each

containing 8 animals. They received 4 different starter diets *ad libitum*: 2 equal diets, with or without in-feed antibiotic avilamycin (+AB, -AB) and 2 equal diets based on cereals and leguminous seeds, with 3% fibre content (LF) or 8% fibre content (HF). Diets meet the requirements of weaning piglets as recommended by the NRC (1998).

Experimental design, sampling and analyses

For each of the dietary regimes mentioned above (SM, +AB, -AB, LF, HF) sixteen piglets were sacrificed at defined days: 6, 4, 2 days preweaning; weaning day; 1, 2, 5, 8 and 15 days postweaning. 384 piglets were used in total.

The whole GIT was removed from the abdominal cavity and the small intestine subdivided in three equals, which were defined as duodenum, jejunum, ileum (from proximal to distal). Entire content of each section was sampled and pooled for 4 animals each time.

Microbial metabolites lactic acid (LA) and volatile fatty acids (VFA) were determined by colorimetric procedure (Haacker *et al.*, 1983) and gaschromatography (Geissler *et al.*, 1976), respectively. For classical microbiology samples were serially diluted and *Enterobacteriaceae* and *Lactobacillus spp.* cultivated on selective agar plates (VRBD, MRS; SIFIN®, Berlin, Germany), according to the laboratory guidelines. *Enterobacteriaceae* were counted manually after 24 h aerobic and *Lactobacillus spp.* after 72 h anaerobic incubation at 37 °C.

For molecular investigations denaturing gradient gel electrophoresis (DGGE) and fluorescence-in-situ-hybridisation (FISH) were applied. DGGE was performed according to Muyzer *et al.* (1993) in 8% polyacrylamide gel using a 30-60% denaturing gradient. Gels were electrophoresed for 16 h at 85 V in 0.5 x TAE buffer at a constant temperature of 60 °C and subsequently stained according to Sanguinetti *et al.* (1994). For FISH we applied oligonucleotide probes EUB-0338 (Amann *et al.*, 1990), EC 1531 (Poulsen *et al.*, 1995), LAB-0158 (Harmsen *et al.*, 1999) and LAB-0722 (Sghir *et al.*, 1998).

Results

Animals

During the entire experiment animals remained healthy and in good condition, one pig died for unknown reason before slaughter. At slaughter days body weights (BW) didn't differ significantly between dietary regimes.

Microbial metabolites

Table 1 shows the content of LA and VFA. LA decreased until 1 day post-weaning and then increased gradually. There was no significant difference between the applied diets. Content of VFA in the small intestine was generally low and we didn't observe any significant influence of the used diets.

Microbiology

The numbers of cultivated bacteria are given in Table 2. We observed the highest colonization of *Lactobacillus spp.* in suckling piglets and a temporary decrease in its counts at weaning time. Their metabolite LA showed a complementary trend. Enterobacterial counts showed a slight increase until 5 days postweaning, but thereafter counts were reduced to the preweaning level. Except at day 1 postweaning we didn't observe any significant differences between diets in the investigated microbial groups. Similar observations about porcine intestinal *Enterobacteriaceae*

Table 1. Content of lactic acid and volatile fatty acids in ileal digesta of piglets pre- and postweaning, fed different diets; mean \pm SD

| Slaughter days | SM | +AB | -AB | LF | HF |
|-------------------------------------|----------------|-----------------|-----------------|-----------------|-----------------|
| Lactic acid (mmol/l) | | | | | |
| -6 | 21.1 \pm 9.4 | | | | |
| -4 | 24.7 \pm 3.0 | | | | |
| -2 | 21.2 \pm 5.2 | | | | |
| 0 | 8.2 \pm 2.7 | | | | |
| 1 | | 7.7 \pm 1.7 | 7.9 \pm 3.3 | 17.4 \pm 19.7 | 7.5 \pm 4.5 |
| 2 | | 16.0 \pm 14.2 | 42.3 \pm 20.4 | 32.2 \pm 18.5 | 30.3 \pm 31.4 |
| 5 | | 9.7 \pm 4.3 | 26.3 \pm 10.5 | 30.1 \pm 26.2 | 25.0 \pm 28.3 |
| 8 | | 11.3 \pm 9.0 | 12.1 \pm 9.8 | 33.3 \pm 26.6 | 53.7 \pm 37.1 |
| 15 | | 28.2 \pm 7.4 | 21.3 \pm 22.5 | 35.9 \pm 24.3 | 23.9 \pm 35.8 |
| Total volatile fatty acids (mmol/l) | | | | | |
| -6 | 12.3 \pm 7.1 | | | | |
| -4 | 9.2 \pm 3.5 | | | | |
| -2 | 13.0 \pm 1.5 | | | | |
| 0 | 8.3 \pm 0.6 | | | | |
| 1 | | 9.3 \pm 4.0 | 9.0 \pm 7.9 | 5.8 \pm 4.4 | 17.4 \pm 15.0 |
| 2 | | 3.7 \pm 3.5 | 7.5 \pm 3.2 | 3.5 \pm 7.0 | 6.8 \pm 3.5 |
| 5 | | 4.9 \pm 2.3 | 8.2 \pm 7.7 | 3.0 \pm 4.1 | 4.8 \pm 4.1 |
| 8 | | 6.8 \pm 4.4 | 4.2 \pm 2.2 | 6.2 \pm 4.6 | 2.1 \pm 2.5 |
| 15 | | 9.3 \pm 2.9 | 5.5 \pm 4.9 | 6.5 \pm 4.5 | 5.1 \pm 2.9 |

and *Lactobacillus spp.* were reported by various authors (Decuypere *et al.*, 1972; Kovacs *et al.*, 1972; Gedek *et al.*, 1993; Mathew *et al.*, 1994; Mikkelsen & Jensen, 1998; Franklin *et al.*, 2002). As a molecular approach we applied DGGE and FISH. The DGGE profile (Figure 1) of the investigated samples (ileal digesta, selection from entire sample set) showed 3 dominant bands in different positions (lower, middle, upper position). Bands in lower and upper positions were detected pre-and postweaning, whereas the middle band was only found postweaning. By 16S rDNA sequencing these bands were identified as uncultured bacterium-clones: the lower band represented uncultured clones p-3301-23G2, p-37-a5 and p-3443-SwA2 belonging to the cluster of *L. amylovorus* (Leser *et al.*, 2002). The middle dominant band was determined as S4D-clone belonging to *E. coli*, which was also found in feces of breast fed human infants (Favier *et al.*, 2002). Unfortunately our attempts to identify the upper band failed up to now.

Using FISH we quantified *Eubacteria*, *E. coli* and *Lactobacillus spp.* in representative samples from our sample set. There was a definite decline of general bacterial counts from 1.0×10^{11} bacteria/g 6 days preweaning to 1.8×10^9 bacteria/g and 1.9×10^9 bacteria/g 5 and 15 days postweaning, respectively. For *Lactobacillus spp.* we used the oligonucleotide probes LAB-0722 and LAB-0158. Despite identical accomplished hybridisation procedure only LAB-0722 was working in our samples. By means of this probe we estimated the number of *Lactobacillus spp.* as 97 % and 77 % of general bacteria 6 days preweaning and 5 days postweaning, respectively. We couldn't detect any *E. coli* before weaning, but already 1.5×10^9 bacteria/g and 9.4×10^8 bacteria/g 5 and 15 days postweaning, respectively.

Table 2. Microbial counts in ileal digesta of piglets pre- and postweaning, fed different diets; mean \pm SD.

| Slaughter days | SM | +AB | -AB | LF | HF |
|---------------------------------------|---------------|----------------------------|---------------|---------------|----------------------------|
| <i>Enterobacteriaceae</i> (log cfu/g) | | | | | |
| -6 | 6.8 \pm 0.8 | | | | |
| -4 | 7.2 \pm 1.0 | | | | |
| -2 | 7.4 \pm 0.4 | | | | |
| 0 | 7.4 \pm 1.4 | | | | |
| 1 | | 6.8 \pm 0.5 ^a | 7.7 \pm 0.7 | 7.5 \pm 0.5 | 8.4 \pm 0.6 ^b |
| 2 | | 7.2 \pm 0.8 | 6.7 \pm 1.0 | 6.8 \pm 1.2 | 8.5 \pm 0.4 |
| 5 | | 7.8 \pm 0.8 | 8.3 \pm 0.3 | 7.8 \pm 0.6 | 8.2 \pm 0.1 |
| 8 | | 7.7 \pm 0.3 | 7.8 \pm 0.3 | 8.2 \pm 1.3 | 7.9 \pm 0.4 |
| 15 | | 7.1 \pm 1.0 | 6.4 \pm 1.4 | 8.2 \pm 0.6 | 6.8 \pm 1.6 |
| <i>Lactobacillus spp.</i> (log cfu/g) | | | | | |
| -6 | 8.9 \pm 0.4 | | | | |
| -4 | 8.6 \pm 0.7 | | | | |
| -2 | 9.0 \pm 0.3 | | | | |
| 0 | 8.8 \pm 0.4 | | | | |
| 1 | | 6.7 \pm 1.0 | 7.8 \pm 0.6 | 7.6 \pm 0.9 | 6.9 \pm 1.3 |
| 2 | | 8.0 \pm 0.8 | 8.9 \pm 0.3 | 8.6 \pm 0.7 | 8.3 \pm 0.6 |
| 5 | | 8.1 \pm 0.5 | 8.7 \pm 0.3 | 8.5 \pm 0.5 | 8.1 \pm 0.7 |
| 8 | | 8.1 \pm 0.6 | 7.9 \pm 0.7 | 8.4 \pm 0.5 | 8.7 \pm 0.4 |
| 15 | | 8.7 \pm 0.2 | 8.0 \pm 1.2 | 8.5 \pm 0.7 | 7.6 \pm 1.5 |

^{a, b} Mean values with unlike subscripts are significantly different (Tukey-test, P<0.05)

Discussion

The aim of our investigation was the characterization of small intestines microflora of piglets during weaning transition and the possible influence of different diets (with/without antibiotics, low/high fibre content).

Our results show that under the previously described experimental conditions the microbial community - and its metabolites as well - in the GIT content of piglets during weaning appear to be quite stable. Dominating bacteria in the investigated time period were *Lactobacillus spp.*, which decreased during weaning process, but re-established to preweaning values soon after, independent from dietary regime. The majority of *Lactobacillus spp.* could be associated with the *L. amylovorus* cluster, as shown by techniques of molecular microbiology (DGGE, 16S rDNA-sequence analyses, FISH). Apparently this group is specific for the porcine GIT as it could be only detected in content of the small and large intestine of swine (and corn silage) up to now. In a recent study Leser *et al.* (2002) were able to identify this *L. amylovorus*-cluster in the GIT of Danish pigs differing in age and feeding regime.

Using FISH we discovered an interesting fact: despite identical hybridisation procedure LAB-0158 (human origin) didn't work in our samples, which indicates the importance of a porcine-specific *Lactobacillus*-group probe. In our study we were able to show that the application of the *Lactobacillus* group-specific oligonucleotide probe LAB-0722 (Sghir *et al.*, 1998) appears to be superior to LAB-0158 in investigating intestinal microecology of weaning piglets.

The *E. coli*-clone S4D could be detected only postweaning with DGGE and FISH, which is contradictory to our results in classical cultivation on agar plates, where *Enterobacteriaceae* were

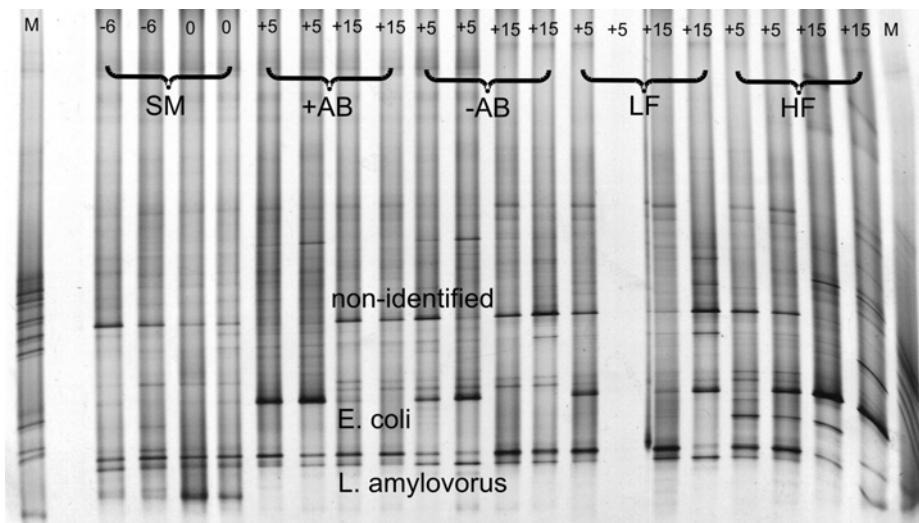


Figure 1. DGGE profile of ileal digesta of piglets pre- and postweaning, fed different diets.

detected already in suckling piglets. This contradiction between molecular and classical approach might be due to the fact, that the density of this bacterial group is lower than the detection limit of the applied molecular approaches ($< 10^6$ bacteria/g) before weaning, whereas with classical cultivation method colony-forming units not necessarily represent single cells but cells in a reproduction cycle (dynamic situation). Additionally this oligonucleotide probe is aiming *E. coli*, whereas on classical agar plates various bacteria belonging to the family *Enterobacteriaceae* are growing and therefore more colony-forming units can be enumerated.

Taking into account the various stressors that appear at this time in practice and are believed to be main inducers of postweaning growth-check - separation from sow and litter; environmental, social and dietary changes - we expected dramatic changes in the porcine GIT. Despite this our investigations indicate, that alterations in intestinal microflora during weaning transition are moderate.

Therefore we conclude that despite the stress occurring at weaning and its quoted impact on GIT-health and performance the porcine gastrointestinal tract has a great capacity to compensate and to adapt successfully to new challenges.

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Impact of grinding and heating of a wheat-pea blend on amino acids ileal digestibility and endogenous losses

L. Lahaye, P. Ganier, J.N. Thibault & B. Sève

INRA, Unité Mixte de Recherches sur le Veau et le Porc - 35590 Saint-Gilles, France

Summary

In order to assess the impacts of industrial-like heat treatment and grinding processes on a wheat-pea mixture, four pigs fitted with an ileorectal anastomosis were included in a 4 x 4 Latin square design. Four diets were compared involving two different particle sizes meals (200 and 500 µm) with or without heat treatment by steam (3 min. 85°C). The isotopic dilution method by labelling of the feedstuffs was used to assess the digestibility and the endogenous losses (EL). Ileal digestibility of energy was higher with the lower particle size but was not influenced by heat treatment. Real digestibility of nitrogen (N) and amino acids (AA) significantly increased ($P < 0.005$) with reduction of the particle size (76.6 to 85.3 for N, 75.7 to 87.3 for lysine and 76.6 to 85.7 for threonine for the 500 and the 200 µm particle size diets respectively). Heat treatment improved ($P < 0.05$) the real digestibility of all essential AA, but to a lesser extent than the decrease in particle size. Nevertheless, this improvement was more marked on the 500 µm than on the 200 µm diet. The decrease in particle size had no effects on N, lysine and threonine EL, but tended to decrease the EL for some other AA (phenylalanine, leucine, valine, glycine and alanine). Lysine was the only AA for which EL were reduced by heat treatment. Threonine was the limiting AA for nitrogen deposition. Both the decrease in particle size and the heat treatment significantly improved the daily nitrogen retention.

Keywords: feed manufacture, amino acids ileal digestibility, endogenous losses

Introduction

The raw material nutritional values are known, but data on nutritional values of the processed feedstuffs are scarce, especially for amino acids (AA) ileal digestibility and more particularly for the associated endogenous losses (EL). However, the technological treatments such as grinding, or extrusion affect the digestibility of the pig diets (Marty et al., 1994; Wondra et al., 1995). Therefore, for better evaluation of animal feeds, the measurement of diet manufacturing impacts upon the nutritive values is of great interest. In the European context, Pea (*Pisum sativum* L.) is the most relevant alternative to soybean meal in cereal-based diets. The technological treatments chose to be tested in this study are milling the first step in diet manufacture, and steam heating treatments, that are generally used to improve the safety or the flowing properties of the meals. In order to get precise values for nitrogen (N) and AA, digestibility measurements have been done at ileal level with distinction between the dietary and endogenous indigestible using the isotopic dilution method by labeling of the feedstuffs.

Materials and methods

Four growing Piétrain X (Landrace x Large-White) with an average body weight of 37.5 kg were fitted with an end-to-end ileo-rectal antevolvular anastomosis. They were fed according to a 4 x 4 Latin square with 4 diets based on a same wheat-pea blend supplemented with lysine, methionine and tryptophan. Diets differed by the technological processes involved for their manufacturing. Thus, we compared diets made of flours with two different particle sizes, 1 fine flour (200 µm)

and 1 coarse flour (500 µm) diet, and for each particle size diet, 1 crude (200c and 500c) and 1 steam heated diet at 85°C during 3 min (200SH and 500SH). Diets were offered at 08h00 and 15h30 in two equal portions mixed with water (1:2, w/v) at the rate of 80 g DM.(BW,kg)^{0.75.d⁻¹. N and AA apparent and standardised ileal digestibility (AID) and (SID) were determined with digesta collected for two days. Nitrogen and AA real digestibility (RID) and EL were determined by collecting digesta 8 hours after feeding a ¹⁵N and chromic oxide labelled diet as described by Hess et al. (1998).}

Results

Energy, N, and AA AID and SID were significantly increased ($P < 0.005$) with particle size reduction but were not influenced by heat treatment (Table 1). For all AA, the heat treatment resulted in higher AID and SID values. For the 500-µm particle size, this improvement was statistically significant at $P < 0.05$ for lysine and at $P < 0.1$ for phenylalanine, leucine, isoleucine. Nitrogen and AA RID significantly increased ($P < 0.005$) with the reduction of particle size (Table 2). The heat treatment improved significantly the RID for N and all essential AA when applied to 500-µm meal. The decrease in particle size had no significant effects ($P < 0.05$) on N, lysine and threonine endogenous losses, but tended to decrease the endogenous losses for some other AA (leucine, valine, phenylalanine, glycine and alanine). Lysine was the only AA for which endogenous losses were significantly reduced by heat treatment. There was a significant negative cumulative effect of particle size reduction and heat treatment on endogenous losses (500c vs. 200SH) for all AA except threonine and lysine. The decrease in particle size ($P < 0.005$) and the heat treatment ($P < 0.05$) independently improved daily N retention (Table 1).

Discussion

Due to slow transit rates of the diets and only partial recovery of the marker (Cr₂O₃) after feeding the ¹⁵N-labelled-diet, real digestibility as measured in this experiment may not reflect the whole digestion process. Nevertheless, it provides indications on the way technological treatments affect the first part of this process. The reduction of diet particle size improved E, N and AA digestibilities in general agreement with literature data (Wondra et al., 1995; Guillou and Landeau, 2000). This effect may be explained by the feedstuff fragmentation, increasing the surface area exposed to the enzymatic attacks and increasing the availability of nutrients (Kaysi and Melcion, 1992). Indeed, cereal and pea starch were previously shown to be much more sensitive to hydrolysis when particle size was decreased (Champ and Colonna, 1993). Our data showed that particle size reduction resulted mainly in reducing dietary protein losses as previously shown with wheat and sunflower (Lahaye et al., 2003). According to literature, decreasing the cereal particle size, from 1000 µm to 500 or 400 µm, results in higher N digestibility (Wünsche et al., 1987; Wondra et al., 1995). Nevertheless, the beneficial effect of reducing the particle size below 400 µm, appears to be low for cereals grains as suggested by our in vitro data (non reported) and data of Healy et al. (1994) on corn-based diets in piglets. Conversely, it was previously shown that micro-grinding of pea flour resulted in higher real digestibility without effects on endogenous losses (Hess et al., 1998).

Therefore the particle size effect should probably be mainly ascribed to the pea fraction. Due to the low anti-nutritional factors (ANF) content of the pea cultivar (Baccara), any heat beneficial effect by inactivation of the ANF seems unlikely. In addition to its denaturation effect on proteins, the steam-heat treatment could have partially disrupted pea seed cells. This could make their contents more accessible to digestive enzymes and act finally as a thermal “grinding” process. This could explain the minor thermal effect observed on the 200-µm particle size. Both the decrease in particle size and the heat treatment significantly improved pig daily nitrogen retention. Because our diets were limiting in threonine, the improvement in N retention was directly related to threonine

Table 1. Technological impacts on apparent ileal digestibility (%).

| Part. size, µm | 200c | 200SH | 500c | 500SH | SEM | Part. size | Heat | Part. size x Heat |
|-----------------------|--------------------|-------------------|-------------------|--------------------|------|------------|------|-------------------|
| Heat treatment | - | 3'85°C | - | 3'85°C | | | | |
| Energy | 77.0 ^a | 77.1 ^a | 72.8 ^b | 71.3 ^b | 0.76 | ** | 0.37 | 0.32 |
| N | 82.2 ^a | 82.7 ^a | 75.2 ^b | 76.8 ^b | 0.60 | ** | 0.13 | 0.37 |
| Ret. N (g/day) | 28.5 ^{ab} | 29.8 ^a | 25.3 ^c | 27.6 ^b | 0.54 | ** | * | 0.41 |
| Essential amino acids | | | | | | | | |
| Lys | 84,9 ^a | 85,0 ^a | 75,7 ^c | 78,9 ^b | 0,69 | ** | * | 0,06 |
| Phe | 84,0 ^a | 84,7 ^a | 78,4 ^b | 80,0 ^b | 0,54 | ** | 0,07 | 0,48 |
| Leu | 83,7 ^a | 84,5 ^a | 77,7 ^b | 79,3 ^b | 0,54 | ** | 0,07 | 0,47 |
| Ile | 82,8 ^a | 83,3 ^a | 76,1 ^b | 77,5 ^b | 0,42 | ** | 0,07 | 0,32 |
| Val | 80,0 ^a | 81,1 ^a | 73,7 ^b | 74,9 ^b | 0,55 | ** | 0,10 | 0,92 |
| Trp | 73,2 ^{ab} | 77,8 ^a | 71,1 ^c | 71,2 ^{bc} | 1,54 | * | 0,17 | 0,20 |
| Thr | 77,3 ^a | 78,1 ^a | 70,0 ^b | 71,1 ^b | 0,82 | ** | 0,27 | 0,83 |

n = 16, within row, means with different superscripts are significantly different at P < 0,05; ** P < 0,005; * P < 0,05

Table 2. Technological impacts on real digestibility (%) and total endogenous losses (g/kg DMI).

| Part. size, µm | 200c | 200SH | 500c | 500SH | SEM | Part. size | Heat | Part. size x Heat |
|-------------------------|--------------------|-------------------|--------------------|--------------------|------|------------|------|-------------------|
| Heat treatment | - | 3'85°C | - | 3'85°C | | | | |
| Real digestibility | | | | | | | | |
| N | 85,3 ^a | 87,1 ^a | 76,6 ^c | 81,6 ^b | 0,64 | ** | ** | 0,05 |
| Lys | 87,3 ^a | 87,4 ^a | 75,7 ^c | 81,5 ^b | 1,16 | ** | * | 0,05 |
| Phe | 85,3 ^a | 87,0 ^a | 79,5 ^c | 83,1 ^b | 0,57 | ** | * | 0,16 |
| Leu | 86,0 ^a | 87,3 ^a | 80,6 ^c | 82,8 ^b | 0,50 | ** | * | 0,41 |
| Ile | 84,8 ^b | 87,0 ^a | 79,5 ^d | 82,6 ^c | 0,62 | ** | * | 0,46 |
| Val | 83,0 ^a | 84,6 ^a | 75,7 ^c | 79,8 ^b | 0,92 | ** | * | 0,24 |
| Thr | 85,7 ^{ab} | 87,6 ^a | 76,6 ^c | 82,6 ^b | 1,32 | ** | * | 0,17 |
| Total endogenous losses | | | | | | | | |
| N | 2,53 | 2,46 | 2,80 | 2,70 | 0,40 | 0,59 | 0,80 | 0,92 |
| Lys | 0,61 ^b | 0,40 ^a | 0,51 ^{ab} | 0,42 ^{ab} | 0,06 | 0,52 | * | 0,39 |
| Phe | 0,59 ^{ab} | 0,52 ^a | 0,82 ^b | 0,75 ^{ab} | 0,08 | * | 0,39 | 0,97 |
| Leu | 0,88 ^{ab} | 0,74 ^a | 1,14 ^b | 0,98 ^{ab} | 0,11 | * | 0,22 | 0,93 |
| Ile | 0,48 ^{ab} | 0,44 ^a | 0,63 ^b | 0,55 ^{ab} | 0,06 | 0,06 | 0,33 | 0,67 |
| Val | 0,75 ^{ab} | 0,68 ^a | 1,04 ^b | 0,78 ^{ab} | 0,09 | * | 0,11 | 0,32 |
| Thr | 0,95 | 0,91 | 1,06 | 1,05 | 0,06 | 0,11 | 0,71 | 0,79 |

n = 16, within row, means with different superscripts are significantly different at P < 0,05; ** P < 0,005; * P < 0,05

sparing resulting from the cumulative effects of the increase in real digestibility and decrease in endogenous losses.

Conclusion

Technological processes involved in diet manufacture act on digestibility values. Thus, without integration of production costs, a 200 vs. 500- μm four of a wheat-pea blend allows a gain of 7 percent units for the N apparent digestibility. Both, grinding and heat treatment seems to improve the rate of real digestibility and to reduce the endogenous losses. Association of both treatments resulted in greater N retention by sparing of threonine.

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Apparent and true ileal protein digestibility of tropical tree foliage and aquatic plants in pigs

P. Leterme¹, W.B. Souffrant², A. Buldgen³, A. Rosales¹, A. Valencia¹, F. Mera¹, R. Ballesteros¹, A. Londoño¹ & P. Sarria⁴

¹ Universidad Nacional de Colombia, carrera 32, Palmira, Valle, Colombia

² FBN, Ernährungsphysiologie "Oskar Kellner", 18196 Dummerstorf, Germany

³ Faculté Universitaire des Sciences agronomiques, Dpt Zootechnie, 5030 Gembloux, Belgium

⁴ Universidad Nacional de Colombia, sede Medellin

Summary

The work aimed to determine the apparent and true ileal digestibility of the amino acids of tropical tree foliage (*Trichanthera gigantea*) or aquatic plant (*Azolla filicoides*) in pigs. The incorporation of 100 or 200 g/kg of either tree foliage or aquatic plant in the pig diet significantly reduced both ileal and fecal digestibilities of the dietary nitrogen and amino acids of the diets ($P < 0.05$). The results are discussed according to the pig digestive physiology and the forage properties.

Keywords: pig, protein, digestibility

Introduction

In the tropics, the small pig owners cannot afford commercial diets. On the other hand, most of the crops they produce are energy sources: sugarcane, cassava, banana or corn. Thus, protein is the limiting factor for small-scale production.

One of the few possibilities they have to provide their animals with protein sources is the "protein bank" system, i.e. the production of tree foliage that can be produced the whole year, at low cost and in sustainable production systems. Another possibility is to recycle pig waste by growing aquatic plants on waste tanks. The protein content of these plants usually ranges from 150 to 300 g/kg.

Although it is common practice in the tropics to feed pigs with tree foliage and/or aquatic plants, very few data are published on their nutritional value in pigs and how to incorporate them in feeding systems. The forages have a low energy density and high bulking properties, which limits dry matter intake (Leterme *et al.*, 2003). Moreover, the high volume also affects the digestive processes when high amounts of forage (> 300 g/kg) are incorporated in the diet of young pigs, as observed by Quirama *et al.* (2002). Some data are available on the growth rate of animals fed with forages but an accurate estimation of their true protein value is still to come.

The aim of the present work was to study the protein value of one species of tree foliage (*Trichanthera gigantea*) and one species of aquatic plant (*Azolla filiculoides*) in young pigs. The forage was incorporated at two levels (100 and 200 g/kg) in a control diet and the effect was measured on the fecal N digestibility and ileal N and amino acid digestibilities.

Material and methods

Fecal digestibility experiment

Animals

24 piglets (22 kg \pm 2) were placed in metabolic cage and adapted to their respective diet.

Diets

A basal diet (600 g corn/kg, 180 g soybean meal, 100 g sucrose, 60 g ground rice hulls and 60 g mineral premix), was first formulated. Thereafter, for each experiment (foliage or aquatic plant), 3 diets were prepared, in order to contain 0, 100 or 200 g/kg of either *Trichanthera* or *Azolla*, the rest being composed of the basal diet. The tree foliage was hand-collected and sun-dried. The aquatic plants were collected with nets and kept one night in Hessian bags before being sun-dried. The amino acid content of the tree foliage and the aquatic plant was the following (g/16 g N): *Trichanthera*: Arg 4.2, His 1.8, Ile 3.4, Leu 6.5, Lys 4.0, Met 1.3, Phe 4.0, Thr 3.6, Trp 0.8, Val 4.3, Ala 4.8, Asp 7.7, Cys 1.2, Glu 8.6, Gly 4.4, Pro 3.9, Ser 3.6, Tyr 2.8. *Azolla*: Arg 6.0, His 2.4, Ile 4.2, Leu 9.6, Lys 5.2, Phe 5.5, Thr 4.6, Val 5.7, Ala 7.4, Asp 11.4, Glu 15.0, Gly 6.3, Pro 5.3, Ser 6.0, Tyr 3.6. The content in Met, Cys, Trp was not determined in *Azolla*.

Methodology

The experiment was divided in 2 trials (tree foliage or aquatic plant). In both cases, the experimental scheme was a completely randomized one: 4 pigs were randomly allocated to one of the 3 diets. They received, for 10 days, the equivalent of 90 g DM.kg⁻¹ W^{0.75}.day⁻¹, divided in 3 meals (7, 12, 17 h). After an adaptation period of one week, the faeces were totally collected for 9 days.

Ileal digestibility experiment

Animals

Twenty piglets (18 kg ± 2) were surgically prepared with an end-to-end ileo-rectal anastomosis according to the method described by Hennig *et al.* (1986). After recovery, they were also fitted with two catheters, in the jugular vein and carotid artery for ¹⁵N-leucine infusion and blood sample collection, respectively. The ¹⁵N-leucine infusion aimed to determine the ileal endogenous N flows. Unfortunately, the results are not available for the present publication.

Diets

A basal diet, similar to that used in the previous experiments, was prepared. Four other diets were prepared, containing 100 or 200 g/kg of either *Trichanthera gigantea* or *Azolla filiculoides*. An N-free diet based on corn starch, sucrose, oil, cellulose and minerals was also prepared.

Methodology

The experimental design was a completely randomized scheme with 4 piglets per diet. The piglets received, for 10 days, the equivalent of 90 g DM.kg⁻¹ W^{0.75}.day⁻¹, divided in 3 meals (7, 12, 17 h). The ileal digesta were collected the last 5 days, in 400 ml ethanol. The ethanol was evaporated at room temperature (30 °C) for 24 h and the digesta were kept in a freezer. At the end, 5 pigs were randomly selected and fed with an N-free diet for 5 days. The ileal digesta were collected the 3 last days for determination of the ileal N flow.

Analysis. The forages were analyzed completely (results not presented here). The faeces were analyzed for their N and energy content whereas the ileal digesta were analyzed for their N and amino acid content, by ion-exchange chromatography.

Results and discussion

The fecal and ileal digestibilities of the dry matter and nitrogen are presented in Table 1. In all cases, the digestibility decreased when forage intake increased. Ly *et al.* (1998) observed the same decrease, with identical diets containing *Leucaena* leaves, a legume tree. The comparison between the ileal and fecal digestibilities of dry matter gives an indication on the rate of fermentation of fibres in the large intestine. The fecal digestibilities were higher for the tree foliage, probably meaning that the tree leaf fibres were better fermented than those of the aquatic plants. This has been confirmed with the in vitro gas method (unpublished results).

The ileal N digestibility decreased more rapidly than the dry matter one, indicating a possible increase in endogenous N losses. The latter were measured during this experiment by means of the ¹⁵N dilution technique but the data are not available for the present communication. The higher endogenous N losses could also be explained by the high bulking properties of the forages, due to their high water-holding capacity. The latter increases significantly the endogenous N losses in pigs.

Table 1. Ileal and fecal dry matter and ileal apparent and true N digestibility of diets supplemented with 0, 100 or 200 g.kg⁻¹ of either tree foliage or aquatic plant.

| | Trichanthera | | | | Azolla | | | |
|----------------------------|-------------------|-------------------|-------------------|--------|-------------------|-------------------|-------------------|--------|
| | 0 | 100 | 200 | SEM | 0 | 100 | 200 | SEM |
| Fecal digestibility | | | | | | | | |
| Dry matter | 84.4 ^a | 79.3 ^b | 76.4 ^b | 1.3* | 83.5 ^a | 69.3 ^b | 65.9 ^b | 3.0*** |
| Nitrogen | 85.1 ^a | 76.5 ^b | 70.4 ^b | 2.2* | 82.3 ^a | 66.9 ^b | 64.4 ^b | 2.2*** |
| Ileal digestibility | | | | | | | | |
| Dry matter | 76.6 ^a | 71.3 ^b | 63.5 ^c | 2.0** | 76.6 ^a | 64.8 ^b | 58.5 ^b | 1.6** |
| Protein | | | | | | | | |
| - apparent | 73.6 ^a | 61.8 ^b | 48.7 ^c | 1.7*** | 73.6 ^a | 60.4 ^b | 53.8 ^b | 1.7** |
| - true | 78.4 ^a | 66.6 ^b | 53.3 ^c | 1.5*** | 78.4 ^a | 65.0 ^b | 58.1 ^b | 1.5** |

a, b, c: in a same row, means with different superscripts differ significantly (P < 0.05)

The amino acid digestibility coefficients followed the same pattern as that of nitrogen (Table 2). However, differences between amino acid were observed. Roughly, the coefficients were slightly higher for the essential amino acids.

The amino acid profile of the tree foliage, given in the “material and method” section, is comparable to that of a cereal. Lysine, methionine and tryptophan may be limiting. The lysine content is better for *Azolla* and according to the FAO (2003), it is also high in methionine-cysteine (4.2) and tryptophan (2.0), which makes that aquatic plant an attractive protein source for monogastric animals. However, the protein content varies according to the growing conditions. Moreover, due to the very high water content, dry matter intake is limited to 0.5 kg/100 kg bodyweight in fresh form and 1 kg in dry form (Leterme *et al.*, 2003). The low digestibility and dry matter intake indicate that these forages may not be the most appropriate protein source for small pigs, i.e. growing animals weighing less than 60 kg. Many data are available in literature to show that adult sows have a much better intake capacity but also that they better digest rough material (Noblet *et al.*, 1998). Experiments are currently conducted to measure the nutritional value and use of these forages in adult sows.

Table 2. Apparent ileal digestibility of the amino acids of diets supplemented or not with 100 g or 200 g/kg diet of tree foliage (*Trichanthera*) or aquatic plant (*Azolla*) in pigs.

| Diets | Control | <i>Trichanthera</i> | | <i>Azolla</i> | | SEM |
|---------------|-------------------|---------------------|-------------------|-------------------|--------------------|-----|
| | | 100 g/kg | 200 g/kg | 100 g/kg | 200 g/kg | |
| Forage level | | | | | | |
| Arginine | 91.4 ^a | 83.3 ^b | 74.9 ^c | 84.2 ^b | 75.1 ^c | 3.2 |
| Histidine | 83.0 ^a | 67.7 ^b | 57.3 ^c | 70.3 ^b | 59.7 ^c | 4.7 |
| Isoleucine | 74.8 ^a | 69.3 ^b | 47.9 ^c | 63.0 ^b | 53.0 ^c | 5.0 |
| Leucine | 75.8 ^a | 62.4 ^b | 50.7 ^c | 65.0 ^b | 57.0 ^{bc} | 4.3 |
| Lysine | 76.7 ^a | 66.1 ^b | 56.6 ^c | 69.4 ^b | 61.9 ^{bc} | 3.4 |
| Phenylalanine | 79.1 ^a | 66.3 ^b | 53.8 ^c | 68.7 ^b | 59.0 ^c | 4.4 |
| Threonine | 69.7 ^a | 55.5 ^b | 44.1 ^c | 57.5 ^b | 48.0 ^c | 4.9 |
| Valine | 70.3 ^a | 53.8 ^b | 40.9 ^c | 57.4 ^b | 45.8 ^c | 5.4 |
| Alanine | 69.2 ^a | 54.6 ^b | 41.8 ^c | 57.3 ^b | 47.3 ^c | 4.9 |
| Aspartic acid | 77.9 ^a | 66.5 ^b | 54.1 ^c | 69.1 ^b | 61.5 ^{bc} | 3.8 |
| Glutamic acid | 82.3 ^a | 72.0 ^b | 60.1 ^c | 75.6 ^b | 69.7 ^c | 3.4 |
| Glycine | 64.0 ^a | 45.2 ^b | 25.2 ^c | 47.6 ^b | 41.0 ^{bc} | 6.3 |
| Proline | 70.7 ^a | 48.7 ^b | 40.6 ^b | 49.4 ^b | 42.8 ^b | 7.1 |
| Serine | 77.5 ^a | 66.5 ^b | 54.1 ^c | 68.3 ^b | 60.7 ^b | 3.9 |
| Tyrosine | 75.6 ^a | 62.0 ^b | 49.6 ^c | 63.1 ^b | 54.5 ^c | 4.4 |
| Total | 77.0 ^a | 63.9 ^b | 51.8 ^c | 66.5 ^b | 58.3 ^c | 4.3 |
| N | 73.6 ^a | 61.8 ^b | 48.7 ^c | 60.4 ^b | 53.8 ^c | 3.6 |

a, b, c: in a same row, means with different superscripts differ significantly (P < 0.05)

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Effect of forage type and supplementation on microbial synthesis and efficiency in the rumen of dairy cows

P. Lund, M.R. Weisbjerg & T. Hvelplund

*Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology,
Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark*

Summary

A wide range of different forages were either fed as the sole feed or supplemented with a fixed amount of concentrate and fed to dairy cows in four separate experiments. Rumen microbial AAN synthesis was calculated based on content of RNA in isolated rumen liquid associated bacteria and in duodenal chyme. Efficiency was calculated based on apparent total tract digested carbohydrates, apparent and true rumen digested OM and true rumen digested fermentable OM. Microbial AAN synthesis varied from 47 g/d for unsupplemented late cut grass silage to 174 g/d for supplemented lucerne hay, and seemed to increase when forages were supplemented. Intake of OM, true rumen digested fermentable OM and total digested carbohydrates could explain 79%, 70% and 72% respectively of the variation in microbial AAN synthesis, whereas fractional rate of passage of liquids and particles only could explain 36% and 6% respectively. Efficiency was clearly not constant as assumed in many protein evaluation systems. Efficiency varied from 15 to 26 g AAN/kg rumen truly digested fermentable OM, and only little of the variance in efficiency could be described from fractional passage rates of liquid and particles and feed characteristics.

Keywords: amino acids, ruminants, microbes

Introduction

Ruminants and rumen microorganisms have a symbiotic relationship, as the rumen ecosystem has the ability to convert fibre and non-protein nitrogen into SCFA and high quality protein, subsequently available for the host. The new protein evaluation systems therefore distinguish between the nitrogen and energy requirements of the rumen microbes and the amino acid requirement of the ruminant. Rumen microbial protein synthesis (MPS) accounts for a major part of the protein that passes to the duodenum and have a high biological value. MPS is therefore both quantitatively and qualitatively important for the amino acid supply of the ruminant animal. Efficiency, defined as MPS pr amount substrate, may vary from 12 to 54 g microbial N pr kg organic matter (OM) fermented in the rumen (NRC, 2001), and despite the key role in protein evaluation, the attempt to relate efficiency to simple feed characteristics has failed. The different protein evaluation systems use different ways to predict MPS. In most cases (Vérité & Peyraud, 1989; Tamminga *et al.*, 1994; Madsen *et al.*, 1995; NRC, 2001), but not all (AFRC, 1992), constant values for the efficiency of MPS are used, despite numerous factors has been shown to affect the efficiency. Hvelplund & Madsen (1985) found a higher microbial efficiency for roughage rich diets compared to concentrate rich diets, and replacing starch with fibre increased efficiency in a study by Huhtanen (1992). Further, higher feeding level and rapid passage rate have been shown to increase efficiency (Robinson *et al.*, 1985). Additionally efficiency is affected by the intraruminal recycling of N by protozoal predation (Jouany & Ushida, 1999).

The present experiment aimed at explaining the variation in MPS and efficiency when a range of forages were fed either alone or supplemented with a fixed amount of concentrate.

Materials and methods

The experiments complied with the guidelines of the Danish Ministry of Justice (Act no. 726, 1993) with respect to animal experimentation and care of animals under study. Four separate 4 x 4 Latin square experiments were conducted (Exp. A, Exp. B, Exp. C, Exp. D) with triple-fistulated Holstein cows. The cows were milked (6:00 and 17:00) and fed twice daily (10:15 and 17:30). Cows late in lactation or dry cows were used in Exp. A and Exp. C, and cows were on average 275 days post partum and yielded 21 kg ECM at the onset. In Exp. B and Exp. D cows in early or mid-lactation were used, and the cows were on average 75 days post partum with an average yield of 27 kg ECM at the onset of the experiments. Yield was however reduced substantially during all the experiments. In Exp. A and B either grass hay (GH), early cut grass silage (EGS), late cut grass silage (LGS) or whole crop barley silage (WCB) was fed ad libitum and in Exp. B supplemented with 4.50 kg DM/day of wheat meal (WM) and 1.32 kg DM/day of soy bean meal (SBM). In Exp. C and D, GH, lucerne hay (LH), maize silage (MS) or pea silage (PS) was either fed alone (Exp. C) or supplemented (Exp. D) with 4.40 kg DM/day of WM and 1.33 kg DM/day of SBM. Although no problems were seen in Exp. D, when PS was fed together with concentrate, feeding PS as the sole feed in Exp. C gave problems. For 3 cows on PS in Exp. C a daily intake of on average 30 kg of PS (8 kg of DM) in the first days of the period was followed by a substantial decrease in feed intake leading to a severe feed deprivation within a few days, and no useful results were therefore obtained for PS in Exp. C. Pea silage was therefore replaced in Exp. C by a first cut clover-grass silage (CGS). Rumen liquid associated bacteria were isolated by centrifugation on three different times (08:00, 14:00 and 20:00), pooled over sampling times within cow and analysed for content of total purines (RNA) and 18 amino acids. During 64 h on day 19 to day 21 in each period, 12 samples of duodenal chyme and faeces were taken and pooled within cow. The collection protocol was arranged to give a representative sample of the diurnal flow. Representative subsamples were analysed for digestibility marker (Cr_2O_3) and nutrient content. Feed intake was determined based on daily allowance and refusals. Intake of fermentable OM was calculated by subtracting intake of end products of fermentation in ensiled feeds (lactate, acetate, butyrate and ethanol) in silage based rations, and true rumen digestion of OM was calculated by subtracting microbial OM from duodenal OM flux. Liquid and particulate passage rates (k_p , %/h) were determined based on disappearance of pulse dosed Co-EDTA and rumen pool and faecal output of indigestible NDF respectively. Rumen microbial amino acid nitrogen synthesis (AAN) was calculated based on content of RNA in isolated rumen liquid associated bacteria and in duodenal chyme, and efficiency was calculated based on apparent total tract digestion of carbohydrates, true rumen digestion of fermentable OM and apparent and true rumen digestion of OM. Statistical analysis within each experiment was done using the GLM procedure in SAS 8.e (SAS Institute, 2000). In total 60 observations were compiled and correlation between either microbial AAN synthesis or efficiency and intake, content and digestibility of different feed fractions and fractional passage rate of liquid and particles was calculated also using the GLM procedure.

Results and discussion

Results from the four separate experiments are presented in Table 1. The effect of supplementation should be evaluated with care, as supplementation is confounded with experiment and therefore intake level and lactation stage.

The AAN:N ratio in isolated bacteria averaged 66% and was similar for all feeds. The ratio was in agreement with the 70% used in the Scandinavian AAT/PBV system (Madsen *et al.*, 1995). Further, only minor differences were found in amino acid profile (data not shown).

Microbial AAN synthesis varied from 47 g/d (unsuppl. LGS) to 174 g/d (suppl. LH), and increased when forages were supplemented. Microbial AAN synthesis constituted on average 42 % of

Table 1. Intake of DM (kg) and AAN (g), AAN content (g/kg OM) and AAN:N ratio in isolated bacteria (%), microbial AAN synthesis (g), microbial efficiency (g AAN/kg) and duodenal flow of AAN (g).

| | Exp. A, - supplementation | | | | | Exp. B, + supplementation ¹ | | | | |
|-------------------------|---------------------------|-------------|-------------|-------------|------------|--|------|------|------|------|
| | GH | EGS | LGS | WCB | SEM | GH | EGS | LGS | WCB | SEM |
| DM intake | 8.35 | 10.5 | 8.18 | 12.7 | 1.5 | 13.7 | 15.3 | 15.5 | 15.5 | 0.83 |
| AAN intake | 94.4 | 169 | 71.9 | 134 | 24 | 225 | 289 | 240 | 239 | 12 |
| AAN in bacteria | 63.9 | 66.1 | 68.1 | 60.4 | 2.4 | 61.1 | 63.3 | 62.1 | 60.5 | 1.8 |
| AAN:N in bacteria | 65.2 | 66.2 | 65.8 | 64.5 | 0.81 | 64.1 | 63.8 | 64.2 | 64.1 | 0.50 |
| AAN synthesis | 56.6 | 67.3 | 47.0 | 86.6 | 9.0 | 122 | 170 | 148 | 163 | 15 |
| Efficiency ² | 35.8 | 17.8 | 19.6 | 16.0 | 7.5 | 21.5 | 29.7 | 22.3 | 27.4 | 4.4 |
| Efficiency ³ | 22.0 | 13.4 | 15.2 | 12.9 | 3.2 | 15.9 | 20.1 | 16.3 | 18.3 | 1.9 |
| Efficiency ⁴ | 22.2 | 16.6 | 20.3 | 14.8 | 3.8 | 15.9 | 23.0 | 18.0 | 19.8 | 2.2 |
| Efficiency ⁵ | 14.6 | 12.1 | 11.9 | 13.8 | 1.7 | 16.3 | 19.0 | 16.7 | 20.2 | 2.1 |
| Duodenal AAN | 180 | 189 | 143 | 192 | 21 | 262 | 343 | 280 | 293 | 14 |
| | Exp. C, - supplementation | | | | | Exp. D, + supplementation ¹ | | | | |
| | GH | LH | MS | CGS | SEM | GH | LH | MS | PS | SEM |
| DM intake | 8.27 | 13.2 | 7.70 | 12.1 | 0.33 | 13.1 | 18.0 | 15.0 | 15.5 | 0.48 |
| AAN intake | 87.8 | 259 | 62.0 | 238 | 8.9 | 249 | 404 | 245 | 296 | 9.3 |
| AAN in bacteria | 60.6 | 72.2 | 65.4 | 67.7 | 0.88 | 62.1 | 67.0 | 62.9 | 64.7 | 0.81 |
| AAN:N in bacteria | 64.8 | 66.6 | 69.6 | 68.2 | 1.3 | 65.9 | 66.7 | 67.4 | 64.7 | 0.89 |
| AAN synthesis | 51.2 | 80.0 | 54.2 | 89.6 | 6.9 | 138 | 174 | 162 | 136 | 8.1 |
| Efficiency ² | 22.5 | 20.2 | 25.8 | 19.7 | 3.2 | 33.2 | 30.5 | 29.3 | 21.4 | 3.7 |
| Efficiency ³ | 16.2 | 15.7 | 18.3 | 15.4 | 1.8 | 21.6 | 20.6 | 19.7 | 16.1 | 1.7 |
| Efficiency ⁴ | 15.8 | 15.6 | 33.4 | 19.2 | 3.3 | 21.6 | 20.6 | 23.9 | 19.0 | 2.0 |
| Efficiency ⁵ | 12.7 | 15.5 | 18.2 | 15.5 | 1.3 | 19.8 | 20.7 | 20.8 | 16.3 | 1.0 |
| Duodenal AAN | 154 | 239 | 146 | 259 | 16 | 303 | 394 | 312 | 302 | 16 |

¹ Exp. B: 5.82 kg DM/d from concentrate, Exp. D: 5.73 kg DM/d from concentrate

² g AAN/kg rumen apparently digested OM

³ g AAN/kg rumen truly digested OM

⁴ g AAN/kg rumen truly digested fermentable OM

⁵ g AAN/kg total apparently digested carbohydrate

duodenal flux of AAN, varying from 32% for unsuppl. GH to 54% for suppl. WCB. AAN synthesis was regressed against intake of N, starch, NDF, OM, fermentable OM and carbohydrates, content of starch and NDF, total digested carbohydrates used in the Scandinavian system, true rumen digested fermentable OM used in the French and Dutch systems and fractional passage rates of liquid and particles. Intake of OM, true rumen digested fermentable OM and total digested carbohydrates could explain 79%, 70% and 72% respectively of the variation in microbial AAN synthesis. Other factors therefore affect the conversion of substrate to rumen microbial outflow, SCFA, and CO₂ and CH₄. The most important factors are believed to be fractional rate of passage of liquids and particles, which alone could explain 36% and 6% of the variation. It is noteworthy that intake of NDF only could explain 1% of the variation, whereas NDF content could explain 59% of the variation.

Efficiency related to total apparently digested carbohydrates was on average 14.3 g AAN/kg (SEM=0.54, N=29) for unsupplemented rations, but 18.9 g AAN/kg (SEM=0.54, N=31) for supplemented rations. The efficiency generally seemed to increase when forages were supplemented. Efficiency related to rumen truly digested fermentable OM was on average 19.9 g/kg (SEM=0.75, N=60). Average efficiencies were similar to the fixed values used in the French PDI system, the Dutch DVE/OBE system and the Scandinavian AAT/PBV system (Vérité & Peyraud, 1989; Tamminga *et al.*, 1994; Madsen *et al.*, 1995), though ways of calculation differ. It is well known that efficiency is dependable upon fractional passage rates of liquid and particles. Despite this, when efficiencies were regressed against these two parameters poor relationships were found, but the best relationship was found for efficiency based on total digestion of carbohydrates: Efficiency (g AAN/kg total digested carbohydrates) = 1.40 (p=0.11) * k_p (particles) + 0.49 (p=0.06) * k_p (liquid) + 9.21 (p=0.0001), $R^2 = 0.18$, CV=21, RMSE=3.4. Additionally, poor correlations were found between feed characteristics and efficiency

Conclusion

Microbial AAN synthesis varied from 47 g/d for unsupplemented late cut grass silage to 174 g/d for supplemented lucerne hay, and seemed to increase when forages were supplemented. The efficiency was clearly not constant as assumed in many protein evaluation systems, but varied from 15 to 26 g AAN/kg rumen truly digested fermentable OM, and only little of the variance could be described from fractional passage rates of liquid and particles and feed characteristics.

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Effect of weaning on urea metabolism in calves

T. Obitsu, S. Watanabe, T. Yoneyama & K. Taniguchi

Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashihiroshima-shi, 739-8528, Hiroshima, Japan

Summary

Three calves were used to investigate the effect of weaning on urea metabolism including production, gut entry and subsequent fate of urea-N. Calves were fed a milk replacer plus ad libitum feeding of a concentrate and hay until 50 days of age. After weaning, the same concentrate and hay were offered in a ratio of 85:15 to support a growth rate of 0.9 kg/d. At 6 (pre-weaning), 9 and 13 (post-weaning) weeks of age, urea kinetics were measured by isotopomer analysis with ^{15}N labeled urea. Nitrogen digestibility decreased but the proportion of urinary N to N intake increased after weaning. Urea entry rate (UER) increased from 6 to 13 weeks of age. Urea entry to the gastrointestinal tract (GER) increased from 6 to 9 weeks of age, but not from 9 to 13 weeks. The proportion of GER to UER increased from 6 (26%) to 9 weeks (60%), but slightly decreased from 9 to 13 weeks of age (46%). The proportion of anabolic use of urea-N to GER (45-50%) was similar across the ages. As a result, the ratio of anabolic use of urea-N to N retention increased with age (7 to 35%). These results indicate that the contribution of recycled urea to N use in calves increases with weaning.

Keywords: urea recycling, rumen development, weaning calves

Introduction

Urea synthesized in the liver is excreted in the urine or enters the gastrointestinal tract (GIT). In ruminants, the urea entering the GIT is hydrolyzed and then converted to microbial protein as a protein source for the host animals. Generally, the rumen is the main site for the urea transfer and use for microbial protein synthesis (Lapierre & Lobley, 2001), but the rumen of the new born calf is immature and only develops rapidly after weaning (Stobo *et al.*, 1966). Thus, the extent of urea metabolism in calves, e.g. hepatic production, GIT entry and urinary excretion, may be different between pre- and post-weaning periods. Recently, the tracer technique that analyzes three labeled species, [$^{15}\text{N}^{15}\text{N}$], [$^{14}\text{N}^{15}\text{N}$], and [$^{14}\text{N}^{14}\text{N}$] urea, has been developed to investigate the urea metabolism in ruminants (Lobley *et al.*, 2000). This approach is particularly suited to studies with young calves, because the alternative, involving surgical catheterization and measurement of net fluxes would depress both growth rate and the development of rumen function. The objective of this study is to investigate the developmental change of urea kinetics during pre- and post-weaning periods in early weaned calves using the non-invasive approach.

Material and methods

One Holstein calf and two crossbreed (Holstein x Japanese Black Cattle) calves were used. The calves were fed 500-600 g/d of milk replacer (digestible energy content (DE) 20.9 MJ/kg and crude protein (CP) content 250 g/kg on a dry matter basis) for the first 20 days of life. After 21 days of age, the calves were fed the same amount of milk replacer plus ad libitum feeding of concentrate (DE:13.3 MJ/kg; CP:220 g/kg) and hay (DE:8.6 MJ/kg; CP:52 g/kg) until 50 days of age. After weaning (50 days of age), calves were offered the same concentrate and hay (2.0 to 3.5

kg/d as fed) in a ratio of 85:15 to support a growth rate of 0.9 kg/d. During pre- and post weaning periods, all feeds were given at 12 h intervals (0900 and 2100).

At 6 (pre-weaning), 9 and 13 (post-weaning) weeks of age, the excreted urine and feces were collected for 4 days for nitrogen (N) balance trials. From the 2nd day of each collection period, double N labeled urea ($[^{15}\text{N}_2]\text{urea}$, 99.4 atom %, Shoko Co., Ltd, Tokyo) was infused via the jugular vein at a constant rate (equivalent to 0.15% of estimated digestible N intake) for 60 h. The urine and feces collected over the last 12 h of the infusion period were used for the measurement of isotopic enrichment of urinary urea-N and fecal N. The enrichments of $[^{15}\text{N}^{15}\text{N}]$ and $[^{14}\text{N}^{15}\text{N}]$ gas liberated under mono-molecular conditions from urinary urea, isolated using a cation exchange resin, were measured by ion ratio mass spectrometer (MAT 252, Finnigan). Urea entry rate (UER), GIT entry rate of urea (GER), and subsequent fates as fecal output (UFE), re-entry to the ornithine cycle (ROC) and anabolic use (ANA), were calculated based on isotopomer analysis (Lobley *et al.*, 2000). Dry matter digestibility, urinary allantoin excretion and plasma urea concentration were also measured. Data was analyzed by the GLM procedure of SAS with age and animal as the main effects. The difference in the means between 6 vs 9 and 13 weeks of age (pre vs post weaning), and between 9 and 13 weeks of age were analyzed as contrasts.

Results and discussion

Feed intake and N balance of calves at 6, 9 and 13 weeks of age are shown in Table 1. Dry matter intake of calves increased from 0.85 kg/d (0.30 kg/d of solid diet) at 6 weeks to 2.7 kg/d at 13 weeks. These intakes supplied 76, 87 and 107% of DE required for 0.9 kg/d body weight gain at 6, 9 and 13 weeks of age, respectively. In contrast, dietary N provided 82, 106 and 133% of the requirement for 0.9 kg daily gain at 6, 9 and 13 weeks, respectively. Body weight gains of calves were 0.60 and 0.94 kg/d during the pre- and post-weaning period, respectively.

As N intake increased from 6 to 13 weeks of age, fecal and urinary N excretion also increased ($P<0.01$). Nitrogen retention in calves increased from 6 to 9 weeks ($P<0.05$) but not from 9 to 13 weeks. Apparent N digestibility decreased with weaning ($P<0.01$), and remained lower thereafter. In contrast, the proportion of urinary N excretion to N intake increased between 9 and 13 weeks. Urinary allantoin excretion, as an indicator of ruminal microbial synthesis, increased with weaning ($P<0.05$), and tended to be higher ($P<0.10$) for 13 than 9 weeks. However, the allantoin excretion per DE intake did not differ between 9 and 13 weeks. Thus, stable rumen function , in terms of the efficiency of microbial synthesis, seemed to be established by 13 weeks.

The results of urea-N kinetics in calves at 6, 9 and 13 weeks are shown in Table 2. Urea entry rate increased from 6 to 13 weeks of age ($P<0.05$). A lower proportion ($P<0.01$) of UER to apparent digestible N was observed before weaning compared with 9 and 13 weeks. The ratio at 6 weeks of age (34%) was also lower than that (45 to 53%) for older growing heifers (body weight 234 kg) reported by Bunting *et al.* (1989). The lower urea production in our pre-weaning calves may reflect not only their N intake but also the composition of N compounds (amino acids vs ammonia) absorbed from the GIT. The increase in digestible N intake between 6 and 9 weeks of age (20.4 g/d) matched the additional UER over the same period (19.2 g/d). In contrast, the increase in UER between 9 and 13 weeks (13.0 g/d) accounted for only 65% of the extra digestible N intake (20.0 g/d). The large increase in UER from 6 to 9 weeks of age probably reflects an inefficient utilization of ammonia by ruminal microbes and subsequent absorption of excess ammonia from the rumen immediately after weaning.

The enhanced urea production after weaning was compensated by increasing urea recycled to the GIT. Indeed, GER at 9 weeks of age was 6.3-fold larger ($P<0.01$) than at 6 weeks of age, but not different between 9 and 13 weeks. The proportion of GER to UER also increased from 6 to 9 weeks of age ($P<0.05$), but slightly decreased between 9 and 13 weeks of age. In contrast, the concentration of plasma urea-N increased from 6 to 13 weeks of age ($P<0.01$). This elevation in the concentration of plasma urea-N may account for a part of the increased GER at 9 weeks

Table 1. Body weight and daily feed intake, nitrogen balance and urinary allantoin excretion of calves at 6, 9 and 13 weeks of age.

| Items | Weeks of age | | | SEM | Contrast ¹ | |
|---------------------------|--------------|------|-------|------|-----------------------|-------------|
| | 6 | 9 | 13 | | weaning ² | 9 vs 13 wks |
| Body weight | 60.3 | 78.6 | 109.3 | 1.8 | <0.001 | <0.001 |
| DM intake, kg/d | 0.85 | 1.97 | 2.65 | 0.06 | <0.001 | 0.001 |
| DE intake, MJ/d | 17.1 | 25.1 | 34.6 | 1.0 | <0.001 | 0.002 |
| N balance, g/d | | | | | | |
| N intake | 34.8 | 66.6 | 91.2 | 3.1 | <0.001 | 0.002 |
| Digested | 30.4 | 50.8 | 70.8 | 2.7 | <0.001 | 0.006 |
| Urinary excretion | 11.9 | 19.2 | 44.2 | 2.9 | 0.005 | 0.004 |
| Retention | 18.5 | 31.6 | 26.5 | 2.8 | 0.036 | 0.266 |
| Proportion to N intake, % | | | | | | |
| Digestible N | 87.6 | 76.3 | 77.7 | 0.9 | 0.001 | 0.346 |
| Urinary excretion | 34.3 | 28.9 | 48.7 | 3.9 | 0.403 | 0.023 |
| Retention | 53.3 | 47.5 | 23.0 | 4.2 | 0.043 | 0.036 |
| Urinary allantoin, g/d | 2.3 | 5.0 | 7.6 | 0.8 | 0.018 | 0.090 |

¹P value

²Comparison between pre- (6 weeks) and post weaning (9 and 13 weeks).

(Lapierre & Lobley, 2001). The expansion of surface area and volume of the rumen, caused by an increase in solid feed intake, may be another factor driving the greater urea entry into the rumen. The slight decline of GER to UER at 13 weeks of age indicates the limited capacity of the urea transfer through the rumen wall.

Fecal output of urea-derived N, ROC and ANA also increased from 6 to 9 weeks of age ($P<0.01$), as GER increased, but were unchanged from 9 to 13 weeks. Although the absolute rates of ROC and ANA were changed by weaning, the proportions of UFE, ROC and ANA to GER were constant over this period. The proportion of ROC to GER for these calves was higher than that for older growing heifer and steers (Bunting *et al.*, 1989, Archibeque *et al.*, 2001, Marini & Van Amburgh, 2003). One explanation for this difference may be that in these younger weaned calves more of the GER enters the small and large intestine, where most of N derived from urea would be directed towards a catabolic fate.

Overall, the ratio of anabolic use of urea-N to total N retention increased with weaning (from 7 to 35%). Enhanced urea production and GIT entry after weaning appeared to improve N balance in the calves by directing urea-N from urinary excretion towards GIT entry and potential anabolic reuse of the N.

Table 2. Urea-N kinetics in calves at 6, 9 and 13 weeks of age.

| Items | Weeks of age | | | SEM | Contrast ¹ | |
|-----------------------------|--------------|------|------|-----|-----------------------|-------------|
| | 6 | 9 | 13 | | weaning ² | 9 vs 13 wks |
| UER ³ | 10.3 | 29.5 | 42.5 | 2.9 | 0.002 | 0.032 |
| GER ⁴ | 2.8 | 17.6 | 19.5 | 1.9 | 0.003 | 0.511 |
| UUE ⁵ | 7.5 | 11.9 | 23.0 | 2.2 | 0.021 | 0.024 |
| UFE ⁶ | 0.2 | 1.7 | 1.3 | 0.2 | 0.004 | 0.237 |
| ROC ⁷ | 1.3 | 7.2 | 9.0 | 1.0 | 0.005 | 0.276 |
| ANA ⁸ | 1.4 | 8.7 | 9.2 | 1.4 | 0.010 | 0.802 |
| Contribution, % | | | | | | |
| UER/DigestibleN | 34.0 | 58.1 | 60.1 | 4.5 | 0.010 | 0.768 |
| GER/UER | 26.4 | 60.0 | 46.0 | 6.2 | 0.025 | 0.183 |
| UFE/GER | 8.0 | 9.5 | 7.0 | 1.6 | 0.916 | 0.324 |
| ANA/GER | 44.9 | 49.7 | 46.4 | 6.0 | 0.760 | 0.720 |
| PUN ⁹ , mg/100mL | 6.7 | 8.2 | 11.6 | 0.5 | 0.006 | 0.009 |

¹P value.

²Comparison between pre- (6 weeks) and post weaning (9 and 13 weeks).

³Urea entry rate.

⁴Urea entry rate to gastrointestinal tract.

⁵Urinary urea excretion.

⁶N from urea excreted in the feces.

⁷N from urea returning to the ornithine cycle.

⁸N from urea used for anabolism.

⁹Plasma urea nitrogen.

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Supplements with different ruminal undegradable protein levels for dairy heifers in tropical pasture: intake, digestibility and plasma urea nitrogen

R.L. Oliveira¹, J.C. Pereira², Ricardo Augusto Mendonça Vieira², Domicio do Nascimento Júnior², Bevaldo Martins Pacheco², M.D. Ribeiro² & F.P. Leonel²

¹ Faculdades Integradas UPIS, Department of Animal Science, Faz. Lagoa Bonita, Planaltina-DF, Brazil

² Universidade Federal de Viçosa-MG, Brazil

Summary

The effects of supplements with different levels (high, medium, low) of ruminal undegradable protein (RUP) by Brown-Swiss heifers grazing *Brachiaria brizantha* cv. Marandu on the intake, digestibility and plasma urea nitrogen concentrations were evaluated. The experiment lasted 90 days, divided in three periods of 30 days, corresponding to May, June and July 2000. Fifteen purebred Brown Swiss heifers (five by treatment), averaging 19.7 months and 394 kg live weight (LW), were used. The pasture availability was monitored to maintain offer of 6% LW, in green dry matter (GDM). The heifers were fed 2.5 kg/head/day of concentrate supplement, offered twice a day. Extrusa was collected in animals fistulated in the esophagus. Indigestible neutral detergent fiber (NDF) in the feeds and feces was used to determine the intake and digestibility. There were no effects of supplements on the pasture, however there were differences among periods, because, as dry season progressed, the forage availability decreased and leaf/colm ratio and senescent material proportion increased. There was no effect of treatments on forage intake or forage + supplement. DM, organic matter (OM) and CP digestibility, and total digestible nutrients (TDN) content were lower for the diets with high RUP content. The animals fed supplement with high RUP content showed average plasma N-urea concentrations lower than those fed supplement with average RUP contents, followed by those fed diets with low RUP.

Keywords: *Brachiaria brizantha, rotational grazing, escape protein, supplementation*

Introduction

In Brazil and in several parts of the world the cattle production is based on tropical pastures, which depends on the raining distribution through the year. During the raining season occurs an intensive forage growth with high production of green leaves that contributes to the animal's high performance. On the other hand, during the dry season, pastures practically cease its growth and decrease quality reducing the cattle productivity. The alternatives for the refereed period would be the feedlot or the pasture supplementation with other roughage of better nutritive values or with concentrate feeds.

When the availability of energy is adequate the microbial protein can offer sufficient protein to maintain stable performance. However, cattle on high rate of weight gain, during the pregnancy or at the beginning of the milking, demands more metabolizable protein than they can obtain from the rumen microbial protein, mainly when limited by energetic contents of forages. In that situation it's necessary to offer a certain level of rumen undegradable protein (RUP) to attend the demand of nitrogen in the small intestine (Buxton & Mertens, 1995).

This experiment had the objective of evaluating intake and digestibility of the nutrients and the concentration of plasma urea nitrogen (PUN) of heifers on *Brachiaria brizantha* pastures

supplemented by concentrated mixtures containing different levels of RUP under system of rotational grazing.

Material and methods

Fifteen brown-Swiss heifers, with 18.7 months and 307.94 kg were kept in *Brachiaria brizantha* rotational grazing, in Silvânia-GO, Midwest Brazil. The total area of the experiment was divided, with electrified fences, in 30 paddocks of 540 m² (ten per treatment) and three central corridors. The cycle of grazing was of 50 days, corresponding to five of grazing and 45 of rest. Five animals were used per treatment. Those animals had access to covered bays where they received water, mineral mixture and the supplements twice a day.

The treatments consisted of three supplements with similar contents of energy and CP, but with high, medium and low levels of RUP. The composition of the *B. brizantha* (esophageal masticate) was obtained from esophageally fistulated steers and the supplements were adjusted for a weight gain of 750g/day (NRC, 1989). The chemical composition of the esophageal masticate and of the ingredients is shown in Table 1. The daily intake, the ratios of each ingredient and the composition of the supplements are shown in Table 2.

Table 1. Organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and total carbohydrate (TC), expressed as DM percentage of esophageal masticate and supplement ingredients; and rumen undegradable protein (RUP), expressed as CP percentage of the esophageal masticate.

| Ingredients | | OM (%DM) | NDF (%DM) | TC (%DM) | CP (%DM) | RUP1 (%CP) |
|--|----------|-------------|--------------|-------------|-------------|---------------|
| <i>Brachiaria brizantha</i> (Extrusa) ² | Period 1 | 88.79 | 52.85 | 74.39 | 12.52 | --- |
| | Period 2 | 87.71 | 54.08 | 76.85 | 8.99 | --- |
| | Period 3 | 88.18 | 54.63 | 77.39 | 9.00 | --- |
| Corn | | 98,83 | 10.51 | 86.50 | 8.30 | 53.02 |
| Corn Gluten Meal | | 98,79 | 9.84 | 31.46 | 66.61 | 60.10 |
| Soybean meal | | 92,87 | 13.56 | 39.91 | 51.82 | 27.59 |
| Urea | | 00,00 | 00.00 | 00.00 | 282.00 | 00.00 |

¹ With 5%/hour of passage rate; ² Obtained from esophageally fistulated steers.

Table 2. Offer (kg/day), ingredient ratios and chemical composition of the supplements.

| Supplement | Offer (Kg/day) | Corn | Corn gluten meal | Soybean meal | Urea | NDF | TC | CP | RUP1 |
|------------|-------------------|-------|---------------------|-----------------|------|-------|-------|-------|-------|
| | | % DM | | | | | | % CP | |
| High RUP | 2.52 | 75.12 | 24.88 | 0.00 | 0.00 | 10.34 | 72.80 | 22.81 | 58.16 |
| Medium RUP | 2.50 | 66.69 | 5.29 | 28.02 | 0.00 | 10.81 | 68.87 | 23.58 | 38.67 |
| Low RUP | 2.50 | 84.43 | 0.00 | 12.00 | 3.57 | 8.87 | 73.04 | 23.28 | 23.42 |

¹ Rumen undegradable protein, considering passage rate of 5%/hour

The experiment lasted three months, from May through July 2000. In the second fortnight of each month the samples for the determination of the intake were taken, when the testing animals received 5g twice a day. In the last six days, the faeces were collected at 8:00 a.m. and 5:00 p.m. The contents of DM, organic matter (OM) and CP of esophageal masticate, of concentrate and of faeces were analyzed in accordance with the AOAC (1990). The concentrations of FDN in esophageal masticate, faeces and supplements according to Van Soest et al. (1991). The total carbohydrates (TC) were determined according to SNIFFEN et al. (1992). The chromium was analyzed in accordance with WILLIAMS et al. (1962).

The fecal production (FP) was estimated by the equation: $FP = Q/C$, where FP = daily fecal production (kg fecal DM/heifer); Q = chromium offered daily to the animal (mg); and C = chromium in the faeces (mg Cr/kg fecal DM). The indigestible NDF (INDF) concentration in the esophageal masticate, in the ingredients of the supplements and in the faeces was determined. These data were used to estimate the DM digestibility (DMD). The DMD and the FP were used to estimate the DM intake (DMI) using the equation: $DMI = FP/(1-DMD)$.

The blood samples for the analysis of PUN were obtained once on each period at 0; 2; 4; and 6 hours after the morning feed.

Results and discussion

No significant differences were found between the intake of DM, CP and fiber in the forage and in the total diet (forage + supplement) by the animals while increasing the percentage of RUP on the supplements (Table 3). In spite of that other researches demonstrated that the incorporation of RUP promoted the reduction on the DM intake. However, Gardner (1968) and Quigley & Bearden (1990) did not report changes on the intake nor daily gain with the increment of RUP in the diet.

Table 3. Average intakes of dry matter (DM), crude protein (CP) and fiber of forage or forage + supplement, by Brown Swiss heifers fed supplements with different levels of ruminal undegradable protein (RUP).

| Item | Treatment | | | VC (%) | <i>P</i> |
|--------------------------|-----------------|-------------------|----------------|--------|----------|
| | <i>High PUR</i> | <i>Medium PUR</i> | <i>Low PUR</i> | | |
| <i>Forage</i> | | | | | |
| DM1 | 4.48 | 4.61 | 4.92 | 9.59 | 0.1598 |
| DM2 | 52.75 | 58.51 | 66.54 | 12.59 | 0.5981 |
| CP1 | 0.44 | 0.47 | 0.51 | 7.79 | 0.3480 |
| CP2 | 5.19 | 5.93 | 6.9 | 12.19 | 0.2102 |
| Fiber1 | 3.09 | 3.17 | 3.39 | 14.57 | 0.7577 |
| Fiber3 | 0.83 | 0.94 | 1.09 | 18.38 | 0.5254 |
| <i>Forage+supplement</i> | | | | | |
| DM1 | 6.73 | 7.11 | 7.42 | 15.31 | 0.6321 |
| DM2 | 79.24 | 90.24 | 100.35 | 12.20 | 0.1479 |
| CP1 | 1.01 | 1.06 | 1.09 | 13.12 | 0.5698 |
| CP2 | 11.97 | 13.37 | 14.77 | 18.23 | 0.2587 |
| Fiber1 | 3.35 | 3.44 | 3.61 | 13.58 | 0.2687 |
| Fiber3 | 0.90 | 1.02 | 1.16 | 14.25 | 0.1958 |

¹ Expresso em kg/dia (*Express in kg/day*).

² Expresso em g/kg PV0,75 (*Express in g/kg LW.75*).

The level of RUP on the supplement influenced the digestibility of DM, OM and CP (Table 4). For heifers fed high levels of RUP the digestibility coefficients were lower, probably the low CP digestibility of the corn gluten meal that escaped of ruminal degradation contributed to a higher faeces excretion of CP as well as DM.

At the same time the TDN intake was lower for the treatments in which the animals received high RUP, probably also influenced by the low ruminal digestion of corn gluten meal. NRC (2001) suggests for this category with weight gain of 750 g/day concentration of 6.25% of NDT in the diet, which was attended by this diet.

Figure 1 demonstrates the PUN curves on the heifer's plasma after the intake of concentrate supplement. It was evident that when the RUP level was diminished; there was an increase of the PUN concentration, resulting in high production of ruminal ammonia. On the other hand, The animals that consumed supplements with high levels of RUP presented PUN concentrations lower than those which consumed supplements with medium RUP contents, followed by those that received diets with low RUP.

The maximum peaks of PUN were higher at the rate that the RUP levels were diminished in the diet. According to Hammond et al. (1997), by increasing the undegradable protein contents in the diet, maintaining similar levels of N, increases the production of ruminal ammonia and, consequently, the level of PUN rises at the same proportion.

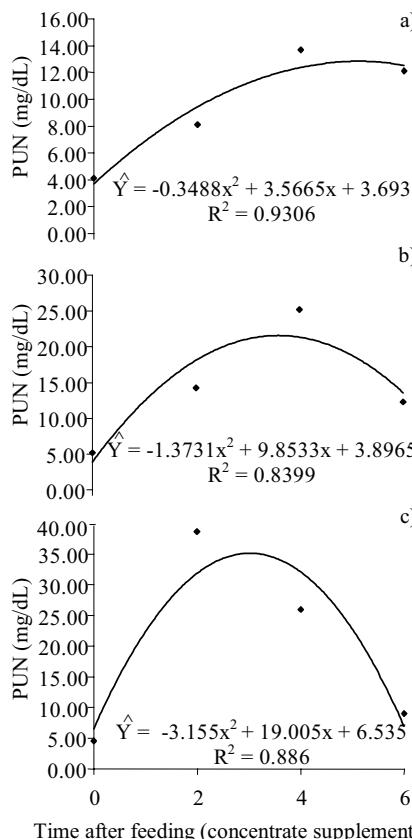


Figure 1. Plasma urea nitrogen (PUN) concentration of heifers after supplementation with different levels of ruminal undegradable protein (RUP). a) treatment with high RUP; b) treatment with medium RUP; c) treatment with low RUP.

Conclusion

The RUP level did not affect the intake of nutrients of forage and of total diet (forage+supplement). However the digestibility of DM, OM and CP of the total diet was lower for animals that received higher RUP. The same situation occurred with the intake of TDN. The PUN concentration of the heifers was higher when the level of RUP was reduced.

Table 4. Coefficients of total apparent digestibility of dry matter, organic matter, crude protein non fiber carbohydrates and total carbohydrates, by Brown Swiss heifers fed supplements with different levels of ruminal undegradable protein (RUP).

| Digestibility (%) | Treatment | | | VC (%) | P |
|----------------------------|-----------|------------|---------|--------|---------|
| | High RUP | Medium RUP | Low RUP | | |
| Dry matter | 57,03b | 60,74a | 61,54a | 3,88 | 0,0016 |
| Organic matter | 59,75b | 62,77a | 63,73a | 3,70 | 0,0107 |
| Crude protein | 56,69b | 61,01a | 63,27a | 7,37 | <0,0001 |
| Total carbohydrates | 61,14 | 62,66 | 63,80 | 11,74 | 0,1851 |
| Non fiber carbohydrates | 90,12 | 89,21 | 88,17 | 13,16 | 0,2321 |
| Total digestible nutrients | 57,23b | 59,64a | 60,15a | 5,17 | 0,0213 |

a,bMeans followed by the same letters differ by Scheffé test

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Effect of fermentation in a liquid diet on nitrogen metabolism in growing pigs

Carsten Pedersen & Jan Erik Lindberg

*Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management,
P.O. Box 7024, SE-750 07 Uppsala, Sweden*

Summary

The effect of fermentation of a barley and wet wheat distillers grain diet on nitrogen metabolism was tested in growing pigs using plasma urea nitrogen (PUN) as indicator.

There were no differences in PUN between non-fermented and fermented diets. Supplementation with lysine resulted in a reduction ($P<0.05$) of PUN, and at 4 and 6 h after the morning feeding a further reduction was found ($P<0.05$) when the diet was supplemented with lysine and threonine. Also, fermentation resulted in an increase in the *in vitro* digestibility for organic matter (+ 3.4 %-units) and nitrogen (+ 2.5 %-units).

It is suggested that, due to interactions between energy and nitrogen metabolism, the fermentation of a cereal based liquid diet may result in a similar utilisation of the dietary nitrogen as a non-fermented liquid diet when fed to growing pigs.

Keywords: *growing pigs, plasma urea nitrogen, PUN, digestibility*

Introduction

In recent years, there has been an increasing interest in the European pig industry to introduce fermented liquid feeding systems in order to reduce the risk pathogen transfer in the food chain. Fermented feed has been shown to improve the performance of piglets (Brooks, 1998), possibly due to the supply of organic acids which have been shown to lower the pH in the stomach. Also, lactic acid inhibits the growth of pathogenic bacteria (Geary et al., 1999). However, concerns have been expressed related to the protein quality of fermented liquid feeds due to the possible microbial use of dietary amino acids.

The aim of this study was to compare the response in plasma urea nitrogen (PUN) in growing pigs fed a non-fermented and a fermented liquid diet based on barley and wet distillers grains.

Materials and methods

Animals and diets

Five castrated pigs (L x Y) fitted with a permanent catheter in *vena jugularis* and with an initial and final body weight (BW) of 33 and 41 kg, respectively, were used. The basal diet (diet A) was composed of (g kg⁻¹): 500 wet wheat distillers grain (WWDG), 238 barley and 262 water (Table 1). Diet A contained 7.6 g digestible crude protein per MJ ME, which was 15% lower than current recommendations for growing pigs in the BW range of 40 to 70 kg (Simonsson, 1994). In diet B the basal diet was supplemented with lysine (1.4 g kg⁻¹ lysine) and in diet D the basal diet was supplemented with lysine (1.4 g kg⁻¹) and threonine (0.34 g kg⁻¹). Diets D-F, were similar in composition to diet A-C and were fermented. In the fermented diets, 80% was replaced with fresh feed every morning and allowed to ferment, which resulted in a fermentation of 7 1/2 and 23 1/2 h in afternoon and morning feeding, respectively. The supplementation of lysine and treonine was planned to be 10% higher than the recommendation for ideal protein for growing pigs (Boisen, 1997).

Table 1. Chemical composition (g kg DM⁻¹) and in vitro digestibility (%) of feedstuffs and diets

| | Barley | WWDG ¹ | Diet A,B and C ^{2,3} | Diet D ² | Diet E ^{2,4} | Diet F ^{2,5} |
|--|--------|-------------------|-------------------------------|---------------------|-----------------------|-----------------------|
| DM | 893 | 95 | 260 | 260 ² | 260 ² | 260 ² |
| Crude protein | 148 | 382 | 168 | 166 | 170 | 172 |
| NDF | 161 | 275 | 171 | 152 | 159 | 158 |
| <i>In vitro digestibility, 2-stage</i> | | | | | | |
| Organic matter | 75.5 | 60.7 | 74.2 | 77.6 | 78.2 | 76.6 |
| Crude protein | 89.1 | 92.6 | 89.4 | 92.4 | 92.5 | 89.1 |
| <i>In vitro digestibility, 3-stage</i> | | | | | | |
| Organic matter | 85.1 | 77.4 | 84.4 | 85.0 | 85.9 | 84.9 |

¹ Wet wheat distillers grain; ² Experimental diet contain 50,82% water, 26,23% barley, 22,95 WWDG and 1% mineral + vitamin mix on feedstuff base; ³ Calculated; ⁴ Same as diet D + 1.4 g kg⁻¹ lysine; ⁵ Same as diet D + 1.4 g kg⁻¹ lysine and 0.34 g kg⁻¹ threonine

Housing and feeding

The pigs were housed in single pens at a constant room temperature of 19°C. The pens had concrete floor and were equipped with at rubber mat to improve the comfort. The pigs were fed at 8 a.m. and 4 p.m. and had free access to water from nipples. Before start in the trial all pigs were fed diet A.

Measurements and analysis

In addition to conventional feed analysis, the pH was measured before every feeding and the *in vitro* digestibility (IVD) of organic matter (IVDOM) and crude protein (IVDCP) in feed ingredients and diets was determined according to (Boisen & Fernández, 1997).

Experimental design and statistical analysis

All diets were tested in a cross over design. Each diet was fed four times and thereafter blood samples were collected at 2, 4 and 6 hours after feeding (Pedersen & Boisen, 2001). To test the differences between fermented/non fermented diets the GLM procedure was used, and using Wilks lambda as parameter. Samples from the same day were considered as repeated measurement. Least square means were used to test difference between diets (SAS, 2001).

Results and discussion

Fermentation resulted in an improved digestibility *in vitro*, with the largest improvement at the ileal (2-stage *in vitro*) compared with the total tract (3-stage *in vitro*) level. The extent of improvement at the ileal level was +2.4 to +4.0 %-units for OM and -0.3 to +3.1 %-units for CP) compared with +0.5 to +1.5 %-units for OM at the total tract level (Table 1). The magnitude of improvement in digestibility of OM due to fermentation was comparable to those reported when supplementing enzyme to barley-based diets (Thacker, 2000), suggesting activation of endogenous enzymes in the fermented feed. In addition, the bacteria in the fermented feed may have contributed to the enhanced digestibility.

Table 2. Total NSP contents in the barley, wet wheat distillers grain (WWDG) and fermented feed (g DM⁻¹) based on barley (91%) and wet wheat distillers grain (9%).

| | Barley ¹ | WWDG ¹ | Diet A-C ² | Diet D-F ³ | Diet D-F ⁴ |
|--------------------|---------------------|-------------------|-----------------------|-----------------------|-----------------------|
| Arabinose | 2 | 28 | 4 | 5 | 5 |
| Xylose | 4 | 54 | 8 | 7 | 10 |
| Mannose | 2 | 7 | 2 | 2 | 3 |
| Galactose | 2 | 5 | 3 | 2 | 2 |
| Glucose | 32 | 8 | 30 | 23 | 18 |
| S-NSP ⁵ | 42 | 102 | 47 | 40 | 38 |
| rabinose | 24 | 45 | 26 | 24 | 21 |
| Xylose | 49 | 71 | 51 | 47 | 43 |
| Mannose | 3 | 13 | 4 | 3 | 3 |
| Galactose | 3 | 6 | 3 | 3 | 3 |
| Glucose | 58 | 83 | 60 | 52 | 46 |
| I-NSP ⁶ | 137 | 218 | 144 | 129 | 116 |
| Total NSP | 179 | 320 | 191 | 169 | 154 |

¹ Mean values from others experiment - analysed in same laboratory; ² Estimated on results from barley and wet wheat distillers grain; ³ Fermented for 7 1/2 h - means from diet D-F; ⁴ Fermented for 23 1/2 h - means from diet D-F; ⁵ Soluble non-cellulosic polysaccharides; ⁶ Insoluble non-cellulosic polysaccharides

One action of added carbohydrate degrading enzymes is to release sugars from the dietary polysaccharides (Chesson & Stewart, 2001), which should result in changes in the digestibility of the dietary NSP fraction (Gill et al., 2000). Interestingly, fermentation of the diet resulted in marked reductions in the dietary content of soluble and insoluble NSP (Table 2). The effect was largest for the insoluble NSP fraction and was higher for diets fermented for 23 1/2 h compared with fermentation for 7 1/2 h. Fermentation for 7 1/2 h decreased the total NSP content with 12%, as compared with 19% when fermenting for 23 1/2 h (Table 2).

The effect of pig, diet and time on the PUN values were significant (Table 3), in agreement with Pedersen & Boisen (2001).

There were no differences in PUN between fermented and non fermented diets at any of the sampling times (Table 4). However, there was a significant effect of adding lysine, and further a significant effect of adding both lysine and threonine on the PUN value (except not after 2h). This was in agreement with the expected effect of adding limiting essential amino acids to pig diets (Boisen, 1997).

Table 3. Statistical analyses of the different diets in the experiment on the plasma urea nitrogen content.

| Time after feeding (h) | 2 | 4 | 6 | Effect of ¹ |
|-----------------------------|------|------|------|------------------------|
| Diet | ** | ** | * | |
| Pig | *** | *** | ** | |
| R ² | 0.91 | 0.93 | 0.86 | |
| Time | | | | ** |
| Time x pig | | | | Ns |
| Time x diet | | | | Ns |
| Non-fermented vs. fermented | | | | Ns |

¹ tested by Wilks' Lambda; Significance: * (P<0.05), ** (P<0.01), *** (P<0.0001) and Ns (non significant)

Table 4. Estimated PUN values by LS-means for the different combinations of diets in the experiment

| Time after feeding (h) | Effect of fermentation | | Effect of supply of amino acids | | |
|------------------------|----------------------------|------------------------|---------------------------------|---------------------|-----------------------------------|
| | Non fermented ¹ | Fermented ² | Non ³ | Lysine ⁴ | Lysine and threonine ⁵ |
| n ⁶ | 10 | 12 | 4 | 10 | 8 |
| 2 | 4.40 | 4.20 | 5.27 ^a | 3.88 ^b | 3.77 ^b |
| 4 | 4.67 | 4.41 | 5.34 ^a | 4.31 ^b | 3.95 ^c |
| 6 | 4.21 | 4.30 | 4.94 ^a | 4.21 ^b | 3.71 ^c |

¹ Diet A, B and C; ² Diet D, E and F; ³ Diet A and D; ⁴ Diet B and E; ⁵ Diet C and F; ⁶ Number of observations
a,b,c Values in the same row with different letters are significant different (p<0.05)

Conclusion

It is suggested that, due to interactions between energy and nitrogen metabolism, the fermentation of a cereal based liquid diet may result in a similar utilisation of the dietary nitrogen as a non-fermented liquid diet with similar composition when fed to growing pigs.

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Influence of the ruminal N-balance on rumen fermentation, microbial protein synthesis, amount of utilisable crude protein and N-excretion

A. Riemeier, P. Lebzien & G. Flachowsky

Institut für Tierernährung, Bundesforschungsanstalt für Landwirtschaft, Bundesallee 50, 38116 Braunschweig, Germany

Summary

Five dairy cows allotted to four groups were used to investigate the influence of the ruminal N-balance (RNB) on rumen fermentation, microbial protein synthesis (MP), utilisable crude protein (uCP) amount and N-excretion. Animals were fed a ration of cornsilage and concentrates (50% : 50% on dry matter basis) which was formulated to meet the requirements of NEL and uCP. RNB amounted to -0,6 g/MJ ME in the basis ration. The other 3 rations were adjusted to RNB-values of -0,3, 0 and 0,3 g/MJ ME which was accomplished by urea supplements.

The RNB increase resulted in a higher NH₃-N concentration in the rumen fluid (1,6-28,5 mg/100 ml) and in the digesta (2,9-11,3 mg/100 ml). Additionally, urea concentration in the milk was increased (13,6-63,0 mg/100 ml). However, the difference of RNB did not show effects on pH-value, volatile fatty acids concentration in the rumen fluid as well as on ruminal digestibility of the organic matter (47,5-50,5%) and on efficiency of MP synthesis (7,7-8,3 g MP/MJ ME; corresponding to 144-148 g MP/kg fermented OM).

The group with the lowest RNB (-0,6 g/MJ ME) showed the highest level of feed protein degradation (UDP= 338 g/d versus 464 g/d, which was the average of the other three groups).

Additionally, the amount of fermented organic matter (FOM) was found with 9,2 kg/d to be significantly lower as compared to group 0 (9,9 kg/d).

The amount of MP which was reduced in this way and the lower amount of UDP resulted in a reduced amount of uCP, which was significant between RNB-group -0,6 and group 0. As compared to the other two groups the difference was nearly 200 g.

Introduction

The German Protein Evaluation System for dairy cows and heifers (GfE 2001) considers the amount of uCP and the RNB for the N-supply of the microbes in the rumen.

The recommendation on the ruminal N-balance has to consider 2 different goals:

- On one hand a N-shortage in the rumen has to be avoided for reasons to ensure a maximal fermentation rate of cell wall carbohydrates and a sufficient microbial protein synthesis.
- On the other hand a surplus of degradable N (RNB too high) would lead to a uneconomic protein utilisation, would induce N-burdens for the animal and the environment and would increase the urea content in milk.

This raises the question on the lowest N-quantity in the rumen, which does not negatively affect the rumen metabolism. Based on the knowledge of today the Committee for Requirement Standards of the Society of Nutrition Physiology (GfE, 2001) starts out from point that 20% of the microbial synthesized protein might be from recycled N, with the consequence that a negative RNB of max. -0,3 g/MJ ME would not affect rumen fermentation negatively.

The aim of present investigations is to reconsider this value in a series of experiments over 4 periods with 5 lactating cows which were fitted with rumen and duodenal cannulae. This was performed by using RNB-values below (-0,6 g/MJ ME) or over (0 and +0,3 g/MJ ME) the value of -0,3 g/MJ ME. As parameters for evalution rumen fermentation, microbial protein synthesis, amount of uCP and N-excretion were studied.

Experimental characteristics

The dairy cows of the German Friesian Breed, which were involved in this experiment, were fitted with a cannula in the dorsal part of the rumen and with a cannula at the proximal duodenum. Average liveweight of the animals was 562 kg ((50) and average milk yield was registered to be 34,5 kg FCM ((8,8). The animals were fed a daily ration consisting of 7,3 kg dry matter of corn silage and 7,3 kg dry matter of concentrate. Considering that the NEL and uCP supply met the requirements, ration were not changed during the experiment. Only the RNB of the ration was increased from -0.6 g/MJ ME to -0.3, 0 or +0.3 g/MJ ME respectively by supplements of different urea amounts to the concentrate.

The experiments comprised 4 periods, in which the animals were fed in exchange on one of the 4 concentrate types. Animals were adapted to the feed over two weeks. In experimental week 3 samples from the rumen fluid were daily taken 3 hours after feeding. In experimental week 4 samples were taken from the duodenal chyme in 2 hours intervals over 5 days. To estimate the digesta flux Cr_2O_3 was used as marker (Rohr et al., 1979).

The portion of microbial N on NAN in the duodenal chyme was analyzed applying the NIR-technique (Lebzien and Paul, 1997). Milk was analyzed for urea concentration once a week.

Results and discussion

$\text{NH}_3\text{-N}$ concentration in the rumen fluid increased from 1,6 to 28,5 mg/100 ml due to higher RNB-values, which is in accordance with results published by Steingass et al. (2001). Beside this, the $\text{NH}_3\text{-N}$ concentration in the duodenal chyme increased from 2,9 to 11,3 mg/100 ml, so did the urea concentration in milk from 13,6 to 63,0 mg/100 ml (see Figure 1), which is in agreement with data by Jilg et al. (1999). Concerning $\text{NH}_3\text{-concentration}$ in the rumen RNB-group -0.6 differed significantly from the others but not from group -0.3. However, concerning $\text{NH}_3\text{-N}$ content in the duodenal chyme values differed not significantly except from those of group 0. The $\text{NH}_3\text{-N}$ -concentration in rumen fluid of group -0.6 which amounted to 1,6 mg/100 ml was found to be remarkably lower than 5,6-8,4 mg/100 ml, which Piatkowski et al. (1990) postulated to be necessary for an optimal rumen fermentation.

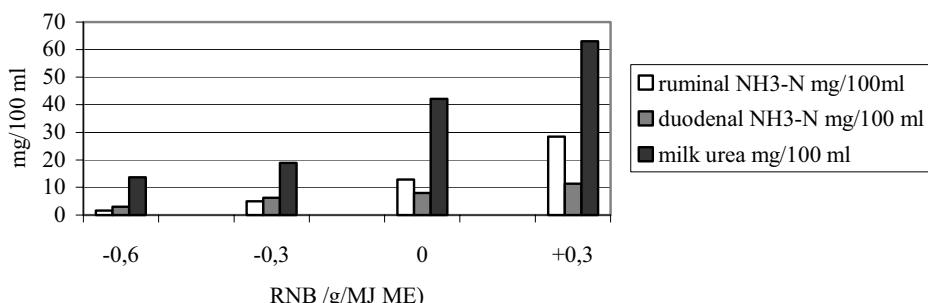


Figure 1. Average $\text{NH}_3\text{-N}$ -concentration in the rumen and the duodenal chyme, as well as urea concentration in the milk as affected by different RNB.

The RNB did not show significant effects on ruminal organic matter digestion and on efficiency of protein synthesis (g MP/MJ ME or g/kg FOM) as indicated in Table 1.

However, the quantity of microbial fermented organic matter (kg FOM) was reduced, when RNB was lowered. Beside this protein degradation increased and the quantity of UDP decreased. Due to the reduced MP and UDP quantity in the RNB-group -0.6 the uCP-quantity was lower than in the other three groups, but only significantly to the group 0 (see Table 1).

In RNB-group -0.6 the quantity of uCP was almost 200 g lower as compared to the average of the other three groups.

Effects on the pH-value in the rumen and the volatile fatty acid concentration due to differences in the ruminal nitrogen balance were not detected, what is in contrast to the results published by Steingass et al. (2001).

Table 1. Ruminal organic matter digestibility, quantity of FOM and flux of the N-fraction passing the duodenum.

| RNB(g/MJ ME) | -0.6 | -0.3 | 0 | +0.3 |
|-------------------|--------------------|---------------------|--------------------|---------------------|
| r. digest. OM (%) | 47,5 | 48,7 | 50,1 | 50,5 |
| FOM (kg/d) | 9,2 ^a | 9,4 ^{ab} | 9,9 ^b | 9,8 ^{ab} |
| NAN (g/d) | 297,4 ^a | 318,0 ^{ab} | 343,1 ^b | 322,0 ^{ab} |
| UDP (g/d) | 338 ^a | 441 ^{ab} | 499 ^b | 451 ^b |
| uCP (g/d) | 1658 ^a | 1792 ^{ab} | 1953 ^b | 1733 ^{ab} |
| MP (g/d) | 1320 | 1382 | 1454 | 1373 |
| (g/MJ ME) | 7,68 | 7,84 | 8,31 | 7,98 |
| (g/kg FOM) | 144 | 143 | 148 | 142 |

mean values in the same line not followed by the same superscript are significantly different, p ≤ 0,05

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Rumen microbial colonization and contamination of feeds*

C.A. Rodriguez¹, J. González¹, M.R. Alvir¹ & R. Redondo²

¹ Universidad Politécnica de Madrid, Departamento de Producción Animal, Ciudad Universitaria, 28040 Madrid, Spain

² Universidad Autónoma de Madrid, Servicio Interdepartamental de Investigación, Cantoblanco, 28049 Madrid, Spain

Summary

The evolution of the colonization (mg microbial DM/ 100 mg total DM) or the microbial contamination (mg microbial N/ 100 mg total N) with rumen incubation time (2, 4, 8, 16, 24 and 48h plus 72h for forages) of 5 feeds (Corn grain, Sunflower meal, Soybean meal, Vetch-oat hay and Lentil straw) was determined using 4 rumen cannulated wethers fed a ration of vetch-oat hay and concentrate (ratio 2:1 DM basis), distributed in 6 meals per day, at an intake level of 80 g DM/Kg^{0.75}. Animals were infused continuously in the rumen with a solution of (NH₄)₂SO₄ (80 mg N/d) with 50% enrichment in ¹⁵N. Labelled adherent bacteria were isolated and used as microbial reference sample. The proportion of microbial DM or N in the undegraded fraction of feed (M) increased with time until reach an asymptotic value. This evolution was established fitting the data to an exponential model ($M=m^*(1-e^{-ft})^j$) of first order ($j=1$) or of order different to 1 ($j\neq 1$). The sunflower and soybean meals presented behaviours that didn't allow the modelling of the evolution of the bacterial biomass. The comparison (test LSD) of their mean values in the different incubation times showed the existence of a growing initial phase of colonization followed by a falling phase, although, differences existed among the two feeds. The soybean meal presented a growing evolution of the colonization until the 16 hours of incubation in the rumen, decreasing later; on the other hand, the sunflower meal evolved increasing until 8 hours of incubation, followed by a decrease until the 16-24 hours, increasing again starting from this incubation time.

Keywords: rumen, microbial contamination, sheep

Introduction

The nylon bag technique is widely used for predicting ruminal degradability of dietary-N in ruminant feeds, being recognised as the official method in most of the current protein systems. However, true degradation values are always biased by the microbial contamination of feed incubated residues (Mathers J.C. *et al.*, 1981). The aim of this work was to determine the evolution of the colonization (mg microbial DM/ 100 mg total DM) and of the microbial contamination (mg microbial N/ 100 mg total N) of different feeds at a level of intake near to that *ad libitum*.

Material and methods

Four rumen cannulated adult wethers were used to determine the evolution of the colonization (mg microbial DM/ 100 mg total DM) or the microbial contamination (mg microbial N/ 100 mg total N) with rumen incubation time (2, 4, 8, 16, 24 and 48h plus 72h for forages) of 5 feeds (Corn grain, Sunflower meal, Soybean meal, Vetch-oat hay and Lentil straw). Wethers were fed a ration of vetch-oat hay and concentrate (0.607 corn grain, 0.3 dehydrated beet pulp, 0.045 soybean meal, 0.02 fish meal, 0.015 bentonite, 0.013 minerals and vitamins) in a 2:1 proportion on a DM basis,

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distributed in 6 meals per day, at an intake level of 80 g DM/Kg^{0.75}, that represent 2.2 times the maintenance requirements for energy and 3.2 times for nitrogen. The rumen microorganisms were labelled with a 50% ¹⁵N-enriched (NH₄)₂ SO₄ solution (80 mg N/d)(ICON Service Inc., New Jersey, USA). Labelled adherent bacteria were isolated and used as microbial reference sample. Statistical analyses were performed with the Statistical Analysis System for Windows software, version 6.12 (SAS Institute Inc., North Carolina, USA). The modelling of the microbial colonization/contamination were carried out by non-linear regression, in which the estimates of the parameters of each pattern were carried out for adjustment of least squares.

Results and discussion

The evolution of the colonization, in function of the incubation time, is shown in figures 1 and 2 and the corresponding to the microbial contamination in figures 3 and 4. In the cases in that it was possible, in the same figures are shown the fitting curves obtained for the group of four animals by non-linear regression. The evolution was established fitting the data to an exponential model ($M=m^*(1-e^{-f^*t})^j$) of first order ($j=1$) or of order different to 1 ($j\neq 1$). Parameters of colonization and contamination kinetics, as well as the model's type used for the non-linear regression and the coefficient of determination (R^2) are presented in table 1.

Nocek & Grant (1987) reported that the microbial contamination showed a curvilinear relationship with regard to the incubation time, being increased with the time of permanence in the rumen until a time starting from which a maximum of contamination is reached, which is not overcome although the rumen incubation time is prolonged. This fact could be conditioned by the readiness of free fixation points that could be in feed particles.

Table 1. Parameters of the kinetics of microbial colonization or contamination and the models used.

| Feed | Model ² | Colonization ¹ | | | | Contamination ¹ | | | |
|----------------|--------------------|---------------------------|------|------|----------------|----------------------------|------|------|----------------|
| | | m | f | j | R ² | m | f | j | R ² |
| Corn grain | 2 | 6.79 | 9.72 | 2.73 | 0.808 | 24.9 | 6.62 | 1.34 | 0.851 |
| Sunflower meal | 1 | N/d | n/d | -- | n/d | 27.0 | 3.13 | -- | 0.943 |
| Soybean meal | 1 | N/d | n/d | -- | n/d | 4.35 | 8.82 | -- | 0.896 |
| Vetch oat hay | 1 | 9.95 | 6.05 | -- | 0.906 | 71.1 | 5.32 | -- | 0.926 |
| Lentil straw | 1 | 7.71 | 3.89 | -- | 0.914 | 64.5 | 4.46 | -- | 0.920 |

¹ m=%; f=%/h; j=adimensional

² 1: simple exponential, 2: logistic

The sunflower and soybean meals presented behaviours that didn't allow the modelling of the evolution of the bacterial biomass. In this way, the comparison (test LSD) of their mean values in the different incubation times (Table 2) showed the existence of a growing initial phase of colonization followed by a falling phase clearly, although, differences existed among the two feeds. The soybean meal presented a growing evolution of the colonization until the 16 hours of incubation in the rumen, decreasing this starting from this hour; on the other hand, the sunflower meal evolved increasing until the 8 hours of incubation, followed by a decrease until the 16-24 hours, increasing again starting from this incubation time. Among the results corresponding to specific feeds two different behaviours can be appreciated: in the forages (vetch oat hay and lentil straw) and in the corn grain, feeds that present all of them high colonization levels, the curves stay growing in a continuous way. On the contrary, in the sunflower and soybean meals the

growing phase of colonization not stays until times excessively high of permanency of the food in the rumen (Table 2).

The evolution of the colonization observed in the sunflower and soybean meals could be indicative of the type of microorganisms associated to the feed particles, in the sense that at high ingestion level would increase the proportion of microorganisms considerably weakly associated to the particles and therefore with a higher possibility of migration toward particles with higher content or readiness of nutrients (Rodríguez C.A. et al., 2000). These feeds did not showed the effect of decrease of the microbial density in the latest stages in incubation, however, in terms of contamination, were growing along the whole studied period (Figure 3).

Table 2. Level of colonization (mg bacterial DM/100 mg DM of residue) of sunflower and soybean meals at different hours of incubation.

| Feed | Hours of incubation | | | | | |
|-----------------------------|---------------------|--------------------|--------------------|-------------------|--------------------|--------------------|
| | 2 | 4 | 8 | 16 | 24 | 48 |
| Sunflower meal ¹ | 2.10 ^{bc} | 2.58 ^{ab} | 2.71 ^a | 1.65 ^c | 1.70 ^c | 2.43 ^{ab} |
| Soybean meal ¹ | 1.52 ^e | 2.48 ^d | 3.85 ^{ab} | 4.56 ^a | 3.33 ^{bc} | 2.87 ^{cd} |

¹ Values in the same row with different superscripts differ ($P < 0.05$)

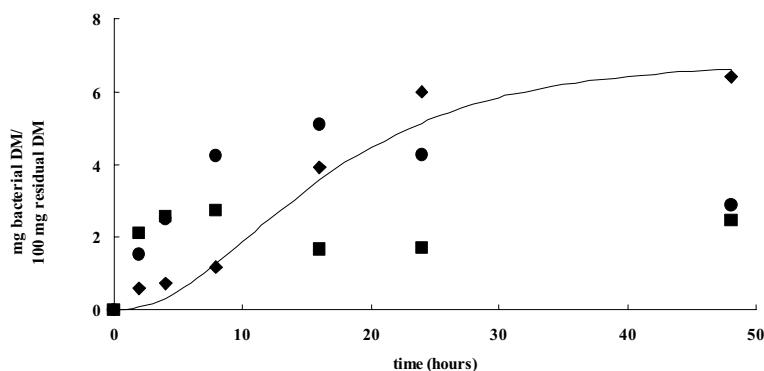


Figure 1. Evolution of the colonization for Corn grain (◆—) Sunflower meal (■) Soybean meal (●).

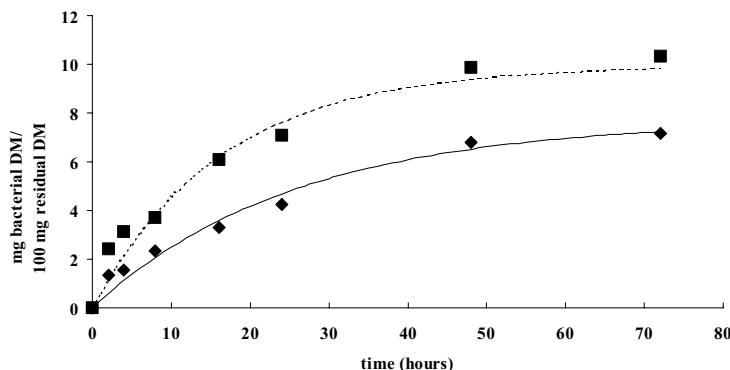


Figure 2. Evolution of the colonization for Lentil straw (◆—) Vetch oat hay (■ ···).

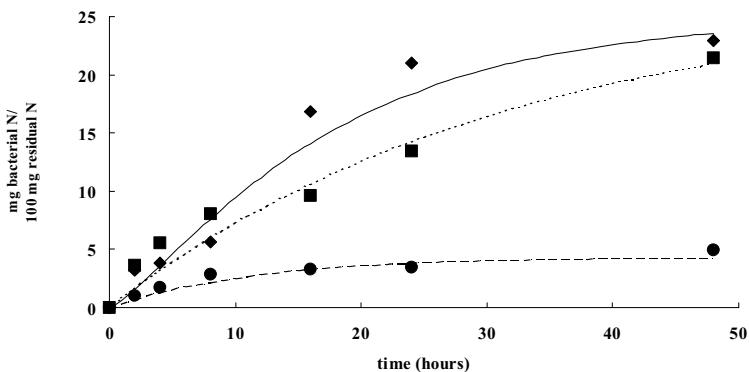


Figure 3. Evolution of the contamination for Corn grain (◆—) Sunflower meal (■···) Soybean meal (●---).

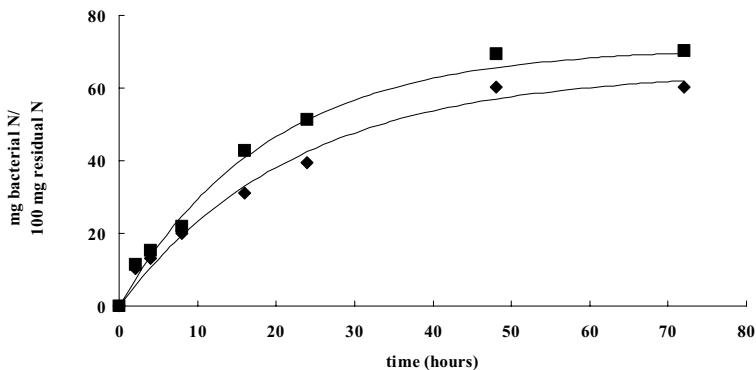


Figure 4. Evolution of the contamination for Lentil straw (◆—) Vetch oat hay (■···).

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Secretion of nitrogen into the gastro-intestinal tract and protein turnover in growing sheep

J. Voigt¹, A. Sandek², K. Krawielitzki², T. Zebrowska³, J. Kowalczyk³, M. Gabel², U. Schönhusen¹ & H. Hagemeister¹

¹ Research Institute for the Biology of Farm Animals, Research Unit Nutritional Physiology "Oskar Kellner", Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

² University of Rostock, Institute for Ecologically-Compatible Animal Husbandry, Justus-von-Liebig-Weg 8, 18059 Rostock, Germany

³ The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland

Summary

Parameters of protein turnover were measured in sheep differing in postruminal secretion and reabsorption of nitrogen. The experiments were carried out with Polish Merino sheep (20-25 kg BW) fitted with rumen and re-entrant cannulas at the duodenum and the ileum. Two groups of animals ($n = 9$) were fed with diets that were nearly isonitrogenous but differed in CF content. (CF in % DM; Group 1, 14.7; Group 2, 24.9). As a result of the higher CF content, the intake of DM was lower in Group 2 than in Group 1 (616 vs. 467 g·d⁻¹). In all individually carried out experiments, one of three animals was labelled with ¹⁵N by intraruminal infusion of ¹⁵N urea. Then duodenal and ileal digesta were exchanged between the labelled animal (No. 1) and the unlabelled ones (Nos. 2 and 3) according to a given scheme during a 48-hour period. The N- and ¹⁵N flow through the intestinal tract and N and ¹⁵N excretion in faeces and urine of the animals were estimated during that period. With these data, secretion and reabsorption of N as well as the kinetic parameters of protein turnover were calculated using the isotope dilution technique, the end-product method, and the 3-compartment model.

Nitrogen retention (104 vs. -56 mg·kg BW⁻¹ x d⁻¹) was higher in Group 1 than in Group 2 ($P < 0.10$). No differences were found in flux (1666 vs. 1458 mg N·kg BW⁻¹·d⁻¹), synthesis (1238 vs. 1087 mg N·kg BW⁻¹·d⁻¹) or breakdown (1134 vs. 1324 mg N·kg BW⁻¹·d⁻¹) of protein. However, the efficiency of protein synthesis (N balance/synthesis·100) was higher in Group 1 (8.8 vs. -5.2 %) ($P < 0.10$). The efficiency of protein synthesis correlated negatively with N secretion in the postruminal tract ($r = -0.99$; $P < 0.001$) and with the ileal flow of endogenous N ($r = -0.95$; $P < 0.01$). In conclusion, high intestinal N secretion adversely affects the efficiency of whole body protein synthesis in growing sheep.

Keywords: whole-body protein turnover, intestinal N secretion, efficiency of protein synthesis

Introduction

Tissue protein retention of growing animals or humans is the result of two opposing, dynamic processes: protein synthesis and protein breakdown. Determination of such kinetic parameters of protein turnover as synthesis and breakdown may be of importance for animal- as well as human nutrition and allow optimising protein gain or minimising protein loss during growth. The efficiency of protein synthesis, i.e. the proportion of synthesised protein that is deposited, is considerably lower in the whole body than in some organs, e.g. muscles (Simon, 1989).

The reason for this is the much higher protein synthesis rates and lower protein deposition rates in some tissues, such as the liver or gastrointestinal tract. Secretion and partial reabsorption of protein in the gastrointestinal tract are part of whole body protein turnover. However, the secreted and

excreted proteins are not measured as protein deposition. It can thus be assumed that protein secretion and the efficiency of whole body protein synthesis are related. Therefore the aim of this study was to analyse whole body protein turnover in growing sheep differing in gastrointestinal secretion and reabsorption of nitrogenous compounds.

Material and methods

Eighteen male lambs (Polish Merino, 20–25 kg BW) were divided into two groups (each group 3 x 3 animals). All of the animals were fitted with rumen and re-entrant cannulas in the proximal duodenum and the distal ileum. Group 1 received a ration containing 14.7 g CF/100 g DM, 16.3 g CP/100 g DM, and 11 MJ ME/kg DM, whereas Group 2 was fed a ration containing 24.9 g CF/100 g DM, 15.7 g CP/100 g DM, and 10 MJ ME/kg DM. The DM intake was 616 g·d⁻¹ and 467 g·d⁻¹ in Group 1 and Group 2, respectively. The diets were offered as six meals per day.

Three sheep were used per experiment. One of them (animal No. 1) was infused intraruminally with ¹⁵N urea to label the metabolic N pool. After 6 d of labelling the duodenal and ileal digesta were exchanged for the following 48 h between the labelled animal and the unlabelled ones as shown in Figure 1:

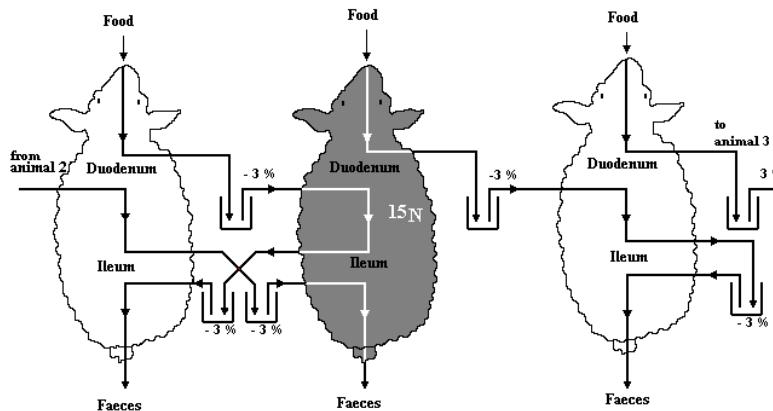


Figure 1. Schematic diagram of digesta exchange between labelled and unlabelled sheep.

During the two-day digesta exchange period, N and ¹⁵N passage along the digestive tract of the animals were estimated in order to calculate the secretion and reabsorption of N using the isotope dilution technique. The details were described before (Sandek et al., 2002). Using the absorbed- and urinary-excreted ¹⁵N in animal 2 and the net-N absorption as well as urinary N excretion of all 3 animals, the turnover rates of whole body protein metabolism were calculated. For this purpose the end-product method and the 3-pool model of Sprinson and Rittenberg (1949) as modified by Krawielitzki et al. (1989) were used. At the end of each experiment, the animals were slaughtered and the whole-body N content was estimated.

Results and discussion

In comparison with Group 2, N retention was significantly higher in Group 1 ($P < 0.10$; Table 1). No difference could be observed in N flux, protein synthesis, protein breakdown or in the reutilization rate of N. However, the fractional deposition rate and the efficiency of protein synthesis (N retention/synthesis·100) were significantly greater in Group 1 ($P < 0.10$). In this Group fed a diet with a lower CF content, N secretion in the small intestine was significantly ($P < 0.10$) lower than in Group 2 given a diet with a higher CF content.

Table 1. Parameters of N secretion and protein turnover in two groups of growing sheep fed a low- (Group 1; n=2) and high- (Group 2; n=3) fibre diet.

| Item | Group 1 | | Group 2 | |
|--|-------------------|------|--------------------|------|
| | Mean | SEM | Mean | SEM |
| N secretion in small intestine (g·kg DMI ⁻¹) | 9.37 ^a | 0.74 | 16.05 ^b | 1.53 |
| N secretion in large intestine (g·kg DMI ⁻¹) | 3.79 | 1.20 | 2.20 | 0.03 |
| Ileal flow of secreted N (g·kg DMI ⁻¹) | 2.67 ^a | 0.63 | 4.70 ^b | 0.34 |
| Protein turnover | | | | |
| Whole body N (g N·kg BW ⁻¹) | 25.8 | 0.2 | 25.1 | 0.7 |
| N retention (mg N·kg BW ⁻¹ ·d ⁻¹) | 104 ^a | 51 | -56 ^b | 23 |
| N flux (mg N·kg BW ⁻¹ ·d ⁻¹) | 1666 | 51 | 1458 | 104 |
| Synthesis (mg N·kg BW ⁻¹ ·d ⁻¹) | 1238 | 87 | 1087 | 94 |
| Breakdown (mg N·kg BW ⁻¹ ·d ⁻¹) | 1134 | 137 | 1144 | 100 |
| Reutilization (%) | 74.3 | 2.9 | 74.4 | 1.7 |
| Efficiency of protein synthesis ¹ (%) | 8.8 ^a | 4.8 | -5.2 ^b | 2.1 |
| Fractional synthesis rate (%·d ⁻¹) | 4.8 | 0.4 | 4.3 | 0.3 |
| Fractional breakdown rate (%·d ⁻¹) | 4.4 | 0.6 | 4.5 | 0.3 |
| Fractional deposition rate (%·d ⁻¹) | 0.4 ^a | 0.2 | -0.2 ^b | 0.1 |

¹N retention/synthesis·100

a, b significant difference between diet groups (P<0.10)

As shown in Figure 2, there existed a significant negative relation between the efficiency of protein synthesis and N secretion in the postruminal tract ($r = -0.99$) and the ileal flow of endogenous N ($r = -0.95$). Hence, it can be concluded that the processes of postruminal N secretion and reabsorption influence whole-body protein turnover significantly. Part of the total synthesised protein is secreted into the intestinal tract. The secretion and reabsorption of protein in the intestine are part of whole-body protein turnover and cause unavoidable protein losses.

Conclusion

In conclusion, the efficiency of whole-body protein synthesis is significantly negatively influenced by N secretion in the postruminal tract. The higher the intestinal N secretion, the lower the efficiency of protein synthesis. Nitrogen secretion is stimulated by dietary factors such as CF content (Sandek et al., 2002) or intestinal passage of NDF (Zebrowska and Kowalczyk, 1991; Lammers-Wienhoven et al., 1998). This loss due to N secretion should be considered in the evaluation of feed proteins for ruminants as has been done by introducing the factor "q" in the energy evaluation system (Van Es, 1975).

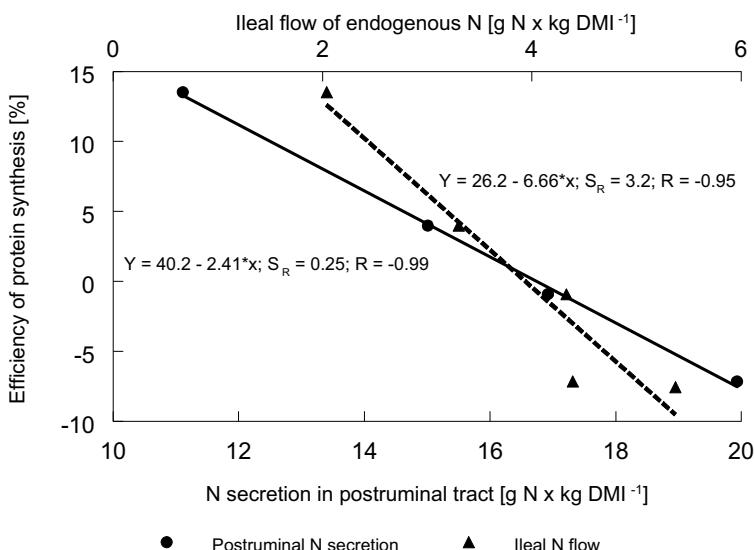


Figure 2. Relations between efficiency of protein synthesis and postruminal secretion of N.

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Use of pymarc as a nitrogen source for grazing dairy calves

J.M. Waweru¹, S.A. Abdulrazak^{1*}, T.A. Onyango², J.O. Ondiek¹ & T. Fujihara³

¹ Department of Animal Science, Egerton University P.O Box 536 Njoro - Kenya

² National Animal Husbandry Research Centre, P.O Box 25 Naivasha, Kenya

³ Shimane University, Matsue-shi 690, Japan

*Corresponding author

Summary

A study was conducted in Ngongongeri farm of Egerton University Kenya to determine optimal levels of pymarc inclusion as a protein supplement to *Chloris gayana* during the dry season. Liveweight gains, intake, diet digestibility, rumen pH and rumen ammonia nitrogen were assessed in the 60 days experiment. Forty dairy calves 65 ± 7 kgs, 20 each of male and female were randomly allocated to a 10-diet treatment in a completely randomized design in a factorial arrangement. The treatment diets were: control, 7.5, 15, 22.5 and 30gDM/kgW^{0.75} pymarc, with or without molasses (PBM and PB). Herbage intake did not differ ($P>0.05$) among the treatments. Total intake, diet digestibility and ADG differed ($P < 0.05$) with supplementation. The results showed that rumen pH did not differ significantly ($p > 0.05$) between the treatments, ranging between 6.97 and 7.17. Rumen NH₃- N control groups PB and PBM had 109.9 and 106.5 mg/l respectively while those supplemented increased linearly ($P<0.05$) to 166.5 and 177.14 mg/l respectively at the highest level of supplementation. It was concluded that pymarc was a good dry season nitrogen supplement.

Keywords: pymarc, molasses, calves, palatability, rumen ammonia nitrogen, rumen pH, average daily gain

Introduction

The demand for animal products in human diet is steadily and substantially increasing with the increasing population which is expected to be higher than the production by the year 2010. This demands an increase of livestock production (output) and productivity (output per unit input) (Delgado *et al.*, 2001). However, lack of adequate quantity and quality of feed is a major constraint especially in the dry season (Walshe *et al.*, 1991). This is evidenced by high calf mortality (15 - 20%), morbidity and low body weight gain of calves at farm levels (Gitau *et al.*, 1994). This scenario has led to diminishing replacement stocks, while delaying age at first service, or more likely the servicing of heifers with a poor weight for age which, inevitably result to poor heifer conception and lactation. By-products like pymarc may have potential to mitigate feed shortage. Extensive work has been done on the nutritional value of by-products such as fishmeal, oilseeds, molasses, bran etc, however, limited work has been reported on pymarc as a supplement for growing calves. The objectives of this experiment were to determine the potential nutritive value of Pymarc based on chemical composition, fibre, minerals, Phenolic concentration, *in-vitro* and *in-Sacco* degradation.

Materials and method

Chemical analysis

Dry matter (DM), ash and nitrogen (N) content was measured according to AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) was determined according to Van Soest *et al.* (1991). Mineral content was determined by atomic absorption spectrophotometry (Varma, 1991). Phenolic compounds were determined as described by Julkunen-Titto (1985).

In-sacco and In-vitro digestibility

The rate and extent of degradation of the Pymarc was determined in fistulated Friesian steers using the nylon bag technique (Ørskov *et al.*, 1980). Samples were incubated *in-vitro* in rumen fluid-buffer mixture in calibrated glass syringes following the procedure of Menke and Steingass (1988).

Feeds and supplements

The calves were grazed on *Chloris gayana* pasture, supplemented with 100g bran and increasing level of pymarc at Control, 7, 15, 22.5 and 30gDM/Kg $W^{0.75}$ with or without molasses (PBM and PB). A mineral lick which contained ca 17.2%, P 12%, NaCl 18.4%, Mg 0.4%, Zn 0.7%, I 0.05%, Cu 0.2 % and Se 0.002%, fresh clean water was offered at all times.

Measurement of intake and digestibility

Intake was estimated from the ratio between the faecal output, collected for the portion attributable to the concentrate and indigestibility of the herbage (Malossini *et al.*, 1995). HI (Kg OM day $^{-1}$) = (D x R/F - 1c x (1 - OMDc))/1 - OMDh). The digestibility of the supplemented animals was calculated by fitting the values to the formular of Malossini *et al.* (1995) assuming no supplement was fed OMDh = 1 - (D x R)/F) / HI (Kg OM/day)

Statistical analysis

Was done using GLM procedure of SAS computer package with initial liveweight as covariates.

Results and discussion

The results of chemical composition indicated a CP of 14 %, NDF 45.08%, ADF 33.91% and ADL 10.50% and were within the ranges reported in other work on Pymarc (Irungu *et al.*, 1981; Kitilit *et al* 1996). Mineral concentration compares and also contrasts with what has been reported in other work with pymarc (Griffin, 1974). Calcium is closely related to phosphorus metabolism in the formation of bones and Ca: P ratio of 2:1 is recommended, Griffins, 1974 reported a ratio of 1.88:1 while ratios of 4.625:1 was obtained in this work. This indicates pymarc is unlikely to be a well-balanced source of minerals. Factors such as soils, climate, stage of maturity and season contribute to variation in the concentration of minerals (Le Houerou, 1980;Topps, 1992).

The results on supplementation of *Chloris gayana* pasture with pymarc diets with or without molasses (PBM and PB) respectively from 7.5 to 30g DM/Kg $W^{0.75}$ did not affect the herbage intake ranging from 1699 to 1899 g/d, it led to a significant linear increase ($p<0.05$) in total DMI. The lack of increase in intake of the basal diet is suggested to be due to adequate content of CP in the basal diet of *Chloris gayana* (72 g/Kg DM), which meant that intake was not limited. Therefore, since the rumen microbes requirements for nitrogen had been met, additional high

quality pymarc had no further stimulating effect on the intake of the basal diet. Gulbransen (1974) reported that supplements substitute part of the basal diet, however, he showed further that the degree of substitution was greater for poor quality forage than high quality forage. The increases in total DMI results are consistent with other work of (Irungu *et al.*, 1981; Kitilit *et al.*, 1996). An increase in intake could probably be a reflection of the small particle size of pymarc, which increase the outflow rate and reduce the rumen retention time hence boosting intake. Minson (1982) reported increased intake by 14 - 77% following provision of supplementary protein. An establishment of a suitable rumen environment that aid in digestion could also explain intake.

Table 1. Intake, ADG, digestibility, pH and Rumen NH₃-N in calves grazed Rhodes grass pasture supplemented with pymarc at 7.5, 15, 22.5 and 30g DM/Kg w^{0.75}.

| | | Inclusion level of pymarc (g DM/Kg w ^{0.75}) | | | | | | |
|--------------------------|---|--|---------------------|---------------------|---------------------|--------------------|---------------------|-------|
| | | Molasses | 0 | 7.5 | 15 | 22.5 | 30 | SEM |
| Diet intake | | | | | | | | |
| DMI (g/d) | B | - | 1699 ^a | 1825 ^a | 1914 ^a | 1913 ^a | 1856 ^a | 0.09 |
| | | + | 1719 ^a | 1870 ^a | 1933 ^a | 1910 ^a | 1899 ^a | |
| | T | - | 2072 ^c | 2304 ^{bc} | 2503 ^{ab} | 2597 ^a | 2595 ^a | 0.09 |
| | | + | 2113 ^c | 2304 ^{bc} | 2414 ^{ab} | 2636 ^a | 2625 ^a | |
| OMI (g/d) | B | - | 1699 ^a | 1825 ^a | 1914 ^a | 1926 ^a | 1856 ^a | 0.09 |
| | | + | 1719 ^a | 1870 ^a | 1933 ^a | 1910 ^a | 1899 ^a | |
| | T | - | 1790 ^c | 2011 ^b | 2287 ^a | 2483 ^a | 2336 ^a | 0.08 |
| | | + | 1785 ^c | 2188 ^b | 2356 ^a | 2538 ^a | 2617 ^a | |
| ADG (g/d) | - | | 157 ^a | 228 ^b | 276 ^c | 293 ^c | 296 ^c | 4.77 |
| | + | | 169 ^a | 241 ^b | 289 ^c | 308 ^{cd} | 330 ^d | 3.97 |
| Digestibility | | | | | | | | |
| DMD g/Kg DM | - | | 565 ^c | 570 ^b | 570 ^b | 570 ^b | 582 ^a | 0.1 |
| | + | | 565 ^c | 570 ^b | 570 ^b | 571 ^b | 573 ^b | |
| OMD g/Kg DM | - | | 571.26 ^e | 581.29 ^d | 597 ^c | 611 ^b | 624 ^a | 0.16 |
| | + | | 570.26 ^e | 580.85 ^d | 596 ^c | 612 ^b | 625 ^a | |
| pH | - | | 7.06 ^a | 7.04 ^a | 7.07 ^a | 6.97 ^a | 7.17 ^a | 0.032 |
| | + | | 7.11 ^a | 7.14 ^a | 7.08 ^a | 6.99 ^a | 6.99 ^a | |
| Rumen NH ₃ -N | - | | 109.9 ^a | 138.2 ^b | 155.9 ^{bc} | 170.1 ^c | 166.5 ^c | 3.84 |
| | + | | 106.3 ^a | 148.8 ^b | 155.9 ^{bc} | 166.5 ^c | 177.14 ^c | |

^{a,b,c} Means within a row with different superscript are significantly different ($P<0.05$); B = basal diet
T = total diet

ADG gain increased linearly ($P<0.05$) and the highest value of 330g/d and 296 g/d were obtained at 30g DM/Kg w^{0.75} for PBM and PB respectively. However, there was no difference ($P>0.05$) at the level at 22.5 DM/Kg W^{0.75} and 30g DM/Kg W^{0.75} level of PBM and PB inclusion. The mean liveweight gain of control groups PBM and PB was 157 and 167 g/day respectively, while those supplemented gained 296 and 330g/d for the two groups respectively. Apparent digestibility of the diet increased significantly ($P<0.05$) from 565 to 582g/KgDM for PB and from 565 to 573g/KgDM for PBM This probably occurred as a result of the higher nutritive value of pymarc and the reduction on the fibre and improved rumen environment - pH of 6.99 - 7.17; and NH₃- N 106.3- 177.14 mg/l.

It was concluded that pymarc is a good nitrogen source for dry season feeding.

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Effect of including purified jack bean lectin in a casein based diet on apparent and true ileal amino acid digestibility in growing pigs

Y.L. Yin^{1,2}, R.L. Huang^{1,2}, C.F.M. de Lange¹ & M. Rademacher³

¹ Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

² Changsha Institute of Agricultural Modernization, the Chinese Academy of Science, Hunan, Changsha, P.O.Box 10, P.R.China 410125

³ Degussa AG, P.O. Box 1345, D-63403, Hanau, Germany

Summary

The effect of including purified Jack Bean lectin (Concanavilin A) in enzymatically hydrolyzed casein (EHC) and regular casein (NC) diets on ileal endogenous amino acid (AA) flow and apparent and true ileal digestibility of AA was investigated in growing pigs. The inclusion of lectin did not influence apparent and true ileal digestibility of CP and AA, and endogenous flow of AA. Pigs fed the EHC based diets had lower apparent ileal AA digestibility than pigs fed the NC based diets, which can be attributed to differences in endogenous ileal AA flows. These data suggest that including purified Jack Bean lectin at the level of 266 mg/kg in a casein based diet did not influence ileal endogenous AA flow, as well as ileal digestibility of AA. Compared with the homo-arginine method, the peptide-alimentation method yielded higher estimates of ileal endogenous AA flows, although these two methods yielded similar estimates of true ileal AA digestibility.

Keywords: growing pigs, lectin, endogenous amino acids

Introduction

It has been demonstrated that incorporation of raw legumes in the diet reduces animal productivity. Protease inhibitors that are present in legumes may contribute to the poor digestion and reduced absorption of nutrients (de Lange et al., 2000). There is increasing evidence that dietary lectin may contribute to reduced animal performance as well. However, there is a scarcity of information on the effect of lectin on ileal endogenous amino acid (AA) flow and ileal AA digestibility. Schulze (1994) studied the effect of added purified Soyabean lectin on ileal endogenous nitrogen (N) flow and observed a linear effect of diet lectin level on endogenous N flow.

A range of methods is available to estimate endogenous ileal AA flows in pigs, while each method has some limitations (Nyachoti et al., 1997a). Recent and more advanced methods (isotope dilution, homo-arginine, peptide-alimentation) all require assumptions when estimating these flows in pigs fed protein-containing diets.

The objectives of this study were to investigate the effect of including purified Jack Bean lectin (Concanavilin A) in the diet on ileal endogenous AA flow, and ileal AA digestibility. Both the homo-arginine method and peptide-alimentation method were used to estimate endogenous ileal AA flows.

Materials and methods

Four Large White castrated male pigs from one litter and with a mean initial live body weight of 25 kg were fitted with a simple a T-cannula at the terminal ileum. The animals were fed one of four experimental diets: (1) an enzymatically hydrolyzed casein (EHC, MW<5000) containing diet, (2) an EHC containing diet supplemented with 266 mg/kg of Jack Bean lectin, (3) a regular casein

(NC) containing diet, and (4) a NC containing diet supplemented with 266 mg/kg of lectin. For the estimation of ileal endogenous AA flows in pigs fed NC diets, a sub-sample of NC was guanidinated according to procedures described by Nyachoti et al. (1997b). The four diets were administered to four pigs based on 4 x 4 a Latin Square design. The pigs were randomly allocated to the initial experimental diets and received their respective diets for 8 d. Pigs were fed twice daily, at 0830 and 1800 h, equal amounts of feed at 2.6 x maintenance energy requirements. In each experimental period, pigs were adjusted to experimental diets for 5 d. On d 6, ileal digesta was collected continuously for 24 h for determining apparent ileal digestibility (AID). At 0830 h of d 8 the HA-NC diets were fed to pigs and ileal digesta was again collected for 24 h from all pigs for determining AID and true ileal digestibility (TID) of AA.

Results and discussion

The addition of lectin did not influence AID of DM, CP and AA (Table 1), endogenous flows of CP and AA (Table 2) and TID of CP and AA (Table 3). In contrast, Schulze (1994) observed a linear increase in ileal endogenous N flow with diet soybean lectin level in young piglets (13 kg BW): up to 0.62 g/d at a diet lectin level of 960 mg/kg. Difference between these two studies may be attributed to various factors. Firstly, the pigs used in current study were heavier than those used by Schulze (1994). With increasing BW pigs may have increased capacity to tolerate the negative effect of dietary lectin. Secondly, across dietary sources, lectins vary in chemical composition and structure and may thus have different effects on animals (de Lange et al., 2000). The effects of feeding different types of lectin to pigs at various stages of development need to be explored further.

Table 1. Apparent ileal digestibility of DM, CP and selected AA (%) for the enzymatically hydrolyzed casein based diet (EHC), EHC based diet supplemented with lectin, normal casein based diet (NC) and NC based diet supplemented with lectin (n= 8 per mean).

| | Casein | | Lectin | | SEM | Probability of effects (P) | | |
|---------------|--------|------|--------|------|------|----------------------------|--------|-----------------|
| | EHC | NC | - | + | | Casein | Lectin | Casein × Lectin |
| Dry matter | 89.9 | 90.6 | 90.2 | 90.2 | 0.31 | 0.135 | 0.996 | 0.401 |
| Crude protein | 89.2 | 92.6 | 91.9 | 89.9 | 0.83 | 0.028 | 0.129 | 0.513 |
| Cysteine | 72.3 | 78.9 | 75.4 | 75.8 | 1.46 | 0.018 | 0.836 | 0.191 |
| Isoleucine | 92.8 | 94.7 | 94.1 | 93.4 | 0.40 | 0.017 | 0.244 | 0.613 |
| Leucine | 94.7 | 96.6 | 96.0 | 95.3 | 0.33 | 0.007 | 0.172 | 0.604 |
| Lysine | 95.2 | 96.4 | 96.2 | 95.4 | 0.41 | 0.072 | 0.213 | 0.994 |
| Methionine | 93.9 | 97.5 | 96.0 | 95.5 | 0.34 | 0.001 | 0.352 | 0.730 |
| Threonine | 86.7 | 90.6 | 89.1 | 88.1 | 0.70 | 0.007 | 0.338 | 0.462 |

Casein type did not affect ileal DM digestibility and TID of AA. However, pigs fed the NC diet had higher AID of AA than pigs fed the EHC based diets (Table 1). The observed lower AID of AA in the EHC diets, as compared to NC diets, can be attributed to higher endogenous ileal AA flows in pigs fed EHC diets (Table 2). Endogenous ileal AA flows were generally higher in pigs fed EHC diets than in pigs fed NC diets. Hodgkinson et al. (2000) demonstrated the stimulating effect of diet EHC level on ileal endogenous N and AA flow in growing pigs: mean ileal endogenous ileal N flows were 1.75, 1.95, 2.85 and 5.74 g/kg DM intake when diets contained 0 (N-free), 5, 10 and 20 % EHC, respectively. Libao (2002) showed that the inclusion of 4.0% additional NC in the diet did not increase endogenous ileal AA flows, which is consistent with

previous observations by Souffrant et al., (1997). This suggests that effects of feeding NC on endogenous gut AA flows are different from EHC. Apparently EHC contains peptides or peptones that have physiological effects on visceral organs, which may increase N secretion into the gut lumen (Seve & Lahaye, 2003). Dietary peptide administration has been shown to increase secretions of hormones involved in regulation of digestive function (Hansen & Holst, 2002). However, casein type did not affect TID of CP and AA. Values for TID ranged between 98 and 111% (Table 3), which are not different from 100% and very close to values reported by Nyachoti et al. (1997b) and Libao (2002).

In conclusion, including purified Jack Bean lectin (Concanavilin A) at the level of 266 mg/kg in a casein based diet did not influence the ileal endogenous CP and AA flow, as well as apparent and true ileal digestibility of CP and AA, in growing pigs between 25 and 50 kg body weight. Compared with the homo-arginine method, the peptide-alimentation method yielded higher estimates of ileal endogenous CP and AA acid flows in pig fed casein based diets, although these two methods yielded similar estimates of true ileal AA and CP digestibility.

Table 2. Endogenous flows of CP and selected amino acids (g/kg DM intake) of pigs fed enzymatically hydrolyzed casein based diet (EHC), EHC based diet supplemented with lectin, guanidinated normal casein based diet (HA-NC) and the HA-NC based diet supplemented with lectin (n=8 per mean).

| | Diet | | Lectin | | SEM | Probability of effects (P) | | |
|---------------|------|------|--------|------|-------|----------------------------|--------|-----------------|
| | EHC | NC | - | + | | Diet | Lectin | Casein × Lectin |
| Crude protein | 24.6 | 14.4 | 18.0 | 20.0 | 2.81 | 0.042 | 0.764 | 0.978 |
| Cystine | 0.37 | 0.25 | 0.32 | 0.31 | 0.045 | 0.120 | 0.950 | 0.673 |
| Isoleucine | 0.89 | 0.46 | 0.69 | 0.67 | 0.100 | 0.022 | 0.870 | 0.648 |
| Leucine | 1.11 | 0.59 | 0.82 | 0.88 | 0.145 | 0.046 | 0.778 | 0.984 |
| Lysine | 0.88 | 0.49 | 0.67 | 0.69 | 0.370 | 0.080 | 0.883 | 0.935 |
| Methionine | 0.25 | 0.13 | 0.19 | 0.19 | 0.030 | 0.034 | 0.978 | 0.753 |
| Threonine | 1.34 | 0.17 | 0.77 | 0.74 | 0.143 | 0.001 | 0.893 | 0.837 |

Table 3 True ileal digestibility of crude protein and selected amino acids (%) for the enzymatically hydrolyzed casein based diet (EHC), EHC based diet supplemented with lectin, normal casein based diet (NC) and NC based diet supplemented with lectin (n= 8 per mean).

| | Diet | | Lectin | | SEM | Probability of effects (P) | | |
|---------------|-------|-------|--------|-------|------|----------------------------|--------|---------------|
| | EHC | NC | - | + | | Diet | Lectin | Diet × Lectin |
| Crude protein | 100.8 | 99.9 | 100.3 | 100.4 | 1.65 | 0.688 | 0.972 | 0.811 |
| Cystine | 111.2 | 101.4 | 109.1 | 103.5 | 5.03 | 0.221 | 0.468 | 0.695 |
| Isoleucine | 101.7 | 99.2 | 100.7 | 100.2 | 1.07 | 0.156 | 0.737 | 0.935 |
| Leucine | 101.0 | 99.7 | 100.5 | 100.3 | 0.89 | 0.356 | 0.879 | 0.771 |
| Lysine | 101.2 | 99.7 | 100.6 | 100.2 | 0.99 | 0.329 | 0.773 | 0.993 |
| Methionine | 98.7 | 99.9 | 99.50 | 99.1 | 0.60 | 0.198 | 0.700 | 0.959 |
| Threonine | 103.0 | 92.6 | 98.4 | 97.2 | 1.89 | 0.008 | 0.661 | 0.905 |

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Protein session 2

Amino Acid Requirement and Metabolism

Intestinal transport of amino acids, peptides and proteins

M. Brandsch¹ & C. Brandsch²

¹ Membrane Transport Group, Biozentrum, Martin-Luther-University Halle-Wittenberg, Weinbergweg 22, D-06120 Halle, Germany

² Institute of Nutritional Sciences, Agricultural Faculty, Martin-Luther-University Halle-Wittenberg, Emil-Abderhalden-Strasse 26, D-06108 Halle, Germany

Summary

In animals, the nutritional needs for amino acids are met by the assimilation of dietary proteins. Digestion of proteins in the lumen of the gastrointestinal tract generates products which are absorbable by enterocytes. These products are mainly amino acids and small peptides. In addition to amino acids and peptides originating from protein digestion, non-proteinogenic free amino acids present in the food such as taurine are also absorbed at the intestinal epithelium. The paracellular pathway for absorption of amino acids and larger molecules is restricted by tight cell junctions. The transcellular route requires transport across two morphologic and functional different membranes of the enterocyte: The apical (luminal) brush border membrane and the basolateral (abluminal, serosal) membrane. At both membranes, multiple transport systems are expressed to handle the different digestion end products. So far, seven different amino acid transport systems in the apical membrane and seven in the basolateral membrane of enterocytes are known on a functional and molecular level. The carriers take up amino acids either against a concentration gradient by using inwardly directed Na^+ - or H^+ -gradients or in amino acid exchange or they catalyse facilitated diffusion. In addition to the amino acid transporters, enterocytes express in their apical membrane an active transport system for di- and tripeptides. In the basolateral membrane, a peptide transport system has been characterized functionally but not yet on a molecular level. Intestinal amino acid and peptide transport is under regulatory control of intracellular and extracellular signals. Under certain circumstances large proteins can be absorbed intact at the intestinal epithelium by endocytotic mechanisms.

Keywords: protein digestion, PEPT1, cell culture

Introduction

The process of protein digestion in the lumen of the gastrointestinal tract of animals generates products which are absorbable by the enterocyte. The end product is a mixture of free amino acids and small peptides contacting the epithelial barrier of the small intestine. Lively debates and speculations on the possible digestive and metabolic transformations which nutrients undergo during their assimilation took place as far back as the late 18th and early 19th century (for review see Matthews, 1975). At the beginning of the 20th century Emil Abderhalden discussed whether proteins are absorbed intact or broken down completely to the level of amino acids (Abderhalden, 1905). He measured the resistance of various peptides versus hydrolysis and speculated already about the maximal size of peptides absorbed in the gut. When the techniques for the determination of amino acids but not peptides in blood had become available the dogma arose that all proteins are digested to their constituent amino acids (Van Slyke & Meyer, 1912). This idea prevailed for the next 50 years. In the late fifties and early sixties of the 20th century peptide transport as a discipline was rediscovered (Newey & Smyth, 1959). First, several authors could prove the appearance of intact dipeptides at the serosal side of the intestinal epithelium. Next it was shown that this uptake was saturable, i.e. carrier mediated, followed by the observation that the uptake in

whole tissue preparation was uphill, i.e. concentrative (for review see Matthews, 1975). So, the quest for the driving force began. It was the use of membrane vesicles that made it possible to resolve the longstanding argument over the energy source for active peptide transport (Ganapathy & Leibach, 1983).

Several studies have indicated that the capacity of amino acid uptake is greater from dipeptides than from mixtures of the respective free amino acids (Rerat *et al.*, 1992; Steinhardt & Adibi, 1986). The two groups of end products are absorbed at different rates in different sections of the small intestine. The absorptive capacity for small peptides is greater in the proximal small intestine than in the distal small intestine whereas in the case of amino acids the absorptive capacity is greater in the distal small intestine than in the proximal small intestine (for review see Matthews, 1975; Ganapathy *et al.*, 1994). These differential gradients along the jejunulo-ileal axis may have importance to the maintenance of protein nutrition.

The current scheme of intestinal assimilation of proteins is given in Figure 1. Luminal digestion of proteins is carried out by gastric and pancreatic proteases. The resultant end products, mostly large peptides, undergo further hydrolysis by a variety of peptidases present in the brush border membrane of the intestinal epithelium. Analysis of luminal contents after a protein meal has shown that amino acids are present in the lumen primarily in peptide form rather than in free form (for review see Ganapathy *et al.*, 1994; Ganapathy *et al.*, 2001).

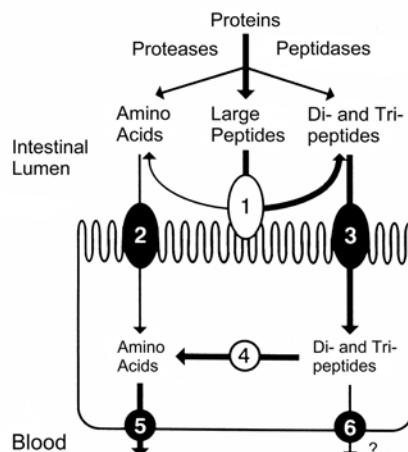


Figure 1. Digestion and absorption of proteins in the mammalian small intestine. Brush border membrane: 1, peptidases; 2, amino acid transport systems; 3, peptide transporter. 4: cytosolic peptidases. Basolateral membrane: 5, amino acid transport systems; 6, peptide transport system.

Free amino acids are absorbed into the enterocyte across the brush border membrane via group-specific amino acid transport systems. Di- and tripeptides are transported across the brush border membrane by the intestinal peptide transporter. Inside the cells the amino acids are either used for cell metabolism or undergo basolateral efflux. The highly active cytosolic peptidases rapidly hydrolyse most of the di- and tripeptides entering the cells and generate free amino acids. The basolateral membrane of the enterocyte possesses group-specific amino acid transport systems which are responsible for the exit of amino acids from the cell into the circulation. The basolateral amino acid transport systems are also capable to take up amino acids from the blood into the enterocyte. Peptides which are resistant to cytosolic peptidases may be transported intact across the basolateral membrane to the blood, but this route contributes very little to the total protein absorptive process.

Early studies on amino acid and peptide transport were often done by feeding proteins to animals and analysing luminal contents, tissues and blood. Perfusion experiments *in vivo* and *in situ*, incubation of everted sacs and rings and the Ussing chamber technique have been used for at least 50 years and are still used in laboratories today. Many results of the past 30 years were obtained in experiments with isolated cells, brush-border membrane vesicles and cultured cell lines such as the human carcinoma cell line Caco-2. Twelve years ago, the era of cloning of mammalian amino acid and peptide transporters began (Kim *et al.*, 1991; Wang *et al.*, 1991; Bertran *et al.*, 1992; Fei *et al.*, 1994). The cloned transport systems are being heterologously expressed in *Xenopus laevis* oocytes, mammalian cells or in yeast cells to study their mechanism, specificity and regulation (for review see Daniel, 1996; Ganapathy *et al.*, 2001).

Today, the first knockout animals are available to study phenotypic physiological and pharmacological relevance of a particular amino acid or peptide carrier (Peghini *et al.*, 1997; Tanaka *et al.*, 1997). Regarding the molecular three-dimensional structures of the proteins in the cell membrane, so far not one of the mammalian amino acid or peptide transporters has been studied by X-ray crystallography (for all currently known structures of membrane proteins see http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html). In the present review selected aspects of intestinal amino acid, peptide and protein transport are discussed from a physiological and nutritional point of view. Recent detailed reviews available in the literature on the topic of amino acid and peptide transport systems, their molecular and structural features and their physiological and pharmacological relevance are by Ganapathy *et al.*, 1994; Daniel, 1996; Fei *et al.*, 1998; Palacin *et al.*, 1998; Ganapathy *et al.*, 2001; Bröer, 2002; Herrera-Ruiz & Knipp, 2003 and in Hediger, 2003.

Entry of amino acids into the enterocyte across the brush border membrane

The transporters responsible for uphill transfer (concentrative transport, accumulation) of amino acids across the intestinal brush border membrane utilize one or more of at least five different driving forces: A Na^+ -gradient, a K^+ -gradient, a Cl^- -gradient, a H^+ -gradient and a membrane potential. Enterocytes possess mechanisms to establish these ionic and electrical gradients across the brush border membrane. The primary mechanism in the generation of these driving forces is the action of the Na^+-K^+ -ATPase, localized in the basolateral membrane of the enterocyte. Several amino acid transport systems are energized for active transport by the inwardly directed Na^+ -gradient. Accordingly, these transport systems are called secondary active symport systems, because they catalyse the transport of their substrate with Na^+ in the same direction. There are several so called tertiary-active amino acid transporters present in the apical membrane of enterocytes, e.g. the H^+ -dependent amino acid transporter PAT1. The intracellular pH in the enterocyte is 7.0-7.3. An inwardly directed H^+ -gradient is established by the activity of the brush border membrane Na^+/H^+ -exchanger, a secondary active system. The exchanger catalyses the entry of Na^+ from the lumen into the cell along its gradient (established by the primary active Na^+-K^+ -ATPase) in exchange for the exit of H^+ from the cell into the lumen. This system is responsible for the acidic microclimate pH of 5.5 to 6.3 at the intestinal epithelium (Lucas *et al.*, 1975).

The classical nomenclature of the multiple amino acid transport systems was originally deduced on the basis of information available for amino acid transport across the plasma membrane of nonpolarized cells (Christensen, 1989; Mailliard *et al.*, 1995). Initial attempts to investigate and categorize amino acid transport systems in the small intestine followed their energetic characteristics, their substrate specificities, their membrane distribution and the species. Classification according to substrates is complicated by the phenomenon of overlapping substrate specificities: Glycine, for example, is at least transported by proteins of the class GLYT1, by the systems $\text{B}^{0,+}$, ASC, asc, L and PAT1 (see below). Furthermore, enterocytes are polarized cells expressing different carriers at their brush border and basolateral membranes for directional

transport. Today, after molecular and functional characterisation of many separately expressed transport proteins and their localization, the classification of the plasma membrane solute carriers (SLC) according to gene families is in progress (for all currently known members of the SLC family see <http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl>; for review see Palacin *et al.*, 1998; Ganapathy *et al.*, 2001; Wagner *et al.*, 2001; Bröer, 2002; Hediger, 2003). At the intestinal epithelium, members of the following gene families have been identified so far: The family of neuronal and epithelial Na^+ -coupled glutamate and neutral amino acid transporters SLC1, the family of Na^+ - and Cl^- -dependent neurotransmitter/amino acid transporters SLC6, the families of the subunits of the heteromeric amino acids SLC3 (coding for the heavy subunits rBAT and 4F2hc) and SLC7 (coding for the light subunits of amino acid transporters and CAT1), the family of monocarboxylate transporters SLC16, the family of H^+ -coupled transporters SLC36 and the family of amino acid and auxin permeases (AAAP-) related transporters SCL38.

Table 1 classifies the different amino acid transport systems in the brush border membrane of absorptive enterocytes. The secondary active systems derive energy from a Na^+ -gradient or other sources such as a K^+ -gradient, a Cl^- -gradient and a membrane potential, but each of these transport systems has its own unique combination of driving forces. The systems B^0 , $\text{B}^{0,+}$, BETA and $\text{X}^{-\text{AG}}$ are obligatorily dependent on a Na^+ -gradient. System $\text{b}^{0,+}$ is considered an amino acid exchanger (antiporter). The H^+ -gradient, along with the membrane potential, provides the driving force for PAT1.

Table 1. Classification of amino acid transport systems in the brush border membrane of the small intestine.

| Transport system | Protein name | Gene family | Amino acids |
|-------------------------|--|-------------|--|
| B^0 | ATB 0 /ASCT2 | SLC1 | neutral |
| $\text{B}^{0,+}$ | ATB $^{0,+}$ | SLC6 | neutral, cationic |
| $\text{b}^{0,+}$ | rBAT/ $\text{b}^{0,+}$ AT ($\text{b}^{0,+}1$) | SLC3/SLC7 | neutral, cationic, cystine |
| | 4F2hc/ $\text{b}^{0,+}$ AT ($\text{b}^{0,+}2$) | SLC3/SLC7 | neutral, cationic, cystine |
| y^+ | CAT1 | SLC7 | lysine, arginine, ornithine |
| BETA | TAUT | SLC6 | taurine, β -alanine, hypotaurine |
| $\text{X}^{-\text{AG}}$ | EAAT3 | SCL1 | aspartate, glutamate |
| “IMINO” | PAT1, LYAAT1 | SLC36 | small, neutral, unbranched, imino |

The major transport system responsible for the transport of neutral amino acids across the intestinal brush border membrane - **system B^0** - interacts only with amino acids having no net charge. Basic and acidic amino acids are not substrates for this transport system. Favoured substrates are alanine, glutamine, leucine and phenylalanine. Imino acids and β -amino acids, though dipolar in nature, are excluded by the carrier. In contrast, **system $\text{B}^{0,+}$** accepts not only most neutral amino acids but also cationic amino acids as substrates (Chen *et al.*, 2003a). It is an apical secondary active system, the coupling ions being Na^+ and Cl^- , the stoichiometry being 2 Na^+ /1 Cl^- /1 amino acid. System $\text{B}^{0,+}$ is also present in blastocysts, oocytes, pituitary and lung. It has a high affinity for hydrophobic amino acids and transports also carnitine. In the mouse colon, an apical tissue selective $\text{B}^{0,+}$ transporter (mCATB $^{0,+}$) has been identified recently. **System $\text{b}^{0,+}$** is a Na^+ -independent heteromeric amino acid transporter with a similar substrate specificity (for a review on historical, functional and structural aspects as well as molecular biology see Palacin *et al.*, 1998; Ganapathy *et al.*, 2001). The heavy subunit is known as rBAT for “related to $\text{b}^{0,+}$ amino acid transporter”. There is also a second heteromeric $\text{b}^{0,+}$ system where the heavy subunit is the type-II glycoprotein 4F2hc. The light subunit $\text{b}^{0,+}$ AT for $\text{b}^{0,+}$ amino acid transporter has been cloned in 1999. System $\text{b}^{0,+}$ is defective in the most common primary inherited aminoaciduria, the cystinuria. **System y^+**

is defined as a system that transports the cationic amino acids lysine, arginine and ornithine by a Na^+ -independent uniport mechanism. Homoserine and cysteine are also transported by this system but only in the presence of Na^+ . It was one of the first amino acid transporters to be cloned (Kim *et al.*, 1991; Wang *et al.*, 1991). Of the several isoforms, CAT1 is the one expressed in the intestine. In mouse and rat CAT1 functions as a receptor for murine ecotropic leukaemia virus. The **BETA system** occupies a unique position because, unlike other transport systems, the main substrates for this system are the non-protein amino acids, taurine, β -alanine and hypotaurine. System BETA (TAUT) has been cloned from placenta but is also the taurine transporter expressed in the intestine (for review see Palacin *et al.*, 1998; Ganapathy *et al.*, 2001). Taurine is the most abundant free amino acid in intracellular water space in many tissues (Huxtable, 1992). The nutritional requirements for taurine in animals are met partly by dietary sources and partly by biosynthesis. Intestinal absorption is obligatory for the maintenance of the high taurine levels in developing animals after birth. The ability of taurine synthesis in most species increases during development. For cats, taurine is essential because of insufficient synthesis capacities also in adult life. Other species do not depend on dietary taurine as adults. For humans, taurine is essential under certain circumstances. The BETA system has an absolute requirement for Na^+ as well as Cl^- . Hence, TAUT belongs together with $\text{B}^{0,+}$ and GLYT1 to the family of Na^+ - Cl^- -dependent transporters (Chen *et al.*, 2003a). We have characterized system BETA in detail in cell lines of human intestinal origin and have shown that it is under regulatory control by protein kinases and by luminal peptide signals such as the *E. coli* heat-stable enterotoxin and guanylin (Brandsch *et al.*, 1995). It is also adaptively regulated in response to taurine concentration and by hypertonic stress at a transcriptional level. Since taurine is an important organic osmolyte the carrier certainly plays a role in regulating osmotic balance in several tissues. **System X^-_{AG}** is defined as a transport system which serves exclusively for the acidic amino acids aspartate and glutamate. The system consists of at least five subtypes (EAATs for excitatory amino acid transporters). EAAT3 is expressed in the brush border membrane of the intestine from where it was originally cloned (Kanai & Hediger, 1992). The uphill transport of acidic amino acids into the intestinal epithelium via system X^-_{AG} is supported by two different driving forces, an inwardly directed Na^+ -gradient and an outwardly directed K^+ -gradient. EAAT3 mediates the cotransport of 1 glutamate, 3 Na^+ and 1 H^+ in antiport with 1 K^+ .

The most recent cloned member of the intestinal amino acid transporters is PAT1, the proton-coupled amino acid transporter 1. PAT1 probably represents the classic **IMINO system** formerly described in intestine. In the last 15 years, several laboratories have produced evidence for the existence of a H^+ -dependent (tertiary active) amino acid transport system in mammalian epithelia. Thwaites and co-workers described H^+ -driven transport of glycine, alanine, imino acids, β -amino acids and others at the apical membrane of Caco-2 cells (Thwaites *et al.*, 1993). The system was shown also to recognize and translocate D-amino acids such as D-serine, D-proline and D-cycloserine with affinity constants similar or even lower than those of the L-isomers. A carrier with these characteristics has been cloned from brain (rLYAAT1, rat lysosomal amino acid transporter 1, for review see Boll *et al.*, 2003). Subsequently, Daniels group identified PAT1 (orthologous to LYAAAT1) from mouse intestine and discussed that this might be the intestinal transporter Thwaites described functionally in Caco-2 cells years before. The groups of Ganapathy and Thwaites described comprehensively the cloning, structure, function and localization of the human PAT1 at the apical membrane of Caco-2 cells (Chen *et al.*, 2003b). The primary substrates for PAT1 in the mammalian small intestine are very likely glycine, L-proline and L-alanine.

Entry of peptides into the enterocyte across the brush border membrane

Mechanism of intestinal H⁺/peptide symport

The existence of specific transport systems for di- and tripeptides in general was a topic of long controversies. This was due to the prevailing view that the protein digestion products are only amino acids. Several factors such as availability of radiolabeled dipeptides, hydrolysis-resistant dipeptides such as glycylsarcosine (Gly-Sar) and an interdisciplinary approach led to the functional characterization of the intestinal and renal peptide transporters (for review see Daniel, 1996; Ganapathy *et al.*, 2001). In 1983 Ganapathy & Leibach reported in the Journal of Biological Chemistry that a H⁺-gradient and the membrane potential are the driving forces for intestinal and renal transport of dipeptides and proposed the model for apical peptide uptake illustrated in Figure 2. According to this, the intestinal and renal apical peptide transport is a tertiary-active process, a H⁺/peptide symporter. In 1994, the rabbit intestinal H⁺/di- and tripeptide transporter PEPT1 was cloned by the *Xenopus laevis* oocyte expression cloning method (Fei *et al.*, 1994). The H⁺-dependence of intestinal peptide transport has now been accepted and the phenomenon has been confirmed with different tissue preparations in a variety of animal species such as *C. elegans*, insects, lobster, eel and other fishes, mouse, rat, rabbit, human (gene family SLC15) and at pig and bovine intestinal brush border membrane (for review see Fei *et al.*, 1998; Wolffram *et al.*, 1998; Chen *et al.*, 1999; Winckler *et al.*, 1999).

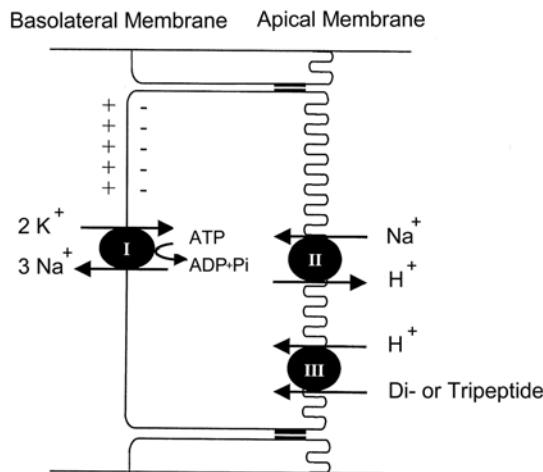


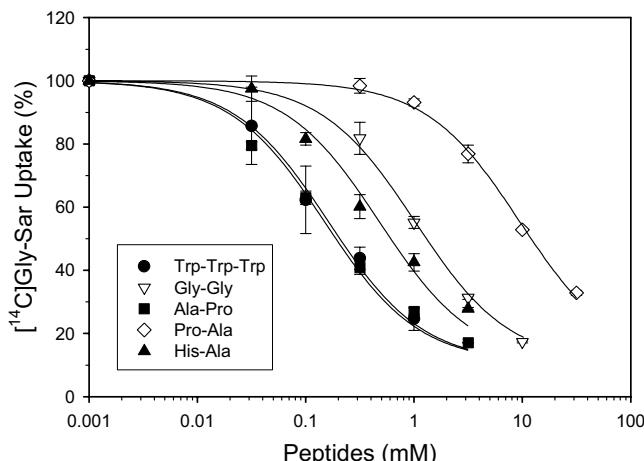
Figure 2. PEPT1 as a tertiary active H⁺/peptide-symporter. I: Na⁺-K⁺-ATPase, II: Na⁺/H⁺ antiporter, III: PEPT1.

Substrate specificity of PEPT1

The substrate specificity of the intestinal H⁺/peptide symporter PEPT1 is under intense investigation in many laboratories mainly for two reasons. It is known that PEPT1 accomplishes the transport of the enormous amount of over 8000 different dipeptides and tripeptides resulting from protein digestion regardless of their size, charge and lipophilicity. Until the exact structural features of the substrate binding site of PEPT1 become available, for example by X-ray crystallography, determination of structure-affinity relationships will have to suffice when investigating the “multispecific” binding site of the transport protein. In this respect, we consider

affinity constants (K_i) for substrates or inhibitors of PEPT1 lower than 0.5 mM as high affinity, between 0.5 and 5.0 mM as medium affinity and above 5 mM as low affinity (Brandsch *et al.*, 2003). The only other available data come from experiments with chimeric mammalian peptide transporters or site-directed mutagenesis studies (Daniel, 1996).

Free amino acids are not transported by PEPT1. In the early stages of investigation of peptide absorption it was believed by some that only dipeptides are taken up. Matthews' laboratory carried out extensive studies in the late sixties and early seventies to determine if peptides larger than dipeptides could be absorbed by the intestinal peptide transporter. There is now general consensus that only di- and tripeptides are substrates for PEPT1, and that the size, i.e. the molecular weight of the peptide is not a limiting factor. The K_i value of Gly-Gly for PEPT1 in Caco-2 cells is 1.1 mM, that of Trp-Trp-Trp 0.15 mM (Figure 3). Cationic and anionic dipeptides are transported by the same system utilized by zwitterionic dipeptides. However, charged dipeptides appear to have a lower affinity compared to structurally similar neutral dipeptides, different transport rates and different stoichiometries (Daniel, 1996).



*Figure 3. Substrate specificity of H^+ /peptide cotransport studied in a competition assay at Caco-2 cells to determine affinity constants. Caco-2 cells express PEPT1 constitutively in their apical membrane (Brandsch *et al.*, 1994, 1997). Uptake of 10 μ M [14 C]Gly-Sar as the labelled hydrolyses-resistant reference dipeptide was measured in monolayer cultures of Caco-2 cells at pH 6.0 in the absence and presence of increasing concentrations of unlabeled peptides (0 - 31.6 mM). Results are shown as mean \pm SEM, n=4.*

A peptide bond in the narrower sense is not an essential structural requirement for transport of a substrate by peptide transporters. δ -Aminolevulinic acid, a precursor of porphyrin synthesis and a compound that possesses a ketomethylene group instead of a peptide bond is transported by intestinal and renal peptide transporters with high affinity (Döring *et al.*, 1998a). In a recent study with Ala- ψ [CS-N]-Pro where the peptide bond is replaced by the isosteric thioxo peptide bond it was found that PEPT1 recognizes the compound with high affinity ($K_i = 0.3$ mM, Brandsch *et al.*, 1998). Döring *et al.* (1998b) reported that ω -amino fatty acids serve as substrates for PEPT1. The required distance between the two charged centers of the compounds for recognition and transport is 500 to 630 pm. Removing the carbonyl function or removing both the carbonyl and the amide group diminishes the affinity completely. Removing the amino terminus, the carboxy terminus or both leads to total loss of affinity (Brandsch *et al.*, 2003).

PEPT1 is conformation specific: We investigated the influence of the substrate backbone dynamics caused by peptide bond *cis/trans* isomerization on the intestinal peptide transport. Only the *trans*

conformer of Ala- ψ [CS-N]-Pro, which exists as a mixture of *cis* and *trans* conformation in aqueous solution, interacted with the transporter and was taken up into the cell (Brandsch *et al.*, 1998). In another series, the K_i values of Xaa-Pro dipeptides at PEPT1 were positively correlated to their *trans* contents (Brandsch *et al.*, 1999). It was concluded that PEPT1 accepts *trans* conformers of zwitterionic Xaa-Pro regardless of size, hydrophobicity and aromatic nature of the N-terminal amino acid.

Pro-Xaa dipeptides are in general not particularly good substrates for peptide carriers. At Caco-2 cells, Pro-Xaa dipeptides display K_i values between 0.5 and > 20 mM. Pro-Ala, Pro-Asp, Pro-Ser, Pro-Glu and Pro-Gly display very low affinity or no affinity at all, respectively. PEPT1 is stereospecific in the sense that L-L-dipeptides and L-L-L-tripeptides display a much higher affinity than dipeptides or tripeptides containing D-amino acids. The affinity constants of D-D-dipeptides consisting of the D-isomers of natural amino acids are in most cases > 30 mM or not measurable. In contrast, for DD-isomers, in which the structure is modified by side chain modifications (D-Ala-D-Lys(Z), D-Lys(Z)-D-Ala, etc.), appreciable affinity constants between 1 and 10 mM were measured (Brandsch *et al.*, 2003).

The second reason for the strong interest in substrate specificity of PEPT1 is due to the fact that many recent studies have shown that several drugs and prodrugs gain entry into the systemic circulation via PEPT1. Much of this development is being driven by the pharmaceutical industry, which looks on peptide transporters as a possible vehicle for drug delivery in general and for purposes of increasing the bioavailability of drugs. For example, β -lactam antibiotics bear sterical resemblance to the backbone of physiologically occurring tripeptides. In papers by the groups of H. Daniel, K.-I. Inui, A. Tsuji, F.H. Leibach, G.L. Amidon, A.H. Dantzig and many others (for review see Taylor & Amidon, 1995; Brodin *et al.*, 2002) it was demonstrated conclusively that certain aminocephalosporins and penicillins are substrates for PEPT1. Recently, we studied the structure-transport relationship of 23 β -lactam antibiotics by measuring both their affinity at PEPT1 and their total transepithelial flux across Caco-2 cell monolayers cultured for 23 days on permeable polycarbonate filters (Bretschneider *et al.*, 1999). The total transepithelial net flux of β -lactam antibiotics is well correlated with the K_i values for Gly-Sar inhibition. Hence, the overall variation of transepithelial flux in the luminal to abluminal direction (mimicking intestinal absorption) is mainly determined by the variation of the affinity at PEPT1. Moreover, from our studies it is most likely that β -lactam antibiotics with a K_i < 14 mM in our assay will have good oral availability from a transport point of view. Other examples for drugs transported by PEPT1 are inhibitors of angiotensin-converting-enzyme and the prodrug valacyclovir, a valine ester of the antiviral agent acyclovir, which is a PEPT1 substrate of high affinity.

Recently, the first high-affinity inhibitors for PEPT1 were developed (Knüttner *et al.*, 2001): For the dipeptide derivative Lys(4-nitrobenzyloxycarbonyl)-Pro (Lys[Z(NO₂)]-Pro) we observed a high-affinity inhibition of [¹⁴C]Gly-Sar uptake in Caco-2 cells. The type of inhibition was competitive. To decide whether Lys[Z(NO₂)]-Pro represents a substrate or an inhibitor of PEPT1, Daniel and co-workers employed the two electrode voltage clamp technique in Xenopus oocytes expressing PEPT1. Lys[Z(NO₂)]-Pro failed to cause any response in current but inhibited Gly-Gln evoked currents. Hence, Lys[Z(NO₂)]-Pro competes efficiently with dipeptides at the binding site of PEPT1 (K_i = 5–10 μ M), but is itself not transported. This compound serves as a starting point to identify the substrate binding domain within the carrier protein PEPT1. The substance interacting with PEPT1 with the highest affinity measured so far is the inhibitor Lys[Z(NO₂)]-Val (K_i = 2 μ M, Knüttner *et al.*, 2003).

Regulation of peptide absorption across the brush border membrane

The ability of the small intestine to absorb amino acids and peptides varies significantly in response to several factors. These factors might be of exogenous or endogenous origin. Regulation of peptide transport has been described as a result of development, diseases, intestinal resection,

inflammation, nutritional status and food composition, hormones and drugs. The underlying mechanisms may be nonspecific (e.g. changes in the absorptive surface area, changes in the physical state of the membrane across which absorption occurs) or specific for a particular carrier. In general, regulation of a membrane transport protein by intracellular or extracellular signals acting from the luminal or serosal side can occur on the transcriptional level, the translational level, by insertion or exertion of carrier proteins from intracellular vesicles ("trafficking"), indirectly via modulation of driving forces (e.g. the Na^+/H^+ -exchanger) or directly by phosphorylation. Virtually all possible regulatory mechanism have been described for PEPT1: The capacity to absorb peptides is maximal at birth and then decreases with age to reach adult levels. Intestinal transport of peptides is upregulated by the presence of high levels of protein in the intestinal lumen. Similarly, short-term restriction of diet increases peptide transport. The intestinal peptide transport is upregulated on the protein expression level at diabetes, after intestinal resection and during inflammation. Insulin, EGF, thyroid hormone, leptin, phorbol esters, cholera toxin, forskolin, the vasoactive intestinal peptide, flavonoids, calcium channel blockers, cyclosporin, the sigma receptor ligand pentazocin, lipopolysaccharides and many other compounds have been shown to modulate intestinal peptide uptakes (for review see Ganapathy *et al.*, 1994; Mailliard *et al.*, 1995; Brandsch, 2000; Meredith & Boyd, 2000; Herrera-Ruiz & Knipp, 2003).

Exit of amino acids across the basolateral membrane

The peptide transport across the brush border membrane of the enterocyte is followed by intracellular hydrolysis of the majority of the transported peptides. In addition to hydrolysing the exogenous peptides arising from absorption from the lumen, the intracellular peptidases of the enterocyte also participate in the breakdown of endogenous proteins. Free amino acids arising in the cells or transported from the lumen into the enterocyte feed into several metabolic pathways, namely degradation, conversion into other amino acids, incorporation into proteins and transport into blood. Glutamine, glutamate and aspartate have been shown to be quantitatively the most important amino acids as respiratory fuel in the intestinal epithelium. The major part of amino acids is transported across the basolateral membrane to the blood. However, certain basolateral amino acid transporters are responsible for the uptake of amino acid from the blood into the enterocytes.

Available information suggests that there are at least seven amino acid transport systems in the basolateral membrane of enterocytes (Table 2).

Table 2. Classification of amino acid transport systems in the basolateral membrane of the small intestine.

| Transport system | Protein name | Gene family | Amino acids |
|------------------|----------------------------|-------------|--------------------------------------|
| A | ATA2 | SLC38 | neutral, imino |
| L | 4F2hc/LAT2 (4F2hc/LAT1) | SLC3/SLC7 | neutral |
| ASC | ASCT1 | SLC1 | alanine, serine, cysteine |
| y ⁺ L | 4F2hc/y ⁺ LAT1 | SLC3/SLC7 | neutral, cationic |
| T | TAT1 | SLC16 | tryptophane, tyrosine, phenylalanine |
| Gly | GLYT1 | SLC6 | glycine |
| asc | 4F2hc/asc-1 | SLC3/SLC7 | alanine, serine, cysteine, D-serine |

System A occurs in nearly all cell types. It is an electrogenic transport system and derives its energy from a Na^+ -gradient as well as from a membrane potential. The orientation of these driving forces *in vivo* is appropriate for active transport of amino acids from blood into the intestinal cells (Ganapathy *et al.*, 1994). The system accepts as substrates small aliphatic amino acids, in particular alanine, serine and glutamine but also imino acids. Of the three currently known isoforms ATA2 is expressed in the intestine. **System L** is the major Na^+ -independent system in the intestinal basolateral membrane for transport of neutral L-amino acids. It acts as an 1:1 amino acid exchanger. It is a heteromeric amino acid transporter (for review see Palacin *et al.*, 1998; Ganapathy *et al.*, 2001; Verrey, 2003). The heavy subunit is the type-II glycoprotein 4F2hc. The light subunit LAT induces system L activity when coexpressed with 4F2hc but is not capable of amino acid transport on its own. Imino acids are excluded by the system. Glutamine and cysteine are also transported. **System ASC** is Na^+ -dependent, electrogenic and active but has a much narrower substrate specificity than system A. It acts as a Na^+ -coupled amino acid exchanger. The short-chain neutral amino acids alanine, serine and cysteine are the preferred substrates for system ASC. The involvement of a Na^+ -gradient and a membrane potential as driving forces for this system indicates that it participates, like system A, in the transport of amino acids from blood into the intestinal cells. **System y^+L** is a heteromeric amino acid transporter. The heavy subunit is 4F2hc, as it is for system L, the light chain is y^+ LAT1. The two subunits are linked by a disulfide bond. The system mediates the Na^+ -independent transport of cationic amino acids and the Na^+ -dependent transport of neutral amino acids. It is likely to mediate the Na^+ -independent efflux of cationic amino acids from the enterocytes to the blood in Na^+ -dependent exchange for entry of neutral amino acids from the blood into the enterocytes (Ganapathy *et al.*, 2001). **System T** (T-type amino acid transporter-1, TAT1), is an aromatic amino acid transporter identified very recently as a member of the “monocarboxylate transporter family” (Kim *et al.*, 2001). The carrier is a Na^+ - and H^+ -independent uniporter. It is strongly expressed in the basolateral membrane of enterocytes. TAT1 transports L- and D-phenylalanine, L-tyrosine and L-tryptophane with affinity constants in the mM range. Glycine supply to human enterocytes is mediated by the basolateral high-affinity **system Gly** (GLYT1). The uptake is Na^+ - and Cl^- -dependent (2 Na^+ , 1 Cl^- , 1 glycine). GLYT1 belongs to the same gene family as TAUT and ATB $^{0,+}$. The transporter exhibits a narrow substrate specificity and accepts only glycine, sarcosine and certain derivatives (Christie *et al.*, 2001). The heteromeric amino acid transport **system asc** is similar to system ASC in its substrate specificity for L- amino acids, but also accepts certain D-isomers. It is Na^+ -independent and acts as an antiporter (Bröer, 2002).

Exit of peptides across the basolateral membrane

In contrast to PEPT1 in the apical membrane, not very much is known about the putative basolateral peptide transporter. There is little doubt that hydrolysis-resistant dipeptides are absorbed across the intestinal epithelium in intact form (Ganapathy *et al.*, 1994). First evidence for a specific, saturable peptide transport system at the basolateral membrane was obtained in studies using vesicles of that membrane. Today's experiments on this topic are mostly performed with Caco-2 cells grown on permeable supports for access to the basolateral membrane. The uptake of peptides and peptidomimetics from the basolateral compartment has been described as saturable but in some studies less sensitive to the medium pH than PEPT1. It does not occur against a concentration gradient (Inui & Terada, 1999). These parameters suggest a facilitative peptide transport system. Altogether, there is not enough evidence in the literature showing unequivocally the existence of a separate peptide transporter in the basolateral membrane. There is, however, general agreement that the rate-limiting step for the intestinal peptide absorption is the transport via PEPT1 at the brush border membrane and that the transport of intact di- and tripeptides *in vivo* across the basolateral membrane is only a small fraction of that at the apical membrane (for review see Ganapathy *et al.*, 1994; Meredith & Boyd, 2000).

Intestinal absorption of proteins

The intestinal epithelium permits the passage of small quantities of proteins resistant to gastrointestinal hydrolysis (Weaver & Walker, 1989). Excessive absorption of antigenic proteins, e.g. because of mucosal defects may result in local or systemic disorders (Gardner, 1988). The permeation of proteins is often protein specific and tightly regulated. It has been known for many years that at the neonatal gut of several mammalian species immunoglobulins (IgG) are absorbed from the colostrum as a form of passive immunisation (Weaver & Walker, 1989). The first step in the selective transfer of IgG is the binding of IgG to luminal Fc-receptors followed by energy-dependent endocytotic mechanisms in coated pits/coated vesicles. Another example is the uptake of lactoferrin, the major iron binding protein in milk, for which a receptor is expressed at the brush border membrane. To a much lower if any extent compared to the newborn, the mature intestinal epithelium retains the ability to absorb proteins by endocytosis. In feeding experiments, the absorption of macromolecules in adult mammals is possibly several orders of magnitude smaller than 0.1 % of the administered dose (for review see Weiner, 1988). An important pathway for access of specific proteins, bacterial antigens and even bacteria to the intestinal lymphoid tissue represents the phagocytotic activity of the follicle-associated epithelial cells (M-cells) and dendritic cells. M-cells take up proteins and release them into the intercellular space where they reach cells of the gut-associated lymphoid tissue (Weaver & Walker, 1989).

The area in intestinal protein absorption that is presently getting the most attention is that of prion protein transport. According to several hypotheses an infection with the pathologic prion protein (PrP^{sc}) by food requires the permeation of the protein through the intestinal epithelial barrier. Several pathways are currently discussed (for review see Shmakow & Ghosh, 2001). A M-cell independent route might be a pericyryptal paracellular uptake of PrP^{sc} at the colonic mucosa (McKie *et al.*, 1999). Rieger *et al.* (1997) identified the human 37-kD laminin receptor precursor (LRP) as interacting with the cellular prion protein PrP. They concluded from their studies that LRP may act as a receptor or coreceptor for PrP^{sc} . LRP occurs at the brush border of the small intestine. Heppner *et al.* (2001) have shown very elegantly *in vitro* that PrP^{sc} are taken up at the intestinal epithelium by M-cells. Hence, one current model assumes that at oral infection with prion proteins, the laminin receptor or its precursor is involved in an endocytotic process at M-cells by acting as a receptor for PrP^{sc} . PrP^{sc} might then reach ileal Peyer's patches and/or enteric autonomic ganglia and efferent fibers of the vagus and splanchnic nerves to retrograde invade areas in the brain (McBride *et al.*, 2001). Similarly, Huang *et al.* (2002) provided strong evidence for a PrP^{sc} transport from the gut lumen to the lymphoid tissue by intestinal migratory dendritic cells.

These examples illustrate that the absorption of intact proteins, although too small to be significant in terms of animal or human amino-nitrogen supply is nutritionally significant.

Conclusion and future direction

It was believed for a very long time that proteins in the intestinal lumen had to be digested completely to generate amino acids in the monomeric form before absorption into the enterocyte. Today, available information strongly support the notion that protein digestion products are absorbed into intestinal cells predominantly in the form of di- and tripeptides. The existence of multiple mechanisms in the small intestine for absorption of amino acids and peptides seems necessary for maintenance of optimal protein nutrition of the organism. Evidence exists for faster absorption of amino acids when given in the form of peptides than in the form of free amino acids, the avoidance of competition during transport, the conservation of metabolic energy, and for functional redundancy at malfunction of single transport systems. Moreover, the intestinal peptide transport system is responsible for the oral availability of many pharmacologically relevant peptidomimetics. In the near future the remaining amino acid transporters will be cloned and

characterized in several mammalian species on a molecular level. Likewise, the substrates for the so called orphan transporters have to be identified. Other areas that need further expansion are the investigation of polymorphisms and genetic defects of mammalian membrane transport proteins and the investigation of their three-dimensional structure by X-ray crystallography.

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Optimal time interval for amino acid supplementation as studied by amino acid oxidation during the postprandial phase

J. Bujko¹, M. Gas¹, M. Krzyzanowska¹, R.E. Koopmanschap², J.A. Nolles² & V.V.A.M. Schreurs²

¹ Department of Human Nutrition and Consumer Sciences, Warsaw Agricultural University, Nowoursynowska 159C, 02-776 Warsaw, Poland

² Human and Animal Physiology Group, Wageningen University, Wageningen Institute of Animal Sciences (WIAS), Haarweg 10, NL-6709 PJ, Wageningen, The Netherlands

Summary

This study examines oxidative losses of methionine when free and protein bound amino acid of the diet are ingested separately in two meals with different time intervals. Measurements were repeated after 3 weeks to study metabolic adaptation. In the experiment 48 rats divided to 4 groups were fed 3 meals a day with different time intervals. The 1st and the 3rd meal were fixed at 09.00 and 16.30 hours, respectively. Depending on the experimental group the 2nd meal was given with 0, 1, 2 and 4 hours interval. The meals provided 45%, 15% and 40% of daily nutritional and energy supply, respectively. For first two weeks of conditioning the diet was complete according to rats' requirements and based on egg-white protein (13.2%). At day 0 of the experiment, the composition of the 1st meal was changed to 5% egg white protein (with bound $1\text{-}^{13}\text{C}$ leucine on the test days) and 8.2 % vegetable protein. The total protein fraction was 50%-deficient in methionine. The 2nd morning meal contained the free methionine as a supplement (with 6.8 mg $1\text{-}^{13}\text{C}$ methionine on the test days). Relative nutritional value of the meals was maintained. The oxidative loss of dietary amino acids was measured as the oxidation of egg white bound [$1\text{-}^{13}\text{C}$]-leucine for the 1st meal and as the oxidation of free [$1\text{-}^{13}\text{C}$]-methionine for the 2nd meal.

The lowest losses of the protein-bound leucine were found at a time interval of 1 hour, and were significantly lower as compare to 0 and 4 hours group. This indicates that the best protein utilization occurs when methionine supplement is given in free form with 1-2 hours interval. After 3 weeks of adaptation period animals fed with both meals mixed improved postprandial utilization to the same level as with 1 or 2 hours interval. Oxidation of leucine did not correspond with free methionine oxidation, which was always significantly lower for mixed than eaten with interval meals. This high level of methionine oxidation may be related to other than protein synthesis functions (e.g. methylation). Higher methionine oxidation with increasing time delay of its supplementation could also result from increasing concentration of the tracer in non labeled amino acids. After adaptation oxidative losses of protein bound leucine and free methionine increased at all time intervals, probably due to slower rate of growth and higher maintenance requirements of bigger animals.

Keywords: $1\text{-}^{13}\text{C}$ methionine, $1\text{-}^{13}\text{C}$ leucine, amino acid supplementation

Introduction

Amino acids from dietary proteins can be utilized for net body protein synthesis or for maintenance processes and non-protein metabolic pathways (e.g. methionine as methyl and sulfur donor) (Orten, 1975). Daily meal feeding is associated with an alternation of postprandial gain and post absorptive mobilization of nutrients. In case of proteins these processes are named as "diurnal protein cycling" (Milward & Rivers, 1988). Free amino acids in the blood have to be kept in small and constant amount due to their toxicity, and when their intake exceeds utilization capacity, the excess is decarboxylated and channeled into energy metabolism. This means that a

substantial part of the dietary amino acids can get lost from the body by early oxidation during the postprandial phase of a meal. The degree of postprandial oxidation will depend on many nutritional conditions, such as the amino acid pattern of the feed protein, the amount of protein per feeding, the feeding frequency, the presence of synthetic amino acids, size of the meals, etc (Schreurs, 1995). Proteins in human and animal diets can be supplemented with free amino acids to meet the amino acids requirements. However, previous studies suggest that dietary amino acids in free form are sometimes less utilized than in protein-bound form. (Schutte, 1992). As the result free supplement reach the body pool faster and in bigger amount causing imbalance that costs more losses of amino acids, and decrease their utilization for protein synthesis. In the nutritional model study with rats it was investigated whether the postprandial loss of dietary amino acids is modulated by the time interval between 50% methionine deficient meal and its supplementation in free form. As the indicator of postprandial protein synthesis the oxidation of protein bound ^{13}C leucine was used since there is no other pathway for leucine metabolism (Schreurs, 1992). Measurements were repeated after 3 weeks for each feeding strategy to study metabolic adaptation.

Materials and methods

On weight basis, 48 young growing WU-rats (50 - 75 grams, 3 - 4 wks, weaned for at least 3 days) were allotted to one of the four "interval" groups, caged individually at a room temperature of 22 °C and conditioned on a light schedule with 16 hours of light and 8 hours dark period from 9 am to 5 pm. Water was always available 'ad libitum'. First two weeks the animals were fed 3 meals a day for 30 minutes with complete diet with protein bound amino acids (13.2%) containing 45, 15 and 40% of daily nutrient and energy supply subsequently, based on the tabulated requirements of rats. The 1st and the 3rd meal were fixed at 09.00 and 16.30 hours respectively. Depending on the experimental group the 2nd meal was given at 9.00, 10.00, 11.00 and 13.00 o'clock with 0 (mixture), 1, 2 and 4 hours interval respectively.

After conditioning period composition of protein in the first meal was changed to 5 % egg white and 8.2% of vegetable protein, 50 % deficient in methionine, which was supplemented in free form in the second meal to meet the requirements.

Measurements

For study body weight development all animals were weighed daily. At the 5th and 26th day of experimental period postprandial oxidation of ^{13}C egg white protein bound leucine and ^{13}C free methionine was studied using "[$^{13}\text{CO}_2$] breath testing" for 5 hours from the tracer ingestion. On the day of breath test for half of the animals egg white in the first meal was changed by highly enriched (ca. 1.48 At%) ^{13}C leucine egg white protein (375 mg dry matter), produced according to Evenepoel et al. (1997). For the rest of the rats part of free methionine in the second meal was replaced by 6.8 mg of ^{13}C methionine (chem. pur. > 99%, isotopic enrich. > 99%, Mass Trace, Woburn, USA). The breath samples were taken every 30 minutes and ^{13}C enrichment was measured on the Finnigan Delta C IRMS, then was recalculated to cumulative recovery of ^{13}C . Total CO_2 -production of animals was measured respirometrically in separate experiments under identical conditions. The results did not differ among the groups because spontaneous movement of animals much more influenced the CO_2 production than the feeding procedure. Therefore, the results were used for calculation of activity factor (2.13) by which CO_2 production, calculated using Brody's formula, was multiplied.

The MANOVA variance analysis and post hoc LSD test (Statgraphics Plus) was used to observe statistically significant differences.

Results

For the whole time of the experiment there was observed steady growth of the animals without significant differences in body weight among the four groups. The rats' mean body weight at beginning was 59g (± 4), at the time of first breath test measurements (before adaptation) 111g (± 4) and after adaptation period 177g (± 6).

MANOVA showed statistically significant influence of time interval and adaptation on postprandial oxidative losses of bound in egg white 1- ^{13}C leucine and free 1- ^{13}C methionine.

The cumulative recovery of ^{13}C (figure 1) shows that the oxidative loss of egg white bound 1- ^{13}C leucine was highest for the longest interval (4 hours), and lowest when the supplementation of free methionine was provided after 1 hour. The oxidative losses in the group with 0 interval were not significantly different from 4 and 2 hours interval groups. After 3 weeks of adaptation, leucine oxidation was increased in all cases with again the highest oxidation for the longest time interval, significantly higher than for the three remaining groups. The differences among the shorter intervals had disappeared.

The cumulative recovery of ^{13}C from free 1- ^{13}C methionine (figure 1) shows that the total oxidative loss was much higher compared to leucine. The lowest values were observed in the group 0, when the deficient and supplemented meals were mixed. The other groups had significantly higher oxidation but did not differ between each other. After adaptation, like for leucine, all values were higher with reminded trends from the pre adaptation measurements.

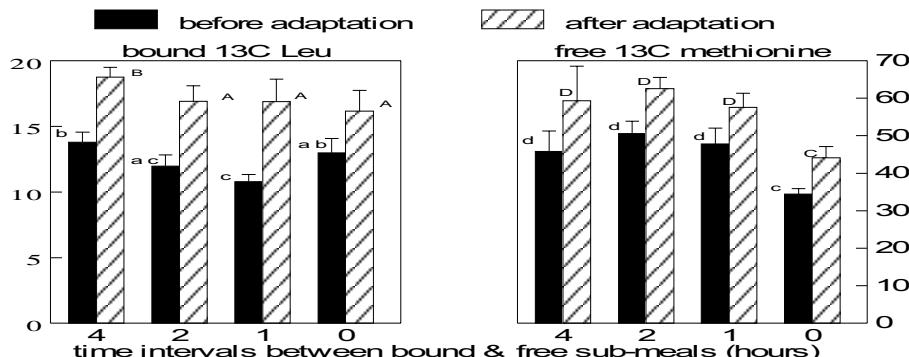


Figure 1. Five hour cumulative recovery of ^{13}C from meals with bound in egg white 1- ^{13}C Leu and free 1- ^{13}C Met before and after 3 weeks of adaptation (% dose of ^{13}C / 5 hours)

(small letters indicate statistically significant differences among groups before adaptation, capital letters indicate statistically significant differences among groups after adaptation).

Discussion and conclusions

Breath test studies with free and protein-bound amino acids indicate that the time interval between the supply of protein-bound and supplemented free methionine influence the postprandial oxidative losses and amino acids utilization for protein synthesis. The lowest losses of the protein-bound leucine were found at a time interval of 1 hour. Assuming that not oxidized leucine reflects postprandial protein synthesis (Schreurs, 1992), it could indicate that the best dietary protein utilization occurs when methionine deficient meal is supplemented with time interval between 1 and 2 hours. The 4 hours interval between methionine deficient meal and its supplementation is too long to show such positive effect, and utilization of dietary protein is similar to 0 time interval (mixture of the two meals). The rats show also physiological ability for improvement of meal protein utilization if time interval between deficient meal and its supplement is less than 4 hours.

After 3 weeks of adaptation to experimental conditions the differences in leucine oxidation between methionine deficient meal and its supplementation in free form mixed together and given with 1-2 hours interval were no longer visible.

Improved protein synthesis, measured with protein bound leucine was surprisingly not proved by the lowest oxidation of the supplemented methionine. Before and after adaptation period significantly lower postprandial oxidation of methionine occurs when deficient meal and its supplementation were mixed together than when were given with investigated time intervals. It could be hypothesized that postprandial protein synthesis requires increased oxidation of methionine e.g. in order to provide sulfur for cysteine. The high level of methionine oxidation may be related to other than body protein synthesis, physiological functions of methionine.

On the other hand, the lowest methionine oxidation, when both meals are mixed together could result from the fact that in this case the same dose of tracer was dissolved in bigger amount of non labeled amino acid than in other groups (even double for 4 h interval group, if both meals do not interact with each other). This could indicate that absolute values of oxidative losses of methionine in this case are much higher, but unfortunately with the used procedure we are not able to recalculate this data for all groups.

After 3 weeks of adaptation period oxidative losses of protein bound leucine and free methionine increased at all time intervals, probably due to slower rate of growth and higher maintenance requirements of bigger animals.

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Influence of time intervals between two consecutive meals on leucine oxidation during postprandial phase

J. Bujko¹, J. Myszkowska-Ryciak¹, J. Keller¹, J. Stankiewicz-Ciupa¹, R.E. Koopmanschap², J. Nolles² & V.V.A.M. Schreurs²

¹ Department of Human Nutrition and Consumer Sciences, Warsaw Agricultural University, Nowoursynowska 159C, 02-776 Warsaw, Poland

² Human and Animal Physiology Group, Wageningen University, Wageningen Institute of Animal Sciences (WIAS), Haarweg 10, NL-6709 PJ, Wageningen, The Netherlands

Summary

The objective of this study was to examine the influence of time intervals between two equal consecutive meals on leucine oxidation during postprandial phase of both meals. Male Wistar rats ($n=48$, 3-4 weeks, 50-60g) were randomly divided into 4 groups. After two weeks of preconditioning each group consumed the same amount of experimental feed during 30 minutes with different intervals between meals (7-A, 3-B, 1-C, 0-D h). On day 19th [$^{13}\text{CO}_2$]-breath test (BT I) with labeled [$1-^{13}\text{C}$] leucine in egg white protein bound form was performed and repeated after 21 days (BT II, day 40). Within each group 6 randomly chosen rats received tracer in the 1st meal and others 6 in the 2nd one, the same animals were used during II BT. Breath samples were taken every 30 minutes for 5 hours and analyzed by IRMS. The results were expressed as cumulative recovery (% dose) and rate of recovery (%dose/h) of ^{13}C in the breath. There was no significant difference in body weight between groups. The cumulative recovery of ^{13}C was significantly lower in case of the 2nd meal compared with the 1st one in groups B, C, D (I BT) and in groups C, D (II BT). There was no significant difference in oxidation level after the 1st meal between groups. In I and II BT after the 2nd meal group A showed statistically higher oxidation comparing with groups B, C, D. During II BT also ^{13}C recovery after the 2nd meal in group B was statistically higher than in groups C and D. 21 days on experimental feeding caused significant increase in oxidation rate in all groups in both meals except the 1st meal in group D. Obtained results suggesting that leucine utilization is better with shorter intervals between meals might be obscured by methodical complications.

Keywords: $1-^{13}\text{C}$ leucine oxidation, meal frequency, rats

Introduction

Schreurs *et al.* (1997) state that meal feeding creates a temporal oversupply of amino acids (postprandial phase) necessary to meet the requirements between meals (postabsorptive phase). Therefore protein metabolism during and between meals is thought to have important influence on the overall efficiency of utilization. The whole body amino acids (AA) economy can be influenced by nutritional conditions. Nutritional strategies should be focused on minimalising of postprandial oxidative losses and channelling into protein synthesis. There are many nutritional factors that may influence protein metabolism of the body, like meal frequency, meal size, time of the day, composition of AA, their physical form (free or protein bound) and time interval between meals (Schreurs *et al.*, 1992; Metges *et al.*, 2000).

We hypothesized that oxidative losses of AA during the postprandial phase of consecutive meals depends on the time interval between the meals. If the next meal is eaten with too short interval after the previous one, while AA oxidation from the first meal is on the high level then overall

oxidative losses are bigger. The interactions between meals might change after a period of adaptation on experimental schedule.

Materials and methods

Individually housed male Wistar-WU rats (3-4 weeks, 50-60 grams) were randomly divided into 4 groups (n=12) with different nutritional treatment and water *ad libidum*. Body weight of individual rats was determined at least 3 times a week between 8.30 and 9.00. The animals were kept on a light (17.00 - 9.00) and dark (red light; 9.00 - 17.00) schedule. During first two weeks of pre-conditioning all groups received meals (pellets, 13% protein based on egg white) twice a day (9.00., 16.30) *ad libitum* for 30 minutes. Daily feed intake was monitored and feeding level was established due to the least eating rats. After two weeks each group received the same amount of experimental food (11g/day, 1,43g protein) for 30 minutes with different time intervals between meals (*Table 1*). On day 19th and 40th [$^{13}\text{CO}_2$]-breath tests with labeled [$1\text{-}^{13}\text{C}$] leucine (6mg) in egg white bound form as an oral tracer were performed in all rodents. Within each group the tracer was introduced to 6 randomly chosen rats in the 1st meal and to 6 others in the 2nd meal. The same animals were used during II BT. Breath samples were taken every 30 minutes for 5 hours and analyzed by IRMS (Isotope Ratio Mass Spectrometer). The results were expressed as cumulative recovery (%dose) and rate of recovery (%dose/h) of ^{13}C in the breath. Production of CO_2 was calculated using Brody's formula multiplied by activity factor established during separate respiratory measurements.

Results of cumulative recovery are presented as a mean value for each group +/- Std. The influence of each variables (time interval, adaptation) was calculated using MANOVA and statistically significant differences between means were estimated using Multiple Range Test. Statistic calculations were made with Stat Graphics 1 Plus 4.0 for Windows.

Table 1. Feeding schedule during the experiment.

| Group | Time interval between meals (h) | Time of the 1 st meal | Time of the 2 nd meal |
|-------|---------------------------------|----------------------------------|----------------------------------|
| A | 7 | 9.00 - 9.30 | 16.30 - 17.00 |
| B | 3 | 9.00 - 9.30 | 12.30 - 13.00 |
| C | 1 | 9.00 - 9.30 | 10.30 - 11.00 |
| D | 0 | 9.00 - 9.30 | 9.30 - 10.00 |

Results

In *Table 2* the average weight at the beginning of experiment and during BT is presented.

Table 2. Average weight (g) in groups during experiment.

| Group | Start of experiment | | I BT (day 19) | | II BT (day 40) | |
|-------|---------------------|-----|---------------|------|----------------|------|
| | Mean | Std | Mean | Std | Mean | Std |
| A | 67 | 3.9 | 133 | 8.3 | 183 | 3.4 |
| B | 69 | 8.3 | 132 | 8.7 | 177 | 7.9 |
| C | 69 | 5.6 | 136 | 10.3 | 182 | 10.1 |
| D | 68 | 6.0 | 129 | 12.7 | 179 | 8.7 |

Results of the total ^{13}C cumulative recovery are presented in *Figure 1*. Leucine oxidation was lower in case of the 2nd meal compared with the 1st one in all groups but the difference was significant in groups C, D (I, II BT) and B (I BT). However there were no significant differences in oxidation level after the 1st meal between groups. In case of the 2nd meal group A showed statistically higher oxidation comparing with other 3 groups (I, II BT). In II BT group B after the 2nd meal showed statistically higher ^{13}C recovery compared to group C and D. The dynamics of ^{13}C recovery after the 1st meal is presented in *Figure 2*.

Three weeks on experimental feeding schedule caused significant increase in oxidation rate in all groups in both meals except the 1st meal in group D, probably due to high Std.

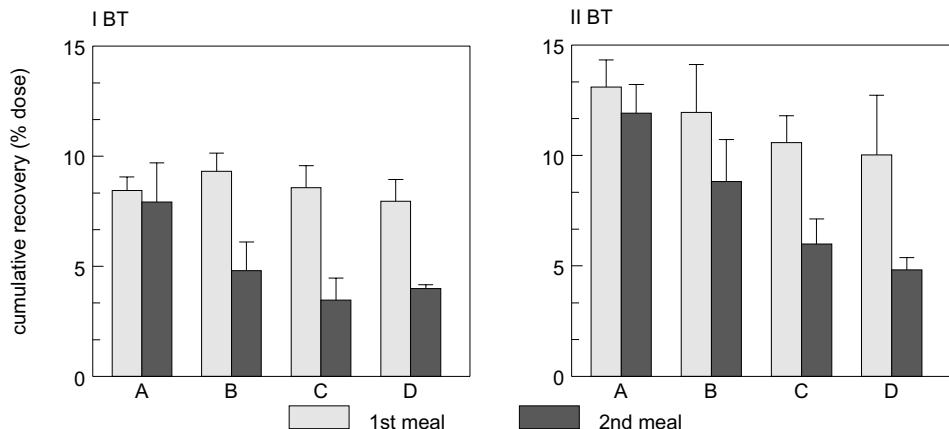


Figure 1. Total cumulative recovery of [1- ^{13}C] leucine in I and II BT.

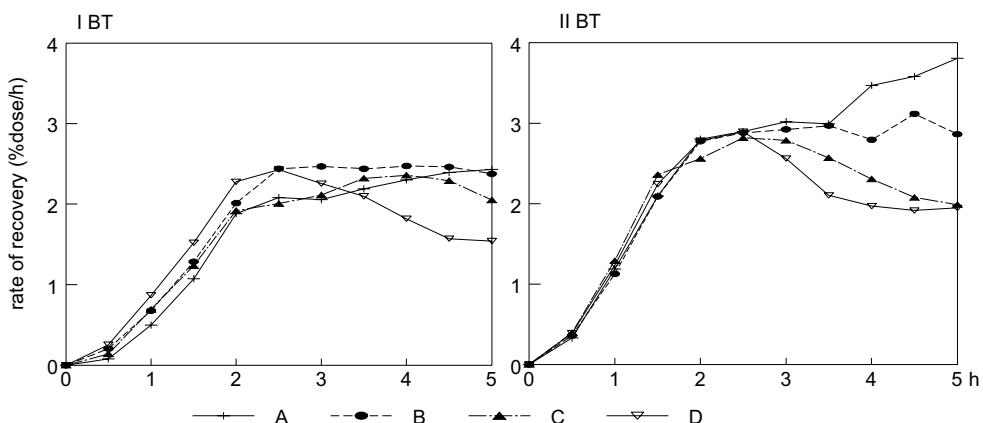


Figure 2. Recovery of [1- ^{13}C] leucine from the 1st meal in I and II BT.

Discussion

Studies on AA utilization in rats (Weijns, 1993; Bujko *et al.*, 1997) suggest that smaller meals served more frequently might improve protein utilization compared with less frequent bigger meals by decreasing postprandial AA oxidation. In this case more AA are available in the body during postabsorptive phase for physiological purposes, for example growth. The large input of AA from meal is more likely to exceed the limits of protein-synthesis capacity of the body but on

the other hand even smaller meals served with too short intervals might cause the same negative effect on protein metabolism.

In this study the patterns of total leucine oxidation were similar in I and II BT and different feeding strategies neither affected the body weight development nor differentiated leucine oxidation during postprandial phase in case of the 1st meal. The total cumulative recovery of ¹³C was similar but the dynamics of oxidation showed different patterns (see *Figure 2*) influenced by introducing of the 2nd meal. In group D oxidation rate was decreasing but not reached the background level while in group A it was still increasing. This tendency was even stronger in case of II BT. Recovery of ¹³C after the 2nd meal seems to be more influenced by the time of supply. Assuming that each BT reflects leucine oxidation after particular meals then absolute oxidation value is higher with increasing time interval between meals. The highest oxidation occurs in group A (no difference between ¹³C recovery after 1st and 2nd meal) what suggests that contrary to others in this group sub-meals might be considered as entirely independent (7h interval). Unexpected low oxidation for the 2nd meal in case of intervals 0, 1 and 3 h might be explained by lower availability of tracer (“queue” effect in alimentary tract). On the other hand lower ¹³C recovery not necessarily means lower oxidation but might be caused by dilution of tracer in larger amount of non labeled leucine. However the tendency to higher oxidation level with increasing time interval between meals is discrepant with previous studies and needs further research.

The higher leucine oxidation level after 21 days of experimental treatment might be explained by different body mass of animals. The II BT was performed on rats bigger by about 34% compared with I BT what might have changed their protein requirements and energetic needs. Higher body weight of animals fed with the same amount of feed like during I BT might have caused the increase of oxidation due to higher maintenance costs.

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The influence of dietary form of [1-¹³C] lysine and [1-¹³C] leucine on their postprandial and postabsorptive oxidation

J. Bujko¹, K. Krupa¹, R.E. Koopmanschap², J. Nolles & V.V.A.M. Schreurs²

¹ Department of Human Nutrition and Consumer Sciences, Warsaw Agricultural University, Nowoursynowska 159C, 02-776 Warsaw, Poland

² Human and Animal Physiology Group, Wageningen University, Wageningen Institute of Animal Sciences (WIAS), Haarweg 10, NL-6709 PJ, Wageningen, The Netherlands

Summary

The purpose of this study was to determine the way different dietary forms of amino acids (aa) influence the level of their postprandial utilisation and to check whether aa oxidation can detect/reflect the rate of protein turnover in postabsorptive phase in humans.

Each substrate (90 mg of L-[1-¹³C]-lysine and 60 mg of L-[1-¹³C]-leucine) was individually ingested by 7 adult persons (two or three times) in three different ways: as a aqueous solution in post-absorptive state or served with the test meal (post-prandial) in either free form (mixed with eggs) or bound to egg white protein. Decarboxylation of amino acids was monitored using a [¹³CO₂]-breath test for 6 hours. The CO₂-production of the subjects was calculated using Schofield equations for basal metabolic rate. The rate of [¹³C]-recovery and its cumulative value were calculated and expressed as % of the dose.

A time lag in maximal oxidation of label between their different forms of administering was observed. For both substrates the label was the fastest recovered in case of its free form in fasted state, then for the aa physically mixed with a meal and the slowest for the aa bound into the protein. This was probably due to the delay in its absorption from the gut. This dynamic of oxidation caused the differences in their magnitude, significant only for leucine. Leucine bound in egg white protein was ca. 12% better utilised than when served in a free form with the same meal, probably due to the larger input to the small free leucine pool, which can overcome the current protein synthesis capacity. In both forms of aa in the meal, the cumulative values were lower for lysine than for leucine, but the difference was significant only in case of the free form (13.43 ± 1.3 and 18.68 ± 0.92 , respectively). That could be the consequence of larger capacity of free amino acids pool for lysine than leucine. Both substrates (as an essential amino acids) administered in post absorptive phase reflect the oxidation of aa from endogenous protein degradation which can be used to determine the rate of protein turnover. However leucine seems to fit better in this as it has simpler catabolism and is far more cost effective.

Keywords: ¹³CO₂ breath test, leucine, lysine, post prandial metabolism

Introduction

Modern nutrition is nowadays increasingly focused on providing indispensable nutrients in required and accurate amounts. In case of animal nutrition it is dedicated to increase the efficiency of production, whereas for human nutrition (mainly sports and enteral) it also stresses the importance of avoiding the increase of total caloric value of the meals by just increasing their nutritional density. The common way of enhancing the quality of poor dietary proteins (deficient in one or more amino acids) is supplementation of insufficient amino acids in free form. In previous studies, Metges et al. (2000) and Schreurs et al. (in preparation, EAAP), both authors independently showed metabolic time lag and catabolic differences for free and protein bound leucine. The conclusion is drawn that the nutritional value of dietary amino acids cannot be

assumed to be independent of their dietary form. In consideration of the above, it was of our interest to establish whether such differences between forms of leucine also apply to other amino acids of more relevance for nutritional practice and more common to be deficient. Lysine was chosen to be the subject of our study along with leucine in order to be able to compare the results, making sure that the conditions of experiment are the same for both amino acids.

Materials and methods

The studies were performed on adult, healthy human subjects. There were three female participants in sets of treatments with labelled leucine (age: 23, 22 and 25 y; body mass: 52, 65 and 66 kg; height: 1.57, 1.76 and 1.66 m). All of them, at least twice, participated in all three different treatments. In case of labelled lysine study there were four subjects: two males (age: 51 and 43 y; body mass: 79 and 76 kg; height: 1.78 and 1.76 m) and two females (age: 25 and 29 y; body mass: 66 and 60 kg; height: 1.66 and 1.71 m). Each subject repeated all types of treatments three times. One female subject participated in both studies. During the experimental period subjects maintained their personal non-specified lifestyle.

Substrates for breath test studies were: L-[1-¹³C]-lysine purchased from Cambridge Isotope Laboratories (Andover, USA) in a form of L-lysine (2HCL and L-[1-¹³C]-leucine obtained from Mass Trace, Inc (Woburn, USA). For both tracers: chemical purity >98%, isotopic enrichment 99%.

The breath tests were divided into three groups of treatments. A) in fasted (postabsorptive) state after oral ingestion of free tracer dissolved in 25 ml of distilled water. The tracer was dissolved in a 25ml of distilled water (additionally, 10 ml of water was used to rinse the vessel) B) in fed state (postprandial) after test meal with [1-¹³C]-aa added to unlabelled eggs C) in fed state after test meal with [1-¹³C]-egg white protein bound with a supplementation of unlabelled aa. Conditions were the same for both leucine and lysine.

The dose of substrate in a free form (treatment A) was calculated to give reliable [¹³C] enrichment of expired CO₂. The minimal oral intake, of 90 mg for L-[1-¹³C]-lysine and 60 mg for L-[1-¹³C]-leucine was served.

In cases of treatments B and C the Test Meal was served. It consisted of two slices of white bread (60 g ± 5 g) and two eggs (110 g ± 10 g) mixed with a pinch of salt and a dose of free form of aa. Eggs were heated on a plate in a microwave (950 Watt) for 4 min and served with bread. Total nutritional value: energy 1113 kJ (± 105 kJ), protein 15.5 g (± 1 g), fat 11.2 g (± 1 g) and carbohydrates 25.8 g (± 2 g). Total content of unlabelled amino acid: lysine 837 mg (± 75 mg) and leucine 1312 mg (± 117 mg). Drinking tea or coffee (without sugar or milk) and water was allowed at any time. Subjects were instructed to avoid corn products (corn on the cob, sweet corn, nachos, tortillas, cornflower etc.), before and between all experiments, as they naturally contain more ¹³C than other products. There were no special requirements regarding their activity except for staying rest. The experiments were performed not more often than every other day.

In order to get each substrate bound in protein, [¹³C]-labelled eggs were produced as described by Evenpoel et al., (1997).

Breath sampling procedure was as follows. After standard breakfast at 8 a.m. at home (two slices of white bread with Orinoko Milk Chocolate Spread from Peeters Producten Roosendaal, The Netherlands), subjects arrived to the laboratory before 10 a.m. Firstly, two blank breath samples were collected. Immediately after oral ingestion of the substrate, time was set at zero (ca. 10.30 a.m.). Thereafter, breath samples were collected after 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min. Subjects used a drinking straw to expire smoothly at the bottom of a 10 ml glass tube (Labco, High Wycombe, UK). Tubes were closed and kept at room temperature until IRMS analysis within a few days.

Calculations and statistics

The [¹³C]-enrichment of labelled egg white as well as the CO₂ in the breath samples was measured at the WIAS-IRMS facility with Finnigan Delta C Isotope Ratio Mass Spectrometer (IRMS) equipped with an Elemental Analyser and a Breath device. The [¹³C]-enrichment of the expired CO₂ was determined as Atom%-excess and expressed as rate of recovery (% dose/h) and cumulative recovery (% dose) using Schofield, (1985) equations for BMR, corrected for activity with a factor 1.5 and an energy equivalent of CO₂=25 kJ/L CO₂ (RQ=0.8)

All data was analysed by multivariate analysis of variance (MANOVA) using SPSS programme 8.0 for Windows. For those measures for which there was a significant interference, post hoc testing of individual means was performed with the tukey test. The differences were considered significant when p<0.005.

Results

The summary of 6-h breath test results is presented in Table 1. For both aa the time lag in maximal oxidation of label between their different forms of administering was observed. For lysine and leucine the label was the fastest recovered in case of its free form - both after 30 min, then for the aa mixed with a meal - both after 105 min - and the slowest for the aa bound into the protein- after 120 and 150 min, respectively. The maximal rate of oxidation was significantly higher in postabsorptive than in postprandial phase but not different between aa.

The recovery of label derived from both aa in free form mixed with a meal was faster, but not statistically significant, than for the protein bound aa, probably due to the delay in its absorption from the gut. This dynamic of oxidation probably caused the differences in their magnitude (cumulative recovery), which was significant only for leucine. Leucine bound in egg white protein was significantly better utilised than when served in a free form with the same meal. Comparison between both aa shows significant difference only in case of treatment B, where cumulative recovery of label derived from lysine is smaller than from leucine.

Table 1. The summary of results of [*I*-¹³C] lysine and [*I*-¹³C] leucine breath tests.

| | | Free, postabsorptive | Free, mixed with meal protein | Protein bound to meal protein |
|---------|------------------------------|-------------------------|----------------------------------|----------------------------------|
| Lysine | Cumulative recovery (Std) | 13.3 (1.4) | 13.4 (1.3) ^a | 14.9 (1.4) |
| | Maximal %dose/h (Std) | 8.1 (1.8) | 4.6 (0.4) | 5.1 (0.5) |
| | oxidation Time of peak (min) | 30 | 105 | 120 |
| Leucine | Cumulative recovery (Std) | 11.6 (0.8) ^A | 18.7 (0.9) ^{b,B} | 16.7 (0.9) ^C |
| | Maximal %dose/h (Std) | 7.4 (0.2) | 6.5 (0.5) | 5.8 (1.0) |
| | oxidation Time of peak (min) | 30 | 105 | 150 |

^{a,B} small letters indicate statistically significant differences (p<0,05) between different aa (rows) and capital letters between the forms of each aa (columns)

Discussion

The main purpose of this study was to establish whether differences between dietary forms (free vs. protein bound) of lysine influences its nutritional value as it holds for leucine and examine potential differences in their catabolism. In general, the results are comparable. Both aa show time lag in breath test response probably as a result of delayed availability for absorption of substrate caused by digestion processes and gastric emptying. Maximal oxidation rate, which is associated

with maximal availability of free substrate for absorption, was reached at the same time (at 105 min) for lysine and leucine given in free form mixed with meal but at different time (at 120 and 150 min., respectively) for protein bound substrates. The latest difference, however noticeable, is minor and not significant. The only significant difference between both amino acids applies to the recovery of label [¹³C] in case of second treatment, where substrates were given in a free form with meal. Not only peak value, but also cumulative recovery is much lower for lysine compared with leucine. Homeostatic control of free amino acids pool in plasma seems to play a key role in this phenomenon. It keeps the levels of aa in the plasma constant and low relative to the amounts ingested by a meal. However, those levels vary for different aa and depend on the physiological condition and anabolic capacity. The plasma pool for free lysine is larger than for free leucine, according to Garrow and James, (2000) it amounts to 195 and 120 (mol/L plasma water, respectively. This could explain why leucine reaches its threshold value earlier and as a consequence its excess is cleared by the catabolic pathways (deamination and decarboxylation) in relative, larger amounts.

Both leucine and lysine (as essential amino acids) administered in post absorptive phase reflect the oxidation of aa from endogenous protein degradation which can be used to determine the rate of protein turnover. However leucine seems to fit better in this as it has simpler catabolism and is far more cost effective.

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Measurement of albumin synthesis in the pig

S. Dänicke¹, E. Swiech², L. Buraczewska² & K. Aulrich¹

¹ Institute of Animal Nutrition of the Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig, Germany

² The Kielanowski Institute of Animal Physiology and Nutrition, ul. Instytucka 3, 05-110 Jabłonna near Warsaw, Poland

Summary

Albumin synthesis was measured in three pigs using a large dose of L-[²H₅ring]phenylalanine combined with unlabelled phenylalanine which was infused intravenously. Blood samples were taken up to 90 min after infusion of the isotope. Isotope enrichment was measured in plasma-free phenylalanine which served as an indicator of the precursor pool, and in albumin-bound phenylalanine using GC-MS. The fractional albumin synthesis rate was estimated at 31%(6% per day, which is on the same order of magnitude as reported by Jahoor *et al.* (1994), who used a priming dose followed by a continuous infusion of stable isotopes in pigs.

Keywords: albumin synthesis, pig, stable isotope

Introduction

Plasma albumin concentration is a poor indicator for the protein synthesis capacity of the liver under many physiological and pathological conditions. The measurement of albumin synthesis in humans using stable isotopes was proven useful for a better understanding of the underlying mechanisms of albumin turnover and liver function under such conditions.

Albumin synthesis can be measured by following the kinetics of the incorporation of stable isotopes into albumin as demonstrated in humans (e.g., Ballmer *et al.*, 1990; Slater *et al.*, 1995), in sheep (Conell *et al.*, 1997) and in pigs (Jahoor *et al.*, 1994).

The aim of the present study was to adapt the flooding dose technique - with L-[²H₅ring]phenylalanine as the tracer amino acid - to measure albumin synthesis in pigs.

Material and methods

The experimental protocol was adapted from Ballmer *et al.* (1990), while the GC-MS-methods for measurement of isotopic enrichment in the precursor and in albumin were used as described by Slater *et al.* (1995).

Three female pigs (39kg ±3 kg) were surgically fitted with permanent intravenous catheters. On the measurement day, a large dose of a mixture of L-phenylalanine and L-[²H₅ring]phenylalanine in physiological saline (104 mmol/L, 11 atom percent excess, APE) was injected intravenously (45 mg phenylalanine per kg body weight). Blood samples were taken at 2, 5, 10, 15, 20, 30, 50, 70 and 90 min after injection and prepared for plasma. Plasma-free phenylalanine APE was assumed to be an indicator for the albumin precursor pool and was determined by GC-MS in the trichloroacetic acid (TCA) soluble fraction of plasma. APE was calculated from the ratio between the peak areas at m/z 239 and m/z 236. Albumin was isolated from the TCA-precipitated plasma protein by differential solubility in absolute ethanol. Purity of the isolated albumin was confirmed by SDS-electrophoresis (Figure 1). After several cleaning and preparation steps, the APE of the albumin bound phenylalanine was determined by using the same GC-MS-instrument. The ratio between the peak areas at m/z 183 and m/z 180 was used for APE-calculations. The fractional

albumin synthesis rate (FSR) was calculated from the ratio between the time-related linear increase in albumin enrichment and the corresponding area under the time-APE curve of the precursor (plasma-free phenylalanine). Albumin FSR is expressed as a percent of the intravascular albumin pool which is synthesized daily.

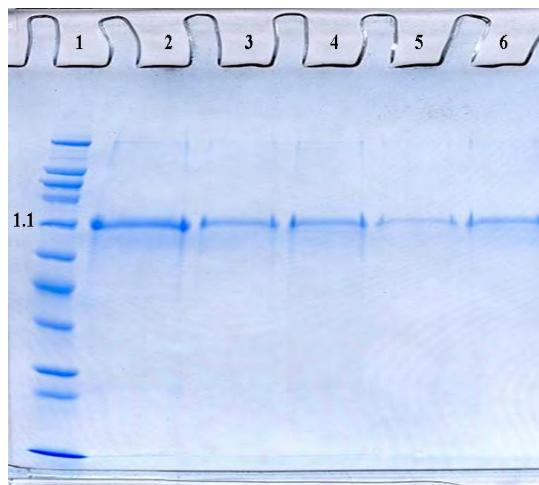


Figure 1. SDS-PAGE (10%) of isolated albumin from pig (1 - protein-standard, 1.1 - bovine serum albumin (MW: 66000), 2 - Albumin-pig 16 (dilution 1), 3 - Albumin-pig 16 (dilution 2), 4 - albumin-pig 16 (dilution 3), 5 - albumin-standard from pig (MW: 68000, 1 µg), 6 - albumin-standard from pig (2 µg).

Results and discussion

The APE of plasma-free phenylalanine decreased in a linear fashion over the measurement period (Figure 2). Enrichment appeared in plasma albumin with a time delay (Figure 2) known as the so-called secretion time, i.e., the time required for albumin to be synthesized and processed in the hepatocyte before being released into circulation. This secretion time was estimated by the extrapolation of the linear increase in albumin APE and was found to be $13\text{min} \pm 7\text{min}$. The fractional albumin synthesis rate was estimated at $31\% \pm 6\%$ per day. This value is on the same order of magnitude as reported by Jahoor *et al.* (1994), who used a priming dose followed by a continuous infusion of stable isotopes.

The absolute amount of daily synthesized vascular albumin mass was estimated by multiplying the fractional albumin synthesis rate with the plasma albumin concentration and the plasma volume. The latter was estimated from a published plasma volume - body weight relationship for growing pigs (Yang & Lin, 1997). Based on these assumptions, the absolute albumin synthesis was estimated at 15 ± 1 g per day.

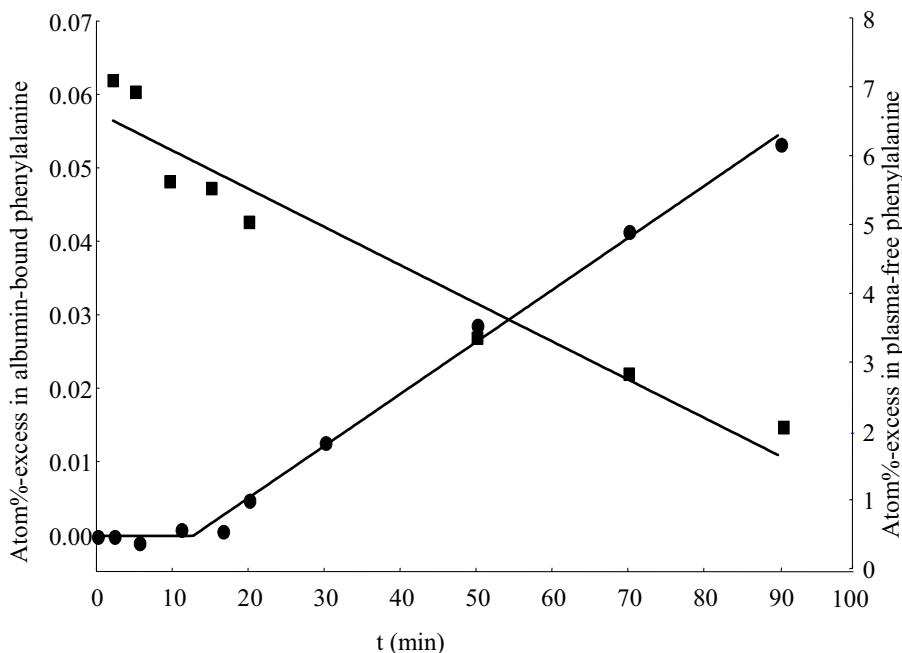


Figure 2. Time course of APE in albumin bound and plasma free phenylalanine of pig 16.
—●—APE in albumin-bound phenylalanine = $(t_s \cdot b + a) \cdot (t \leq t_s) + (a + b \cdot t) \cdot (t > t_s)$, where t = time in min, t_s = secretion time of albumin, a = intercept on ordinate and b = slope of the APE increase in albumin-bound phenylalanine;
—■—APE in plasma free phenylalanine = $a - b \cdot t$, where t = time in min, a = intercept on ordinate, b = slope of APE-decrease in plasma phenylalanine.

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Dietary cysteine cannot replace dietary methionine for protein accretion in growing broiler chickens

A.A. Fatufe & M. Rodehutscord

Universität Halle-Wittenberg, Institut für Ernährungswissenschaften, Emil-Abderhalden-Str. 26, 06108 Halle (S.), Germany

Summary

In a 14-day dose-response study, we studied the effect of increasing sulfur-containing amino acid (SAA) concentration in the diet on feed intake, growth rate, feed conversion and composition of gain in male growing broilers. SAA were supplemented to two basal diets (calculated to be equal in Met and Cys with 4.5 g/kg total SAA) in 7 graded levels by replacement of equivalent masses of NEAA with either pure DL-Met or a 1:1 mix of DL-methionine and L-cysteine. An upper level of 10.5 g/kg SAA was achieved. Broilers responded non-linearly to increasing concentration of SAA with increased feed intake, improved growth rate and better feed conversion and a plateau was achieved in all cases. The 1:1 mix of Met and Cys failed to match the growth performance of Met-alone diets in the range of suboptimal SAA supply. SAA intake and feed conversion were similar between the two alternatives. Response in protein accretion was also non-linear over the entire range of SAA intake. Content of Met but not Cys increased in gained body protein with increased SAA intake. 95% of y_{max} in protein accretion were achieved with a SAA concentration of 5.9 (Met alone) and 8.1 g/kg (1:1 mix of Met+Cys), respectively. The maximum in marginal efficiency was 88% in case of Met alone and 45% in case of the 1:1 mix of Met and Cys. It is concluded that cysteine cannot replace at all dietary methionine for protein accretion.

Keywords: broiler, sulphur containing amino acids, efficiency, utilisation, accretion

Introduction

Sulphur containing amino acids (SAA) are the first limiting amino acids under most dietary conditions for broiler chicks. Recommendations for SAA by scientific committees (GfE, 1999; NRC, 1994) are usually stipulated on the basis of methionine (Met) and total SAA. This implies that cysteine (Cys) can replace Met for protein accretion. There are conflicting reports in the literature on the replacement value of Cys for Met in growing animals. Even though many dose-response studies have already been conducted on this subject, the efficiency of utilisation of SAA for protein accretion has not been investigated. This study was conducted, therefore, to determine (1.) how incremental SAA intake affects SAA utilisation, and (2.) whether Cys can replace Met for protein accretion.

Materials and methods

A basal diet clearly deficient in SAA and mainly based on maize, peas, field beans, wheat gluten and free amino acids was used. The basal diet was calculated to be 10% in excess in all essential amino acid (GfE, 1999) except in SAA content. The basal diet was mixed as a single lot and divided into 4 portions. The 4 portions were modified to conduct 4 experiments, and only two of these experiments are reported herein. Two basal diets (table 1) calculated to be equal in N, essential amino acids and metabolisable energy but slightly different in non-essential amino acid (NEAA) content were supplemented in 7 graded levels with SAA at the expense of equivalent mass of an NEAA mix either as pure DL-Met or as 1:1 mix of DL-Met and L-Cys. Intended levels

of SAA were confirmed by analysis. Analysed concentrations of SAA in diets with only DL-Met supplementation were 4.2, 4.6, 5.0, 5.5, 6.1, 7.3, 8.0 and 10.3 g/kg diet while the values for the diets containing the Met:Cys mix were 4.5, 5.0, 5.5, 6.0, 6.5, 7.5, 8.5 and 10.5 g/kg. Diets were pelleted through a 3-mm die. Male broiler chickens (Ross) were fed *ad libitum* for 14 d beginning on d 8 post hatch. Each diet was allocated to three pens of 10 birds each. All birds were killed by carbon dioxide asphyxiation on d 21, and representative control animals were also killed on d 8 for baseline body composition measurement. Body homogenates were freeze-dried, and analyses were made in defatted and finely ground material. SAA content in samples was determined after oxidation according to VDLUFA standard methods (VDLUFA, 1997). Accretion of protein and amino acids was determined by comparative whole body analysis.

Table 1. Composition and analysis of the basal diets.

| Ingredients g/kg | Pure Met diet | Met:Cys 1:1 diet |
|--------------------------|---------------|------------------|
| Maize | 483.5 | 483.5 |
| Field beans | 130 | 130 |
| Peas | 130 | 130 |
| Wheat gluten | 60 | 60 |
| Maize starch | 75.37 | 74.27 |
| Soya oil | 40 | 40 |
| Glycine | 2 | 2 |
| Premix ¹ | 10 | 10 |
| Dicalcium phosphate | 26 | 26 |
| Limestone | 8 | 8 |
| Salt (NaCl) | 3 | 3 |
| EAA ² | 28.09 | 28.09 |
| NEAA MIX1 ³ | 4.04 | - |
| NEAA MIX2 ⁴ | - | 5.14 |
| Analysed contents (g/kg) | | |
| Dry matter | 902 | 897 |
| Crude Protein | 209 | 211 |
| ME MJ/kg ⁵ | 13.2 | 13.2 |
| Methionine | 1.7 | 1.9 |
| Met + Cys | 4.2 | 4.5 |

¹ Supplied per kg of diet: vitamin A 12000 IU; cholecalciferol 3000 IU; vitamin E 42 mg; vitamin K2 mg; vitamin B₁ 2 mg; vitamin B₂ 6.6 mg; vitamin B₆ 5mg; vitamin B₁₂ 0.02 niacin 36 mg; pantothenic acid 15 mg; nicotinic acid 36 mg; biotin 0.15 mg; folic acid 1 mg; BHT 120 mg; choline chloride 700 mg; Ca 0.23 g; Mg 0.071 g; Fe 60 mg; Mn 61 mg; Zn 51 mg; Cu 5 mg; I 0.62 mg; Se 0.20 mg

² Comprises (in g): L-threonine 4.4; L-tryptophan 0.7; L-valine 5.1, L-isoleucine 1.7; L-arginine 4.69; L-lysine HCl 8.5; L-histidine 0.5; L-leucine 1.7; L-phenylalanine 0.8

³ Amino acid composition (g/100g): L-glutamic acid 50.5 g, L-aspartic acid 24.75 g, L-alanine 24.75 g

⁴ Amino acid composition (g/100g): L-glutamic acid 53.75 g, L-aspartic acid 21.41 g, L-alanine 24.84 g

⁵ Calculated

The response to increasing SAA concentration and SAA intake, respectively, was described by a sigmoidal function (Gahl et al. 1994). The marginal efficiency of SAA utilisation (Δ SAA accretion/ Δ SAA intake) was calculated as the first derivative of the sigmoidal function for SAA accretion depending on SAA intake.

Results

Table 2 shows the SAA intake, body weight gain and feed conversion of chickens fed the two SAA alternatives. Birds clearly responded to incremental SAA intake with distinct differences between the SAA alternatives (figure 1). To achieve 95% of y_{max} in protein accretion, a SAA concentration of 5.9 (Met) and 8.1 g/kg (Met+Cys) was needed (left panel, figure 1). The response in protein accretion was non-linear over the entire range of SAA supply. Met but not Cys concentration in gained protein was affected by SAA supply. The marginal efficiency of supplemented SAA was calculated as a first derivative of methionine accretion depending on SAA intake (right panel, figure 1). The maximum in marginal efficiency was 88% in case of Met alone and 45% in case of the 1:1 mix of Met and Cys.

Table 2. SAA intake, body weight gain and feed conversion of male broilers fed two SAA alternatives (M: Met alone, M+C: Met:Cys 1:1; n=3 pens of 10 birds per treatment, growing from d 8 to 21).

| Treatments | BW at start (g) | | SAA intake (g) | | BW gain (g) | | Feed/gain (g/g) | |
|-------------------------------|-----------------|-------|----------------|--------|-------------|--------|-----------------|-------|
| | M | M+C | M | M+C | M | M+C | M | M+C |
| B ¹ + ... g/kg SAA | | | | | | | | |
| 0.0 | 107.0 | 105.9 | 1.34 | 1.37 | 150.8 | 137.2 | 2.41 | 2.26 |
| 0.5 | 107.0 | 107.2 | 1.84 | 1.95 | 218.0 | 172.0 | 1.90 | 2.26 |
| 1.0 | 106.9 | 106.3 | 2.42 | 1.91 | 342.2 | 204.7 | 1.42 | 1.75 |
| 1.5 | 105.9 | 106.2 | 4.04 | 3.02 | 532.0 | 286.3 | 1.37 | 1.76 |
| 2.0 | 107.2 | 107.6 | 4.43 | 3.84 | 555.0 | 364.6 | 1.31 | 1.61 |
| 3.0 | 106.1 | 106.3 | 5.47 | 5.41 | 565.8 | 543.4 | 1.33 | 1.33 |
| 4.0 | 106.7 | 107.2 | 6.18 | 6.36 | 572.9 | 548.3 | 1.35 | 1.36 |
| 6.0 | 106.4 | 107.8 | 6.80 | 7.73 | 491.7 | 555.1 | 1.35 | 1.33 |
| Pooled SEM | 0.26 | 0.26 | 0.403 | 0.462 | 33.5 | 35.3 | 0.12 | 0.09 |
| <i>P (ANOVA)</i> | | | | | | | | |
| SAA | | | | <0.001 | | <0.001 | | 0.001 |
| SOURCE | | | | 0.394 | | <0.001 | | 0.208 |
| SAA (SOURCE) | | | | 0.024 | | <0.001 | | 0.892 |

¹ Basal diet for supplementation of Met alone contained 1.7 g/kg Met and 4.2 g/kg SAA while the basal diet for supplementation of 1:1 mix of Met:Cys contained 1.9 g/kg Met and 4.5 g/kg SAA.

Discussion and conclusions

The response to increasing SAA intake in both SAA alternatives was non-linear and, therefore, the efficiency of SAA utilisation is not constant even in the range of marginal SAA supply. This needs consideration in future modeling of amino acid requirements. The maximum in the efficiency of utilization was achieved with dietary SAA concentrations that were lower than those needed for high protein accretion (figure 1). Dietary cysteine completely failed to replace dietary methionine for protein accretion. The consistently lower and almost halved growth performance observed with the 1:1 mix of Met:Cys as compared to pure Met at the marginal range of SAA supply despite the similarity in SAA intake is an indication that the response can be almost exclusively attributed to methionine supplementation and not SAA supply. Based on growth and feed conversion data, Huyghebaert and Pack (1996) concluded that Cys has a relative efficacy of about 60% compared to Met. In their study the range in dietary SAA supply and in response, respectively, was lower than in the present work.

Since cysteine cannot replace methionine for protein accretion (at this growth phase) and a cysteine content of less than 2.6 g/kg is unlikely to occur in diets based on practical ingredients, requirements for SAA may be more accurately expressed with the focus on methionine. More attention should be paid to the non-linearity in efficiency of utilisation depending on intake, and how to include this into dietary allowances.

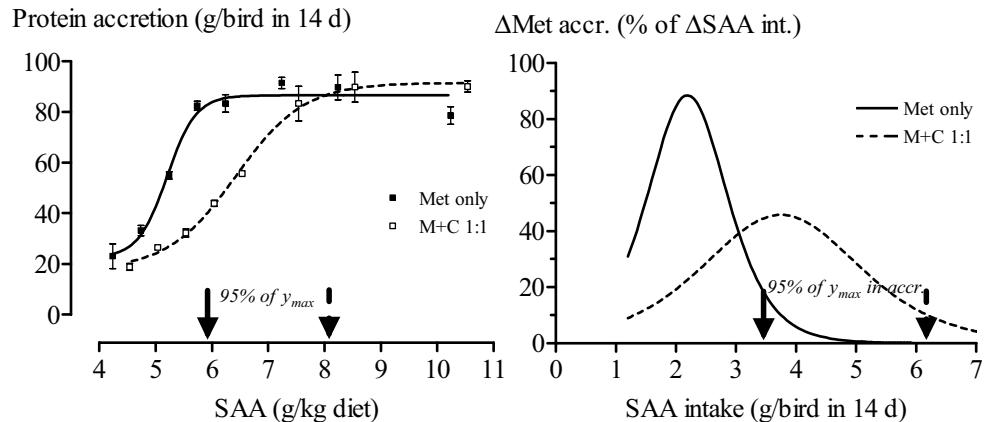


Figure 1. Response of broiler chickens in protein accretion (Av. and SEM) and marginal efficiency of SAA utilisation ($n=3$ pens of 10 birds per treatment, growing from d 8 to 21). Arrows show the x -value needed to achieve 95% of y_{max} in the different measurement.

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Lysine requirement in adult miniature pigs estimated by an indicator amino acid oxidation method

Ulf Hennig¹, Gunda Backes², Klaus J. Petzke², Angelika Elsner², Peter Junghans¹, Michael Dernö¹, Gerd Nürnberg¹ & Cornelia C. Metges^{1,2}

¹ Research Units Nutritional Physiology, and Genetics and Biometrics, Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany

² German Institute of Human Nutrition, 14558 Bergholz-Rehbrücke, Germany

Summary

Lysine requirement values were derived by an indicator amino acid oxidation (IAAO) method from measurements of leucine oxidation and balance in minipigs fed wheat gluten (WG) based diets at two different lysine intakes (WG 2.7 g; WG+Lys, 6.6 g lysine·× kg⁻¹ diet). ¹³C bicarbonate recoveries were used to correct for incomplete recovery of ¹³C in breath. The dietary lysine concentration significantly affected the ¹³C bicarbonate recoveries. The daily ¹³C-leucine balances were dependent of the dietary lysine level and were: WG -46.0(19.7, WG+Lys 20.0±19.7 mg·kg⁻¹·d⁻¹ ($P<0.05$). Lysine requirements were 105 mg × kg⁻¹ × d⁻¹ and 84 mg × kg⁻¹ × d⁻¹ when the ileal leucine losses are considered or omitted, respectively. This is in reasonable agreement with the lysine requirement of about 86 mg × kg⁻¹ × d⁻¹ in adult conventional swine.

Keywords: lysine requirement, leucine oxidation, ileal losses

Introduction

The classic nitrogen balance method to assess the requirements of amino acids (AA) in adult pigs is characterized by several methodological limitations. A method frequently used in humans is the indicator amino acid oxidation (IAAO) method (Zello et al. 1993) which is based on the concept that an imbalanced AA supply caused by the deficiency of an indispensable AA results in the oxidation of any surplus of the other indispensable amino acids. The requirement is met at that test AA intake when the oxidation rate of the tracer AA reaches a minimum. The aim of this investigation was to estimate the requirement of lysine using ¹³C-leucine as IAA in adult miniature pigs with consideration of the quantitative importance of ileal leucine losses .

Material and methods

Experimental design and diets

Ten male castrated minipigs (strain Minilewe) were purchased at a mean age of 8 wk and a mean body weight (BW) of 8 kg and they were fed a commercial diet ("Masta Universal") until the pigs each reached a BW of 23 kg. The pigs were randomly assigned to two feeding groups. and fed wheat gluten - wheat starch based diets without or with supplementation of crystalline lysine (WG, 2.7 g; WG+Lys, 6.6 g lysine·× kg⁻¹ diet, respectively) in amounts of 40 g DM × kg^{0.75} BW × d⁻¹. Pigs were fitted with ileo-ileal reentrant cannulas to collect ileal effluent during the last three days of the experiment and catheters were implanted into the jugular vein and carotid artery for tracer infusion and blood sampling, respectively. On days 7 and 9, respectively, 10 h fasted and 10 h fed intravenous tracer protocols with L-[1-¹³C]-leucine (99 atom %; 3.5 µmol × kg⁻¹ × h⁻¹; priming dose 5.25 µmol × kg⁻¹ × h⁻¹) and [¹³C]-sodium bicarbonate (99 atom %; 3.5 µmol × kg⁻¹ × h⁻¹; priming dose 5 µmol × kg⁻¹ × h⁻¹) to estimate leucine oxidation rates were performed. Leucine

oxidation rate was calculated based on plasma α - ^{13}C -ketoisocaproic acid enrichment and ^{13}C in breath- CO_2 corrected by the $^{13}\text{CO}_2$ recovery factor in breath.

Table 1. Experimental design.

| Dietary group | WG | WG+Lys |
|--|-----|--------|
| Lysine, g $\times \text{kg}^{-1}$ diet | 2.7 | 6.6 |
| Ileo-ileal cannula, d | -18 | -18 |
| Catheter, d | -3 | -3 |
| ^{13}C -leucine, d | 7 | 7 |
| ^{13}C -bicarbonate, d | 9 | 9 |

Mean BW of pigs on the day prior to the tracer infusions was 23.3 ± 0.5 kg. During the first 4 h of the infusions no feed was given while from 12.00 h to 18.00 h pigs were fed half-hourly meals as shown in Figure 1.

Sample collection and analysis

Blood and breath samples were collected during the infusions according to the Figure 1.

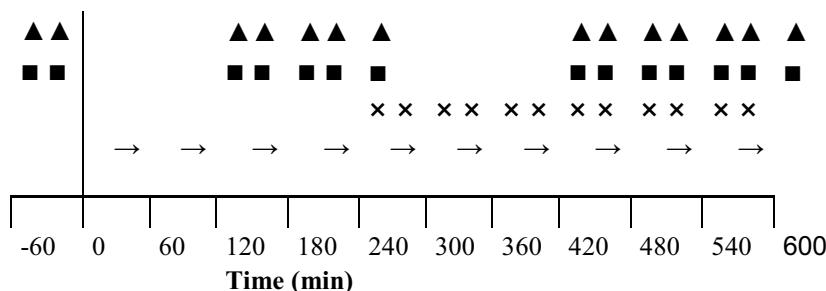


Figure 1. Sampling protocol of 10h ^{13}C -leucine and ^{13}C -bicarbonate infusions (\rightarrow i.v. infusion; \times small meals; ■ blood; ▲ breath).

Before the infusions baseline samples were drawn and during the last 2 and 3 h of the fasted and fed state samples were drawn every 30 min and plasma was separated. Breath samples were collected in gas tight bags using a face mask and transferred into evacuated tubes. The plasma α -[^{13}C]-ketoisocaproic acid enrichment, considered to represent the enrichment of the intracellular leucine pool, was measured in quinoxalinol-t-BDMS derivates by electron-impact GC-MS (Matthews *et al.*, 1982). Enrichments of ^{13}C in breath were analyzed by isotope ratio mass spectrometry and expressed as AP excess (APE). The APE was calculated by taking the difference between the enrichment of each breath sample and the basal breath sample. The determination of ileal leucine losses has been performed as previously described in Backes *et al.* (2002). The $^{13}\text{CO}_2$ production has to be corrected for incomplete recovery of $^{13}\text{CO}_2$ in breath. The recovery (R) of $^{13}\text{CO}_2$ was calculated as the ratio of the $^{13}\text{CO}_2$ output to the amount of [^{13}C]-bicarbonate infused. An individual value for each pig was derived for the fasted and fed states each which was used to correct the $^{13}\text{CO}_2$ production measured during [^{13}C]-leucine infusion.

The leucine oxidation rates per kg BW were calculated using the equation:

$$\text{Oxidation rate } (\mu\text{mol} \times \text{kg}^{-1} \times 30 \text{ min}^{-1}) = \frac{\text{$_{13}$CO}_2 \text{ production } (\mu\text{mol} \times \text{kg}^{-1} \times 30 \text{ min}^{-1})}{\text{Plasma } [^{13}\text{C}] \text{-KIC enrichment (MPE) / 100}}$$

where:

$\text{$_{13}$CO}_2$ production = VCO_2 ($\mu\text{mol} \times \text{kg}^{-1} \times 30 \text{ min}^{-1}$) \times $\text{$_{13}$CO}_2$ breath enrichment (APE) \times 1/R
and VCO_2 is the volume of CO_2 produced.

R is the recovery factor of ^{13}C in breath.

$$R (\%) = \frac{\text{VCO}_2 \text{ (mmol} \times \text{kg}^{-1} \times \text{h}^{-1}) \times \text{$_{13}$CO}_2 \text{ breath enrichment (APE) / 100}}{[^{13}\text{C}] \text{-bicarbonate infused } (\mu\text{mol} \times \text{kg}^{-1} \times \text{h}^{-1})}$$

CO_2 production in each pig was determined on a separate occasion by means of gas exchange measurement using the same feeding pattern as applied during isotope infusions. 24h leucine oxidation rates were extrapolated from the measured fasted and fed oxidation rates assuming that the animals were in the fasted and fed state for 12 h each. Lysine requirement was derived graphically from the intersection with the zero balance line.

Statistical analysis. Data were analysed by ANOVA using the SAS GLM procedure and comparison of means. The significance level was set at $P < 0.05$.

Results and conclusion

^{13}C bicarbonate recoveries were in general agreement with values derived in human subjects, and dependent on dietary lysine concentration and metabolic states (fed vs. fasted) with lower values with the low lysine diet (WG), and significantly higher in the fed vs. the fasted state (Table 2; $P < 0.05$). It was rather unexpected that the $\text{$_{13}$CO}_2$ bicarbonate recovery was significantly lower with the lower dietary lysine intake. This implies that more carbon is fixed in the body with marginal lysine supply which might be a consequence of the imbalanced amino acid pattern in the WG diet. In this fixation process carbon could be sequestered via anaplerotic reactions to replenish citric acid cycle intermediates. The daily ^{13}C -leucine balances were significantly affected by the dietary lysine level (Table 2).

Table 2. ^{13}C bicarbonate recovery rates and leucine balance data of minipigs fed two different lysine levels (LSM \pm SE).

| | WG | WG+Lys |
|--|------------------------|-------------------|
| ^{13}C recovery (%) | | |
| fasted | 69.1 ± 3.3^1 | 82.5 ± 3.3 |
| fed | 85.9 ± 3.3 | 90.6 ± 3.3 |
| Daily leucine balance ($\text{mg} \times \text{kg}^{-1} \times \text{d}^{-1}$) | | |
| with consideration of ileal leucine loss | $-46.0 \pm 19.7^{1,2}$ | 20.0 ± 19.7 |
| without | -29.1 ± 21.3^1 | 48.2 ± 21.3^2 |

¹ diet groups are significantly different at $P < 0.05$.

² significantly different from zero.

Based on these data we estimate that the lysine requirement of adult minipigs is $105 \text{ mg} \times \text{kg}^{-1} \times \text{d}^{-1}$ when the ileal leucine losses are considered and added to the oxidative losses, while it is $84 \text{ mg} \times \text{kg}^{-1} \times \text{d}^{-1}$ when these losses are omitted. These values are in reasonable agreement with the lysine requirement of about $86 \text{ mg} \times \text{kg}^{-1} \times \text{d}^{-1}$ in adult swine (McLarney et al., 1996).

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Influence of protein and threonine level on liver threonine dehydrogenase activity and efficiency of threonine utilization in diets for rat and chicken

F. Liebert¹ & C.W. Lee²

¹ Institute for Animal Physiology and Animal Nutrition, Georg-August-University Goettingen, Kellnerwer 6, 37077 Goettingen, Germany

² Institute of Genetic Engineering, Changwon National University, 9 Sarim-dong, Changwon City, Gyeongnam, 641-773, Korea

Summary

Threonine oxidation rate and threonine efficiency were investigated in laboratory rat and chicken fed diets with graded levels of protein and threonine supply. The increase in threonine (Thr) content from 0.28 to 0.72% in a diet containing 12.0% CP (crude protein) caused a gradual increase in threonine dehydrogenase (TDG) activity in rat liver. Similar, but more pronounced results were observed after 18.0% CP in the diet. Both protein levels in combination with the highest threonine supplementation increased liver TDG activity significantly, indicating enhanced threonine catabolism. Parameters of efficiency of threonine utilization, derived from parallel nitrogen balance studies, gave also an indication of threonine oversupply after the highest level of threonine supplementation by decreasing threonine utilization rate significantly. At lower levels of threonine addition, the efficiency of threonine utilization was not significantly affected. In the chicken liver, up to 0.60 % dThr (true digestible threonine) in the 18.5% CP diet no significant effect on the TDG activity was observed. However, TDG activity in the liver was increased after application of the diet containing 22.5% CP (0.60% dThr). In this case threonine supplementation reduced the efficiency of threonine utilization, indicating the end of the limiting range of threonine. In conclusion, the results indicate that in vitro TDG activity in the liver of rat and chicken has an indicator function for the dietary supply of threonine.

Keywords: amino acid efficiency, threonine dehydrogenase, threonine utilization, rat, chicken

Introduction

The efficiency of threonine utilization is of special interest relating to actual studies about threonine requirement of growing animals. The level of threonine degradation by different pathways is an important factor corresponding to the dietary supply of this essential amino acid. Threonine is catabolized by the liver enzymes threonine dehydratase (TDH) as cytosolic enzyme and threonine dehydrogenase (TDG) as mitochondrial enzyme. TDG-pathway is responsible for more than 80 percent of the total threonine catabolism in the liver.

The present study was conducted, to elucidate whether in vitro TDG-activity in the liver is in connection with the efficiency of threonine utilization in diets for rats and growing chicken.

Material and methods

Totally 36 male albino rats (Wistar, SPF; 50-70g body weight) and 24 male growing chicken (Cobb 500, day 20-25) were used in N-balance studies. Additional a growth trial with chicken of the same genotype (day 17-30) was conducted. The semisynthetic diets were based on wheat gluten (rats) and wheat/wheat gluten (growing chicken) with different supplements of crystalline amino acids (tables 1, 2).

Table 1. Composition of basal diets for rats (g/ kg diet).

| Ingredients | 12% CP | 18% CP |
|-------------------|----------------------|----------------------|
| Wheat gluten | 148.00 | 223.00 |
| Wheat starch | ad 1000 | ad 1000 |
| Sucrose | 100.00 | 100.00 |
| Cellulose | 50.00 | 50.00 |
| Soybean oil | 55.00 | 50.00 |
| Mineral premix | 60.00 | 60.00 |
| Vitamin premix | 20.00 | 20.00 |
| DL-Methionine | 1.60 | 3.80 |
| L-Threonine | 0 / 1,45 / 4,45 | 0 / 1,05 / 3,05 |
| L-Isoleucine | 0.76 | 1.20 |
| L-Leucine | 2.58 | 3.90 |
| L-Lysine·HCl | 5.44 | 8.23 |
| L-Phenylalanine | 1.51 | 2.30 |
| L-Tryptophan | 0.50 | 0.80 |
| L-Valine | 1.25 | 1.90 |
| Amino acid ratios | Lys [1] : Thr [0.40] | Lys [1] : Thr [0.40] |
| ME (MJ/ kg DM) | 16.54 | 16.46 |

Table 2. Composition of basal diets for chicken (g/ kg diet).

| Ingredients | 18.50 % CP | 22.50 % CP |
|-----------------------------|---|---|
| Wheat | 588.80 | 739.20 |
| Wheat gluten | 94.20 | 118.30 |
| Wheat starch | 213.30 | 30.10 |
| Soybean oil | 28.00 | 40.00 |
| Premix(Vitamins, minerals) | 10.00 | 10.00 |
| Mono calcium phosphate | 20.50 | 18.00 |
| CaCO ₃ | 12.00 | 13.00 |
| NaCl | 3.00 | 3.00 |
| MgO | 0.40 | 0 |
| Celite | 10.00 | 10.00 |
| L-Threonine | 0.93 | 0 |
| L-Lysine·HCl | 6.14 | 7.71 |
| DL-Methionine | 1.49 | 1.87 |
| L-Tryptophan | 0.24 | 0.30 |
| L-Arginine | 2.83 | 3.62 |
| L-Isoleucine | 0.97 | 1.20 |
| L-Glycine | 7.20 | 3.70 |
| Amino acid ratios | Lys [1] : Thr [0.53] : Gly [1,30] : Gly+Ser [2,02] | Lys [1] : Thr [0.44] : Gly [0,89] : Gly+Ser [1,61] |
| ME _N (MJ/ kg DM) | 15.40 | 15.06 |

Threonine was the limiting amino acid in the basic diets. Two protein levels (12 vs. 18% CP) with three levels of threonine were compared in the rat experiments, two protein levels (18,5 vs. 22,5% CP) and two levels of threonine in experiments with growing chicken. The efficiency of threonine utilization was determined by the help of N-balance data based on an exponential N-utilization model for growing animals (Liebert and Gebhardt 1988, Thong and Liebert 2003). Livers of individual animals were homogenized in ice cold 0.25M sucrose solution and isolation of liver mitochondria fraction from the homogenate after the method of Schneider and Hogeboom (1950). The mitochondria solution was incubated in a metabolic shaker after a modified method of Bird et al. (1984). After protein precipitation aminoacetone and glycine were determined in the supernatants. The formation of aminoacetone and glycine (nmol/30min/mg protein) in the liver was used as indicator of the TDG-activity.

Results and discussion

The results with laboratory rats (table 3) show a gradual and partly significant increase of total TDG-activity in rat liver corresponding to the increase of threonine level from 0.28% (A) to 0.72% (C) in the diets containing 12.0% CP. Diets containing 18.0% CP and 0,42% (D) to 0,72% (F) threonine indicated the same trend, but at a higher level of TDG-activity. After application of the diets with the highest level of threonine supplementation (C resp. F) the TDG-activity was significantly increased, indicating enhanced threonine catabolism. The TDG-activity was almost exclusively modulated by aminoacetone accumulation, glycine accumulation was altered only after diet F. Threonine efficiency parameters are obviously responding to threonine oversupply and decrease significantly after diets C and F.

In chicken (table 4) an addition of 0.15% L-Threonine to the basal diet (18.5% CP) had no effect on TDG-activity. The similar supplementation to the diet with increased protein content (22.5% CP) increased the TDG-activity significantly. The response of the efficiency of threonine utilization was more pronounced for both levels of protein supply. The significant decrease of this parameter indicates the end of the limiting range of threonine in these diets.

Table 3. Effect of protein and threonine content in the diet on liver TDG-activity and Thr-efficiency of rats.

| Diet | CP-/ Thr-content | Mean liver weight (g) | TDG activity (nmol/ 30 min/ mg protein) | | | Thr-efficiency (bc ⁻¹)* |
|------|-----------------------|--------------------------|---|--------------------|---------------------|--|
| | | | Aminoacetone | Glycine | Total activity | |
| A | 12.0% CP 0.28% Thr | 9.3 ± 0.8 | 7.70 ^a | 0.86 ^a | 8.56 ^a | 502 ^{ad} |
| B | 12.0% CP 0.42% Thr | 9.7 ± 0.5 | 9.96 ^{ac} | 0.91 ^{ac} | 10.87 ^{ac} | 527 ^{ad} |
| C | 12.0% CP 0.72% Thr | 10.1 ± 0.6 | 12.22 ^{bc} | 0.77 ^a | 12.99 ^{bc} | 288 ^b |
| D | 18.0% CP 0.42% Thr | 10.5 ± 0.7 | 10.59 ^{ac} | 1.02 ^{ac} | 11.60 ^{ac} | 578 ^{ac} |
| E | 18.0% CP 0.52% Thr | 11.1 ± 1.2 | 13.13 ^{bc} | 0.60 ^a | 13.74 ^{bc} | 674 ^c |
| F | 18.0% CP 0.72% Thr | 10.3 ± 1.0 | 15.16 ^b | 1.42 ^{bc} | 16.57 ^b | 445 ^d |

* Data from N-balance trial, exponential N-utilization model

Different superscripts within a row indicate significant differences (p ≤ 0,05; Tukey test)

Table 4. Effect of protein and threonine content in the diet on liver threonine dehydrogenase activity of chickens.

| Diet | CP-/Thr-content | Mean liver weight (g) | TDG activity (nmol/ 30 min/ mg protein) | | | Thr-efficiency (bc ⁻¹)* |
|------|-------------------------|-----------------------|---|-------------------|--------------------|-------------------------------------|
| | | | Aminoacetone | Glycine | Total activity | |
| A | 18.5% CP 0.45% dThr* | 34.2 ± 4.0 | 2.58 ^a | 1.71 ^a | 4.29 ^a | 144 ^a |
| B | 18.5% CP 0.60% dThr | 37.8 ± 4.8 | 2.78 ^a | 1.49 ^a | 4.27 ^a | 126 ^b |
| C | 22.5% CP 0.45% dThr | 40.8 ± 2.0 | 3.90 ^{ab} | 2.62 ^a | 6.52 ^a | 177 ^c |
| D | 22.5% CP 0.60% dThr | 37.5 ± 4.9 | 5.88 ^b | 5.57 ^b | 11.45 ^b | 162 ^d |

* true digestible threonine (AminoDat 1.1, Degussa 1997)

** Data from N-balance trial, exponential N-utilization model.

Different superscripts in the row indicate significant differences ($p \leq 0.05$; Tukey test).

In rats at 12%CP the TDG-activity was significantly increased after threonine supplementation above the requirement. The N-balance study indicated a significant decrease of the efficiency of threonine utilization at this level of threonine supply. At the higher dietary protein level (18% CP) similar results have been observed, indicating an increased catabolism of threonine at the highest level of threonine supplementation (0.72% total threonine in the diets). In growing chicken at 18.5%CP the increase of threonine supply did not change the TDG-activity in the liver, however the decreasing efficiency of threonine utilization already indicated changes in the limiting order of the diet. At the higher protein level (22.5%CP), corresponding with the same dietary threonine concentration (0,60% true digestible threonine) like in the 18.5%CP diet, a significant increase of liver TDG-activity was observed.

It can be concluded that the in vitro determination of liver TDG-activity corresponding to protein and threonine supply in the diet gives important indications in relation to parameters of total threonine utilization in laboratory rat and growing chicken. These informations are important for the additional metabolic indication of the limiting range of threonine in threonine utilization studies.

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Oxidation of dietary excess leucine in rats fed [$\text{U}-^{13}\text{C}$]-L-leucine as a tracer

M. Miura, R. Sakai & T. Kimura

Institute of Life Sciences, Ajinomoto Co., Inc., 1-1 Suzuki-cho Kawasaki-ku, 210-8681, Kawasaki-shi, Japan

Summary

There are few observations on the metabolism of amino acids, including their oxidation to carbon dioxide, taken in excess over their requirements. The aim of the present study was to estimate the portion of an amino acid added in diet that was being oxidized. We focused on L-leucine (leu) in this study. Rats were fed diets containing leu (0 - 30%) for 14 days. The labeled diets containing [$\text{U}-^{13}\text{C}$]-leu as a fraction of additional leu were fed on the last day only. Expired breath CO_2 was trapped in NaOH solution and precipitated as BaCO_3 to calculate total CO_2 production. Isotope ratio of the CO_2 regenerated from BaCO_3 was measured by an NDIR spectrometer, and then, $^{13}\text{CO}_2$ production from [$\text{U}-^{13}\text{C}$]-leu and the proportion of oxidized leu were calculated. Both $^{13}\text{CO}_2/\text{CO}_2$ and $^{13}\text{CO}_2$ production were increased as leu intakes increased. A linear phase followed by a plateau phase was observed for the plot of the percentage of oxidized leu against actual leu intake. By applying broken-line analysis, the intersection was found to be at the intake of 8.9g leu/kg BW.

In another experiment, rats fed diets containing leu over 10% ($> 8.2\text{g leu intake/kg BW}$) had significant growth inhibition. The similarity of these values suggests that the adverse effects of excessive leu intake may be related to the metabolic overload. Non-oxidized leu could have been retained in the animal's bodies, and it was calculated that this portion was rose as leu intakes increased. These are novel findings for the research of metabolism with the ingestion of excess amino acids and could serve as the basis for the development of methods to measure metabolic limits for macronutrients.

Keywords: leucine, excess, metabolism

Introduction

The requirements of some amino acids have been studied in healthy adults, by a number of groups using an intravenous ^{13}C -labelled amino acid tracer approach (24hr indicator amino acid oxidation and/or balance approach). Generally, in these methods, $^{13}\text{CO}_2$ excretion in the breath by oxidation of the indicator ^{13}C -amino acid declines in concordance with graded supplement of the amino acid being evaluated for its requirement. And some amino acids, for example, L-phenylalanine has been studied for its oxidation during continuous and graded infusion into humans (Zello *et al.*, 1990) and neonatal piglets (House *et al.*, 1997). But there are few observations concerned with metabolism of excess amounts of amino acid over the requirements by oral administration, including oxidation to carbon dioxide.

The aim of the present study was to measure the ratio of $^{13}\text{CO}_2/\text{CO}_2$ in the breath of rats fed diets containing excesses of a single amino acid, L-leucine (leu), and to estimate the portion of oxidized added amino acid at the end of a 14 day-period of excess feeding.

Material and methods

We used male F344 rats (180-200 g, Charles River Japan Inc.) maintained with lighting from 21:00 to 09:00h. Rats consumed tap water *ad libitum*. Diets were prepared based on the AIN-93G composition (NRC, 1992) with some modification, in which both dextrinized cornstarch and sucrose were substituted by cornstarch, and crystalline leu (Ajinomoto Co., Inc., Tokyo, Japan) was added in the graded levels of 2.5, 5, 7.5, 10, 15, 20, 30% in each diet at the expense of cornstarch. [U-¹³C]-leu (U-13C6, 98%+, CIL, MA, U.S.A.) was mixed into the additional leu at an adequate ratio of total addition. Rats were fed each diet for two weeks with training to complete each meal within 9 hrs (09:00-18:00h).

The labeled diets were prepared in equal amounts of food intakes of the day before the administration and fed independently ($n=2 - 4$ per diet) in tight-sealed boxes at 09:00h on the last day only. Breath of rats in the boxes was aspirated by vacuum pumps immediately and independently at the flow rate of 1L/min, and the expired breath CO₂ was collected in NaOH solution for 24 hrs. Carbon dioxide in the air was removed by passing through both soda lime and NaOH solution columns, and then, the CO₂-free air was supplied to rats in the sealed boxes. The absorbed CO₂ was precipitated as BaCO₃ by the method of Benevenga *et al.* (1992) and daily CO₂ production was calculated from the recovery of BaCO₃. To obtain the isotope ratio (¹³CO₂/¹²CO₂), expired breath CO₂ was regenerated by the addition of trichloroacetic acid (TCA) to each BaCO₃ precipitate, and the regenerated CO₂ gas was measured by an isotope selected nondispersive infrared (NDIR) spectrometer (UBiT-IR300, Otsuka Electronics Co., Ltd., Osaka, Japan). For each CO₂ sample placed in the sample side of the inlet, delta ¹³CO₂ was measured against a standard gas sample of known ¹³CO₂ content in the reference side of the inlet. Total ¹³CO₂ production of each animal was calculated by using both total CO₂ (¹³CO₂ + ¹²CO₂) production and ratio of ¹³CO₂/¹²CO₂. The amount of ¹³CO₂ due to natural abundance was calculated by ¹³CO₂ production per metabolic body weight (BW^{0.75}) of rats fed basal diet. By subtracting this natural ¹³CO₂ from total production, net ¹³CO₂ production from [U-¹³C]-leu was estimated in each animal. The proportion of oxidized tracer [U-¹³C]-leu was calculated as follows:

$$\text{Ox (\%)} = \frac{P\ (\text{¹³CO}_2)}{I\ ([\text{U-}^{13}\text{C}]\text{-leu}) * 6} * 100 \quad (1)$$

where Ox is percent of oxidized [U-¹³C]-leu; P (¹³CO₂), the ¹³CO₂ production from tracer [U-¹³C]-leu (mmol); and I ([U-¹³C]-leu), the intake of [U-¹³C]-leu in diets (mmol). The constant 6 accounts for the maximum ¹³CO₂ production from [U-¹³C]-leu, which contains six of ¹³C-atom in the molecule. We considered that the oxidation of tracer [U-¹³C]-leu was equal to the oxidation of tracee, additional leu in diets.

After termination of 24hr-breath test, blood samples were collected from the inferior vena cava of all rats. Blood samples containing EDTA were centrifuged to obtain blood plasma samples. Each blood plasma and a 5% TCA solution was mixed (1:2) and centrifuged again. Amino acids in the resulting supernatant were evaluated using an automatic amino acid analyzer (L-8800, Hitachi, Tokyo, Japan).

Regression analysis and ANOVA were performed on MS Excel 2000 (Microsoft Corporation), and a change of tracer oxidation was evaluated by the broken-line analysis of Anderson & Nelson (1975).

Results and discussion

In the range of leu additions from 0 to 30% in diets, the ratio of ¹³CO₂/¹²CO₂ in the breath increased linearly ($r^2=0.9928$), and ¹³CO₂ production corrected by each animal's metabolic body weight (BW^{0.75}) also tended to increase as leu intakes increased. However, it seemed that the

changes of oxidation reached a plateau phase at more than 10% of leu in diets (> ca. 9g leu/kg BW). Therefore we have evaluated the biphasic changes by applying broken-line analysis to the plot of leu oxidation (%), Y against the actual intakes of additional leu (g leu/kg BW, X). As a result, a linear phase ($Y=2.4435X+35.669$, $r^2=0.2245$) following by a plateau phase ($Y=-0.0148X+57.514$, $r^2=0.0007$) was shown and the intersection of the two phases was found to be at the dose of 8.9g leu/kg BW in the same plot (Figure 1). It might be thought that the capability of enzymatic oxidation of excessive leu (or alpha-ketoisocaproate, KIC) was altered around the intersection in its biphasic changes, but a cause of the alteration was not clear. Concentrations of leu and the other amino acids in blood plasma, which was taken at the termination of the 24hr-breath test, did not show any significant changes ($P>0.05$) between all of leu diet groups. These results showed that there was no accumulation of leu in blood stream. Therefore, additional and excessive leu was thought to be deaminated and decarboxylated to isovaleryl-CoA via KIC on the metabolic pathway of leu, or to be directly incorporated into proteins. And, it must be important to confirm the rise of blood leu concentration just after meals, and also the fall just before next meals, because diets were fed to rats only for 9hrs.

In our previous study, rats fed diets containing leu over 10% in the diet, which was equivalent to an actual intake of 8.2g leu/kg BW, had statistically significant growth inhibition (unpublished). The fact that the two limit doses were almost the same, suggests that at least a part of the adverse effects of excessive leu intake may be related to the metabolic overload of leu in animals. The portion of non-oxidized leu could have been retained in the animal's bodies, and it was calculated that this portion was rose as leu intakes increased (Figure 2).

These are novel findings for the research of metabolism with the ingestion of excess amino acids and could serve as the basis for the development of methods to measure metabolic limits for macronutrients. Further investigations regarding excretion of ^{13}C , that came from metabolism of [$\text{U-}^{13}\text{C}$]-leu, into urine and feces, fractional rate constants, and contribution of each position in the carbon skeleton of leu to oxidation or metabolism, are in progress.

Oxidation, % of dietary leu

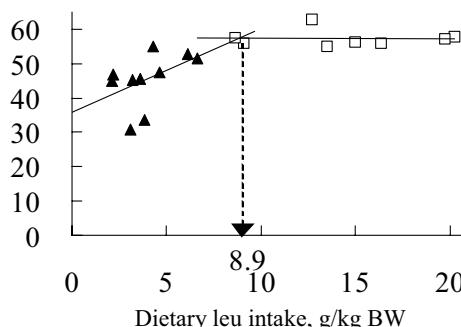


Figure 1. Broken line analysis for oxidation of dietary leu fed to rats. Fitted line for linear phase: $Y=2.4435X + 35.669$, $r^2 = 0.2245$; for plateau phase: $Y = -0.0148X + 57.514$, $r^2 = 0.0007$, where X is the dietary leu intake (g/kg BW), Y is oxidation % of the dietary leu. An arrow indicates the amount of leu intake (8.9 g/kg BW) at the intersection between linear and plateau phases. "Dietary leu" is not including proteinous leu in casein protein in the diets.

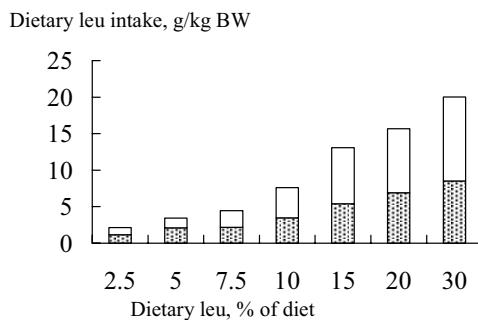


Figure 2. Oxidation of dietary leu in rats. Values are means, n=2 or 4. Opened columns shows oxidized portion and closed one shows the other portion of dietary leu. "Dietary leu" is not including proteinous leu in casein protein in the diets.

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Post prandial oxidative losses for free amino acids in the diet: studies on interactions with dietary protein and on long term adaptation

J.A. Nolles, A.M. Verreijen, R.E. Koopmanschap & V.V.A.M. Schreurs

Human and Animal Physiology group, Wageningen Institute of Animal Sciences (WIAS), Wageningen, the Netherlands

Introduction

Dietary proteins deficient in one or more amino acids are commonly supplemented with free amino acids to meet tabulated requirements. The supplemented free amino acids are supposed to be utilized in the same way and with the same efficiency as the amino acids derived from dietary proteins. A general trend in many studies is the so-called 'Dietary protein paradox' a negative relation between the appearance rate of free amino acids in the body pools and their utilization efficiency. For high protein meals the true availability of amino acids might be even lower than for meals with moderate protein content (Morenz et al., 1997, Moundras et al., 1993). Also proteins with differences in rate of digestion, as Casein and Soya, cause a difference in appearance rate for their amino acids. For soya protein a higher postprandial appearance rate and a higher oxidative loss is seen (Boirie et al., 19970, Dangin et al., 2001).

In contrast to proteins, that have to be digested first, free dietary amino acids can be absorbed immediately and may therefore show a high appearance rate shortly after a meal. Postprandial oxidative losses are higher for meals with amino acids supplied in the free form as compared to meals with protein bound amino acids (Dangin et al., 2001, Metges et al., 2000). Thus far little is known about the mechanisms that cause differences in postprandial oxidative losses and whether some metabolic adaptation might occur on long term exposure to free amino acid diets. Results with long term exposure to free amino acid diets are hard to predict. This is illustrated by the fact that both decreases in food intake and growth performance (Officer et al., 1997) as well as an increase in food intake and growth performance are observed (Forsum & Hambraeus, 1978).

In this study we have tried to elucidate differences in postprandial oxidation between free and protein bound amino acids both after short and long term exposure. By labeling the different dietary forms their fate of the different dietary forms could be traced independently. By this approach we were also able to study potential interactions between the oxidation of free and protein-bound amino acids when both are mixed in one meal.

Materials and methods

Animals, feeding and diets

Metabolic oxidation of free or egg white bound amino acids was studied with 38 male Wistar (WU) rats (Harlan, The Netherlands) of about 300 g. The rats were fed twice a day for 30 min, between 9.00 - 9.30 and 16.30 - 17.00. During the experimental period the animals got 8.5 g of the experimental feed per meal. This sub-maximal amount was always eaten completely but was adequate for normal growth. Only the amino acid source (21%) was different for the experimental diets, but always based on the amino acid pattern of egg white protein. Water was always available '*ad libitum*'.

Studies were performed with four experimental groups of animals with different diets. Diet I contained egg white bound amino acids only. For the breath tests with this diet the egg white protein intrinsically labeled with [1-¹³C]-leucine has been used. Diet II contained the same pattern of amino acids as diet I but supplied in free form. Free [1-¹³C]-leucine was used as intrinsic label

of this diet. In addition two 50-50 mixtures of both diets were used, for breath testing mixture either free or egg white bound [$1\text{-}^{13}\text{C}$]-leucine was used.

Breath test substrates and breath test procedure

The breath test substrate used in this study was L-[$1\text{-}^{13}\text{C}$]-leucine (chemical purity of > 99%, isotopic enrichment > 99%) obtained from ARC (Amsterdam). In the test meals the labeled material was placed instead of the non-labeled material. In case of free leucine labeling 6 mg of [$1\text{-}^{13}\text{C}$]-Leucine was used per meal. Labeled egg white was produced as described previously (Evenepoel, 1997). For diets in which intrinsically labeled egg white was required the total amount of [$1\text{-}^{13}\text{C}$ -Leucine] ingested was calculated to be also about 6 mg. The enrichment of the intrinsically labeled protein was measured by Isotope Ratio Mass Spectrometer (IRMS) analysis. The breath tests were performed on day 5 and day 26 after switching to the experimental diets. Day 5 was the first day the meal was eaten completely. At the day of the experiment the rats were placed in an air tight cage at 08.30 am. After half an hour (at 09.00 am) the air in the cage was sampled (blank). Feed was available for 30 min. Over a period of five hours every 30 min. the air was sampled. Immediately after each sample the rats were transferred to a cage with fresh air. The CO_2 of the air samples was analyzed for [^{13}C]-enrichment by IRMS-analysis. The CO_2 production of the animals during the meal and the post prandial period was measured the day prior to the experiment but under identical nutritional conditions.

Statistics

The data are shown as mean \pm SE and analyzed by SPSS statistical program with a General linear model and post hoc Tukey test. Differences are supposed to be significant for $P < 0.05$.

Results and discussion

Breath tests, 5 days after change over to experimental diet

Diets with 100% free versus protein bound amino acids

Measurements 5 days after a change over from a commercial diet to the experimental diets (see figure 1) clearly show that post prandial metabolism of the free amino acids in the diet is different from protein-bound amino acids. Expiration rate of label from [$1\text{-}^{13}\text{C}$]-leucine depends on its dietary form. As compared to the protein-bound form the free [$1\text{-}^{13}\text{C}$]-leucine shows a faster increase and a higher maximal value. At the end of the experiment (5 hours) expiration rate is similar in both cases, but not yet returned to zero values. As far as measured the cumulative recovery or oxidative loss is higher when dietary amino acids are ingested in free form. This indicates that postprandial metabolism of free amino acids is less efficient in channeling the dietary amino acids towards a proper physiological utilization.

50 -50 % Mixtures of diets with free and protein-bound amino acids

A striking result of this study was that metabolism of free and protein-bound amino acids present in the same meal do not show any interaction (see figure 1). The curves of the 50% groups did not differ from the 100% curves. The shape of the recovery curve is designated exclusively by the nature of the label. The presence of amino acids in another dietary form does not influence the oxidation rate of the labeled form of the amino acid. This indicates that measurements with a breath test substrate in a specific dietary form represent only that part of the meal in that specific dietary form. Results are shown to be independent of their relative abundance.

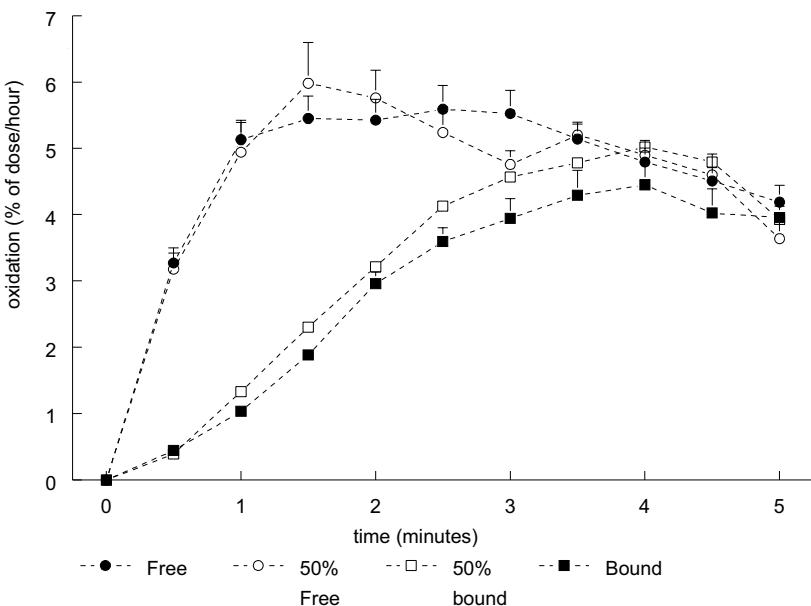


Figure 1. Oxidative losses, expressed as % of the methionine dose recovered per hour.

In long term studies metabolic adaptation might play a crucial role in the final results. Metges et al (2000) claimed already full metabolic adaptation in humans after six days. However, in recent studies in our laboratory with rats we observed important changes in metabolism of free amino acids between 5 and 26 days. The direct cause of these amino acid sparing changes might be related to a change in amino acid absorption rate, stomach emptying, gut kinetics, amino acid pool size, metabolic capacity for oxidation and or protein synthesis.

Adaptation effects 5 days compared to 26 days after change over to experimental diet

Diets with 100% free amino acids

This study shows that oxidative losses decrease in time. The oxidative losses 26 days after change over to free amino acids are lower compared to the oxidative losses 5 days after changing over (see figure 2). Especially the increase in oxidation rate during the first hour is tempered. The maximal level of the oxidation is lower and the position of the peak is postponed for a few hours.

The values of oxidation suggest that the utilization efficiency of free amino acids can increase with time. The observed time lag between the oxidation peak at day 5 and day 26 suggests that rats slow down the handling of the feed after adaptation. This might indicate that an improved utilization is not necessarily an adaptation on metabolic capacity, but rather an adjustment of the appearance rate in the metabolic pool to the metabolic capacity. This is in line with the hypothesis that free amino acids have a high appearance rate, and therefore can exceed the metabolic capacity. The rats need to slow down the resorption otherwise the appearance rate will be too high and metabolic capacity will be too small to incorporate dietary amino acids efficiently (Boirie et al., 1997).

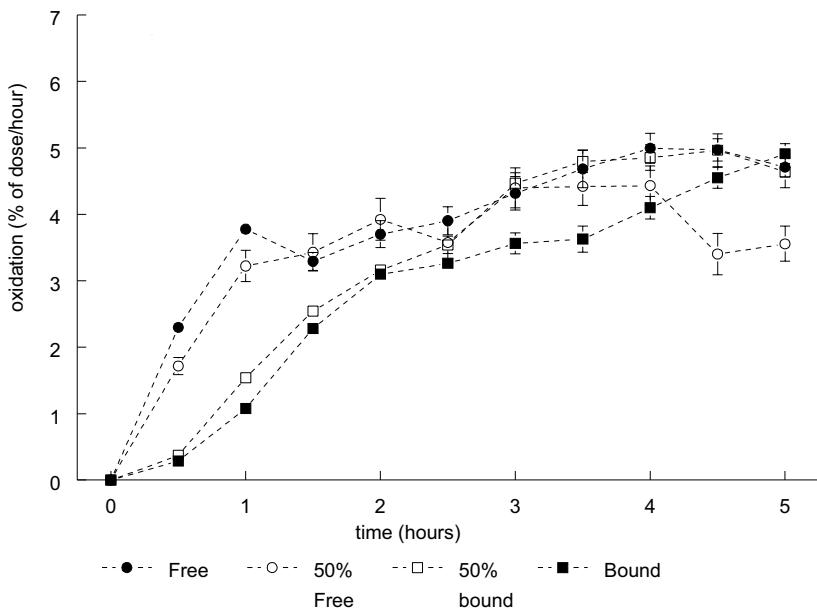


Figure 2. Oxidative losses, expressed as % of the methionine dose recovered per hour.

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Essential amino acid requirements for maintenance in adult sows

F.X. Roth, B.M. Jahn & W. Schönberger

*Technische Universität München, Fachgebiet Tierernährung und Leistungsphysiologie,
Hochfeldweg 6, 85350 Freising-Weihenstephan, Germany*

Summary

Balance experiments on adult sows were carried out to estimate their essential amino acid requirements for maintenance. Purified diets based on crystalline amino acids as sole source of N were fed, in which one amino acid was made specifically deficient in four steps of 100, 60, 30 or 0 % of a presumed maintenance level, respectively. N retention decreased linearly ($P < 0.01$) as the respective amino acid was gradually omitted from the basal diet. From the relationship between N retention and amino acid intake the daily amino acid requirements to maintain N equilibrium were estimated to be (mg/kg BW^{0.75}) lysine 38, methionine + cystine 37, threonine 40, tryptophan 11, isoleucine 20, leucine 24, valine 23, phenylalanine 17 and phenylalanine + tyrosine 44.

Keywords: *amino acid, N equilibrium, adult sows*

Introduction

The amino acid requirements of pigs include two components, a requirement for maintenance and a requirement for protein accretion or lactation. The pattern of amino acids required for each of these components is quite different (Fuller et al. 1989). There are only few estimates of the amino acid requirements for non-producing adult swine (Baker et al. 1966 a, b). Therefore, it was of interest to evaluate the maintenance requirement of essential amino acids for adult sows in maintenance metabolism. The approach used in the present experiments was to determine the relationship between the N retention and amino acid intake for each amino acid when it was given at different rates but was always limiting. The amount of each amino acid needed to maintain N equilibrium was calculated as the x-intercept of the linear regression relating N accretion to amino acid intake.

Material and methods

In N balance experiments the amino acid requirements to maintain N equilibrium in adult sows with body-weights between 171 and 252 kg were measured. The tested amino acids were Lys, Met, Met + Cys, Thr, Trp, Ile, Leu, Val, Phe and Phe + Tyr. On average four sows were used to test one or a pair (Met + Cys or Phe + Tyr) of amino acid. A chemically defined protein-free diet was fed, in which the respective amino acid was made specifically deficient in four steps. The deficient amino acid was given in descending order of their dietary levels in four balance periods. The first period included an adjustment feeding of 15 d followed by a 5 d collection period, the following three periods consisted of a 9 d preliminary feeding period each followed by a 5 d collection period each. Sows were kept in farrowing pens and fed twice a day, thereby urine was collected with sterile bladder catheters and feces by stimulated defecation.

The basal diets consisted of cornstarch, sucrose, glucose cellulose, vegetable oil, minerals, vitamins and crystalline amino acids. Basal amino acid supply was based on 120 % of the pattern estimated by Fuller et al. (1989) for growing pigs. The following average dietary concentrations were used (g/kg): Lys 1.329, Met 0.352, Cys 1.352, Thr 1.962, Trp 0.374, Ile 0.526, Leu 0.958, Val 0.767, Phe 0.705, Tyr 0.655, His 0.416, Ala 16.838, Arg 0.549, Asp 25.149, Glu 27.789, Gly 0.081, Pro 1.298, Ser 0.081. To make one amino acid under study first limiting, 100 %, 60 %, 30

Table 1. Dietary concentrations to make the respective amino acid first limiting (g/kg)

| Limiting amino acid | Balance periods | | | |
|---------------------|-----------------|-------|-------|-------|
| | 1 | 2 | 3 | 4 |
| Lysine | 1.121 | 0.705 | 0.393 | 0.081 |
| Methionine | 0.748 | 0.465 | 0.252 | 0.040 |
| Cystine | 0.672 | 0.389 | 0.176 | 0.040 |
| Threonine | 1.655 | 0.888 | 0.505 | 0.121 |
| Tryptophan | 0.318 | 0.191 | 0.095 | 0.000 |
| Isoleucine | 0.432 | 0.245 | 0.104 | 0.041 |
| Leucine | 0.825 | 0.560 | 0.361 | 0.162 |
| Valine | 0.653 | 0.424 | 0.253 | 0.081 |
| Phenylalanine | 0.624 | 0.416 | 0.208 | 0.081 |
| Tyrosine | 0.659 | 0.439 | 0.220 | 0.000 |

% and 0 % of the respective basal level was adjusted in the diets of the four consecutive balance periods, while the N content was maintained constant through addition of Ala, Asp and Glu, each supplying one third of the additional N required. In Table 1 the dietary omissions of the respective amino acids under study to make them first limiting are given. Omissions were made of both Met + Cys, or of Met alone, maintaining the same total sulphur amino acid content as the control diet by addition of extra Cys. Similarly, Phe + Tyr were both omitted from one diet and Phe alone from another, with additional Tyr to keep the total aromatic amino acids constant as in the control diet. On average, dietary supply was 320 mg N/kg BW^{0.75} · d⁻¹ and at an energy level of 13,0 MJ ME/kg diet daily ME intake amounted to 0.45 MJ/kg BW^{0.75} to reach the full maintenance requirement of N and energy. Mean rates of N balances were calculated by analysis of variance. For each amino acid a regression analysis was made to estimate the relationship between daily N accretion and daily amino acid intake.

Results and discussion

The response of N retention to the dietary levels of each amino acid under study is shown in Figure 1. Respective regression equations describing N retention as a function of amino acid intake are summarized in Table 2. N retention decreased linearly as the respective dietary amino acid(s) was gradually omitted from the presumed maintenance level to nearly zero amino acid intake. According to the regression equations a zero intake of Lys, Met + Cys, Thr, Trp, Ile, Leu, Val, Phe or Phe + Tyr resulted in a body N loss of 42, 128, 107, 79, 64, 18, 86, 53 and 76 mg/kg BW^{0.75}, respectively. N losses were attributed to different urinary N losses without change of fecal N excretion. Negative N retention was highest by dietary removal of Met + Cys, Thr, Val, Trp and Phe + Tyr and lowest for Ile, Phe, Lys and Leu. The removal of Met alone did not influence N balance, indicating a low maintenance requirement for Met compared to Cys.

From the regression equations the maintenance requirements were calculated as the amino acid intakes required for zero N retention and the values are given in Table 3. These presented data for adult sows correspond largely with results found for growing pigs (Fuller et al. 1989, Heger et al. 2002, 2003) using similar diets but calculating extrapolation from positive to zero N balance. The presented data are based on the assumption that the maintenance amino acid requirement is a function of metabolic body weight. However, for practical application there is evidence that feeding level, protein level and type of dietary ingredients may influence the endogenous intestinal amino acid losses and therefore significantly contribute to the maintenance needs.

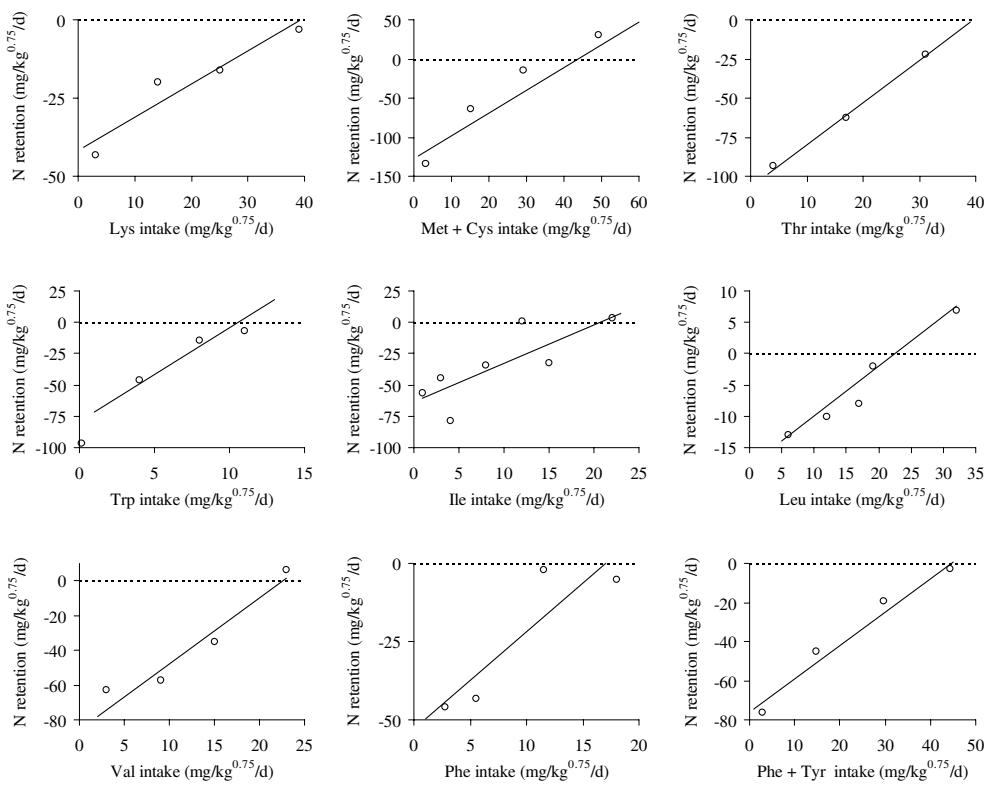


Figure 1. The relationship between N retention and amino acid intake of adult sows fed diets with specific deletions of individual amino acids (\circ = average of $n = 4$).

Table 2. Regression equations relating N retention (y , mg/kg $BW^{0.75}$) to amino acid intake (x , mg/kg $BW^{0.75}$) in adult sows fed on descending levels of the respective limiting amino acids.

| Limiting amino acids | Regression equation | $s_{y(x)}$ | R^2 | P |
|--------------------------|---------------------|------------|-------|-------|
| Lysine | $y = -42 + 1.1 x$ | 14 | 0.53 | 0.002 |
| Methionine + cystine | $y = -128 + 3.5 x$ | 27 | 0.84 | 0.001 |
| Threonine | $y = -107 + 2.7 x$ | 15 | 0.76 | 0.001 |
| Tryptophan | $y = -79 + 7.5 x$ | 24 | 0.64 | 0.001 |
| Isoleucine | $y = -64 + 3.1 x$ | 25 | 0.45 | 0.001 |
| Leucine | $y = -18 + 0.8 x$ | 13 | 0.22 | 0.030 |
| Valine | $y = -86 + 3.8 x$ | 20 | 0.67 | 0.001 |
| Phenylalanine | $y = -53 + 3.1 x$ | 19 | 0.52 | 0.002 |
| Phenylalanine + tyrosine | $y = -76 + 1.7 x$ | 25 | 0.61 | 0.001 |

Table 3. Mean estimates of amino acid requirements to maintain N equilibrium in adult sows and compared to growing pigs (mg amino acid/kg BW^{0.75} · d⁻¹)

| Amino acid | Requirements for N equilibrium | | |
|--------------------------|--------------------------------|---------------|----------|
| | Adult sows | Growing pigs | |
| | | Present study | 1) 2) |
| Lysine | 38 | 36 | 39 |
| Methionine + cystine | 37 | 49 | 46 |
| Threonine | 40 | 53 | 49 |
| Tryptophan | 11 | 11 | 16 |
| Isoleucine | 20 | 16 | 18 |
| Leucine | 24 | 23 | 33 |
| Valine | 23 | 20 | 23 |
| Phenylalanine | 17 | 18 | - |
| Phenylalanine + tyrosine | 44 | 37 | 43 |

¹⁾ Fuller et al. (1989)

²⁾ Heger et al. (2002, 2003)

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Investigations on the valine requirement of lactating sows

D.A. Roth-Maier & B.R. Paulicks

*Division of Animal Nutrition, Department of Animal Sciences, Technical University Munich,
Hochfeldweg 6, 85350 Freising-Weihenstephan*

Summary

In order to determine the requirement of valine (Val) for lactating sows a total of 72 lactations (35 days long) of sows of the German Landrace, nursing 10-12 piglets was examined. The sows were assigned to 6 treatments ($n=12$), which differed only in the Val content of the lactation feed (g Val/kg feed: 4.5, 5.5, 6.5, 8.5, 10.5, 14.5, resp.) derived by adding crystalline L-Val to a basic feed mixture (4.5 g native Val/kg, 3.5 g apparent ileal digestible Val/kg). Feed composition, nutrient content (155 g CP, 10.1 g Lys, 13.4 MJ ME/kg), housing conditions, feeding during gestation, body weight, farrowing month, and so forth, were kept constant or were distributed equally between the treatments.

Feed intake (kg/d) of the sows during lactation averaged 2.9, 4.3, 4.6, 4.6, 4.9, and 5.1 for treatment 1 to 6, resp. With 4.5 and 5.5 g Val/kg body weight losses of the sows during lactation were higher (1207 and 805 g/d, resp.) and milk production was lower (7076 and 8686 g/d, resp.) compared with the average of treatments 3 to 6 (625 g/d body weight loss; 9263 g milk/d), which showed no differences. Milk protein content and milk Val content were lower and milk fat content was higher with 4.5 and 5.5 g Val/kg than with higher Val supply. Amino acid pattern in milk was unaffected. Piglets' daily weight gain was also lower (146 and 171 g/d versus 200 g/d) and even when starter feed was offered additionally to the suckling, the piglets were not able to compensate this retarded growth performance and had significant lower weights at weaning (6.6 kg and 7.5 kg vs. 8.5 kg).

As a result of the present investigation Val contents of at least 6.5 g/kg feed, i.e. 5.5 g apparent ileal digestible Val per kg feed, must be recommended for lactating sows to avoid consisting depressions in feed intake, lactation yield, and piglet performance.

Keywords: sow, valine, requirement

Introduction

For a high performance of lactating sows an adequate nutrient supply, particularly also with essential amino acids, is necessary. The amount and the dietary content of lysine, sulfur-containing amino acids, or threonine required by the lactating sow was confirmed in several investigations. For valine (Val), recommendations are divergent and vary between 5.3 g/kg (Rousselot and Speer, 1980) and 12.0 g/kg (Moser et al., 2000). Therefore, in the present investigation the Val requirement of lactating sows was determined using several performance parameters and physiological metabolites.

Material and methods

Multiparous sows (German landrace) with litters of 10 to 12 piglets were observed over a total of 72 lactations. They were randomly assigned to 6 treatments according to age, genetics, farrowing month, and body weight. During gestation all sows were fed with 2.4 kg of a commercial gestation feed mixture (114 g crude protein and 10.9 MJ ME per kg; day 60 to day 84 of gestation) and from day 85 to day 110 depending on the body weight with 2.6 to 3.0 kg of a special gestation feed

mixture (115 g crude protein, 4.8 g Val, 11.3 MJ ME per kg). From day 110 of gestation the sows received a lactation feed based on wheat, oats, corn starch, sugar, soybean meal, fish meal, soybean oil, and a mineral-vitamin-premix, which covered the nutrient recommendations (NRC, 1998) for lactating sows (155 g crude protein, 10.1 g lysine, 13.4 MJ ME per kg) with the exception of Val, which amounted to only 4.5 g native Val per kg (i.e. 3.5 g/kg apparent ileal digestible Val). This basic feed mixture was supplemented with up to 10.0 g crystalline L-valine deriving feed mixtures with 4.5 to 14.5 g valine per kg (cf. Table 1). Feed intake and body weight of the sows and piglets were recorded on several days. Milk yield was determined on day 21/22 of lactation using the weigh-suckle-weigh-method. Milk samples were examined for their fat, protein, urea, and amino acid contents. Blood samples of the sows were detected for their contents of urea and amino acids. Data were statistically evaluated by analysis of variance; treatment means were compared using Student-Newman-Keuls test ($P<0.05$). For further details see Paulicks et al. (2003) and Roth-Maier et al. (2003).

Results

In Table 1 some of the most important experimental parameters are listed in response to the valine (Val) content of the diet. In treatment 1 (4.5 g Val/kg diet) feed intake of the sows was significantly reduced amounting to only 2.9 kg/d compared with more than 5 kg/d in treatment 6 (14.5 g Val/kg diet). Consequently body weight losses of the sows during lactation (1207 g/d) and milk fat content (8.9 %) were highest, daily milk yield and milk protein content were lowest for treatment 1 (7076 g/d, 3.83 %, resp.). Also sows with 5.5 g Val/kg diet (treatment 2) showed inferior performance data compared with the animals with higher Val supplementations. Animals of treatment 6 showed the best performance for all these parameters.

The number of piglets weaned and the piglet survival rate showed no influence of the treatment, but the growth performance of the piglets was significantly affected by the valine content of the sow's feed. For treatment 1 and 2 daily weight gain and weanling weight of the piglets amounted to only 146 g/d, 171 g/d, 6.58 kg, 7.52 kg, resp. whereas the corresponding data in treatments 3 to 6 averaged 200 g/d and 8.35 kg. The differences in growth performance of the piglets were observed throughout the whole experiment, including week 4 and 5 with supplemental piglet feeding when piglets in treatment 3 to 6 tended to consume more feed (4990 g/litter) than those in treatment 1 and 2 (3630 g/litter). There were no statistical differences between the treatments due to a high standard deviation.

The contents of amino acids in milk were significantly lower with 4.5 g Val/kg diet than for higher dietary Val contents. Highest amino acid concentrations were observed in the milk of sows in treatment 4, amounting to 3.50 g lys, 0.90 g met, 0.65 g cys, 1.86 g thr, 2.38 g val, 3.91 g leu, 1.87 g ile, 1.91 g phe, 1.69 g tyr, 1.29 g his, 1.62 g ala, 2.23 g arg, 3.85 g asp, 9.48 g glu, 1.56 g gly, 4.94 g pro, and 2.30 g ser per kg milk. There were no significant differences between treatments 3 to 6. In the ratio of the total protein content in milk, there were no differences in amino acid contents between the treatments. Also the amino acid pattern was similar for all treatments (lys : met+cys : thr : val : leu : ile : phe+tyr : his : ala : arg : pro : ser = 1 : 0.43 : 0.53 : 0.68 : 1.10 : 0.53 : 1.02 : 0.37 : 0.46 : 0.66 : 1.45 : 0.66).

In blood plasma the concentration of free Val reflected the dietary Val supply increasing from 9.2 to 131.7 mg/l between treatment 1 and 6. For all the other amino acids in blood plasma no significant effects of the treatment were observed. Most amino acids tended to be lower in plasma with 4.5 g Val/kg diet. Lysine averaged 44.9 mg/l plasma for treatments 2 to 6 and was in the following ratio to the contents of the other amino acids in blood plasma: lys : met+cys : thr : trp : leu : ile : phe+tyr : his : ala : arg : pro : ser = 1 : 0.75 : 0.98 : 0.07 : 0.63 : 0.44 : 0.82 : 0.40 : 1.51 : 0.35 : 0.93 : 0.44. Urea contents in milk and blood plasma were not significantly different between the treatments and averaged 186 mg/l milk and 280 mg/l plasma. Milk urea concentration tended to be lower for treatment 1 and 4 than for the other treatments.

Table 1. Selected parameters of growth and lactational performance of sows and piglets in response to the dietary valine supply.

| | | | | | | |
|-------------------------------|-------------------|--------------------|--------------------|-------------------|-------------------|--------------------|
| Val (g/kg feed) | 4.5 | 5.5 | 6.5 | 8.5 | 10.5 | 14.5 |
| Feed intake (kg/d) | 2.92 ^b | 4.30 ^a | 4.59 ^a | 4.55 ^a | 4.85 ^a | 5.07 ^a |
| Sows' body weight loss (g/d) | 1207 ^a | 805 ^b | 769 ^b | 729 ^b | 529 ^b | 472 ^b |
| Milk production (g/d) | 7076 ^b | 8686 ^a | 9289 ^a | 8945 ^a | 8847 ^a | 9972 ^a |
| Valine in milk (g/kg) | 1.90 ^c | 2.08 ^{bc} | 2.26 ^{ab} | 2.38 ^a | 2.37 ^a | 2.26 ^{ab} |
| Urea in milk (mg/l) | 155 | 206 | 204 | 166 | 180 | 204 |
| Piglet weaning weight (kg) | 6.58 ^c | 7.52 ^b | 8.32 ^a | 8.30 ^a | 8.52 ^a | 8.85 ^a |
| Valine in blood plasma (mg/l) | 9.2 ^d | 17.9 ^d | 20.6 ^d | 45.4 ^c | 77.0 ^b | 131.7 ^a |
| Urea in blood plasma (mg/l) | 260 | 291 | 267 | 269 | 293 | 298 |

^{a,b,c} means with different superscripts differ significantly ($P<0.05$, Student-Newman-Keuls test)

Discussion

The main effect of the present feeding experiment was the decreased feed intake of sows with 4.5 and 5.5 g Val/kg diet. This up to 40 % lower nutrient supply reduced milk yield and impaired growth performance of the piglets, which were not able to compensate this nutrient deficiency by consuming more piglet starter feed. As all the other factors with influence on feed intake (c.f. Verstegen et al., 1998) were identical for all the treatments, this effect must have been due to the valine content of the diet.

The mechanism of the regulation of feed intake in connection with amino acid imbalances are not completely clear up to now. The most important regulation happens presumably in particular areas of the brain, where biochemical changes are responsible for the depression in feed intake caused by dietary amino acid imbalances (Tews et al., 1991). Cusick et al. (1979) reported that rats deprived of Val exhibited neurological signs attributed to disrupted protein synthesis in the red nuclei. Amino acids must be transported into the brain via blood plasma to induce reactions in the brain. Hutchison et al. (1983) found no change in the Val concentration of the brain in Val-deficient rats, but a lower Val concentration in blood and reduced feed intake. This might be valid for sows, too, as in the present investigation plasma Val level was significantly decreased with lower Val supply.

The increase of the Val content in milk was linear up to 6.5 g Val/kg diet. Up to 8.5 g Val/kg the increase was slower and then formed a plateau. In blood plasma there was a slow increase in Val content up to 6.5 g Val/kg diet and afterwards a significant increase with higher Val supply. Similar observations are known from experiments examining the requirements for other amino acids (Kirchgessner et al., 1993, 1998). The explanation is, that as far as the respective amino acid is limiting, the supplement is used for protein synthesis in liver or mammary gland. Supplements, that exceed protein synthesis capacity, increase plasma concentrations. On the other hand, an amino acid supply, which maximizes the amino acid content in milk, can be regarded as the requirement of the lactating animal for that amino acid. In the present investigation this was the case for a dietary Val content of 8.5 g/kg.

Another variable to determine amino acids requirement is the concentration of urea in milk and blood plasma defining the requirement as the lowest urea value with increasing amino acid supply. In the present experiment urea contents in blood and milk were the lowest for 6.5 and 8.5 g Val/kg diet confirming the results derived from the other parameters.

In conclusion, the results from the present study indicate, that lactating sows need dietary valine concentrations of at least 6.5 g/kg (i.e. 5.5 g/kg of apparent ileal digestible valine) to avoid severe depressions in milk yield and growth performance of the piglets. For an optimal amino acid supply

for the piglets by the sow's milk, 8.5 g Val (7.5 g apparent ileal digestible Val) per kg feed must be recommended, particularly for sows nursing large litters.

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Differential response to 2-hydroxy-4(methylthio) butanoic acid and DL-methionine above requirements on broiler and pig performance and iron metabolism

M. Vázquez-Anon, M. Wehmeyer, C. Wueling, T. Hampton, C.D. Knight & J.J. Dibner

Novus International, Inc. 530 Maryville Center Drive, St. Louis, MO, 63141, USA

Summary

One broiler and one pig study evaluated the response to supplementation above Met requirements of 88% solution of HMB (Alimet® Feed Supplement. Novus International, Inc. St. Louis, MO) and DL-methionine, dry 99% (DLM; Degussa Hulls). In the broiler study, birds were fed a diet deficient in methionine and cysteine (M+C) and five levels of the two M sources were added on an equimolar activity basis for each diet (0.2, 0.5, 1.0, 1.5 and 2.0). Bird performance to M supplementation followed a quadratic response. Birds responded to the first level of M ($P < 0.05$) by increasing BW, feed intake, and feed efficiency equally for both sources. Performance reached a plateau at the next level of supplementation (0.5 %) and further supplementation induced a gradual ($P < 0.05$) reduction in BW, feed intake and feed efficiency, indicating M toxicity. The response to M supplementation differed among sources. The negative impact on BW (1.2 vs 1.4 kg; $P < 0.001$) and feed efficiency (1.8 vs 1.7; $P < 0.001$) was more severe and occurred sooner (1.0 vs 1.5 %; $P < 0.001$) in birds fed DLM than birds fed HMB. These differences in response to M over supplementation resulted in higher concentration of plasma M, homocysteine (HCY) and hepatic iron in birds fed DLM than HMB, indicating lower toxicity for HMB and DLM. In the weanling pigs study, the experiment compared live performance, iron content in spleen and plasma M of pig fed a basal diet sufficient in M +C and supplemented with 1, 2 or 4% additional M activity from HMB or DLM. Average daily gain and average daily feed intake showed a negative linear and quadratic response ($P < 0.05$) for M dose but were not affected by source of M activity. Plasma M and splenic iron content increased linearly with M dose ($P < 0.05$), and both were higher for the DLM 2% than the HMB 2% treatment but not at the 4% dose. Results from both studies demonstrated that HMB is less metabolically toxic than DLM in chickens and pigs.

Keywords: methionine, 2-hydroxy-4-methylthio-butanoic acid, toxicity, iron, broilers, pigs

Introduction

Methionine is one of the most toxic of the essential amino acids. The negative impact on growth and mechanisms of methionine (M) toxicity at the tissue level are described extensively for rats (Mitchell and Benevenga, 1978) with additional reports describing toxicity chicks and pouls (Katz and Baker, 1975). The toxicity of HMB relative to DLM has been reported to be lower in chicks fed crystalline amino acid diets (Katz and Baker, 1975). In contrast to the numerous reports that examine M toxicity in rats and poultry, there are few reports that examine the toxicity of M in commercial swine (Edmonds and Baker, 1987) and none that examine the relative toxicity of HMB and DLM. The impact of excess M activity from HMB and DLM on performance and tissue responses in chicken and pigs is evaluated in this study.

Materials and methods

Poultry study

In the broiler study, 792 Ross x Ross male chicks were raised in battery cages during the first 36 d of age. Mashed corn soy diets were deficient in M+C, and included a starter (16d) and grower (19d). Five supplemental levels of the two M sources were added on an equimolar activity basis for each diet (0.2, 0.5, 1.0, 1.5 and 2.0 %). The first level of supplementation was defined to supply M at requirements and the rest of the levels supply M in excess. Ten chicks per pen were housed in 72 batteries and allowed ad libitum access to feed and water. Feed consumption and BW was measured at d 35 of age. Blood, liver, kidney, and spleen were removed from one bird per pen, weighed and freeze for later determination of free M, HMB and HCY in plasma, and iron content in tissues. The effect of M source and level of supplementation was analyzed as a 2 x 5 factorial with a basal treatment using the general linear model procedure in SAS (1992).

Swine study

Forty-two pigs were assigned to 7 treatments. The treatments included a control corn soy basal diet with 3 levels of supplementation (1.0, 2.0 and 4.0%) for each M source. Two pigs per pen were housed in 21 elevated pens and allowed ad libitum access to feed and water and feed consumption and BW gain were measured weekly for 28 days. After 14 days one pig from each pen was killed and the remaining pigs were killed after 28 days on treatment. Liver and spleen were removed, weighed, frozen and lyophilized for later iron content analysis. Experimental data were subjected to ANOVA procedures appropriate for randomized complete-block designs using the general linear models procedure of SAS. Main effects of the model included M treatment, room and replicate with initial BW for the pen as a covariate. Linear and quadratic effects were tested.

Results and discussion

Poultry study

Bird response to M supplementation followed a quadratic response (Figure 1). Birds responded to the first level of M (0.2 %; P < 0.05) by increasing BW, feed intake, and feed efficiency equally for both sources, indicating the basal diet was deficient in M. Performance reached a plateau at the next level of supplementation (0.5 %) and further supplementation induced a gradual (P < 0.05) reduction in BW, feed intake and feed efficiency, indicating M over supplementation. The response to M supplementation differed among sources. The negative impact on BW (1.2 vs 1.4 kg; P < 0.001) and feed efficiency (1.8 vs 1.7; P < 0.001) was more severe and occurred sooner (1.0 vs 1.5 %; P < 0.001) in birds fed DLM than birds fed HMB. Changes in breast, leg, liver, kidney and spleen weights followed the same response. All tissue weights increased during first and second level of supplementation and started to decrease when level of DL M was at and above 1 %, and at and above 1.5 % for HMB fed birds. The differences in the response to M over supplementation among the two sources might be due to the differences in how the two sources are metabolized at the tissue level. Plasma free M and HCY concentrations increased exponentially with level of supplementation but the magnitude of the increase differed among sources (Figure 2). At and above 1% M supplementation, plasma free M and HCY from birds fed DLM increased exponentially but birds fed HMB only observed a gradual increase in plasma M and HCY concentration.

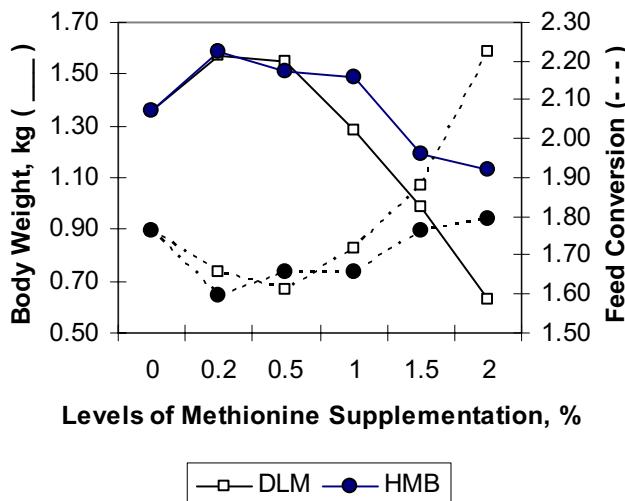


Figure 1. Effect of methionine source and level of supplementation on body weight and feed conversion. There was a significant source ($P < 0.0001$), level ($P < 0.0001$) and source by level ($P < 0.0001$) interaction. HMB = 2-hydroxy-4-methylthio-butanoic acid. DLM = DL Methionine.

Differences among the two sources have been previously observed and reflect the two M sources are metabolized differently. HMB, once absorbed and taken up by the tissues is rapidly converted to M by the enzymes described by Dibner and Knight (1984). The M derived from HMB is further utilized by the cells and only secreted into the blood stream in conditions of excess supply of M, mostly by the kidney and to less extent by liver and small intestine (Lobley et al, 2001). When over-supplemented, DLM accumulates in plasma prior to its uptake and further metabolism by the tissues, and D Met is the isomer responsible for this accumulation (Vázquez-Añón et al., 2000). In this study, approximately 25 % of the free Met in plasma was in the form of D in birds fed DLM. High elevation of plasma M has been associated with reduction in feed consumption. In this study, feed intake and BW gain depression coincided with elevation of plasma M

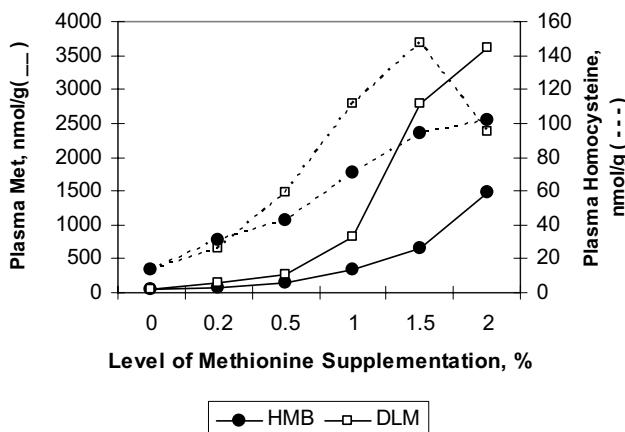


Figure 2. Effect of methionine source and level of supplementation on plasma free methionine and homocysteine concentration. There was a significant source ($P < 0.0001$), level ($P < 0.0001$) and source by level ($P < 0.01$) interaction. HMB = 2-hydroxy-4-methylthio-butanoic acid DLM = DL Methionine .

concentration, at 1 % for DLM but not until 1.5 % for HMB fed birds. Increases in plasma HCY above normal levels has been associated with heart failure in humans and impaired growth in rats. In the present study, increases in plasma HCY might have contributed to the impaired growth observed at high levels of M supplementation and might explain the differences in performance between the two sources. In birds fed HMB, plasma HCY concentration was significantly lower than in birds fed DLM and might have contributed to alleviate the negative impact of M over-supplementation. In this study, iron content increased in all tissues when levels of supplementation were at and above 1.5 %. Hepatic iron content was lower for birds fed HMB than DLM (634 vs 455 ppm; $P < 0.02$), indicating lower toxicity for HMB. No differences were observed in spleen and kidney iron content with M source.

Pig study

Performance results are summarized from 0-28 days of study (Table 1). Average initial BW was 21 kg and did not differ due to M treatment. For the overall 28 day period, M+C intake was increased quadratically, resulting in a quadratic reduction in ADFI, ADG and final BW ($P < .05$). Feed to gain was not significantly affected by M dose for this period, nor were there any differences in performance measures between HMB and DLM for the 2 and 4% levels of supplementation.

Spleen weight was not affected by treatment after 14 days (Table 1); however, there was a linear and quadratic increase in spleen iron content ($P < .05$) that was greater for 4% DLM than the corresponding HMB treatment ($P < .01$). Liver weight was linearly reduced with M dose after 14 days of treatment, and liver iron concentration was greater for the 4% HMB than the corresponding DLM treatment ($P = .03$). Spleen weight was still unaffected by treatment after 28 days and spleen iron content was linearly increased ($P < .05$). The spleen iron concentration was greater for the 2% DLM than the 2% HMB treatment group ($P = .03$), however, there was no difference due to M source for the 4% dose level. Liver weight was linearly and quadratically reduced at 28 days

Table 1. Performance of pigs fed 0, 1, 2, or 4 % excess Methionine activity as DL-Methionine (DLM) or 2-hydroxy-4-methylthiobutanoic acid (HMB).

| | Methionine Source and Level, % ^a | | | | | | | | HMB vs DLM | |
|---------------------------------|---|-------|-------|-------|-------|-------|-------|------|-------------|-------------|
| | Basal | DLM | DLM | DLM | HMB | HMB | HMB | SEM | Level 2% | Level 4% |
| | | 1% | 2% | 4% | 1% | 2% | 4% | | | |
| n= | 4 | 4 | 3 | 3 | 4 | 3 | 3 | | | |
| (0-28 days) | | | | | | | | | | |
| ADG ^{b,c} , g | 819 | 734 | 714 | 392 | 780 | 729 | 321 | 50 | .76 | .33 |
| ADFI ^{b,c} , g | 1546 | 1479 | 1450 | 931 | 1719 | 1424 | 867 | 92 | .78 | .63 |
| M+C Intake ^{b,c} , g/d | 10.6 | 24.9 | 38.7 | 45.3 | 29.1 | 38 | 39.8 | 3.2 | .83 | .26 |
| Feed:gain, g/g | 1.91 | 2.03 | 2.02 | 2.42 | 2.24 | 1.95 | 2.78 | 0.24 | na | na |
| Spleen Wt, g | 72.7 | 72.3 | 72.3 | 85.3 | 61.2 | 58.5 | 49.8 | 10.8 | na | .32 |
| S Fe, ug/g | 105.9 | 123.3 | 430.9 | 605.1 | 138.7 | 104.4 | 528.0 | 123 | .03 | .32 |
| Plasma Met, mM/dl | 2.9 | 20.6 | 185.5 | 204.8 | 8.7 | 56.6 | 150.8 | 42 | .01 | .50 |

^a Data are least squares means (SAS, 1996) with 1 barrow and 1 gilt/pen from 0-14 days and 1 barrow or gilt/pen from 14-28 days.

^b Linear response of methionine dose ($P < .05$).

^c Quadratic response of methionine dose ($P < .05$).

^d na = not applicable as treatment effect was not significant ($P < .10$).

(P<.05) but there was no effect of M dose on liver iron content at this time. There was no effect of treatment on packed cell volume at 14 or 28 days of study.

Conclusions

From the results of this study we conclude that over supplementation of M in practical diets impairs growth in pigs and broilers and the severity of the effect differs among sources. Less severe reduction in animal performance, lower plasma M and HYC, and lower iron content in tissues demonstrate HMB is less metabolically toxic than DLM in chickens and pigs.

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Assessment of the optimum level of leucine to stimulate milk protein yield in the dairy cow

M. Vestergaard, C.F. Børsting, S. Michalak, N.B. Kristensen, K. Sejrsen & T. Hvelplund

*Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology,
Research Centre Foulum, Blichers Allé 1, P.O.Box 50, DK-8830 Tjele, Denmark*

Summary

The aim of the experiment was to study the influence of leucine (LEU) infusion on yield and composition of milk. An intra-duodenal infusion experiment was performed with 4 multiparous mid to late-lactation Holstein cows arranged in a Latin square design with 4 periods. Cows were milked twice daily (06.00 and 17.00 h) and a TMR supplied at 06.30 and 17.30 h. The basal TMR was made low in LEU and other amino acids and, therefore, all cows were supplemented by an infusate containing lysine, methionine and histidine in order to fulfil the expected requirements of these three amino acids (7.4, 2.5, and 2.6% of the amount of amino acids absorbed in the intestine (AAT)). The TMR contained 90 g AAT/Feed Unit Cattle (FU_c), 136 g digestible crude protein/FU_c and 7.4% AAT-LEU (87% of expected requirement). The increasing levels of LEU (8.4, 9.4 and 10.4% AAT-LEU) were obtained by infusing increasing levels of LEU (i.e., LEU7=0, LEU8=17.7, LEU9=35.8, and LEU10=52.8 g/day). The daily amount of LEU infused was based on the cow's initial DMI. The L-form of LEU was mixed in 8 L water and infused by peristaltic pumps for 20-22 h each day of the 7-day infusion periods. Feed intake,orts, milk yield and milk composition (protein, fat, lactose and urea) and somatic cell counts (SCC) were recorded daily. The responses in these parameters were measured over the last 4 days of each infusion period. Blood was sampled on day 5 to 7. Plasma was analysed for LEU, insulin, glucagon, growth hormone and IGF-I. The results showed that LEU infusion increased LEU concentration in plasma from 76 to 115, 115, and 175 µmoles/l for the 4 levels, respectively. Daily dry matter intake (DMI), milk yield, ECM, protein and fat percentages and protein and fat yield did not change over the 4 weeks of experiment. LEU infusion had no effect on either DMI, milk yield or milk composition. In agreement with a lack of response in milk production, the concentrations of the 4 metabolic hormones were not affected. The results suggest, that additional leucine could not affect protein percentage or protein yield, and thus indicates that leucine cannot be considered a major limiting amino acid for dairy cows under the present conditions.

Keywords: amino acid, leucine, intra-duodenal infusion, dairy cows, protein yield

Introduction

Through the last decades attempts have been made to make it possible to predict the amount of amino acids (AA), which are absorbed in the small intestine. In the Nordic countries, the "AAT-PBV"-system (Hvelplund & Madsen, 1990) has been widely adapted in ration formulating for dairy cows. The next logical step has been to describe the AA-composition in the feeds and in the ration (Misciattelli et al., 2002), although the ration AA-content is not directly reflected in the AA-content of the digesta in the intestine. It is important though to find out how to combine different feedstuffs in a dairy cow's ration to optimise milk production and reduce N-losses to the environment. One such attempt is to identify, which AA are limiting milk production. The first-limiting AA is dependent on: 1) AA-composition of the feed, 2) the degradation rate of the feed protein, 3) the extent of microbial AA synthesis, and 4) intestinal digestibility of undegraded and microbial AA (Misciattelli et al., 2002). In a recent review (Misciattelli, 2001), it was concluded

that lysine, methionine and histidine often is expected to be first limiting for N-balance and milk yield. These AA have already been studied in some detail (Pisulewksi et al., 1996; Robinson et al., 1999; Varvikko et al., 1999; Korhonen et al., 2000). However, also proline, leucine and probably phenylalanine and iso-leucine seem to be limiting in some feeding situations (see Misciattelli et al., 2002). The requirement for leucine has been estimated (Iburg & Lebzien, 2000).

As some AA infused into abomasum or duodenum will increase the plasma concentrations of these AA (Vicini et al., 1988; Kröber et al., 2001), it is possible that some AA may act by influencing the endocrine system. Several AA given intravenously are known to affect the release of metabolic hormones, like growth hormone and glucagon (Kuhara et al., 1991). Thus, metabolic hormones were measured to elucidate this possible route of action.

The purpose of the present experiment was to investigate if leucine could be a limiting AA for milk production of dairy cows.

Material and methods

An intra-duodenal infusion experiment was performed with 4 multiparous mid to late-lactation Holstein cows, arranged in a Latin square design with 4 periods. At start of the infusion experiment, the 4 cows were 121, 133, 245, and 337 days in milk, produced 24.6, 31.7, 27.5, and 20.0 kg milk/day, and weighed 620, 655, 652, and 630 kg, respectively. Cows were milked twice daily (06.00 and 17.00 h) and a TMR supplied at 06.30 and 17.30 h. The TMR contained on a DM-basis: clover-grass silage (45.9%), barley (19.7%), canola cake with 13% fat (10.5%), malt sprouts (18.8%), beet molasses (4.4%) and mineral mixture (0.6%).

When using infusion of individual AA into the duodenum as a method of studying first-limiting AA, it is important that other AA are not limiting. Thus, as the basal TMR was made low in LEU and consequently also in other AA, all cows were supplemented by an infusate containing lysine, methionine and histidine in order to fulfil the expected requirements of these three AA (7.4, 2.5, and 2.6%, respectively, of the amount of AA absorbed in the intestine [AAT]).

The TMR contained 0.94 Feed Unit Cattle (FU_c)/kg DM, 90 g AAT/ FU_c , 136 g digestible crude protein/ FU_c and 7.4% AAT-LEU (87% of expected requirement). The increasing levels of LEU (8.4, 9.4 and 10.4% AAT-LEU) were obtained by infusing increasing levels of LEU (i.e., LEU7=0, LEU8=17.7, LEU9=35.8, and LEU10=52.8 g/day). The daily amount of LEU infused was based on the cow's initial DMI. The L-form of LEU was mixed in 8 L water and infused into the duodenal cannula by peristaltic pumps for 20–22 h each day of the 7-day infusion periods.

Feed intake,orts, milk yield and milk composition (protein, fat, lactose and urea) and somatic cell counts (SCC) was recorded daily. The responses in these parameters were measured over the last 4 days of each 7-day infusion period.

Blood was sampled from a jugular vein at 09.00 h on day 5 to 7 as well as each hour from 10.00 to 14.00 h on day 6. Blood was cooled, divided and treated with appropriate anti-coagulants and aprotinin (samples for glucagon analysis), centrifuged, and the plasma frozen. Plasma was analysed for LEU (09.00 h, day 6) by gas chromatography. Plasma was also assayed for insulin, growth hormone (GH) (09.00 to 14.00 h, day 6), and insulin-like growth factor-I (IGF-I) (09.00 h, day 5 to 7) by specific TR-IFMA (time-resolved immunofluoro-metric assays), and for total glucagon (09.00 h, day 5 to 7) using a commercial RIA kit (DPC, Cat. no. KGND1).

The statistical model included the fixed effects of cow, period and LEU level as appropriate for a Latin-square design. There were no missing observations. GH and insulin concentrations were \log_e -transformed before analysis to obtain approximately normal distribution of the errors. The procedure MIXED of SAS (SAS Inst. Inc., Cary, NC) [with 'sample number' as repeated for the hormone data] was used to test (type 3 F-tests) for treatment effects and to generate LS Means and standard errors (SEM). There were generally no effects of period (see below), but for most traits the effect of cow was significant.

Results and discussion

LEU infusion increased LEU concentration in plasma from 76 to 115, 115, and 175 (moles/l for the 4 levels, respectively. These values are comparable to the levels observed in another study (Kröber et al., 2001). Across treatments, DMI, milk yield, ECM, protein and fat percentages and protein and fat yield did not change over the 4 weeks of experiment.

LEU infusion had no significant effect on either DMI, milk yield or milk composition (Table 1) in agreement with results from Kröber et al. (2001). This suggests that the LEU7 treatment will supply sufficient leucine. At the LEU7 treatment, cows received 113 g LEU/day, which is somewhat lower than the estimated requirement of LEU (158 g/day) for a 600-kg cow yielding 27 kg of milk/day according to Iburg & Lebzien (2000).

In agreement with a lack of response on milk production, plasma concentrations of the metabolic hormones were not affected by the LEU-infusion (Table 1). Likewise, others found no effects of LEU infusion on insulin and thyroid hormones (Kröber et al., 2001).

Table 1. Dry matter intake (DMI), milk yield, energy-corrected milk yield (ECM), milk composition, somatic cell count (SCC), and metabolic hormone concentrations in blood of Holstein dairy cows infused with increasing amount of leucine (LEU) into the duodenum.

| Trait | LEU7 | LEU8 | LEU9 | LEU10 | SEM | P-value |
|--|-------|-------|-------|-------|------|---------|
| DMI, kg/day | 18.3 | 18.0 | 18.2 | 17.9 | 0.17 | 0.55 |
| Milk, kg/day | 26.5 | 26.7 | 27.3 | 27.3 | 0.51 | 0.63 |
| ECM, kg/day | 27.3 | 27.1 | 28.1 | 27.9 | 0.46 | 0.45 |
| Fat% | 4.20 | 4.08 | 4.17 | 4.15 | 0.04 | 0.26 |
| Protein% | 3.50 | 3.49 | 3.46 | 3.44 | 0.03 | 0.63 |
| Lactose% | 4.61 | 4.63 | 4.71 | 4.62 | 0.04 | 0.27 |
| SCC, (1,000 | 447 | 289 | 165 | 421 | 123 | 0.42 |
| log _e -insulin, pM ¹ | 4.1 | 4.0 | 4.0 | 4.1 | 0.12 | 0.71 |
| Glucagon, pg/ml ² | 65 | 65 | 70 | 65 | 1.7 | 0.23 |
| log _e -GH, ng/ml ¹ | -0.91 | -0.61 | -0.86 | -0.42 | 0.18 | 0.19 |
| IGF-I, ng/ml ² | 94 | 93 | 90 | 95 | 2.7 | 0.82 |

¹Mean of the 6 daily samples in each of 4 periods

²Mean of the 3 days sampled in each of 4 periods

Conclusion

The results suggest that additional leucine do not affect protein percentage or protein yield, and indicates that 7.4% LEU-AAT fulfilled the requirement. Thus, leucine cannot be considered a major limiting amino acid, because this proportion of LEU-AAT is found in most rations for dairy cows under practical conditions.

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Protein session 3

Post-absorptive Metabolism of Amino Acids

Post-absorptive metabolism of amino acids

G.E. Lobley¹ & H. Lapierre²

¹ Rowett Research Institute, Greenburn Road, Aberdeen, AB21 9SB, U.K.

² Agriculture and Agroalimentaire Canada, Lennoxville, Québec, Canada J1M 1Z3

Summary

Amino acids (AA) are catabolised by various tissues as they journey from absorption to the site of anabolism. Such catabolism varies between tissues and in response to nutritional, physiological and environmental factors that alter the qualitative and quantitative patterns that flow in and out of various organs. The digestive tract can oxidise most essential AA, except threonine, but for some (e.g. lysine) such catabolism only occurs from enteral sources. AA oxidation appears sensitive to dietary protein supply while low net absorption of threonine is probably linked to poorly reabsorbed endogenous secretions into the lumen. The liver regulates concentrations in arterial plasma by removing AA not used by peripheral tissues, rather than active control of post-splanchnic supply. Use of AA for muscle protein gain does not appear to be limited by the amount provided by either arterial supply or transport into the cells. Instead, there may exist a sensing mechanism that links extracellular concentrations or the relative rates of AA intracellular influx (or efflux) to regulatory components of the translation cascade. Control of this cascade involves both AA and insulin, either separately or in combination. Unlike muscle, the mammary gland appears able, under appropriate conditions, to utilise more than 90% of the AA that is supplied to and enters the cells. The efflux of AA in the udder may be less important than in muscle.

Keywords: *essential amino acids, oxidation, digestive tract, liver, muscle, mammary gland*

Introduction

Following absorption from the lumen of the small intestine, amino acids (AA) are partitioned between ‘anabolic’ (protein gain or secretion) and ‘catabolic’ (oxidation but to also include synthesis of obligate metabolites) fates. In animal production, or during recovery from injury and disease, the aim is to direct more AA into anabolism and reduce the amount catabolised. Because of the interactive nature of amino acid and protein metabolism within mammals, processes or treatments that impact on anabolism will have the opposite effect on catabolism and vice versa. Much attention has focussed, quite correctly, on regulation of protein gain through impacts on protein synthesis and degradation (Attaix *et al.*, 2001; Davis *et al.*, 2002) but the efficiency of transfer of dietary protein into body protein may be limited by factors that lead to obligate or linked oxidation (or loss) of AA. Such efficiency limitations relate to a number of important issues. For example, where in the body are the AA catabolised? How much of any catabolism is vital to maintenance of viable biological systems and how much represent removal of ‘excess’ and, therefore, can be manipulated? Furthermore, how are these events altered by the nutrition, age and physiological status of the animal? Do the same mechanisms operate in both ruminants and non-ruminants? These series of questions need to be addressed before our understanding would allow manipulations targeted on reducing AA catabolic losses and hence improve the efficiency of protein deposition.

The structure of this short review involves following the fate of absorbed AA as they journey through, and passed, key regulatory sites, notably the digestive tract and liver, to the target organs, muscle, and mammary gland. How the splanchnic organs impact on both the absolute amounts and pattern of AA presented to the target tissues will be discussed, as will and how nutrient supply

and hormone interactions determine the overall anabolic response. Hypotheses will be advanced as to why AA catabolism occurs across specific organs and how overall regulation between peripheral and splanchnic tissues might be achieved.

The digestive tract

Early studies in rats showed that non-hepatic tissues could oxidise both non-essential AA (NEAA) and the branch-chain AA (BCAA; Miller, 1962). Subsequent studies have clearly demonstrated that the digestive tract of both ruminants and non-ruminants has the ability to catabolise NEAA. For example, in both sheep and pigs there is extensive removal and oxidation of both glutamate and glutamine (Heitmann & Bergman 1978, 1981; Reeds *et al.*, 2000; Reeds & Burrin 2001; van der Schoor *et al.*, 2001). Indeed the demands of the gut for these two AA are often so large there is net removal across the portal-drained viscera (PDV; Stoll *et al.*, 1999; Gate *et al.*, 1999; Lapierre *et al.*, 2000). In these circumstances, catabolism is the equivalent of dietary supply plus additional sources of these NEAA supplied in the artery from synthesis elsewhere in the body, particularly muscle (Bruins *et al.*, 2003). Such demand is linked, in part, to use of the AA carbon as an energy source. Thus, in young pigs the contribution of glutamate plus glutamine to CO₂ production across the PDV exceeded (54%) that derived from the sum of enteral and systemic glucose utilisation (44%; Stoll *et al.*, 1999). Other roles are also important. For example, glutamine is needed to support both the gut-associated lymphoid tissue and the overall immune system (Newsholme 2001) and to provide precursor-N for nucleic acid biosynthesis, with the latter requiring approximately 6% of flux in sheep (Gate *et al.*, 1999).

Although extensive use of NEAA by the digestive tract may have consequences for the overall protein economy of the animal, particularly the need to conserve N for appropriate transamination and re-amination reactions, the more critical question is whether the essential AA (EAA) are oxidised by the digestive tract? The answer to this appears to depend on the AA. For example, in both ruminants and pigs there is extensive oxidation of leucine (and probably the other BCAA), with substantial impact on supply to other tissues. For example, in sheep fed just above maintenance leucine oxidation across the PDV decreased net supply to the animal by 23% (Lobley *et al.*, 2003), similar to values observed for young pigs offered a high protein (1.8 g/kg BW/h) diet (van der Schoor *et al.*, 2001). Interestingly, when pigs were offered a low protein diet (0.9 g/kg BW/h) leucine oxidation declined to zero. Similarly, when metabolisable protein supply to dairy cows was raised by 17% this caused an increase from 20 to 30% in the proportion of absorbed leucine oxidised across the PDV (Lapierre *et al.*, 2002). Changes in both diet quality (altering the proportions of forage:concentrate) and quantity (fed versus fasted) also impacted on leucine catabolism in sheep (Pell *et al.*, 1986; Lobley *et al.*, 1996b). The leucine used for oxidation by intestinal tissues can be derived from either the lumen or the arterial supply (van der Schoor *et al.*, 2001), which either means rapid transport from both sources to the site of oxidation, or that the mucosal and serosal cells have similar regulatory mechanisms.

Factors other than nutrition can impact on AA catabolism. For example, leucine oxidation across the digestive tract was increased by 24-38% by the presence of intestinal roundworms (Yu *et al.*, 2000) but was reduced by 18% when normal lambs were treated with flavomycin to reduce the populations of certain bacteria (Macrae *et al.*, 1999). In consequence, there were corresponding inverse responses (reduction and improvement, respectively) in net absorption into the portal vein. Thus, leucine catabolism appears sensitive to the metabolic pressures exerted on the digestive tract.

Leucine, and the other BCAA, are rather unusual in that the primary enzymes for their catabolism, the BCAA-transaminase and -dehydrogenase, are widely distributed across tissues, including liver, muscle, fat, mammary gland as well as the intestine (Goodwin *et al.*, 1987; DeSantiago *et al.*, 1998). So, although the digestive tract has a substantial, and variable, impact on the amount of

leucine available to the animal, does this also extend to the other EAA? Here the evidence and the impact is less certain.

In piglets, lysine supplied enterally was oxidised with high protein diets; this reduced lysine supply to the liver by 8% and represented 31% of whole body lysine oxidation (van Goudoever *et al.*, 2000). As with leucine, when a low protein diet was offered lysine oxidation by the PDV declined to zero. In contrast to leucine, however, there was no oxidation of systemic lysine either in pigs, regardless of the level of protein in the diet (van Goudoever *et al.*, 2000), or in sheep (Lobley *et al.*, 2003). While kinetic data based on free AA infusions into the lumen may be compromised, either because the site of absorption is shifted to the upper small intestine or because micro-organisms dwelling in the digestive tract lumen may metabolise free AA, these data suggest that catabolism of enteral-derived lysine does occur and is sensitive to supply. Furthermore, recent studies have shown that the intestinal tissues of neonatal piglets contain the mitochondrial enzyme lysine ketoglutarate reductase, necessary to oxidise lysine, at approximately 25% the activity of the liver enzyme (Pink *et al.*, 2003). One immediate challenge is to resolve the mechanism by which enterocytes can sense and respond to luminal supply of lysine without involvement of systemic AA supply, as appears to be the case with leucine. This is a particular challenge because the majority of the total AA flux through intestinal cells is derived from the arterial supply (MacRae *et al.*, 1997b; van Goudoever *et al.*, 2000).

For both leucine and lysine oxidation across the PDV, there are sufficient data to indicate qualitative and quantitative similarities between ruminants and non-ruminants. For other AA the information is less complete and more uncertain. For example, based on systemic infusion of tracer, there was no oxidation of phenylalanine across the ovine PDV, assessed from no appearance in the portal vein of either $^{13}\text{CO}_2$ or labelled tyrosine in sheep (Lobley *et al.*, 2003). In contrast, with young pigs, more than 40% of enteral phenylalanine supply was oxidised during the absorptive process (Bush *et al.*, 2003). This latter study was not able to separate catabolism between first-pass absorption and recycled phenylalanine in the arterial supply but, in view of the observations with leucine and lysine, the more probable explanation is that this represents oxidation of the enteral source.

In contrast to lysine and phenylalanine, methionine from the arterial supply can be oxidised by the ovine intestinal tract (Lobley *et al.*, 2003), but only to a limited extent, with consequent net supply to the liver reduced by 9%. In the young piglet, however, methionine use by the intestine has been estimated as much greater, 31% of total requirements (Ball 2002). Although it is attractive to postulate that all EAA may be oxidised, either within the lumen of the small intestine or during transport through the enterocytes, this is not the case for threonine, at least, where no PDV oxidation occurs regardless of whether the labelled AA is infused at either enteral or systemic sites (Burrin *et al.*, 2001).

These various data leave a series of further questions and conundrums. The most important of these is ‘why does the digestive tract oxidise EAA?’. Of course, some of the oxidation may be due to microbial metabolism as substantial quantities of bacteria inhabit the small intestine, even in pigs, and influence AA availability (Le Guen *et al.*, 1995; Torrallardona *et al.*, 2003). To ascribe all the enteral oxidation to bacterial action is difficult, however, given the sensitivity to level of protein supply (van Goudoever *et al.*, 2000; van der Schoor *et al.*, 2001) and instead the involvement of tissue metabolism must be considered. Would actions of the latter involve common or specific mechanisms? For example, is there an obligate need for oxidation associated with the high rates of protein turnover in intestinal tissue (Lobley *et al.*, 1994; Bush *et al.*, 2003) or the proliferative demands of tissue replacement (Attaix & Meslin 1991)? If so, why is there no apparent catabolism of threonine? Alternatively, should each AA be considered separately for key aspects of intermediary metabolism? The wide distribution of leucine catabolic enzymes has already been mentioned and this may link to the involvement of this AA in acute regulation of nutrient-hormone sensitivity (Garlick & Grant, 1988). Such a mechanism would require that the modulator can reach the target cells but then be quickly removed from the system, possibly by

catabolism within these cells. A more mundane, but still important, role may link to the role of leucine as a major transamination source (Cheng *et al.*, 1985; de Lange *et al.*, 1992), with oxidation a consequence of increased intracellular concentrations of the oxo-acid. The degradative pathways of lysine produce glutamate and the importance of this AA in digestive tract metabolism is well-established (Reeds *et al.*, 2000; van der Schoor *et al.*, 2001). While at first sight it may appear counter-intuitive to utilise a key essential EAA to produce an NEAA, the 5-carbon skeleton intermediate formed is essential also for the biosynthesis of arginine and proline. In the young pig, these are both co-essential AA (Brunton *et al.*, 1999), with the intestine as a major (or exclusive) site of the synthesis of both (Ball *et al.*, 2002). These demands may outweigh the need to conserve lysine. In the case of methionine there is involvement in one-carbon metabolism, with key functions in cell proliferation including synthesis of nucleotides and regulation of gene expression (Wajed *et al.*, 2001). Indeed, rates of trans-methylation reactions are as high in intestinal tissue as in kidney and liver (Lobley *et al.*, 1996a). Prevention of homocysteine from entering the trans-sulphuration pathway (and thus oxidation of the main part of the methionine carbon chain) depends on supply of methyl groups from methyltetrahydrofolate and/or betaine, shortage of these will result in catabolism. Alternatively, catabolism of methionine is also necessary for the synthesis of polyamines, again vital for proliferative tissues with short cell half-lives (Attaix & Meslin 1991). The situation with phenylalanine is puzzling, however, as evidence from the rodent indicates that the digestive tract tissues do not contain phenylalanine hydroxylase (Ayling *et al.*, 1974; Davis *et al.*, 1992) and certainly not in the amounts necessary to induce the substantial catabolism observed in the pig (Bush *et al.*, 2003). Perhaps, microbial degradation may play a more important role for this EAA.

Direct oxidation is not the only route by which AA are lost across the intestine. Another major mechanism involves the endogenous secretions: these comprise saliva, gastric juices, bile, pancreatic secretions, sloughed epithelial cells and mucins (Tammenga *et al.*, 1995). Due to their inherent metabolic functions (including forming part of the innate immune defence and digestive enzyme activity) some of these protein are resistant to degradation and pass beyond the ileum, with consequent non-availability to the animal of the constituent AA. Estimation of endogenous losses is complex and a variety of techniques have been approached. One of the simplest is to use protein-free diets and measure AA flows at the ileum (Nieto *et al.*, 2002). This assumes that the presence of protein does not impact on the secretions and the approach is better suited to non-ruminants than ruminants, where continued rumen microbial synthesis occurs with plasma and salivary urea providing the N source. Another technique involves long-term labelling of body protein and monitoring isotope appearance in digesta from various parts of the intestine (de Lange *et al.*, 1992; van Bruchem *et al.*, 1997; Hess *et al.*, 1998, 2000; Ouellet *et al.*, 2002; Zuur *et al.*, 2003). This approach is constrained by isotope costs, selection of the appropriate precursor pool (Leterme *et al.*, 1998; Ouellet *et al.*, 2002) and estimation of the digestibility of the endogenous secretions. Nonetheless, such approaches have shown that 19-33 g of endogenous N enter the pig digestive tract daily (Souffrant *et al.*, 1993), with similar values for sheep (Van Bruchem *et al.*, 1997) while dairy cow estimates reach 90-100 gN/d, with approximately 18-20% of faecal N being of endogenous (non re-absorbed) origin (Ouellet *et al.*, 2002). Such losses impact on both the absolute quantity and the profile (or balance) of AA available to the animal, because poorly digested endogenous proteins can contain substantial quantities of individual AA, as is the case with mucins that contain large amounts of threonine and proline (Lien *et al.*, 1997).

In non-ruminants, the differential impact of the combination of endogenous losses plus AA oxidation can be assessed from the amounts and proportions of absorbed AA (net portal flux) compared with those in the diet or digested in the small intestine (Table 1; Le Floc'h *et al.*, 1999; van Goudoever *et al.*, 2000). These shifts can be exaggerated when protein intakes are low and, indeed, net absorption of threonine in piglets has been reported as zero, even when it is the most abundant EAA in the diet (van Goudoever *et al.*, 2000). In contrast, the net absorption of the other EAA (from appearance in the portal vein) ranged from 16-40% of intake. Even at high protein

Table 1. Flux EAA (mmol/h) from the diet or disappearance from small intestine (SID), and net portal-drained viscera (PDV) in pigs and sheep. Values in parentheses are proportion of EAA supply¹.

| | Leucine ¹ | Phenylalanine ¹ | Threonine ¹ |
|-------------------|----------------------|----------------------------|------------------------|
| Pig | | | |
| Diet ² | 4.71 (26.5) | 2.02 (11.0) | 3.50 (13.8) |
| PDV ² | 2.59 (15.4) | 1.62 (14.8) | 1.25 (11.4) |
| Body ³ | (12.2) | (12.5) | (13.0) |
| Sheep | | | |
| SID ⁴ | 2.44 (15.3) | 1.21 (11.1) | 1.71 (16.1) |
| PDV ⁴ | 1.33 (14.7) | 1.08 (13.2) | 1.19 (17.9) |
| Body ⁵ | (10.6) | (10.1) | (17.8) |

¹ Expressed as percentage of total isoleucine + leucine + lysine + phenylalanine + threonine + valine

² Le Floc'h et al (1999)

³ Campbell & Taverner (1988)

⁴ MacRae et al (1997)

⁵ MacRae et al (1993)

intakes, absorption of dietary threonine recovery was still only 16%, while values for the other EAA were 2- to 3-fold greater (van Goudoever *et al.*, 2000). Low supply of threonine (or any other EAA) will automatically limit the efficiency of the conversion of feed protein to animal product.

For ruminants, comparison of net portal appearance with dietary protein composition is meaningless due to N inter-conversions in the rumen. Instead, comparisons are better made against either duodenal flows or the disappearance from the lumen of the small intestine (MacRae *et al.*, 1997b; Berthiaume *et al.*, 2001). As for non-ruminants apparent losses are smaller (10%) for some AA (e.g. phenylalanine; Table 1), with lower oxidation and contribution to non-reabsorbed endogenous proteins. For other AA, including leucine and threonine, known to be substantially oxidised or present in high proportion in mucins, the apparent losses across the PDV are larger (30-45%) and this impacts on the pattern of absorbed AA compared with the profile in body protein (Table 1). Direct interpretations of these ratios are compromised, however, by the inflows of endogenous proteins, both pre- and post-duodenum (Ouellet *et al.*, 2002). Nonetheless, the impact of secretions that arise from sources such as the fore-stomachs, the pancreas and hind-gut, or oxidations that occur across the same tissues, can be assessed by comparison of net uptakes across the mesenteric-drained viscera (MDV, representative of net uptake from the small intestine) and the PDV (Seal & Parker, 1996; MacRae *et al.*, 1997b). In both dairy cows (Berthiaume *et al.*, 2001) and sheep (Lobley *et al.*, 2003), there were significant differences between the EAA (Table 2) with low ratios for threonine, the BCAA and tryptophan, as well as proline. These data indicate that endogenous losses and oxidation also distort the pattern of AA supplied to the liver in ruminants. It is noteworthy that the most marked change in ratio is with tryptophan, an AA rarely reported because of technical constraints. Why the demand for this EAA is so high is unclear but the digestive tract of non-ruminants contains tryptophan hydroxylase (Kobayashi *et al.*, 1991).

Table 2. Ratio of PDV:MDV appearances in lactating dairy cows and growing sheep.

| | His | Leu | Lys | Met | Phe | Thr | Try | Pro |
|--------------------|-------------------|--------------------|-------------------|--------------------|-------------------|---------------------|-------------------|--------------------|
| Cows ¹ | 0.75 | 0.68 | 0.72 | 0.66 | 0.76 | 0.38 | - | - |
| Sheep ² | 0.71 ^b | 0.61 ^{de} | 0.71 ^b | 0.67 ^{bc} | 0.81 ^a | 0.63 ^{bcd} | 0.48 ^e | 0.66 ^{bc} |

¹ Berthiaume et al (2001)

² Lobley et al (2003). Values with unlike superscripts differ by P<0.01

Many of the above considerations have been based on transfers of free AA, but over the past decade there has been a lively debate on whether peptides should also be considered when quantitative balances across the digestive tract are attempted. Such measurements are technically demanding and this lead, in no small part, to problems with some of the early studies where the summation of free AA, ammonia and peptide absorption greater exceeded digested N plus urea entry into the gut (Webb, 1999). More recent studies have either reduced (Rémond *et al.*, 2000) or eliminated (Backwell *et al.*, 1997) peptides as a major contributor. Nonetheless, there may be circumstances where the animal does rely more on peptide absorption but first careful attention should be paid to the N balance across the digestive tract to see if there is a need to invoke other forms of N.

In both ruminants and non-ruminants, there is little doubt that digestive tract metabolism alters the quantitative and qualitative supply of AA to the rest of the body. These demands are associated with both direct oxidation of AA plus losses due to endogenous secretions. Under certain circumstances, this overall 'catabolism' appears sufficient to constrain productive responses and would need to be included in future predictive models of AA needs. More data are needed to quantify factors that influence these losses. With such information it will be possible to reduce the impact on production with either additional supply to match the needs of the digestive tract or by manipulation of animal husbandry to reduce the demands of these metabolic processes.

Liver

As the major site for ureagenesis in the body the liver is often considered the most important organ for AA catabolism, but this is an over-simplification. In addition to the already discussed impact of the digestive tract, a wide range of other tissues can catabolise BCAA (Goodwin *et al.*, 1987). Indeed, in ruminants hepatic catabolism of leucine can be less than across the intestine (Lobley *et al.*, 1996b; Lapierre *et al.*, 2002). In addition, threonine is oxidised in the pancreas (Le Floc'h *et al.*, 1997) and lysine in the mammary gland (Mabjeesh *et al.*, 2000) and muscle (Pink *et al.*, 2003). Nonetheless, the liver does play a dual role in both maintaining arterial plasma AA concentrations within physiological bounds and yet allowing appropriate quantities to reach peripheral tissues to support anabolism. In terms of this review the key question asked is: how 'active' is the role of the liver in determining such supplies to the periphery? In other words, 'does the liver regulate exactly the amount of absorbed AA available to the peripheral tissues (controller)?' or 'does the liver remove those AA not used by the peripheral tissues (responder)?'. The answer to this question then determines how AA supply for anabolism is regulated and what mechanisms may be involved.

Of course, life is not that simple and the complexity of the liver means that elements of both approaches are involved. First, removal of AA by the liver does not necessarily mean these are directed towards oxidation and ureagenesis. Instead, such removals may support vital metabolic functions. For example, the liver synthesises a number of export proteins and, although this alters in response to both short and long-term nutritional (Jahoor *et al.*, 1996; Connell *et al.*, 1997) and environmental (Jahoor *et al.*, 1999) events, a basal synthesis is maintained, even under fasting

conditions (Connell *et al.*, 1997). These requirements account for 1-16% of the AA extracted by the liver (Jahoor *et al.*, 1999; Raggio *et al.*, 2002). These relatively modest requirements do not necessarily represent a net drain to either the liver or the animal, however, as these export proteins are 'internal' (as opposed to those associated with milk secretion, fibre production or even secretion into the lumen of the digestive tract) and the AA can be recovered following degradation at a number of tissue sites (Maxwell *et al.*, 1990). Other processes that are obligate and, therefore, require 'active' control include conversion of AA to other metabolites that perform key functions. These would include synthesis of cysteine from methionine to support albumin and glutathione synthesis (Jahoor *et al.*, 1995; Shoveller *et al.*, 2003). Similarly, in ruminants offered forage diets synthesis of hippurate, involving glycine, can account for as much as 17% of N intake under extreme conditions (Lowry *et al.*, 1993). This essential detoxification mechanism explains why, even under more normal conditions, the liver can remove glycine from both dietary and endogenous sources to yield a negative hepatic balance, i.e. net outflow is less than net inflow (Lobley *et al.*, 2001).

Nonetheless, for absorbed EAA the majority is either used for anabolism (mainly peripheral tissues) or is catabolised, with the liver playing a dominant role for some AA but not others. For example, with animals at zero N retention a number of AA, notably histidine and phenylalanine are completely removed by the liver (Wray-Cahen *et al.*, 1997; Le Floc'h *et al.*, 1999; Lobley *et al.*, 2001), thus none is net available to support peripheral tissue growth. This means that, under the same conditions, other AA that show a net positive balance across the liver must be catabolised elsewhere in the body (Harris *et al.*, 1992; Le Floc'h *et al.*, 1995; Bequette *et al.*, 1996). Data from lactating cows (Figure 1) provides the same picture, where the net post-splanchnic supply of histidine, phenylalanine and methionine matches closely with the amounts extracted across the mammary gland and secreted in milk protein (Lobley & Lapierre, 2001).

At first glance, such data would support the 'controller' theory, where liver metabolism determines the net supply of histidine, methionine and phenylalanine to peripheral tissues in the exact amounts necessary to support anabolism. An alternative explanation is that the liver is the dominant post-digestive tract site for oxidation of these three EAA (Raiha *et al.*, 1973; Hryb & Feiglsson, 1983;

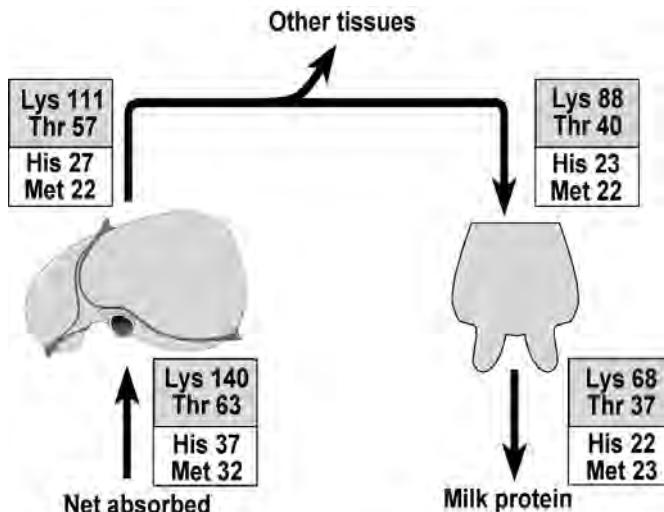


Figure 1. Net (g/d) absorption into portal vein, post-liver supply, uptake by the mammary gland and secretion as milk protein for four EAA, histidine (His), methionine (Met), Lysine (Lys) and threonine (Thr). Data from Lobley & Lapierre (2001); Blouin *et al.* (2002); Berthiaume *et al.* (2002).

Lichter-Konecki *et al.*, 1999), and their metabolic fates are then simply partitioned between use for peripheral tissue anabolism and hepatic catabolism i.e. without the need for ‘active’ control. Another point against the ‘controller’ theory is that for most other EAA (lysine, threonine and the BCAA) post-hepatic net supplies exceed those removed by the mammary gland and that appear in milk protein (Figure 1). For these AA, therefore, a substantial proportion of the post-splanchnic supply must be oxidised in tissues, other than liver, and post-splanchnic supply supports both anabolic demand and peripheral tissue catabolism. This observation would be more logical if the liver played the role of ‘responder’.

Indeed, more direct support for the ‘responder’ theory is obtained hepatic removal is expressed relative to the total inflow of AA to the liver rather than the net amount absorbed. In practice, only a small portion of the AA supply to the liver via the portal vein comes from first pass absorption, most is from arterial inflow to the splanchnic tissues and derived from endogenous or non-first pass sources. To this must be added hepatic artery inflows that represent AA recycled from post-liver sources. Thus, for growing sheep offered 1.2 x maintenance only 6-19 % of portal flow is from first pass absorbed EAA (see Figure 2; Lobley *et al.*, 2001).

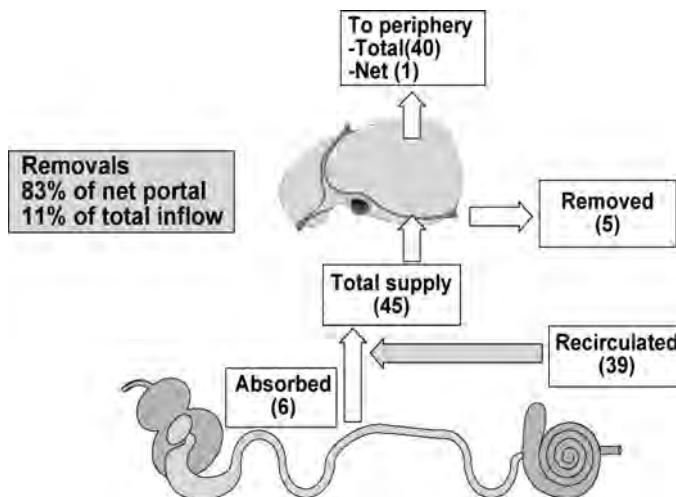


Figure 2. Absolute transfers of methionine ($\mu\text{mol}/\text{min}$) across the splanchnic tissues in sheep offered 1.2 x maintenance intake. From Lobley *et al.* (2001).

At higher intakes, as observed with dairy cows, these values are only 7-38 % (Berthiaume *et al.*, 2001; Blouin *et al.*, 2002). In consequence, of the total amount presented to the liver (including a 3-20% contribution from the hepatic artery) only a small fraction is removed (Figure 2). Interestingly, this fraction appeared to be relatively constant for each EAA across a wide range of total inflows, as shown for phenylalanine (Figure 3; Wray-Cahen *et al.*, 1997; Lobley *et al.*, 2001). The fraction of total inflow removed was between 0.08 to 0.29 for all EAA, except for the BCAA with values close to zero.

Such data allow the response of the system to be modelled (Hanigan *et al.*, 1998) and for certain consequences to be predicted. First, following absorption of a bolus dose of AA, a decreasing absolute amount (but the same fraction of total inflow) is removed on each circulation through the liver. If, on any pass some of the absorbed AA is removed by other (peripheral) tissues, then less is returned to the liver and a smaller absolute quantity is extracted and catabolised. This concept would imply that a proportion of absorbed AA is removed during first pass through the liver but then the requirements (and use by) the peripheral tissues then determines how much is returned to the liver for possible extraction on each subsequent pass. Based on the data available

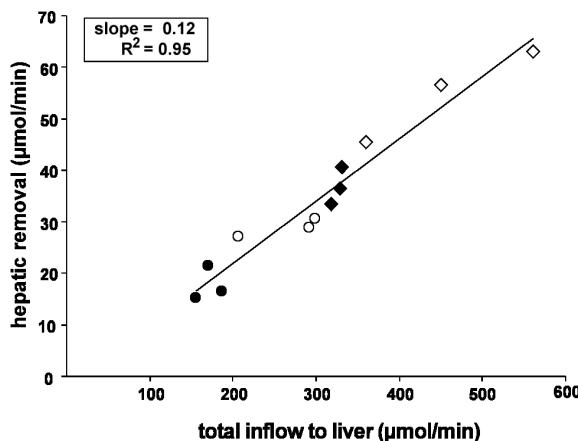


Figure 3. Response in liver extraction of phenylalanine ($\mu\text{mol}/\text{min}$) to increasing hepatic inflows produced by 4.5 h infusions of the AA, equivalent to 1- to 4-fold basal absorption at 1.2 \times maintenance intake. From Lobley *et al.* (2001).

(Lobley *et al.*, 2001), then for sheep fed close to nitrogen equilibrium 50% of a phenylalanine dose would be removed in 3 min, but 10% would still remain after 12 min. This agrees well with an observed half-life of 4 min from direct measurements (Figure 4).

Thus, provided the basal hepatic inflows (plasma flow \times concentrations) are known, or can be reasonably estimated, then the amount and time over which absorbed AA are available to peripheral tissues can be predicted, at least for those AA that are catabolised predominantly by the liver. In consequence, current steady-state models can be extended to non steady-state conditions and the impacts of meal feeding patterns on potential anabolic performance predicted.

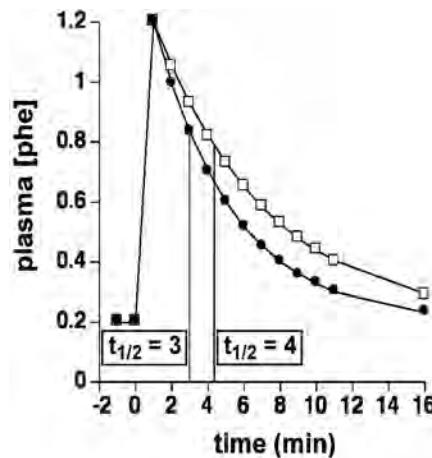


Figure 4. Predicted (●) changes in arterial plasma concentrations (μM) following absorption of a bolus dose of phenylalanine based on the concept of the liver being a 'responder' and from the fractional clearance rates reported by Lobley *et al.* (2001). This is compared with observed (□) changes in plasma concentrations following injection of a bolus dose (115 μmol) of [$1-^{13}\text{C}$]phenylalanine into the jugular vein of 4 sheep (unpublished results).

This approach also allows the question of where hormones act to be assessed. For example, addition of growth hormone to animal systems can increase protein anabolism without altering intake (Bush *et al.*, 2003). Obviously this also means less AA catabolism and lowered urea production. If the impact of the hormone is exclusively on the tissues (i.e. removal of post-splanchnic supply) then the hepatic fractional extraction will not alter (but absolute removals will be lower due to tissue use). Alternatively, if the hormone also acts to down-regulate liver metabolism (and catabolic pathways) then the fraction extraction will decrease.

Finally, the concept would mean that there is always finite removal of AA by the liver (even under conditions of zero absorption) because plasma concentrations, and hence hepatic inflow, are maintained by release from protein breakdown. This would fit with, and help explain, the negative protein (AA) balance that occurs during fasting or sub-maintenance intakes (Lapierre *et al.*, 1999). Similarly, the impact of a limiting AA on utilisation of other EAA is easy to explain through changes in their removal by peripheral tissues leaving those in excess to be returned to the liver for removal on subsequent passes.

This concept would indicate that the liver plays a relatively minor role in direct control of supply to peripheral tissues, but does regulate the time over which peripheral tissues are exposed to absorbed AA. This also allows short-term control over arterial AA levels and helps prevent metabolic disturbances caused by supra-physiological concentrations.

One interesting follow-up is that the model would also explain, when AA are provided by jugular infusion and milk protein yield is increased, why post-hepatic supply of some EAA is negative (Table 3, (Berthiaume *et al.*, 2002). The AA supplement would raise arterial plasma concentrations with a consequent increase in total inflow to the liver, with absolute removal then increased as a result of the constant fractional removal. This scenario has analogies with fasting, when endogenous (post-splanchnic) mobilisation raises, or maintains, supply to the liver and there is negative post-hepatic supply. A similar situation occurs with use of the 2-hydroxythiomethylbutanoic acid (HMB) that is converted into methionine in peripheral tissues (particularly the kidney) and reduces the dependency of the animal on dietary methionine. In consequence, the liver can remove 75% (dairy cows, (Lapierre *et al.*, 2003) or all (sheep; (Wester *et al.*, 2000) of absorbed methionine, but still maintain productive performance.

The data in Figure 1 and Table 3 indicate that for some EAA oxidation occurs in peripheral tissues and this can be a substantial portion of net absorption. Quite why some EAA are oxidised predominantly in the liver and others elsewhere in the body is not entirely clear. Although some of this oxidation occurs within the mammary gland, this can vary with supply (Bequette *et al.*, 1996) and, therefore, may not provide an obligate function. Part of the role of this extra supply of AA could be to provide substrate N for synthesis of NEAA or transamination with oxo-acids, as shown for leucine in muscle (Cheng *et al.*, 1986, 1987; Tessari *et al.*, 1999) and lysine in the mammary gland (Lapierre *et al.*, 2003). Alternatively, some of these AA may regulate their own utilisation (and that of others) through modulation of endocrine action, as discussed in the following section.

Peripheral tissues

To the question ‘what regulates AA metabolism at the peripheral tissues?’ the obvious answer often given is ‘nutrient supply’. But what exactly does this mean? In terms of total supply, as assessed by arterial plasma (blood) flow and AA concentration, this is considerably in excess of net uptake (gain) across leg tissues (Biolo *et al.*, 1995; Bush *et al.*, 2003; Hoskin *et al.*, 2003) or milk output across the mammary gland (Bequette *et al.*, 2000). Even when N intake is below ‘maintenance’, arterial supply is still sufficient, in theory, to support substantial anabolic gains (Hoskin *et al.*, 2003), yet net retention is negative.

Muscle

Attempts to relate arterial AA inflow to the ovine hind-quarters with retention, have yielded only moderate correlations (R^2 0.73) and with a low coefficient (0.18) based on threonine, leucine and valine kinetics (Hoskin *et al.*, 2003; Lobley 2002). Such a low coefficient implies a constraint, other than total supply to the tissues, for example AA transport into the cells. If, indeed, transport were a limitation then strategies to either increase the number of AA transporters (via natural or applied genetics) or their activity (by endocrine manipulation, for example) would be desirable. To measure AA transport *in vivo* is difficult and, for rodents, has required sequential slaughter studies (Banos *et al.*, 1973; Hundal *et al.*, 1986). An alternative, indirect approach based on trans-organ arterio-venous preparations, allied to tissue biopsy samples or analysis of secreted proteins, has been developed for larger species, including humans (Biolo *et al.*, 1995). Although the method has limitations, it has been applied to sheep hind-quarters (Hoskin *et al.*, 2003) and the mammary gland in both goats (Bequette *et al.*, 2000) and cows (Zuur *et al.*, 2002). In terms of the ovine hind quarters (a mixture of muscle, skin, bone and fat), EAA inward transport was approximately 30–50% of arterial inflow (histidine was lower at 14%), but still exceeded net retention by 2.5 to 11-fold (Hoskin *et al.*, 2003; Lobley *et al.*, 2003). Even at sub-maintenance intakes, inward transport was still greater than the AA retention observed at the supra-maintenance intake. Similarly, in dairy cows, inward transport of methionine into the mammary gland exceeded milk protein output by 2.5-fold (Zuur *et al.*, 2002).

Nonetheless, AA inward transport does increase in response to anabolic stimuli, such as insulin administration in humans (Biolo *et al.*, 1995) and level of nutrition in sheep (Hoskin *et al.*, 2003). Indeed, the relationship between protein gain and inward transport is moderately good (R^2 0.92) in response to nutritional changes but with a coefficient of only 0.28 for the composite data based threonine, isoleucine and valine (Lobley 2002). Two important aspects of these data need to be considered. First, the fact that inward transport exceeds net anabolism means that AA either efflux from the cell (outward transport), or are released in other forms (e.g. peptides) or are catabolised. Although catabolism is a fate for the BCAA in muscle (Harris *et al.*, 1992; Tessari *et al.*, 1995), the major route of loss from the cell is efflux as free amino acid. Therefore, mechanisms that slow of the efflux of AA may have effects on anabolism by increasing the amount of intracellular substrate available.

*Table 3. Impact of intra-jugular infusion of EAA mixture on net transfers across the splanchnic bed (TSP) and output in milk protein in dairy cows (from Berthiaume *et al.*, 2002 and unpublished).*

| | Leu | Lys | Met | Phe | Thr |
|----------------|------|-------|------|-------|-------|
| Control | | | | | |
| PDV | 36.7 | 26.7 | 9.1 | 19.0 | 18.7 |
| Liver | 2.3 | 1.7 | -1.0 | -8.1 | -0.3 |
| TSP | 39.0 | 28.4 | 8.1 | 10.9 | 18.4 |
| Milk | 28.4 | 21.6 | 7.1 | 11.3 | 13.7 |
| Infused | | | | | |
| PDV | 30.1 | 29.9 | 8.5 | 18.7 | 19.0 |
| Liver | -7.3 | -15.4 | -7.6 | -20.6 | -21.2 |
| TSP | 22.8 | 14.5 | 0.9 | -1.9 | -2.2 |
| Infused | 19.3 | 16.9 | 4.9 | 9.1 | 15.7 |
| Milk | 33.0 | 25.0 | 8.3 | 13.1 | 15.9 |

The second issue relates to the mechanism by which inward transport impacts on retention. This may relate to control of protein synthesis, another process that greatly exceeds the rate of net anabolism (Bush *et al.*, 2003; Hoskin *et al.*, 2003). Indeed, the correlation between AA transport and protein synthesis over the wide intake range investigated with sheep is good, with a slope of 0.90 and an R^2 of 0.83 (Lobley, 2002) and similar correlations have been reported across the human fore-arm (Zanetti *et al.*, 1999). While, in theory, tissue protein synthesis can be maintained without inflow of AA from plasma, by just using the intracellular pools within a closed loop, these data imply that some control on the process is exerted by inward transport, or that inward transport may be regulated by protein turnover. As a note of caution, such a relationship may only exist under anabolic conditions as in hyper-catabolic patients protein synthesis can be increased without accompanying alterations in the rate of inward transport (Biolo *et al.*, 2002). Nonetheless, it appears that although arterial inflow, inward transport and protein synthesis all exceed rates of AA retention (Figure 5) any, or all, of these processes may link mechanistically to control of protein deposition. This raises the next question - what mechanism(s) might be involved?

One concept is that the changes in the rates of inward and outward transport and those of protein deposition would alter intracellular AA concentrations, the substrates for protein synthesis. In practice, however, in both humans (Kobayashi *et al.*, 2003) and sheep (Hoskin *et al.*, 2003) intracellular concentrations can remain essentially constant despite wide changes in arterial levels. Indeed, it has been argued (Wolfe & Miller 1999) that the high rates of inward (and outward) transport provide a sensitive mechanism through which intracellular AA concentrations are maintained constant, or at least are much less variable than plasma values. This theory would require that AA are sensed either at the extracellular level or as part of the transport processes, with the latter possibly driven in response to alterations in the intracellular:extracellular gradient.

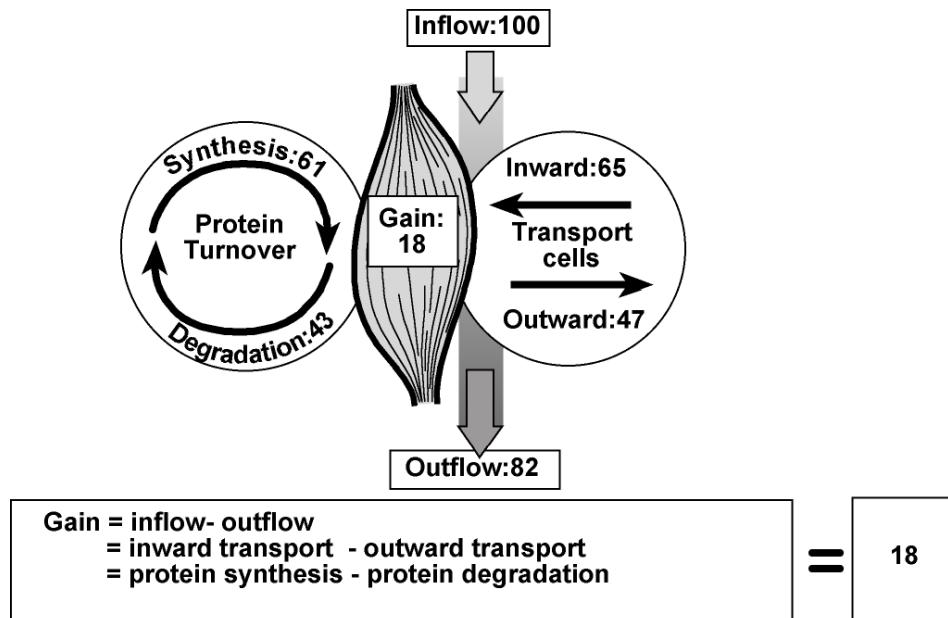


Figure 5. The responses in inward transport, outward transport, protein synthesis, protein degradation and retention in tissue proteins across the hind-quarters of sheep following an increase in arterial inflow of 100 $\mu\text{mol}/\text{min}$ of isoleucine, threonine and valine. From Hoskin *et al.* (2003) and Lobley (2002).

In terms of the molecular mechanisms that link nutrient supply and endocrine action to protein metabolism much attention has been focussed on two components of the cascade that regulates mRNA translation. The first is the eukaryote initiation factor 2B (eIF2B), a protein involved in the guanine nucleotide exchange of eIF2 to permit binding of met-tRNA to the 40S ribosomal subunit at the initiation of translation. A second critical stage involves formation of the 48S ribosomal complex through binding of the mRNA to the 43S pre-initiation complex. This requires the presence of eIF4F, formed by the association of eIF4E and eIF4G with the availability of eIF4E regulated by phosphorylation of a 4E-binding protein (4E-BP1) (Kimball, 2002).

When either AA or insulin is administered to post-absorptive rats eIF2B activity is not apparently altered, although muscle protein synthesis is increased (Anthony *et al.*, 2000; Davis *et al.*, 2000). At first sight this would preclude a major role for eIF2B in regulation of nutrient responses but under conditions when circulating AA concentrations are reduced to below basal, as occurs during haemodialysis, then both eIF2B activity and protein synthesis are depressed (Kobayashi *et al.*, 2003). Provision of supplemental AA restored both eIF2B and protein synthesis to control values. This suggests a permissive role for eIF2B (Kobayashi *et al.*, 2003), whereby other eIFs can only operate provided arterial AA concentrations and/or inflow to the tissues achieve minimum values. Because these changes occurred even though intracellular AA concentrations were unaltered, this links either extracellular concentrations or rates of transport to regulation of the activity of this initiation factor.

The formation of the active eIF4F complex appears to be governed by several factors, but notable amongst these are insulin and AA. For example, in rats fed a 25% protein meal muscle protein synthesis (assessed *in vitro*) was elevated, as was the extent of phosphorylation of 4E-BP1 (thus making more eIF4E available to bind to eIF4G) and p70 S6 kinase, another key component of regulation of translation (Balage *et al.*, 2001). When insulin secretion was blocked, however, protein synthesis rates were similar to fasted rats and 4E-BP1 remained in a hypo-phosphorylation state (and complexed with eIF4E), with only low amounts of eIF4F formed. With a 0% protein diet there were no increases in protein synthesis or phosphorylation status compared with fasted rats, even though insulin concentrations were elevated. These data indicate that both insulin and AA status are critical to alter muscle protein synthesis (Yoshizawa *et al.*, 1998). In young pigs maintained with a euglycaemic and euaminoacidaemic clamps, however, insulin and AA could separately stimulate protein synthesis, with synergistic effect when supplied together (Davis *et al.*, 2002). These apparently contradictory findings between rats and piglet may reflect the different experimental approaches. With the clamp technique the principle is to maintain plasma concentrations but at the expense of altering nutrient fluxes. If, as suggested, flows into (and from) cells are critical in regulation of protein synthesis then clamp procedures may simulate a feeding response, with more phosphorylation of 4E-BP1 and s70 S6 kinase, increased formation of the eIF4F complex and elevated muscle protein synthesis (Yoshizawa *et al.*, 1995; Kimball *et al.*, 2002). In addition, differences in the concentrations of insulin from either a basal diet (pigs) or after pharmacological suppression of release (rats) may alter sensitivity to AA supply.

The potential interaction between insulin and AA in regulation of muscle protein synthesis has focussed attention on the role of leucine, because this AA has the greatest ability to enhance the sensitivity to this hormone (Garlick & Grant, 1988). In fasted rats given a bolus of leucine, equivalent to a total day's intake, muscle protein synthesis was enhanced, with hyperphosphorylation of E4-BP1 and s70 S6 kinase and increased formation of the eIF4E:eIF4G complex (Anthony *et al.*, 2002). This was accompanied, however, by an increase in insulin and so direct (or non-associated) effects could not be fully established. Nonetheless, the same study showed that in diabetic rats a leucine-induced increase in protein synthesis occurred (albeit to a lesser extent) but without changes in insulin, nor in phosphorylation of either 4E-BP1 or s70 S6 kinase. The conclusion was that leucine-enhanced protein synthesis in muscle was as a consequence of both insulin-dependent and -independent mechanisms (Anthony *et al.*, 2002). While such observations involving large single doses of AA must not be over-interpreted, these

data provide a mechanistic basis for how leucine may potentiate responses to insulin and, as such, act as signal of nutrient status to peripheral tissues.

Mammary gland

It is unclear if these kinetic observations and molecular mechanisms uncovered for muscle can be extended to the mammary gland. A value of 400:1 is often quoted as the relationship between blood flow (l/d) and milk output (kg/d), this would suggest that total inflow is a key factor, perhaps with similarities to the extraction by the liver and use by muscle. This is over-simplistic, however, as blood flow can be increased while milk production is decreased (Bequette *et al.*, 2000). Neither do changes in total AA supply (inflow) appear to act as main regulators (Cant *et al.*, 2002). Thus, when arterial concentration (and total inflow) of leucine was reduced markedly, while those of other AA were increased (by infusion), there was little effect on milk protein output compared with appropriate controls (Bequette *et al.*, 1996). Leucine may not be the best AA to use for such comparisons, however, because it is catabolised by the mammary gland and this metabolic fate was reduced to 'spare' sufficient to maintain milk protein synthesis (Bequette *et al.*, 1996). Better is to examine an AA that is not metabolised by the mammary gland. Thus, in goats receiving a histidine-deficient infusion of AA, the arterial histidine concentrations were reduced 9-fold and, as blood flow was only increased by 33%, total arterial inflow to the gland decreased by 85% (Bequette *et al.*, 2000). Yet, milk protein output was only reduced by 20%. In consequence, mammary vein plasma histidine concentrations were as low as 2 µM and the sensitivity of the gland to the 'limiting' AA was markedly improved. Interestingly, the largest effect of reduced total inflow was on efflux, which was reduced by 80% compared with a decrease of only 34% for inward transport. Furthermore, with efflux then less than 10% of inward transport (compared with 30% under control conditions) more than 90% of histidine that entered the mammary cells was secreted in the form of milk protein (Bequette *et al.*, 2000).

The massive metabolic drive associated with milk production has probably involved development of specific regulatory mechanism unique to the mammary gland, with the 'set point' for milk output maintained by regulation of mammary blood flow and activity of the AA transporter systems (Cant *et al.*, 2002). Indeed, the above data imply that the mechanisms by which AA are transported into the gland can operate over extremely wide physiological concentrations and would provide only a limited 'constraint'.

Conclusions

Partition of AA between anabolic and catabolic fates occurs at several major sites within the body. There seems little doubt that the digestive tract tissues have the ability to oxidise some, but not all, EAA. For some this appears to be restricted to lumen sources and how this might be regulated in response to supply in the digesta is unknown and clearly should be a major target for future research in order to devise strategies to minimise losses at this site. That the digestive tract appears to function adequately when this oxidation is suppressed would suggest the mechanism is not to fulfil obligate needs and, therefore, would be a sensitive and appropriate target for manipulation. Although alteration in ration processing (including exclusion of anti-nutritional factors) may assist in reducing endogenous secretions, an alternative solution is to use supplements of specific AA to cover these losses. For hepatic metabolism, if the 'responder' theory is correct then it is necessary to determine if the fractional extractions can be altered by treatment, e.g. hormonal status, that then influences the hepatic catabolic pathways. If this is not a major control mechanism then perhaps attention should be turned to what influences the basal level of arterial AA concentrations and thus total inflow to the liver. For peripheral tissues, particularly muscle, the metabolic and molecular links that occur between supply and retention need to be better understood. This is an area that requires good nutritional and metabolic designs to maximise use of the rapid advances

being made at the molecular level. For the mammary gland it is clear that, under extreme conditions at least, very efficient use (perhaps the highest of any biological system) is made of AA that enters the cell. If the same mechanisms function under 'normal' dietary conditions then clearly intracellular events hold the key and the same rigorous molecular approaches adopted for other tissues must be applied to the mammary gland.

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Using wool growth to estimate skin protein turnover in sheep at pasture

N.R. Adams, J.R. Briegel, S.M. Liu, A.C. Schlink & M.R. Sanders

CSIRO Livestock Industries, Private Bag #5, PO Wembley, WA 6913, Australia

Summary

Changes in wool growth rate and liveweight often occur at different times in sheep run on pastures in highly seasonal climates. This study measured skin protein mass and wool growth rate each month during a period of dramatic seasonal changes in pasture quality and quantity, in order to explore the underlying drivers of change. The fractional rate of protein synthesis (FSR), measured at the beginning and end of the experimental period, showed that a constant proportion of the protein synthesized in the skin was secreted as wool protein. Wool growth rate was poorly related to skin mass (indicated by the weight of a skin trephine biopsy), but was related to both the concentration of protein in the skin and the FSR. The increase in protein synthesis rate in skin preceded the increase in skin mass. Furthermore, the estimated skin protein synthesis rate reached a maximal value before there was any significant increase in liveweight. In a second experiment, changes in the volume of follicular bulb tissue followed the same pattern as skin mass, but were of greater magnitude. This pattern reflected the changes in skin protein concentration observed in the first experiment.

We conclude that measurements of skin protein mass and wool growth rate provide useful insights into skin protein turnover in sheep at pasture. Skin protein turnover appeared to be out of synchrony with the remainder of the body, as reflected in changes in liveweight. The independence indicates that competition between wool and other protein pools for amino acids varies throughout the year.

Keywords: synthesis, follicle, liveweight

Introduction

Wool growth is often conceptualised as protein deposition, like muscle growth, but the processes differ biologically; wool growth reflects only protein synthesis. A relatively constant proportion of the protein synthesized in the skin of sheep is secreted as wool protein, regardless of breed or nutritional state (Adams *et al.* 2000a). Therefore, it is possible to estimate changes in the FSR of skin protein in Merino sheep by measuring wool growth rate and the mass of skin protein. This paper reports these values in sheep under differing pasture conditions and examines the inferences that can be drawn about protein turnover in the skin.

Materials and methods

Experiment 1 utilized 34 4-year-old Merino wethers grazed on annual grass / clover pasture at Bakers Hill, Western Australia. Initial measurements were carried out during autumn as the sheep grazed limited, dead, poor quality pasture. Two weeks after the opening rainfall for the season (May 11, 1998), germination resulted in sparse short green pasture, but almost no dead pasture. Further significant rainfall occurred on May 22 and mid-June, so that short green pasture was available throughout June and July. Low temperatures limited pasture growth until August when the spring flush began, resulting in abundant green pasture by September. No significant rainfall occurred after the first week of September, and the last sampling occurred on mature, green pasture.

Sheep were weighed every 2 weeks between April and September. Midside wool patches approximately 100 cm² were clipped on April 6th, and wool samples collected from the patch on May 1st, May 25th, June 29th, August 3rd, August 31st and September 25th. The area of the patch was measured by tracing it on a plastic sheet to correct for variations in patch size. On the same day, triplicate skin biopsies were collected by trephine (1 cm diameter, 0.785 cm² in area) from midside skin on the other side of the sheep. The biopsies were weighed, frozen in liquid nitrogen, and kept at -70°C until analysis. The concentration of protein in the skin biopsies was measured as described by Adams *et al.* (2000b).

Wool samples were washed twice in wool-scouring detergent and weighed. Clean wool was considered to be 90% protein. All characteristics were compared by correcting to the amount per cm² of skin.

Fractional synthesis rate (FSR) of skin protein was measured in 16 sheep on May 22nd, and the same sheep were measured again on September 24th. The sheep were fitted with bilateral jugular catheters the day before the study, and returned to the field. Animals were mustered at 9.30am the next day and infused over 10 min with 0.27g/kg^{0.75} liveweight L-phenylalanine including about 10% of [L-ring-d₅] phenylalanine. Fractional protein synthesis rate were measured as described by Adams *et al.* (2000b). An abstract of these results was presented by Briegel *et al.* (2000).

Experiment 2 reports further measurements on samples from the study described by Schlink *et al.* (2000). In brief, skin biopsies were collected at 4-week intervals from 12 wethers grazed on annual pasture at Bakers Hill, WA between September 1992 and October 1993. Biopsies were weighed after fixation in formalin followed by 70% ethanol, and serial vertical histological sections were prepared and stained with haematoxlyin and eosin (Schlink *et al.*, 2000). A minimum of 35 follicles from each sample was measured to estimate the volume of the follicle bulb calculated as the product of the square of the radius and the height of the dermal papilla, multiplied by 3.14 (Maddock & Jackson, 1988).

Results were analysed statistically by repeated measures ANOVA, or by regression using the general linear model of Systat 10 (Wilkinson, 1998).

Results

As shown in Table 1, liveweight in Expt 1 declined after May 1st, presumably due to the loss of dry pasture after the opening rains. Liveweight increased slowly on the short green pasture, such that animals still weighed less on June 29th than they did at the start of the experiment. Liveweight then increased more rapidly as pasture growth rate increased. The weight of the skin biopsy was relatively constant from the beginning of the experiment until June 29th, increased rapidly by August 3rd, and then remained constant. The concentration of protein in the skin, and consequently the mass of protein in the skin, followed a similar pattern (Table 1).

In contrast to both liveweight and skin biopsy weight, wool growth from the midside patch increased substantially by June 29th, and reached a maximum on August 31st (Table 1). The ratio of wool protein to skin protein therefore also increased on June 29th and increased further by August 3rd. This implies that skin protein FSR increased before the mass of protein in the skin, since wool protein/skin protein mass reflects the skin protein FSR.

Wool protein made up 18% of the total protein synthesized in the skin in May, and 19% in September (Briegel *et al.*, 2000).

Changes in liveweight and skin biopsy mass during Expt 2 followed a similar pattern to Expt 1, with liveweight lagging behind skin mass by approximately 2 months (data not shown). The average volume of follicle bulb tissue followed an identical pattern to skin biopsy weight (Fig. 1), but the amplitude of change in bulb volume between March and October (210%) was greater than that of the skin biopsy mass (146%).

Table 1. Mean (\pm s.e.m.) monthly values for skin and wool protein and liveweight, in sheep grazing annual pastures during the period of pasture germination and growth.

| Date | SP ¹ conc. (mg.g ⁻¹) | SP mass (mg.cm ⁻²) | WP ² growth (mg.day ⁻¹ .cm ⁻²) | WP / SP (day ⁻¹ x100) | Liveweight (kg) |
|----------------------------|--|-----------------------------------|---|-------------------------------------|--------------------|
| 1 st May | 93 \pm 5 | 16.0 \pm 1.8 | 0.51 \pm 0.01 | 4.0 \pm 0.3 | 64 \pm 2 |
| 25 th May | 103 \pm 5 | 16.3 \pm 1.9 | 0.56 \pm 0.02 | 4.2 \pm 0.3 | 61 \pm 2 |
| 29 th June | 97 \pm 5 | 17.0 \pm 1.5 | 0.73 \pm 0.02 | 5.5 \pm 0.5 | 62 \pm 2 |
| 3 rd August | 110 \pm 8 | 21.6 \pm 4.1 | 1.13 \pm 0.03 | 6.7 \pm 0.4 | 65 \pm 2 |
| 31 st August | 109 \pm 8 | 21.9 \pm 2.7 | 1.18 \pm 0.04 | 6.6 \pm 0.5 | 71 \pm 2 |
| 25 th September | 118 \pm 9 | 21.2 \pm 2.4 | 1.09 \pm 0.04 | 6.1 \pm 0.4 | 74 \pm 2 |

¹ Skin Protein

² Wool Protein

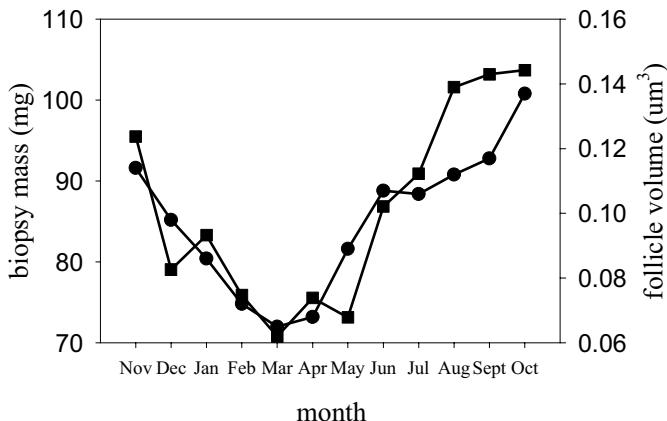


Figure 1. Changes in biopsy mass (■) and wool follicle volume (●) in sheep through the year in sheep on annual pastures.

Discussion

Changes in skin protein mass depend on the synthesis and degradation rates. Skin protein synthesis rate can be estimated from wool growth, because the proportion of protein synthesized in the skin that was secreted as wool remained constant between May and September, despite a doubling of wool growth rate (Briegel *et al.*, 2000). Changes in the relative protein FSR over time can therefore be inferred by dividing wool protein mass by skin protein mass. In Expt 1, wool growth rate commenced to increase by June 29th, while skin protein mass was not increased at this time, so the ratio of wool to skin protein increased (Table 1). This indicates that the increase in skin protein FSR preceded the increase in skin protein mass.

Skin mass *per se* was poorly related to wool growth. For example, stepwise regression of the May sampling in Expt 1 showed that wool growth rate was related to both the FSR and the concentration of protein, but not to the mass of the skin biopsy. It is likely that skin protein concentrations reflect characteristics of the wool follicles. In Expt 2, the amplitude of change in follicular volume (210%) was greater than that of skin mass (146%). However, this was still less than the changes in wool growth rate (286%; Schlink *et al.* 1999), so changes in follicular mass

alone were not enough to account for the total increase in wool synthesis. It is probable that the rate of protein synthesis in follicles also increased.

Liveweight increase was slow before August 31st while skin protein mass was maximal that this time. Thus, skin protein mass responded more rapidly than the body as a whole. This is consistent with skin being a labile tissue that responds more rapidly to change in nutrient supply than tissues such as muscle (Waterlow, 1995).

In Merino sheep, wool growth rate indicates the amino acid drive on skin protein synthesis, because photoperiod has little effect on wool growth rate. The supply of amino acids for synthesis comes from both diet and protein degradation. Liveweight loss in May was probably accompanied by increased whole-body protein degradation, increasing the supply of amino acids available to drive protein synthesis rate. Faster pasture growth after mid-August resulted in increased liveweight gain. The increased energy supply would be expected to affect the supply of amino acids to skin by decreasing the degradation rate of muscle and by sparing amino acids from oxidation. Further measurements of wool growth rate and skin protein mass may open up opportunities to exploit differences in the timing of requirements for amino acids by skin, gut and muscle in sheep on pasture.

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Certain amino acids regulate growth hormone (GH) binding to cultured pig hepatocytes

J.M. Brameld & P.J. Buttery

Division of Nutritional Biochemistry, University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough, LE12 5RD.

Summary

Previous studies have demonstrated effects of certain amino acids (arg, pro, thre, tryp, val) on GH-stimulated IGF-I mRNA expression by cultured pig hepatocytes. The present study was aimed at investigating one possible mechanism for this effect, translation of the GH receptor (GHR) gene, using GH-binding as a measure of GHR protein. Removal of arg, pro, thre or tryp reduced specific binding of bGH to cultured pig hepatocytes. There was no effect on intracellular DNA, protein, glycogen or free glucose, but changes in the intracellular free amino acid pool were observed, although the significance is not known. Certain amino acids may therefore interact with the GH-IGF axis by regulating translation of GHR mRNA.

Keywords: amino acids, growth hormone binding, hepatocytes

Introduction

The mechanisms of how amino acids influence gene expression are still unclear. We have previously shown that decreasing concentrations of arg, pro, thre, tryp and val inhibit GH-stimulated IGF-I mRNA in a dose-dependent manner, with little or no effect on GH-receptor (GHR) mRNA (Brameld *et al.* 1999). Three mechanisms are possible for these amino acid effects: 1) inhibition of translation of GHR mRNA into protein; 2) reduced transcription of the IGF-I gene via interactions with unidentified transcription factors; or 3) reduced stability of IGF-I mRNA, via regulation of IGF-I mRNA-binding proteins. The studies described here were carried out to investigate the first of these mechanisms, regulation of translation of GHR.

Material and methods

Pig hepatocytes were isolated and cultured in 60mm dishes (9 per treatment per pig - 3 NSB, 3 Total and 3 DNA etc) as described previously (Brameld *et al.* 1999). Hepatocytes (1.56×10^7 viable cells per dish) were cultured overnight in Hepes buffered Williams' medium E supplemented with 2g/L BSA and 100 nmol/L insulin (basal medium), with or without the various nutrients (glucose, arginine, proline, threonine, tryptophan, valine or leucine). The cells were then maintained on the same basal medium (with or without glucose and individual amino acids), in the presence or absence (control and lacking glucose only) of 3,3',5-tri-iodothyronine (T_3 , 10nmol/L) and dexamethasone (Dex, 100nmol/L). GH-binding or intracellular concentrations of DNA, protein, glycogen and free glucose and amino acids were measured 24 hours later. Bovine GH (bGH) was radiolabelled with ^{125}I via the lactoperoxidase method and purified via gel filtration (Sephadex G50 Fine). GH binding to cultured hepatocytes in the presence (NSB) or absence (Total) of excess unlabelled bGH was as previously described (Niimi *et al.* 1990; 1991), in order to calculate specific binding. The effects of removing individual nutrients on intracellular concentrations of DNA, protein, glycogen, free glucose and amino acids were also investigated in separate dishes, in order to check cell viability and the specificity of any effects. DNA measurements were carried out as previously described (Rago *et al.* 1990). Intracellular protein

concentrations were determined using the Lowry method (Lowry *et al.* 1951), adapted for use on a 96 well plate format. Intracellular free glucose concentrations were measured using a plasma glucose kit (Boehringer Mannheim), also adapted for use on the plate reader. Glycogen content was estimated via the glucose released following digestion with α -amylase and amylo-glucosidase, using the same plasma glucose kit. Glucose concentration was then multiplied by 0.9 (162/180) to convert free glucose to anhydroglucose, as in glycogen. Intracellular free amino acid concentrations were determined following 5-sulphosalicylic acid deproteinisation using an amino acid analyser (Biochrom 20) and lithium buffer system.

Four pigs were used to provide the replication for these experiments, as well as 3 dishes per treatment per measurement. Data was subjected to analysis of variance (ANOVA) using the Genstat 6 statistical package. Means were compared to controls by Dunnett's test. Differences of $p<0.05$ were considered significant, while differences of $p<0.10$ were considered as tending towards significance.

Results

There was a tendency ($p=0.089$) for non-specific binding (NSB) to be reduced when glucose (including glucose and T3/Dex) was removed from the medium compared to control (data not shown). Total binding was reduced ($p<0.001$) by the removal of glucose, glucose and T3/Dex, arginine, proline, threonine and tryptophan (data not shown), with no effect of removing T3/Dex, leucine or valine alone ($p>0.05$). There were no effects ($p=0.155$) on DNA concentrations (data not shown), thus indicating that the numbers of cells on the dishes remained constant. Specific Binding (total minus NSB per μg DNA), was reduced ($p=0.008$, Table 1) by the removal of glucose and T3/Dex, arginine, proline, threonine, tryptophan and valine, with no effect of glucose alone, T3/Dex alone or leucine.

Table 1. Effects of removing individual nutrients and/or T3/Dex on specific binding and intracellular protein, glycogen and free glucose concentrations in cultured pig hepatocytes. Mean values are shown along with the standard error of the differences of the means (SED), no. of degrees of freedom (DF) and the ANOVA p-value.

| Treatment | Specific binding (cpm/ μg DNA) ^a | Specific Binding (% control) | Protein ($\mu\text{g}/\mu\text{g}$ DNA) | Glycogen ($\mu\text{g}/\mu\text{g}$ DNA) | Glucose ($\mu\text{g}/\mu\text{g}$ DNA) |
|-----------------|---|---------------------------------|---|--|---|
| Control | 316 | 100 | 55.46 | 126.5 | 0.972 |
| - T3/Dex | 213 | 69.7 | 56.34 | 171.7 | 1.077 |
| - Gluc | 239 | 76.6 | 55.78 | 130.9 | 0.315* |
| - Gluc & T3/Dex | 158* | 43.7* | 54.6 | 105.9 | 0.25* |
| - Arg | 115* | 37.0* | 52.78 | 125.7 | 1.08 |
| - Pro | 113* | 31.3* | 51.86 | 127.6 | 1.08 |
| - Thre | 161* | 48.6* | 54.71 | 130.5 | 1.081 |
| - Tryp | 149* | 44.5* | 53.39 | 105.4 | 1.116 |
| - Val | 195+ | 57.4* | 55.47 | 109.3 | 1.026 |
| - Leu | 250 | 79.2 | 54.61 | 138.2 | 1.022 |
| SED | 49.9 | 11.65 | 1.85 | 18.71 | 0.099 |
| DF | 25 | 25 | 25 | 25 | 25 |
| p-value | 0.008 | <0.001 | 0.353 | 0.064 | <0.001 |

^a Specific binding is (Total - NSB)/DNA

* $p<0.05$: Significantly different from the control value (Dunnett's test)

+ $p<0.10$: Tends to be different from control value (Dunnett's test)

Table 2. Effects of removing individual nutrients and/or T3/Dex on intracellular free amino acid concentrations (nmoles/µg DNA) in cultured pig hepatocytes. Mean values are shown along with the SED, no. of degrees of freedom (DF) and the ANOVA p-value.

| AA conc. (nmoles/ µg DNA) | Treatment | Leu | | | | | | | | SED | DF | p-value |
|---------------------------------|-----------|-------|--------|-------|---------------|--------|--------|-------|--------|-------|-------|---------|
| | | Cont | T3/Dex | Gluc | Gluc & T3/Dex | Arg | Pro | Thre | Trypt | | | |
| Asp | 21.76 | 23.73 | 10.48+ | 14.50 | | 36.56* | 30.61+ | 28.09 | 23.94 | 26.55 | 22.23 | 3.905 |
| Thre | 7.12 | 8.07 | 6.58 | 8.04 | | 10.57* | 11.70* | 4.83* | 11.55* | 9.86 | 10.01 | 0.934 |
| Ser | 21.58 | 19.36 | 19.40 | 19.15 | | 22.29 | 22.28 | 24.49 | 25.20 | 26.53 | 23.92 | 2.25 |
| Asn & Glu | 64.8 | 69.6 | 36.6* | 36.2* | | 83.3 | 81.2 | 87.2 | 80.6 | 79.3 | 70.1 | 8.79 |
| Gln | 33.3 | 43.0 | 9.7+ | 8.2+ | | 37.1 | 37.1 | 42.5 | 36.7 | 42.1 | 33.7 | 10.22 |
| Gly | 40.43 | 42.88 | 33.16+ | 36.37 | | 39.07 | 38.18 | 40.36 | 41.00 | 42.65 | 41.47 | 2.73 |
| Ala | 56.9 | 56.3 | 21.8* | 18.6* | | 61.5 | 63.0 | 78.3 | 70.9 | 69.1 | 68.0 | 10.35 |
| Val | 8.61 | 7.02 | 6.53+ | 7.22 | | 6.58 | 7.34 | 8.07 | 9.54 | 8.04 | 10.01 | 0.94 |
| Met | 3.83 | 4.00 | 2.51* | 3.06 | | 3.08 | 3.18 | 3.21 | 3.18 | 3.55 | 3.29 | 0.266 |
| Ile | 3.82 | 3.43 | 2.70+ | 3.16 | | 3.63 | 3.56 | 4.16 | 4.23 | 3.87 | 4.04 | 0.47 |
| Leu | 8.29 | 8.16 | 5.95* | 6.98 | | 7.86 | 7.18 | 7.62 | 8.08 | 7.74 | 6.21 | 0.53 |
| Tyr | 4.52 | 4.86 | 3.27* | 4.03 | | 3.57* | 3.64* | 3.85 | 3.84 | 4.06 | 4.45 | 0.250 |
| Phe | 4.74 | 4.96 | 3.14* | 4.18 | | 3.37* | 3.63* | 3.88 | 3.87 | 4.08 | 4.46 | 0.317 |
| Orn a | 7.50 | 6.74 | 4.83* | 5.52* | | 4.74* | 6.54 | 6.17* | 6.05* | 6.60 | 6.93 | 0.339 |
| Lys | 11.07 | 11.33 | 8.12 | 9.55 | | 10.09 | 10.23 | 10.68 | 10.48 | 11.09 | 12.01 | 0.606 |
| Pro a | 5.80 | 5.38 | 4.47 | 6.21 | | 6.69 | 4.11+ | 7.91+ | 8.76+ | 7.80 | 7.60 | 1.02 |

* p<0.05: Significantly different from the control value (Dunnett's test)

+ p<0.10: Tends to be different from control value (Dunnett's test)

a Only detected in the first 2 experiments (n=19 (ornithine) and 20 (proline) out of a possible 40 samples), therefore only those included in the analyses
Arginine, Histidine and Tryptophan were below the limit of detection in all of the samples

There were no effects ($p=0.353$, Table 1) on intracellular protein concentrations, indicating that there were no dramatic effects on protein metabolism (synthesis or degradation). There was a tendency for glycogen content to vary with treatments ($p=0.064$, Table 1), but this appeared to be due to a slight increase with the removal of T3/Dex alone, rather than a depletion in any of the nutrient restricted groups. Intracellular free glucose content was reduced ($p<0.001$, Table 1) when glucose (including glucose and T3/Dex) was removed from the medium.

There were dramatic fluctuations in the intracellular free amino acid pools (Table 2). In particular, the removal of glucose from the medium resulted in varying decreases in the intracellular free concentrations of most of the amino acids, the only ones apparently not affected being threonine, serine, lysine and proline. The removal of arginine from the medium resulted in decreases in intracellular free ornithine, phenylalanine and tyrosine, and increases in aspartic acid and threonine (Table 2). Proline had a very similar effect resulting in decreases in intracellular free proline, phenylalanine and tyrosine, and increases in aspartic acid and threonine (Table 2). Removal of threonine decreased intracellular free threonine and ornithine and tended to increase proline. Removal of tryptophan decreased intracellular free ornithine and increased (or tended to increase) threonine and proline. Removal of valine or leucine appeared to have no effect on intracellular free amino acid concentrations.

Discussion

The presence of glucose and the combination of T₃ and Dex had previously been shown to increase GHR mRNA (Brameld *et al.* 1999) and, as expected, removal decreased GH-binding. Leucine, unlike the other amino acids being studied, had no effect on GH-stimulated IGF-I mRNA (Brameld *et al.* 1999), and, as expected, removal had no effect on GH-binding. Removal of certain individual amino acids (arg, pro, thre, tryp and val) reduced specific binding of bGH to cultured pig hepatocytes, with no effect on intracellular DNA, protein, glycogen or free glucose. There were fluctuations in the intracellular free amino acid pools, such that removal of an amino acid tended to reduce its intracellular free concentration. Removal of arg, pro, thre and tryp also resulted in imbalances of the intracellular free amino acid pool, such that some amino acids were increased while others were decreased, but the significance of this is not known. Certain amino acids may therefore interact with the GH-IGF axis by regulating translation of GHR mRNA, but the mechanism is yet to be elucidated.

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The effect of exogenous purine supply on plasma concentration and urinary excretion of purine derivatives in growing goat kids

*T. Fujihara & S.A. Abdulrazak**

Faculty of Life and Environmental Science, Shimane University, Matsue-shi 690-8504, Japan

** Present address: Dept. of Animal Science, Egerton University, P. O. Box 536, Njoro, Kenya*

Summary

The aim of the present study was to investigate the effect of exogenous purine supply on plasma level and urinary excretion of purine derivatives (PD) in growing kids when they were given milk replacer (MR) to which purine nucleosides has been added. Three Japanese Saanen kids (twin: male & female, female; birth wt., 3.3, 2.2 and 4.3 kg respectively) were used. They were separated from their dams on the 4th day after birth, and then, reared by bottle-feeding of MR for 24 weeks in metabolism cages. Feeding level throughout the experimental period was at the requirement level (about 150g live weight gain per day) for energy and protein. The daily amount of exogenous purine (adenosine: guanosine, 0.464: 0.536/mol) supplied experimentally were 0, 0.4, 0.8 and 1.2 mmol/kg^{0.75} on 4-, 12- and 20-week-old. Each level of purine was added to the diet for 3 consecutive days and urine was collected on the 2nd and 3rd days for each level. About 10ml jugular blood was collected on the 3rd day at 0, 3 and 5 hours after morning feed. The results obtained were as follows; 1) The growth rate of animals was favorable and the average daily gain was 131.0 ± 7.4 g/d during the experimental period, and then, creatinine clearance (l/d) was normal. 2) The urinary PD excretion during no purine supply period tended to decrease with an advance of growth of the animals. 3) The urinary PD excretion was clearly increased with an increase of exogenous purine supply. 4) There was a peak of plasma PD level at 5 hours after morning feed in each level of exogenous purine supply on 4, 12- and 20-week-old. 5) The recovery of purine (as urinary PD per supplied PD) at 4, 12 and 20 weeks was 31.1, 28.3 and 39.9% (0.4mmol/kg^{0.75}), 39.3, 42.3 and 51.7% (0.8mmol/kg^{0.75}) and 37.9, 39.6 and 40.7% (1.2 mmol/kg^{0.75}), respectively. Results indicate that growing goat kids are able to incorporate an exogenous purine base to provide an increasing demand of nucleic acids in the body, however for metabolizing an excess amount exogenous purine the activity of xanthine oxidase seems to be insufficient.

Keywords: *exogenous purine, urinary PD excretion, growing kids*

Introduction

The relationship between the volume of exogenous purine entered into small intestine and urinary excretion of purine derivatives (PD) in ruminants has been reported (Fujihara et al., 1987; Chen et al., 1990). However in young ruminant, this relationship is not always clear, because they incorporate directly exogenous purine to provide an increasing demand of nucleic acids for their growth. Therefore, it seems that the effect of exogenous purine on metabolism of nucleic acids could be unstable in growing ruminants. The aim of the present study was to investigate the effect of exogenous purine supply on plasma level and urinary excretion of PD in growing kids when they were given the purine-free diet to which purine nucleosides has been added.

Materials and methods

Animals and diet

Three Japanese Saanen goat kids (twin: male & female, female; birth wt., 3.3, 2.2 and 4.3 kg) were used. They were separated from their dams on the 4th day after birth, and then, bottle-fed with milk replacer (MR: purine-free) for 24 weeks in metabolism cages.

Experimental procedure

The body weight of animals was measured every 5 days during the experimental period. The feeding procedures were the same as described earlier (Fujihara et al. 2003b), and the feeding level throughout the experimental period was at the requirement level (about 150 g live weight gain per day) for energy and protein (NRC, 1981). The daily amount of exogenous purine (adenosine: guanosine, 0.464: 0.536/mol) supplied experimentally were 0, 0.4, 0.8 and 1.2 mmol/kg^{0.75} on 4-, 12- and 20-week-old. Each level of purine was added to diet for 3 consecutive days (see Figure 1). Urine was collected on the 2nd and 3rd days for each level, and about 10 ml jugular blood was collected on the 3rd day at 0, 3 and 5 hours after morning feed.

Sampling and analytical procedure

About 100-150ml of urine was collected in 10% H₂SO₄ solution to adjust pH value below 3.0 to prevent a disappearance of PD during collection (Fujihara et al. 1991). The samples of urine and blood plasma were stored in deep freezer (urine: -20°C, plasma: -40°C) until analysis. Nitrogen (N) in the urine was determined by Kjeldahl method, and purine derivatives (PD) in urine and blood plasma was analyzed by the methods of Fujihara et al. (1987) and Young and Conway (1942).

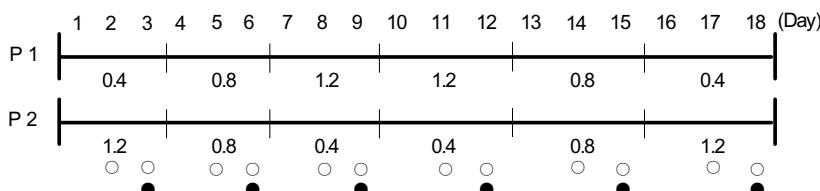


Figure 1. Experimental schedule; (*: Dose (mmol/kg^{0.75}/day); ○: Urine sampling ●: Blood sampling.

Results and discussion

There were no retardation in growth of animals throughout the experimental period, but instead an average daily gain was 131.0±7.4g during 24 weeks was recorded. Urinary N (g/kg^{0.75}/d) tended to vary among individuals and also sampling days during the entire experimental period. Little effect ($p>0.05$) of exogenous purine on urinary N excretion was observed. There were no obvious changes in creatinine clearance before and after exogenous purine supply, and this clearly shows the physiological status in bottle-fed kids as it is in one fed ordinary feed.

Urinary PD excretion

As shown in Figure 2, the urinary PD excretion (mmol/kg^{0.75}/d) of goat kids fed MR alone, which would be as endogenous excretion, tended to decrease along with the growth. The values for allantoin agreed our previous results (Fujihara et al., 2003a), and fairly lower than those in milk-

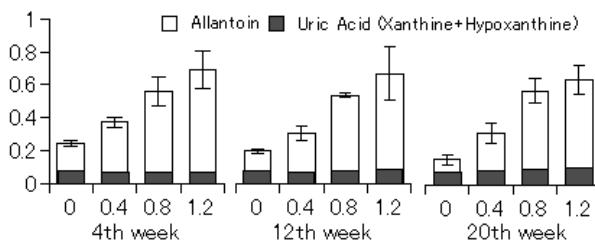


Figure 2. Urinary PD Excretion (mmol/kg^{0.75}/d) * Dose (mmol/kg^{0.75}/d).

fed young goats reported by other workers (Lindberg, 1989; Matsumoto et al., 1991). The increase of urinary PD excretion with an increase in exogenous purine, confirms a positive correlation between purine absorption and urinary PD excretion as described earlier (Fujihara et al., 1987; Chen et al., 1997). Urinary PD excretion, however, did not change along with an advancing of age in each supplementary level of purine nucleoside. The calculated endogenous PD in the urine tended to decrease with an increase of exogenous purine, and this would be due to an increase of purine supply in the body (Chen et al., 1990). Urinary allantoin excretion obviously increased with an increase of exogenous purine regardless of the other PD change in each level of exogenous purine, findings that are in agreement with results of Fujihara et al., (1991).

Urinary recovery of PD

The average recovery of PD into urine of kids after supplied with purine nucleoside, tended to increase with an advance of growth of animals (Table 1). The urinary recovery of PD tended to be low in 0.8 mmol/kg^{0.75}/d than in 1.2 mmol/kg^{0.75}/d at 4, 12 and 20 weeks after birth. It is likely that the contribution of endogenous PD to total urinary PD decreased with increased exogenous purine supply (Chen et al., 1990). The values were fairly lower than those reported by Chen et al. (1997), in sheep nourished through intragastric infusion, with urinary recovery of PD as 67.3% after supplemented purine nucleoside. The difference could be due to increase in tissue uptake of purine nucleoside during the early stage of growth of kids in the present study.

Table 1. Recovery (%) of exogenous purine.

| Dose (mmol/kg ^{0.75} /d) | 4 weeks | 12 weeks | 20 weeks | Recovery |
|-----------------------------------|---------|----------|----------|----------|
| 0.4 | 31.14 | 28.37 | 39.91 | 33.14 |
| 0.8 | 39.31 | 42.38 | 51.70 | 44.46 |
| 1.2 | 37.91 | 39.58 | 40.69 | 39.39 |
| Recovery | 36.12 | 36.78 | 44.10 | - |

Plasma PD concentration

The average plasma PD level was 45.0±2.4umol/l in goat kids fed MR alone at 4, 12 and 20 weeks (Figure 3). These results are comparable to those of our previous study in sheep nourished with intragastric infusion (50umol/l, Chen et al., 1997), and defaunated young goats (40.9umol/l, Fujihara et al., 2003a). The uric acid: total PD ratio in plasma was relatively constant in all the level of exogenous purine, although the concentrations of other PD fairly changed with changes in the level of exogenous purine. The plasma levels of xanthine + hypoxanthine tended to increase with an increase of exogenous purine, and this would be due to insufficient in xanthine oxidase activity to metabolize absorbed purine nucleoside in early growing kids. As a whole, it can be

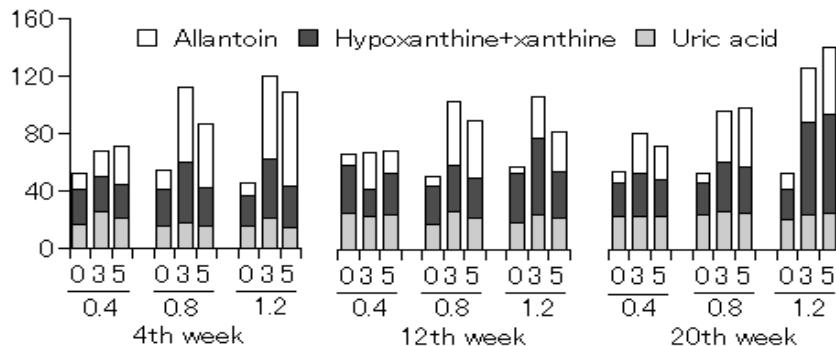


Figure 3. Plasma PD concentration ($\mu\text{mmol/l}$) at 4, 12 and 20 weeks (*: hours after feeding **: Dose ($\text{mmol/kg}^{0.75}/\text{d}$)).

considered that exogenous purine nucleoside is easily metabolized, and reflected in plasma PD concentration in goat kids during a relatively short time after feeding.

Conclusion

The growing goat kids are clearly able to incorporate an exogenous purine base to provide an increasing demand of nucleic acids in the body, however for metabolizing an excess amount exogenous purine the activity of xanthine oxidase seems to be insufficient some-times.

Acknowledgement

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Effect of diet on the partitioning of amino acids between body components of growing beef cattle fed at similar level of metabolisable energy intake

E.J. Kim¹, N.D. Scollan¹, M.S. Dhanoa¹ & P.J. Butterly²

¹ Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK

² Department of Nutritional Biochemistry, School of Bioscience, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

Summary

Eighteen Hereford x Friesian steers were reared from approximately 155 to 250, 350 and 500 kg live weight on either grass silage alone or a mixture of grass silage and concentrate (60:40 on metabolisable energy basis) in a comparative slaughter experiment designed to determine the amounts of individual amino acids (AA) accreted in different body components during growth. The dissected and bulked fractions were: head, hide, feet and tail; heart and lung; intestinal tissues; liver and gall bladder; non-carcass fat depots; bladder and kidney; and carcass. Nitrogen (N) accretions in the empty body weight between 250-500 kg were linear and no significant differences between diets were detected for total body-N at each slaughter point. This was reflected in a linear accretion of total and essential AA in the EBW in each fraction. The main difference between feeding all forage, silage compared to silage and concentrate was to increase the rate of tissue accretion, but it did not influence the accretion of AA in the total body or in various organs and tissues at selected empty body weights.

Keywords: amino acid accretion, growth, forage

Introduction

The metabolisable protein requirements for growing beef cattle were established from empirical formulas using nitrogen loss and tissue accretion (Agricultural and Food Research Council, 1992; NRC, 1996). However, compared to non-ruminants, little information is known about the amount of specific amino acids absorbed and the net requirement of individual tissues and organs for these amino acids at different stage of growth (MacRae *et al.*, 1993; Oldham *et al.*, 1997). This is despite the fact that tissue masses vary both absolutely and relatively during growth and therefore requirements of individual amino acid (AA) may also change. On the other hand, while there have been excellent reviews on amino acids requirements of ruminants (see review of Boisen *et al.*, 2000), there is a distinct lack of information on the rates of protein growth, in particular of growing animals fed on different diets. A comparative slaughter study was performed to assess the accretion of protein and amino acids of body tissues in growing steers fed on either forage or forage and concentrate offered at similar levels of metabolisable energy intake.

Material and methods

Eighteen Hereford x Friesian steers (av. 155 kg liveweight) were randomly assigned to two dietary treatments; grass silage alone or a mixture of grass silage and a barley/soya concentrate (80:20 DM basis) in the ratio of 60:40 (on a ME basis), and one of 3 slaughter liveweights, 250, 350 or 500 kg. Each animal received 800 kJ ME per kg $M^{0.75}$ per day. At slaughter, the animals were dissected into 7 different fractions (F1-F7) components; 1) head, hide, feet and spinal cord

(HHFT), 2) heart, lungs, trachea, spleen, thymus and diaphragm, 3) oesophagus, rumen, reticulum, omasum, abomasum, intestines and pancreas, 4) liver and gall bladder, 5) mesenteric, omental, thymus fat and kidney knob channel fat, 6) genitals, empty bladder and left kidney, 7) left half carcass. Samples were frozen (-20°C) before analysis. Amino acid composition of the tissue samples were determined on hydrolysates using an amino acid analyser (Biochrom 20, Pharmaci Biotech Ltd., England). Relationships between amino acid accretion and empty body weight (EBW) were analysed by allometric model: $\log_e Y = \log_e a + b \log_e X$. The relationships between AA and total N accretion were also examined. The full model with intercept and slope for each diet was used to conduct parallel line analysis in order to compare diets. Predicted means for total and individual amino acids in each fraction were calculated at 200, 350 and 500 kg EBW.

Results

Actual ME intakes were 791 and 822 (s.e.d 15.6) kJ/kg M^{0.75} per day on silage alone and silage-concentrate, respectively. Feeding silage-concentrate mixture resulted in higher gains of liveweight ($P < 0.01$), carcass ($P < 0.01$), carcass fat ($P < 0.05$) and protein ($P < 0.01$) as reported by Kim *et al.* (2000). Nitrogen (N) accretions in the EBW between 155 and 500 kg live-weight were linear and no significant differences between diets were detected for total body-N at each slaughter point. The linear accretion responses were reflected in total and essential AA accretion in the EBW (Table 1) (pooled across diets) with adjusted correlation (r^2) averaging 0.93 across the fractions. Hence, for most fractions b values were high (typically 0.9), except for gut fraction ($b = 0.64$) reflecting the low accretion of AA in this tissue relative to for example carcass ($b = 0.89$). AA

Table 1. Relationships ($\log_e Y = \log_e a + b \log_e X$) of total and essential amino acid-N (Y-axis; g) in whole empty body and selected fractions, and empty body weight (X-axis; kg) and fitted values at specified empty body weights.

| | Whole | Carcass | Liver | Intestine | HHFT |
|----------------|------------------|--------------|---------------|--------------|--------------|
| EBW (kg) | Total-AA (g) | | | | |
| 250 | 6401 | 4353 | 102.2 | 211.9 | 1553 |
| 350 | 8507 | 5868 | 136.5 | 264.5 | 2015 |
| 500 | 11501 | 8053 | 185.6 | 334.8 | 2657 |
| a | 59.73 | 31.98 | 0.929 | 6.070 | 21.49 |
| Log a (s.e.) | 4.09 (0.124) | 3.47 (0.191) | -0.07 (0.320) | 1.80 (0.391) | 3.07 (0.300) |
| b (s.e.) | 0.85 (0.022) | 0.89 (0.034) | 0.85 (0.056) | 0.64 (0.069) | 0.78 (0.053) |
| r ² | 0.99 | 0.98 | 0.93 | 0.85 | 0.93 |
| Residual s.d. | 0.0284 | 0.0437 | 0.0730 | 0.0889 | 0.0686 |
| EBW (kg) | Essential-AA (g) | | | | |
| 250 | 3163 | 2285 | 58.5 | 108.6 | 620 |
| 350 | 4219 | 3078 | 78.6 | 135.4 | 814 |
| 500 | 5725 | 4222 | 107.6 | 171.1 | 1087 |
| a | 27.85 | 17.00 | 0.479 | 3.172 | 7.011 |
| Log a (s.e.) | 3.33 (0.125) | 2.83 (0.196) | -0.74 (0.350) | 1.15 (0.400) | 1.95 (0.248) |
| b (s.e.) | 0.86 (0.022) | 0.89 (0.035) | 0.87 (0.062) | 0.64 (0.070) | 0.81 (0.044) |
| r ² | 0.99 | 0.98 | 0.93 | 0.84 | 0.96 |
| Residual s.d. | 0.0285 | 0.0448 | 0.0800 | 0.0909 | 0.0568 |

Table 2. Amino acid composition of selected fractions (g/kg empty body weight).

| Essential amino acid | Empty body | Carcass | Liver | Intestine | HHFT |
|----------------------------|------------|---------|-------|-----------|-------|
| Cysteine | 0.17 | 0.100 | 0.006 | 0.005 | 0.053 |
| Methionine | 0.22 | 0.181 | 0.006 | 0.006 | 0.023 |
| Threonine | 0.52 | 0.412 | 0.012 | 0.015 | 0.074 |
| Valine | 0.72 | 0.552 | 0.017 | 0.020 | 0.117 |
| Isoleucine | 0.54 | 0.422 | 0.012 | 0.015 | 0.077 |
| Leucine | 1.03 | 0.803 | 0.027 | 0.026 | 0.153 |
| Phenylalanine | 0.43 | 0.327 | 0.011 | 0.011 | 0.069 |
| Lysine | 1.88 | 1.500 | 0.036 | 0.046 | 0.262 |
| Histidine | 1.07 | 0.908 | 0.020 | 0.020 | 0.115 |
| Arginine | 3.67 | 2.536 | 0.050 | 0.084 | 0.929 |
| Total essential amino acid | 10.25 | 7.746 | 0.196 | 0.250 | 1.867 |
| Total amino acid | 20.40 | 14.800 | 0.334 | 0.491 | 4.414 |

composition (g/kg EBW) in each fraction was not different between diets. As far as net AA requirements are concerned, the major sites of protein deposition were in the carcass, HHFT, intestinal tissues, heart and lung, liver representing 72, 22, 2.4, 2.0, 1.6% (Table 2). The relationship between accretion of essential AA in the EBW and intestinal tissues and empty body-N accretion is given in Table 3. The relationships were similar to those noted above against EBW.

Discussion

In contrast to the abundance of results on total body protein deposition few studies have examined the distribution of protein accretion between different body components and little information is available on the AA composition of these gains. Such information is essential for the partial construction of more mechanistic growth models describing requirements of specific AA. The regression equations presented in Table 1 and 3 show that within a fraction similar “b” values are noted for individual essential AA and total AA but across fractions they may differ (for example compare total v. intestine; Table 3). Similar observations were noted by MacRae *et al.* (1993) in a study on sheep growing between 25-55 kg liveweight, and the importance of such observations in relation to measurements of protein kinetics were fully discussed. Diets did not affect any of the parameters reported. The main difference between feeding all forage, silage compared to silage and concentrate was to increase the rate of tissue accretion (Kim *et al.*, 2000), but it did not influence the accretion of AA in the total body or in various organs and tissues. These differences in tissue accretion rate may relate to a higher efficiency of utilisation of absorbed AA on the silage-concentrate diet (MacRae *et al.* 1995), although such relationships could not be established in this study.

Acknowledgements

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Table 3. Relationships ($\log_e Y = \log_e a + b \log_e X$) of essential amino acid-N (Y-axis; g) in empty body (Total) and intestine, and total-N accretion (X-axis; g) in the empty body.

| Essential amino acid | Fraction | a | Log a (s.e.) | b (s.e) | r ² | Residual s.d. |
|----------------------|-----------|--------|---------------|--------------|----------------|---------------|
| Cysteine | Total | 0.0040 | -5.52 (0.419) | 1.06 (0.047) | 0.98 | 0.0516 |
| | Intestine | 0.0030 | -5.81 (0.649) | 0.76 (0.072) | 0.87 | 0.0800 |
| Methionine | Total | 0.0067 | -5.01 (0.340) | 1.03 (0.038) | 0.98 | 0.0419 |
| | Intestine | 0.0009 | -7.06 (1.380) | 0.89 (0.154) | 0.66 | 0.1710 |
| Threonine | Total | 0.0362 | -3.32 (0.246) | 0.95 (0.027) | 0.99 | 0.0303 |
| | Intestine | 0.0050 | -5.30 (0.830) | 0.81 (0.092) | 0.83 | 0.1020 |
| Valine | Total | 0.0556 | -2.89 (0.251) | 0.94 (0.028) | 0.99 | 0.0309 |
| | Intestine | 0.0150 | -4.20 (0.727) | 0.73 (0.081) | 0.83 | 0.0896 |
| Isoleucine | Total | 0.0296 | -3.52 (0.334) | 0.97 (0.037) | 0.98 | 0.0412 |
| | Intestine | 0.0087 | -4.75 (0.872) | 0.75 (0.097) | 0.78 | 0.1070 |
| Leucine | Total | 0.0686 | -2.68 (0.379) | 0.95 (0.042) | 0.97 | 0.0467 |
| | Intestine | 0.0330 | -3.41 (0.777) | 0.68 (0.086) | 0.79 | 0.0958 |
| Phenylalanine | Total | 0.0250 | -3.69 (0.145) | 0.97 (0.016) | 0.99 | 0.0178 |
| | Intestine | 0.0088 | -4.73 (0.682) | 0.72 (0.076) | 0.85 | 0.0841 |
| Lysine | Total | 0.0898 | -2.41 (0.160) | 0.99 (0.018) | 0.99 | 0.0197 |
| | Intestine | 0.0327 | -3.42 (0.730) | 0.73 (0.081) | 0.83 | 0.0900 |
| Histidine | Total | 0.0279 | -3.58 (0.211) | 1.05 (0.024) | 0.99 | 0.0260 |
| | Intestine | 0.0129 | -4.35 (0.787) | 0.74 (0.088) | 0.81 | 0.0971 |
| Arginine | Total | 0.1142 | -2.17 (0.210) | 1.03 (0.023) | 0.99 | 0.0258 |
| | Intestine | 0.0273 | -3.60 (0.711) | 0.81 (0.079) | 0.86 | 0.0877 |

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Recovery in urine of allantoin administered intravenously at differentiated levels of feeding sheep

J. Kowalczyk¹, M. Czauderna¹, J.J. Pajak¹, J.A. Strzelenski² & J. Skomial¹

¹ *The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland*

² *Research Institute of Animal Production, 32-083 Balice, Poland*

Summary

Recovery in urine of allantoin infused intravenously in different amounts was investigated in sheep given rations at above-, below- or maintenance level. The experiment was carried out on 9 wethers of about 45 kg BW divided into 3 groups fed a diet consisting of meadow hay, ground barley and rapeseed oilmeal. Group I was fed at maintenance level, group II at 20% less, and group III, at 20% above maintenance level. A solution of 0.2% NaCl without allantoin as a control was infused continuously into the jugular vein for 4 days into the first sheep, a solution containing 0.26 % allantoin into the second, and one containing 0.52 % allantoin into the third sheep of each group. Allantoin was determined in collected daily samples of urine. The direct recovery in urine of intravenously infused allantoin was proportional to the amount of allantoin infused but was surprisingly low, about 20%, expressed as a percentage of the allantoin infused, independently of feeding level or amount of allantoin infused. These preliminary findings justify further study on the recycling and metabolism of allantoin in ruminants, providing new data for evaluation of allantoin excreted in urine as a predictor of microbial protein synthesis in the rumen.

Keywords: *allantoin, microbial protein, rumen, blood*

Introduction

Precise estimation of the size of microbial protein synthesis in the rumen is an important factor of modern feed evaluation systems and can be achieved by indirect methods using different markers such as purine bases, phosphatidylcholine, diaminopomelic acid, ³⁵S, ¹⁵N. However, the results of such estimations obtained by different procedures are not consistent (Illg and Stern, 1994; Berchielli *et al.*, 1995a,b; Djouvinov et Todorov, 1998). The currently most popular method is measuring the amount of allantoin excreted in the urine, since microbial protein synthesized in the rumen contains purines metabolized to allantoin, uric acid, xanthine, and hypoxanthine, which are thought to be the end-products of purine metabolism that are excreted in urine (Topps and Elliot, 1965; Antoniewicz *et al.*, 1980, 1981; Chen *et al.*, 1993). However, endogenous excretion of allantoin varies among goat, sheep, cattle, and buffalo in terms of correlation coefficients between excreted allantoin and the amount of microbial protein synthesized in the rumen (Chen *et al.*, 1990a; 1996). Chen et al. (1990b) and Kahn and Nolan (2000) reported that small amounts of allantoin can be recycled from the blood to the digestive tract *via* saliva or directly to the digestive tract walls.

The aim of the present experiment was to determine the proportion of allantoin excreted in urine related to the amount of allantoin infused into the jugular vein of sheep, as such data could provide information about the suitability of allantoin as a marker of the extent of microbial protein synthesis in the rumen.

Material and methods

The experiment was carried out on 9 wethers of 45 kg average BW, divided into 3 groups and fed twice daily with a diet containing 12% crude protein and consisting of meadow hay, ground barley and rapeseed oilmeal (Table 1). Group I was fed at the maintenance level (1.85% BW), group II, 20% less, and III, 20% more than the maintenance requirement.

Table 1. Diet composition and nutrient contents in feeds, %.

| Component | Proportion | Dry in the diet | Crude matter | Ash protein | Ether extract | Crude fibre |
|------------------|------------|--------------------|-----------------|----------------|------------------|----------------|
| Meadow hay | 70.75 | 87.15 | 9.30 | 6.02 | 2.16 | 27.56 |
| Ground barley | 25.39 | 89.40 | 11.57 | 2.19 | 2.09 | 5.10 |
| Rapeseed oilmeal | 3.86 | 90.58 | 35.30 | 6.65 | 4.89 | 11.91 |

After 14 days of feeding the sheep the respective diets the animals were placed in metabolic cages and urine was collected quantitatively for 6 days using the device described by Kowalczyk *et al.* (1996) Starting on the second day of urine collection continuous infusions into the jugular vein using a peristaltic pump were begun; for four days one animal of each group received controlled amounts of: sterilized 0.2% NaCl solution sheep one of each group (control), sheep two received 0.26% allantoin in 0.2% NaCl, sheep three, 0.56% allantoin in NaCl. Urine was also collected for one day after the infusion was stopped. Allantoin in urine samples representing daily collections (four days of allantoin infusion and 1 day after infusion) from each sheep was determined by HPLC according to Czuderna and Kowalczyk (1997).

Results and discussion

The mean amount of allantoin excreted daily in urine increased significantly with the amount of infused allantoin ($P < 0.01$) as well as with the level of feeding ($P < 0.05$) as shown in Table 2. This increase might result from the higher nitrogen and energy intake that favors higher microbial protein synthesis in the rumen and from higher nitrogen and possibly allantoin recycling depending on diet intake and composition (Kowalczyk *et al.*, 1975).

Table 2. Average amount of allantoin secreted daily in urine, g.

| Feeding level | Concentration of allantoin in infused solution | | | |
|--------------------|--|-------------------|-------------------|-------------------------|
| | 0% (control) | 0.26% | 0.52 % | difference ¹ |
| Maintenance | 0.84 ^a | 1.05 ^a | 1.30 ^a | *** |
| 0.8 of maintenance | 0.77 ^b | 1.10 ^b | 1.36 ^b | *** |
| 1.2 of maintenance | 0.87 ^c | 1.26 ^c | 1.59 ^c | *** |

¹ - differences in rows, $P < 0.01$

a,b,c - differences in columns, $P < 0.05$

Kahn and Nolan (2000) reported that only a small amount of intravenously injected labeled [¹⁴C]allantoin was transferred from blood to the digestive tract with saliva and about 80% of injected [¹⁴C]allantoin was recovered in urine during 12 h after tracer injection, increasing to 94% after 4 days. Allantoin C also passed through the blood bicarbonate pool, suggesting that allantoin is degraded in the gastrointestinal tract. These authors suggest that the net flux of allantoin through the blood should be a better predictor of rumen microbial outflow than urinary allantoin excretion. In our experiment with continuous four-day intrajugular infusion of allantoin, recovery in urine of allantoin infused intravenously, calculated by deduction of the amount of allantoin excreted by animals not infused with allantoin was surprisingly low (Table 3) as it ranged from 18.5 to 24.7% of the total amount of intravenously administered allantoin.

Table 3. Recovery in urine of the allantoin infused into the blood.

| Level of feeding | Concentration of allantoin infused solution, % | |
|----------------------------|--|------|
| | 0.26 | 0.52 |
| Maintenance: | | |
| allantoin infused, mg/d | 1602 | 3400 |
| recovery, mg | 376 | 792 |
| recovery, % | 24.7 | 23.3 |
| 0.8 of maintenance: | | |
| allantoin infused, mg/d | 1589 | 3194 |
| recovery, mg | 334 | 590 |
| recovery, % | 21.0 | 18 |
| 1.2 of maintenance: | | |
| allantoin infused, mg/d | 1730 | 3195 |
| recovery, mg | 387 | 719 |
| recovery, % | 22.4 | 22.6 |

Recovery in urine of infused allantoin, expressed as a per cent of the infused amount, did not differ between levels of feeding or amount of infused allantoin ($P > 0.05$), indicating that only about 20% of blood allantoin was excreted with urine but about 80% was recycled into the digestive tract and then degraded or metabolized in different compartments of the body. Low recovery in urine of allantoin infused into the blood weakens the value of allantoin as a marker of microbial protein production in the rumen. The preliminary results of this experiment justify further study on the recycling and metabolism of allantoin in the organism of ruminants to provide new data for evaluation of allantoin excreted in urine as a predictor of microbial protein synthesis in the rumen.

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Lysine utilisation by the mammary gland

H. Lapierre¹, E. Milne², J. Renaud¹ & G.E. Lobley²

¹ Agriculture and Agri-Food Canada, Lennoxville, QC, Canada, J1M 1Z3

² Rowett Research Institute, Aberdeen, UK AB21 9SB

Summary

Uptake of lysine by the mammary gland is usually higher than lysine output into milk protein and may contribute to synthesis of non-essential (NE) amino acids (AA) that are otherwise extracted at less than requirements. This hypothesis was tested with 4 cows infused with [¹⁵N]lysine for 7 h, with the isotopic enrichment of various AA measured in arterial plasma and in milk casein produced during the last hour of infusion. The enrichment of NEAA was higher (but lysine lower) in casein than in arterial plasma: arginine > aspartate > glutamate > alanine > serine. Label also appeared (but to a lesser extent) in the branch-chain AA, histidine and phenylalanine. These data indicate that much the N from extra uptake of lysine by the mammary gland, relative to milk lysine output, is transferred to other AA.

Introduction

Although lysine is often a first limiting amino acid (AA) in rations fed to high producing dairy cows in both Europe and North America (Rulquin 1993; Schwab *et al.*, 1996), uptake by the mammary gland is usually at least 20% greater than output in milk protein (Guinard & Rulquin, 1994). This suggests that lysine may play a critical role within the mammary gland. One possibility is to provide N for the synthesis of non-essential (NE) AA, as uptake of these by the mammary gland is often smaller than required for the observed milk protein output (Guinard & Rulquin, 1994). The original objectives of this study were to quantify, within the mammary gland, the transfer of lysine N to other AA and to examine whether such transformations varied with lysine supply.

Materials and methods

Four lactating dairy cows, averaging 168 days in milk, were used in a cross-over design, with two treatments. Cows were fed a basal diet (grass silage, 29.4%; corn silage: 29.4%; ground corn: 22.0%; corn gluten meal: 7.8%; fatty acid calcium salts: 4.9%; mineral & vitamin premix: 2.2%; grass hay 4.3%) balanced to provide lysine at only 5.1% of metabolizable protein supply, i.e. 70% of the actual recommendations (NRC, 2001). Control cows received no infusion while treated cows received a portal vein infusion of lysine (8 mmol/h; 37.5 mL/h) for 6 days. At least 3 mo before the start of the experiment, the cows were surgically implanted with chronic catheters into the portal vein, one hepatic vein and a mesenteric artery for blood sampling, and two distal mesenteric veins for p-aminohippurate (pAH) infusion.

On d 6 of lysine supplementation, the site of infusion was switched to one mesenteric vein to allow blood collection from the portal vein for the treated cows. All cows received a 7.5-h intra-jugular infusion of [2-¹⁵N]lysine (0.235 mmol/h), preceded by a priming dose (0.235 mmol). Hourly blood samples (*n*=6) were collected simultaneously from the arterial, the portal and the hepatic catheters and by venipuncture from a mammary vein, from 2 to 7 h after the onset of the labelled lysine infusion. Plasma flows for the splanchnic tissues were determined from downstream dilution of

pAH while those for the mammary gland were estimated with the Fick principle, using phenylalanine and tyrosine as the marker AA. Plasma lysine isotopic enrichments (IE; as atom % excess, ape) and concentrations (by isotope dilution ; Calder et al., 1999) were determined as MTBSTFA derivatives using a quadrupole gas chromatograph-mass spectrometer (GC-MS). In addition, extra blood samples were collected from the artery 7 h after the beginning of the infusion, and the cows were milked at 6 and 7 h using oxytocin to assist emptying of the gland. The IE of various AA in milk casein (after 18h hydrolysis in 6M HCl) and in the arterial free pool, both sampled 7 h after the beginning of the infusion were determined by GC-combustion isotope ratio MS of the tBDMS derivatives. These values are presented as ape x 1000.

Unfortunately, during period 2 of the cross-over design, unseasonably hot weather depressed both feed intake and milk production in all cows. At the same time, the two control cows from period 1 became ill (not related with treatment: one mastitis and one pneumonia), with consequent halving of milk production. So the second objective, examination of the impact of changing lysine supply on mammary metabolism, could not be pursued. Instead, the study was modified to examine in the two healthy cows if differences existed in the transfer of the transfer of N from the 2- and 5-atoms. After a respite of 14 days, the two cows were again infused with lysine for 8 d (8 mmol/h). On day 5, [2-¹⁵N]lysine was infused, intra-jugular, for 7 h (0.47 mmol/h) preceded by a priming dose (0.47 mmol). On day 8, [5-¹⁵N]lysine was infused for 7 h (0.47mmol/h) preceded by a priming dose (0.47 mmol). On both days, cows were milked with oxytocin 6 and 7 h after the beginning of the labelled lysine infusion and arterial samples were also collected at 7h. Analyses were as described for period 1.

Results

As the planned study was only conducted in period 1, with n=2 per treatment, there were no significant responses. Data are presented as mean ± SD for this period. Milk and protein yields averaged $27.4 \pm 0.4 \text{ kg d}^{-1}$ and $860 \pm 84 \text{ g d}^{-1}$. Whole body (n = 4) and net flux of lysine flux across the portal-drained viscera (PDV; n=3), the splanchnic tissues (TSP, n=3), the mammary gland (n=4) and in milk (n=4) averaged 44.9 ± 9.4 , 30.9 ± 15.7 , 27.2 ± 6.4 , -23.6 ± 1.6 and $20.3 \pm 2.0 \text{ mmol/h}$. The uptake to output ratio across the mammary gland was unaffected by the increment in lysine supply and averaged 1.16 ± 0.06 . As there was no treatment effect for the enrichment of the AA in milk protein, the data were combined for the period (Table 1). Of the mammary arterial ¹⁵N lysine uptake, $66 \pm 13\%$ was recovered as milk protein lysine.

Within the 2 cows examined with the two isotopes of lysine, there were no differences in the pattern of label transfer to other AA, in either the arterial free pool or in casein (data not shown). Furthermore, although the dose infused in period 2 was doubled that in period 1, with increased IE for most AA, the ratio of the IE of each AA to lysine in either sample site (plasma or casein) was not affected. Therefore, data across all periods and treatments were combined and the IE each AA relative to lysine compared between the arterial free pool and casein: a transfer of lysine N within the mammary gland would lead to higher relative ratio in the casein compared with the artery. This was the case for all AA, except tyrosine (Table 2).

Discussion

Lysine transfers between the PDV, TSP and mammary gland followed the patterns recently reported (Blouin et al., 2000). Specifically, only a small fraction of absorbed lysine was removed across the liver (<10%), with the mammary gland removing 85% of post-splanchnic supply but with only 86% of this uptake secreted as milk protein lysine. As lysine removed by the mammary gland support both constitutive and export protein synthesis, the fact that only 66% of uptaken [¹⁵N]lysine was recovered as casein lysine indicates substantial synthesis of constitutive mammary proteins occurs, as previously reported (Bequette et al., 1994). The increase in IE of AA relative

Table 1. Isotopic enrichment (IE) of AA in arterial plasma and casein of cows infused with [2-¹⁵N]lysine (period 1).

| AA | IE arterial free pool (ape x 1000) | | IE milk casein (ape x 1000) | | Proportion of ¹⁵ N / total ¹⁵ N in milk (%) | |
|-----|---------------------------------------|-------|--------------------------------|------|--|------|
| | Mean | SD | Mean | SD | Mean | SD |
| His | 3.5 | 2.1 | 4.9 | 2.0 | 0.79 | 0.27 |
| Ile | 2.2 | 1.0 | 3.9 | 1.3 | 0.54 | 0.13 |
| Leu | 2.6 | 1.5 | 3.5 | 1.1 | 0.82 | 0.20 |
| Lys | 386.4 | 112.0 | 234.9 | 34.4 | 82.91 | 4.52 |
| Met | - | - | 1.4 | 0.2 | 0.08 | 0.01 |
| Phe | 1.2 | 0.4 | 7.4 | 3.5 | 0.67 | 0.28 |
| Thr | 1.7 | 0.4 | 2.2 | 0.1 | 0.13 | 0.15 |
| Val | 1.0 | 0.5 | 3.4 | 2.4 | 0.57 | 0.35 |
| Ala | 3.4 | 1.0 | 6.2 | 2.4 | 0.74 | 0.24 |
| Arg | 2.9 | 1.1 | 10.6 | 1.8 | 1.34 | 1.56 |
| Asn | 0.9 | 0.3 | - | - | - | - |
| Asp | - | - | 13.4 | 3.9 | 3.68 | 0.65 |
| Glu | 2.6 | 1.3 | 9.0 | 3.0 | 5.87 | 1.53 |
| Gly | 1.5 | 0.4 | 1.4 | 0.5 | 0.11 | 0.06 |
| Pro | 0.2 | 1.0 | 3.0 | 2.9 | 0.75 | 0.72 |
| Ser | 3.1 | 1.2 | 3.8 | 2.6 | 0.73 | 0.53 |
| Tyr | 4.8 | 1.5 | 2.6 | 1.7 | 0.27 | 0.21 |

Table 2. Ratio of IE of each AA to the IE of lysine in the arterial free pool or in the milk protein in dairy cows infused with [¹⁵N]lysine (all periods).

| AA | Site | P | | | |
|-----|------|--------|--------|--------|----------------------|
| | | Artery | Casein | SEM | (effect of the site) |
| His | 0.71 | 1.89 | 0.18 | <0.001 | |
| Ile | 0.63 | 1.62 | 0.14 | <0.001 | |
| Leu | 0.71 | 1.42 | 0.10 | <0.001 | |
| Lys | 100 | 100 | - | - | |
| Phe | 0.50 | 2.73 | 0.23 | <0.001 | |
| Thr | 0.44 | 1.02 | 0.12 | 0.01 | |
| Val | 0.36 | 0.99 | 0.22 | 0.06 | |
| Ala | 0.98 | 3.00 | 0.26 | <0.001 | |
| Arg | 0.71 | 6.07 | 0.93 | 0.01 | |
| Asp | 0.31 | 5.63 | 0.33 | <0.001 | |
| Glu | 0.63 | 4.01 | 0.23 | <0.001 | |
| Gly | 0.51 | 0.76 | 0.10 | 0.09 | |
| Pro | 0.19 | 0.75 | 0.23 | 0.09 | |
| Ser | 0.81 | 2.02 | 0.30 | 0.01 | |
| Tyr | 0.99 | 1.16 | 0.24 | 0.65 | |

to lysine in casein compared with arterial free AA indicates that extensive metabolism of lysine does occur within the mammary gland. For NEAA that are extracted by the mammary gland in insufficient quantities to support milk output, lysine provides a source of N to support the necessary biosynthesis: arginine > aspartate > glutamate > alanine > serine. The two pathways of lysine catabolism both lead to the 2-N appearing in glutamate which should show the second highest enrichment, but this did not occur for casein due to conversion of poorer-labelled glutamine to glutamate during the acid hydrolysis. As in many other tissues, extensive transamination between aspartate and glutamate occurred in the mammary gland. Of particular interest was the label transfer into essential AA. For the branch-chain AA, this is not too surprising as these also act as N sources for transamination pathways. Labelling in phenylalanine and histidine were more unexpected and this may represent either simple N-exchange via transamination or true net biosynthesis within the udder, both these options would involve metabolism of the appropriate oxo-acids. Finally, the excellent agreement between the ratios of lysine in milk protein:mammary gland lysine uptake (0.86 ± 0.05) and lysine ^{15}N :total ^{15}N in casein (0.83 ± 0.05) suggests that most of the extra lysine N taken up by the udder can be accounted by transfers into other AA. Overall, these data indicate that the udder of the dairy cow has even greater metabolic potential than generally realised and that mechanisms have developed to utilise available N sources within the body.

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Influence of high altitude and of forage from alpine origin on protein composition and renneting properties of cow's milk

F. Leiber, D. Nigg, C. Kunz, M.R.L. Scheeder, M. Kreuzer & H.-R. Wettstein

Institute of Animal Science, Animal Nutrition, ETH Zurich, CH-8092 Zurich, Switzerland

Summary

A two factorial design was applied to separate the effects of high altitude and alpine forage quality on milk protein and cheese-making properties. Milk was analysed for protein composition, plasmin and plasminogen content, and rennet coagulation properties. High altitude caused slightly decreased milk protein contents, a decreased plasminogen content and significantly impaired rennet clotting time. Hay of alpine origin, when compared to hay of lowland origin, led to significant lower milk yield and total protein concentration. Whey protein and κ -casein proportions of total protein were significantly reduced, casein/total protein ratio was elevated and rennet coagulation properties were partly impaired by the alpine hay, whereas plasmin and plasminogen-derived activity were not clearly affected.

Keywords: altitude, forage quality, milk protein composition, plasmin, cheese-making

Introduction

Milk production on alpine sites is one form of using forage resources in less favoured areas, often combined with the production of an unconventional cheese of high quality. However, high altitude and insufficient forage quality may impair milk protein synthesis and composition, with the consequence of undesired side-effects on cheese yield and quality. Although genetically limited to a small variation, casein and its fractions can be affected by environmental factors such as season and ambient temperature (Kroeker et al., 1985; Lacroix et al., 1994; Reichardt et al., 1995). Particularly, a deficit in energy supply, as it is common on high altitude pastures, might be detrimental for protein synthesis and even casein proportion of total milk protein (Reichardt et al., 1995). A decreased casein proportion leads to lower cheese yield (Melilli et al., 2002) and may also modify the processes of coagulation and cheese ripening. The aim of this experiment was to study independently of each other the influences of high altitude and alpine origin of the forage on milk protein content and composition, proteolytic activity and rennet coagulation properties of milk.

Materials and methods

With a two-factorial design, the effects of altitude and of forage quality were tested simultaneously. Four groups of 3 lactating Holstein and 3 Brown Swiss cows each were investigated in the experiment. The groups were balanced according to stage of lactation, energy-corrected milk yield, protein content and κ -casein genotype of the cows. Two groups were fed with hay ad libitum, one of them (G1) at ETH research station 'Weissenstein' at 2000 m a.s.l. and the second (G2) at ETH research station 'Chamau' at 400 m a.s.l. A third group (G3) was paired to G1 and kept at 'Chamau' for evaluating the impact of a decreased food intake, which may occur under alpine conditions (Christen et al., 1996). All these groups were fed with hay alone either from alpine (AH) or from lowland origin (LH) following a change-over design. After one week of adaptation to the hay diets the hays were fed to half of the cows within each group in the sequence alpine-lowland-alpine, and in the sequence lowland-alpine-lowland to the other half. Each period

within sequence lasted for 21 days. Thus the average forage quality for each group was equal and the different forages were tested under both, alpine and lowland conditions. The alpine hay had higher fiber and lower crude protein contents and a clearly lower digestibility compared to the lowland hay. Consequently, the net energy supply from the alpine hay was reduced from 5.8 to 5.1 MJ NEL/kg DM. A fourth group (SR) was fed with a silage ration and concentrates corresponding to milk yield.

All cows were tethered in barns. In the last week of each 21-day period, milk was sampled at every milking. Cows were weighed and blood samples were taken twice in every sampling week. All individual milk samples were analysed for major milk constituents, including protein content, with infrared technique using a Milkoscan 4000 (Foss Electric, Hillerød, Denmark). Over the whole week, aliquot milk samples were pooled for each animal. Proportions of milk protein fractions were quantified with RP-HPLC (Merck-Hitachi, Darmstadt, Germany) on a C4 column (Vydac, Hesperia, USA), adapting the method of Bordin et al. (2001). Plasmin and plasminogen-derived activity were measured modifying the method of Richardson & Pearce (1981) on a microplate fluorescence reader (Tecan, Männedorf, Switzerland). Rennet clotting properties were evaluated with a Lattodinamografo (Foss, Padua, Italy) after skimming and adding chymosin (Maxiren, Gist-Brocades, Seclin Cedex, France). Rennet clotting time (RCT) is the time (min) from adding chymosin until coagulation begins. K20 is a measure of the dynamics of coagulation and expresses the time (min) from the start of the coagulation until a defined curd firmness is reached. Plasma metabolites were measured with commercial photometric test kits (for β -hydroxybutyrate from Sigma, Buchs, Switzerland; for glucose from Roche, Basle, Switzerland). Statistical analyses were done using the procedure 'Mixed' of SAS with two models. The first included forage, group, breed, protein genotypes and period as fixed and animal within breed \times group as random effect. All interactions were considered. Initial values obtained two weeks prior to the experiment were used as a covariate. This model was applied on the groups G1-G3. For inclusion of SR, a second model considering 'diet type' instead of hay and group was applied. In Figure 1 only the values for SR were derived with this model.

Results and discussion

The difference between G1 and G2 gave the best estimate of the altitude effect because forage intakes were similar between these ad libitum-fed groups. Only in the initial week of the whole experiment, G1 showed severe intake refusals, which were mirrored in the paired G3 cows. In both groups this led to a persistent negative energy balance, indicated by elevated plasma β -hydroxybutyrate, reduced glucose levels and by slight body weight losses. Consequently, results where G1 and G3 are similar but different from G2 are interpreted as to have been influenced more likely by the initial undernutrition than directly by the altitude.

Altitude did not cause a reduced milk yield (Fig. 1). Milk protein content declined in both G1 ($P<0.05$) and G3 and therefore an effect of the initial undernutrition is assumed. Correspondingly the casein content of milk was lower in G1 and G3 than in G2 ($P<0.05$). Casein/total protein proportion was lower in the alpine group (G1) than in the lowland hay-alone groups. Within the casein fraction, the κ -casein was lower in the milk of the alpine group; all other casein fractions were rarely affected by altitude. Plasminogen derived activity of the alpine group was decreased ($P<0.1$), but this was not accompanied by alterations of the plasmin activity. A clear negative effect of high altitude on the renneting properties was found, which is expressed in prolonged rennet clotting time ($P<0.05$) and a trend towards retarded dynamics (K20).

Both hays were not sufficient in quality to cover the energy and protein requirements of the lactating cows, and consequently the milk yield of the groups G1-G3 was drastically lower for both hays than in the group, which was constantly fed on silages and concentrate (SR). The hay with alpine origin further depressed milk yield compared to the lowland hay. Additionally the milk protein content was reduced ($P<0.001$) by use of the alpine instead of the lowland hay on

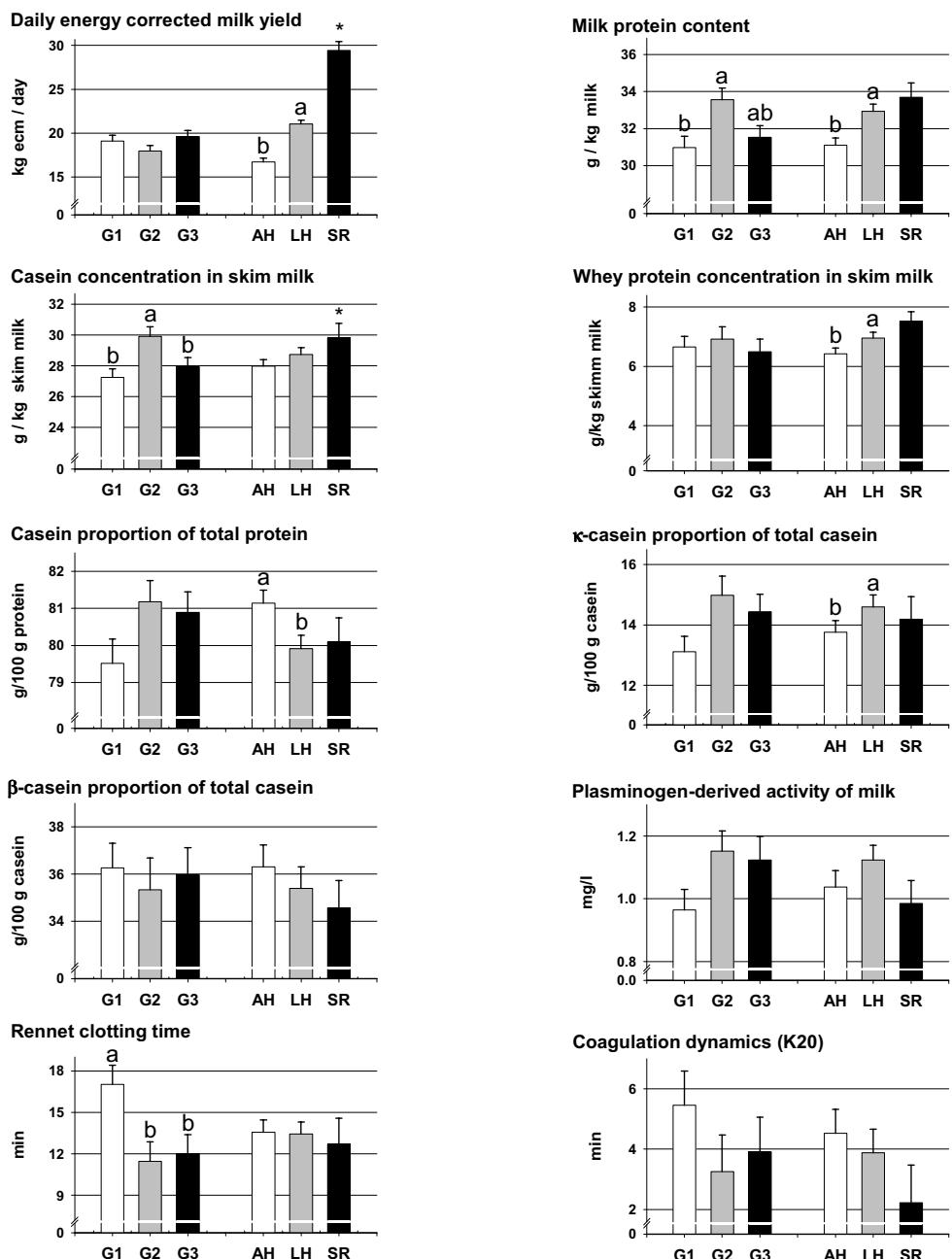


Figure 1. Influence of group treatment and diet type on milk yield and composition. Group treatments are: G1: alpine site, ad libitum; G2: lowland site ad libitum; G3: lowland site, paired to G1. Diet types are: AH: alpine hay; LH: lowland hay; SR: silage ration with concentrates. Bars carrying different letters are significantly different within the group or the hay factor at $P>0.05$. Asterisks above the SR bars indicate significant differences between the silage ration and the two hay types.

average of both altitudes. This decrease in milk protein content was relatively more pronounced in the whey proteins ($P<0.01$) than in the caseins, which resulted in a higher ($P<0.01$) casein/total protein ratio of the milk produced from the alpine hay. Within the caseins, κ -casein was significantly reduced ($P<0.05$), whereas β -casein was slightly increased when comparing the alpine hay with the lowland hay. On the basis of the Van-Slyke formula (Melilli et al., 2002), the reduced milk protein concentration which is caused by the alpine hay type would lead to a reduction of cheese yield of 260 g/100 kg milk when the casein/total protein ratio would be constant. Considering the higher casein proportion with the alpine hay in the formula, the reduction is only 205 g/100 kg milk.

Casein content of milk was positively affected ($P<0.05$) by the silage ration. For the other traits related to milk protein quality, silage ration was never different from lowland hay and one factor which may have led to the observed changes was probably the particularly low supply of nutrients from the alpine hay. Similar to altitude, the type of feed did not influence the plasmin activity of milk, but the plasminogen-derived activity was elevated by the use of any hay compared to the silage ration, ($P<0.1$ for the lowland hay type). The rennet clotting time was not affected by the diet type, whereas the dynamics of coagulation was weaker with the hays, especially with the alpine hay, compared to the silage ration.

This study shows, that, although small, significant changes in the protein composition can be evoked by the diet. The extent of the difference of 1% in the casein proportion caused by changing the hay type is more or less equal to the phenotypical difference which occurs between the κ -casein AA and AB genotypes in several studies (Reichhardt et al., 1995) and also was found in our study. As shown, these alterations have a relatively small impact on the cheese yield. The higher casein proportion caused by the alpine hay did not lead to improved rennet coagulation properties, this probably because of the concomitantly reduced milk protein content. Although altitude had no significant influence on milk protein content and composition, the significantly delayed rennet clotting time seems to reflect the adverse trends in protein concentration and casein proportion.

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Is intestine really involved in threonine catabolism in pigs?

Nathalie Le Floc'h & Bernard Sèze

UMR Calf and Pig, INRA F-35590 Saint Gilles, France

Summary

In pigs, threonine (thr) is massively utilised by the splanchnic tissues composed by the portal drained viscera (PDV) and the liver. Two experiments were conducted to estimate the contribution of the splanchnic tissues to thr metabolism and especially catabolism in growing pigs. In the first experiment, 4 pigs were continuously infused with L-[1-¹³C]-thr through a jugular catheter. PDV and total splanchnic viscera extracted 14.3 and 18.8% of arterial thr input respectively. In a second experiment, 6 pigs were continuously infused with L-[1-¹³C]thr and ¹⁵N-glycine in the duodenum for 8 hours. Splanchnic tissues extracted 60% of newly infused labelled thr : 88% of which were extracted by PDV so that thr extraction by the liver was low. Thr dehydrogenase (TDG) activity - the main enzyme involved in thr catabolism in pig - was not detected in the small intestine mucosa. *In vivo* estimations of thr catabolism through the TDG pathway showed that 1.6 % and 7.7 % of [1-¹³C]-thr sequestered by the PDV and the liver were degraded in glycine through the TDG pathway. Among splanchnic tissues both the liver and the pancreas can degrade thr in glycine. Finally our data suggest that thr catabolism was only a minor component of total thr utilization by the splanchnic tissues.

Keywords: pigs, threonine, gastrointestinal tract

Introduction

In pigs, threonine (thr) has been identified to be the first limiting amino acid for maintenance (Fuller *et al.*, 1989) suggesting that thr utilisation for other processes than muscle protein synthesis and deposition is high. This implies that thr irreversible losses through catabolism and digestive endogenous losses are important. It is now established that a part of thr requirement is associated to splanchnic metabolism (Bertolo *et al.*, 1998). Several authors have shown that more than 50% of dietary thr was sequestered by the portal drained viscera or PDV (Rérat *et al.*, 1992; Stoll *et al.*, 1998;). However the fate of dietary thr during its first pass removal by the PDV remains controversial even if experimental evidences have suggested that thr intestinal metabolism would be associated to mucin synthesis and endogenous losses (Ball *et al.*, 1999) or catabolism (Stoll *et al.*, 1998).

The objectives of this work were to measure *in vivo* thr sequestration and catabolism by the PDV and the liver. Such an approach requires the association of both tracer infusion and measurement of labelled thr net appearance in the portal vein and the hepatic vein. These techniques were used to measure thr utilization by the splanchnic viscera in order to 1. estimate the contribution of the splanchnic viscera (PDV and the liver) to total thr metabolism and 2. to determine if thr catabolism could be or not a major contributor to thr metabolism in the splanchnic area. A first experiment was conducted to measure arterial thr sequestration during a constant intravenous infusion of L-[1-¹³C]-thr. In a second experiment we studied thr metabolism during intraduodenal L-[1-¹³C]-thr infusion. Arterial thr sequestration rate determined in the first experiment was used to calculate tracer first pass removal by the PDV and the liver. Thr catabolism through the thr dehydrogenase (TDG) - the main enzyme involved in thr catabolism into glycine - pathway was also investigated in the splanchnic tissues by combining a constant infusion of ¹⁵N-glycine to estimate *in vivo* thr catabolism in the PDV and the liver and measuring TDG activity in the different tissues composing the splanchnic area.

Materials and methods

Animals and infusion protocols

Exp. 1 : The experiment was conducted on 4 female pigs (30.5 ± 2 kg live weight) surgically prepared for chronic insertion of catheters in the portal and the hepatic veins and in the carotid artery for blood sampling. A jugular catheter was used for [1^{-13}C]-thr continuous infusion ($12.04 \mu\text{mol/kg/h}$) for 8 hours. Ultrasonic blood flow probes (Transonics, Ithaca, NY) of 14 (14 SB) and 5 (5 RB) mm i.d. were placed around the portal vein and the hepatic artery respectively for continuous blood flow recording.

Exp. 2 : Six female pigs were surgically prepared for chronic insertion of catheters and flow probes as described for exp.1 except that the jugular catheter was replaced by a duodenal catheter for [1^{-13}C]-thr ($25.5 \mu\text{mol/kg/h}$) and ^{15}N -glycine ($1.45 \mu\text{mol/kg/h}$) 8-h continuous infusions.

Pigs were hourly fed with a commercial standard diet ($100 \text{ g/kg of BW}^{0.75}$ per day) in order to ensure a near steady state for nutrients necessary to simplify the study of thr metabolism. Four blood samples were taken from portal and hepatic veins and carotid artery during the two last hours of infusion for plasma thr and gly concentration and enrichment measurements.

Analytical procedures

Plasma AA were analysed by ion exchange liquid chromatography (LC 5001 Biotronik). Plasma and tissue ^{15}N and ^{13}C -glycine and ^{13}C -thr were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-MS) after derivatization with ethyl chloroformate (ECF). Results are expressed in mole percent excess (MPE, %) taking in account the two and four fold dilution of carbons from glycine and thr respectively in the ECF derivative. TDG specific activity in liver, pancreas and intestine was measured as the rate of aminoacetone formation *in vitro* as described by (Le Floc'h *et al.*, 1994).

Calculations

During constant intravenous infusion of [1^{-13}C]-thr (exp. 1), the rate of arterial thr sequestration by the PDV was calculated as:

$$\text{Rate Seq-art, \%} = 100 * \text{PF}_{\text{PV}} * (([\text{thr}]_{\text{pv}} * E_{\text{pv}}) - ([\text{thr}]_{\text{A}} * E_{\text{A}})) / (([\text{thr}]_{\text{A}} * E_{\text{A}}) * \text{PF}_{\text{PV}}))$$

where PF_{PV} is the plasma flow recorded in the portal vein.

During intraduodenal infusion of ^{13}C -thr (exp 2), total tracer sequestration was calculated as:

$$\text{ID infusion rate} - \text{PF}_{\text{PV}} * (([\text{thr}]_{\text{pv}} * E_{\text{pv}}) - ([\text{thr}]_{\text{A}} * E_{\text{A}}))$$

The same equation was used for ^{15}N -glycine infused intraduodenally.

Tracer first pass removal was calculated as the difference between total tracer sequestration and the flux of labelled thr recycled from artery.

Thr sequestration by the liver was calculated in the same way considering that the input was the sum of artery and portal vein and the output was the hepatic vein flux.

Under steady state conditions, thr and glycine disposal rate (DR) in the PDV and the liver was calculated from [1^{-13}C]-thr or ^{15}N -glycine total sequestration divided by threonine or glycine enrichment in the portal vein (considered as representative of the whole PDV enrichment) and the liver respectively. DR corresponds to the sum of two metabolic fluxes: catabolism and incorporation into proteins.

The rate of threonine degradation in the PDV and the liver through the TDG pathway was calculated by multiplying the ratio of ^{13}C -glycine to ^{13}C -thr enrichments ($\text{FC}_{\text{thr}(\text{gly})}$) measured during the intraduodenal [$1-^{13}\text{C}$]-thr infusion by glycine DR calculated during the ^{15}N -glycine infusion.

Statistical analysis

Data were submitted to variance analysis according to the general linear model (GLM) procedure of SAS (1989) and means were compared by protected *t*-test.

Results and discussion

In pigs intravenously infused with [$1-^{13}\text{C}$]-thr, we estimated that 14.3 % of arterial thr was extracted by the PDV and 18.8 % for whole splanchnic tissues. This is little much higher than values reported by Stoll *et al.* (1999) for phenylalanine which concentration in mucosal protein are lower than for thr.

Table 1. Thr sequestration by the PDV and the liver in 6 pigs infused with [$1-^{13}\text{C}$]thr in the duodenum (values are means \pm se for 6 pigs).

| | Total ^{13}C thr Sequestration | Sequestration of ^{13}C thr from infusion $\mu\text{mol/kg/h}$ | Thr DR |
|-------|---|---|------------------|
| PDV | 17.4 ± 2.6 | 13.3 ± 1.8 | 174.8 ± 46.1 |
| Liver | 2.86 ± 1.2 | | 40.2 ± 26.5 |

After correction of labelled thr sequestration by arterial sequestration (*Table 1*) we calculated that 53.5 % of newly infused thr was sequestered by the PDV ($13.3 \mu\text{mol/kg/h}$ of $25.5 \mu\text{mol/kg/h}$). PDV tissues preferentially used thr from the lumen since 75 % of thr sequestered by the PDV came from the infusion. By comparison thr sequestration by the liver was much lower (5.3 % of liver input that corresponds to arrival of the tracer by hepatic artery and portal vein). Compared to the whole PDV, the utilisation of thr by the liver was much lower confirming the statement of Rérat *et al.* (1992) who previously showed that dietary thr seems to be poorly taken up by the liver compared to the other essential AA. This shows that among splanchnic tissues, the PDV rather than the liver are involved in thr metabolism as it was already concluded for leucine (Yu *et al.*, 1990) and phenylalanine (Stoll *et al.*, 1997).

As expected TDG activity was found in the liver and the pancreas but was not detected in the mucosa of the small intestine (*Table 2*). We calculated that 1.6 % and 7.7 % of thr sequestered and metabolised by the PDV and the liver respectively were degraded through the TDG pathway. This pathway was previously shown to represent 70-80 % of total thr catabolism (Ballevre *et al.*, 1990). Therefore we conclude that that the high rate of thr sequestration by the PDV was not explained by thr catabolism through known metabolic pathways.

The difference between thr disposal rate (*Table 1*) and thr catabolism (*Table 2*) was supposed to correspond to thr incorporation into protein. Assuming that degradation through the TDG pathway represented 75% of total catabolism and on the basis of an average thr content of 3.9 g/100 g for visceral proteins (Bikker *et al.*, 1994), we estimated that 372 g of protein were synthesised per day. This very high value suggests that thr may be lost through an unknown pathway in the PDV. In conclusion, our results have shown an important disappearance of thr across the splanchnic tissues especially the PDV whereas the liver seems to be less involved in thr metabolism. Thr is probably not degraded by the intestine at least by known enzymes. This suggests that most of thr

Table 2. Thr catabolism through the TDG pathway (values are means \pm se for 6 pigs).

| | TDG activity μmol/min/g | DR _{gly} μmol/kg/h | Ox _{thr-gly} μmol/kg/h | FC _{Thr - Gly} % |
|-------|----------------------------|--------------------------------|------------------------------------|------------------------------|
| PDV | 5.62 \pm 2.4 | 437.8 \pm 110 | 2.74 \pm 0.59 | 0.64 \pm 0.16 |
| Liver | 1.46 \pm 0.54 | 220.6 \pm 73.3 | 3.50 \pm 2.33 | 1.34 \pm 0.88 |

visceral metabolism could be explained by the incorporation of thr in the tissue and secretory proteins of the digestive tract with minor participation of catabolism pathway. However the existence of other pathways can not be excluded.

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Effect of tryptophan and acetate on milk production in late lactating dairy goats

T.G. Madsen¹, L.K. Sjölander¹, L. Misciattelli² & M.O. Nielsen¹

¹ Department of Anatomy and Physiology, The Royal Veterinary and Agricultural University, Grønnegaardsvej 7, DK-1870 Frederiksberg C, Denmark

² Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Grønnegaardsvej 7, DK-1870 Frederiksberg C, Denmark

Summary

The objectives of the present experiment were to study how mammary supply of essential amino acids (EAA), especially tryptophan (Trp), and acetate affects milk synthesis in dairy goats. Four goats were restrictedly fed a diet calculated to meet approximately 90 % and 75 % of daily requirements for energy and absorbable amino acids, respectively, and randomly allocated to the 4 treatments in a balanced 4 x 4 Latin Square design. The treatments consisted of intravenous infusions of solutions of saline as control (C), sodium acetate (A), and essential amino acids (EAA) with or without Trp (+T & -T). The EAA and acetate infusions were calculated to be isoenergetic. Simultaneous arterio-venous blood sampling over the udder was performed during the last 24 h of infusion. Blood O₂ and CO₂ were measured immediately and used to calculate the respiratory quotients (RQ) across the udder. Plasma concentrations of acetate, glucose, and β-hydroxybutyrate (BHB) were determined. Milk production was recorded the last day of infusion, and milk fat, protein and lactose contents were measured. Infusion of energy (A, +T & -T) increased milk and milk protein production, but no difference was found between the A and EAA infusions or between the two EAA profiles (+T & -T). Thus, mammary supply of energy yielding substrates (A as well as EAA's) can limit milk and milk protein synthesis in mid to late lactation, but Trp does not seem to be a limiting factor on a short term basis at this stage of lactation. When acetate was infused the milk fat yield increased, probably through increased de novo fat synthesis, as indicated by the increased mammary RQ.

Keywords: ruminants, milk protein, energy, acetate, tryptophan

Introduction

Over the last decades an increased attention has been given on identifying amino acids limiting milk protein synthesis. Increasing protein supply has in general been shown to increase milk protein production, but whether the main effect of additional amino acid supply is due to a specific need for amino acids or other effects like increased mammary energy supply and effects on transcription regulation is still unclear (Hanigan *et al.*, 2001a). If mammary energy supply is a main determinant for protein synthesis, it might be possible to increase efficiency of mammary amino acid utilization by increasing supply of acetate, the main energy supplier to the mammary gland. Indeed, close arterial acetate infusion has been shown to affect protein synthesis positively (Maas *et al.*, 1995). On the other hand, increased supply of a number of specific amino acids have been found in some situations to have a positive effect on milk protein production without increasing energy supply (Rulquin *et al.*, 1993; Hanigan *et al.*, 2001a), but not always (Seymour *et al.*, 1990). These results demonstrate our inadequate understanding of mammary amino acid metabolism. In theory all the essential amino acids can limit protein synthesis, and one of the least investigated amino acids has been Trp, partly because of the difficulties involved with analysing

Trp content in proteins. However, according to the model described by Hanigan *et al.* (2001b) Trp was the most limiting EAA for milk protein synthesis.

It was therefore the objective of the present study to evaluate; 1) how mammary supply of EAA and acetate affects mammary nutrient uptake and milk synthesis, and 2) whether Trp is a limiting amino acid in dairy goats in mid-late lactation.

Material and methods

Four crossbred Saanen-Landrace dairy goats (46 ± 2.4 kg live weight) in mid to late lactation (179 ± 15 days in milk) were fed restrictedly, a diet consisting of artificially dried grass hay (54.5 % DM), barley (29.9 % DM), rape seed (7.1 % DM), urea (0.8 % DM), and tallow (7.5 % DM), meeting approximately 0.90 and 0.75 of daily requirements for energy and absorbable amino acids, respectively, based on pretrial milk yield (1495 ± 246 g/d). Values are presented as mean \pm SEM. The goats were randomly allocated to the 4 treatments in a 4×4 Latin Square design. Treatments consisted of 4 isoosmotic intravenous infusions (945 g/d); C) saline (39.9 g/L) as control, A) sodium acetate (0.68 M), and two EAA profiles resembling milk protein composition (0.21 M) with Trp (+T) and without Trp (-T). The two EAA and the acetate infusions were calculated to have the same theoretical ATP yield if completely oxidized in the oxidative pathways (6.8 mol ATP/L of infusion solution).

Infusion periods lasted 4 days separated by 3 days where the goats were fed the same amount of concentrate as during the infusion period and hay ad libitum. During the last 24 hours of infusion, milk production was recorded and simultaneous arterio-venous blood samples were withdrawn every 4 hours from catheters inserted into one of the exteriorized carotid arteries and both milk veins.

Whole blood O₂ and CO₂ were measured right after sampling on acid-base laboratory equipment (ABL 300, Radiometer, Denmark). Milk samples were analysed by infrared spectroscopy on a Milkoscan 104 (Foss Electric, Denmark). Commercial spectrophotometrically kits were used to analyse for acetate (Boehringer Mannheim, Germany) and glucose (Sigma-Aldrich, Denmark), and BHB analyses were done according to Cant *et al.* (1993).

Data were analysed statistically using the mixed model procedure of SAS version 8.2 (SAS Institute Inc., Cary, NC, USA). Goat and period were included as random effects and treatment as fixed effects. Results are expressed as least squares means (LS-means) with standard error of mean (SEM). Sum of squares for treatment effects were further divided into single degree freedom contrasts to compare effects of acetate infusion (C vs. A), energy infusion (C vs. A, +T & -T), protein infusion (A vs. -T & +T), and infusion of EAA with and without Trp (+T vs. -T).

Results and discussion

Infusion of energy (A, -T & +T) tended to decrease feed intake, but increased milk and milk protein production, however, no difference were found between the acetate and EAA infusions, see table 1. These results indicate that energy supply was limiting milk and milk protein synthesis in the animals on the control treatment, whereas there was no indication of a specific protein limitation in the present study. In agreement, Trp did not in this short term study limit production, however, because of the relatively short infusion periods it is possible that the endogenous AA output from body pools covered the amino acid deficits and amino acids therefore in longer terms could become limiting (Iburg & Lebzien, 2000). The finding that energy seemed to be the main determinant of milk production in this study is additionally supported by the close relationship between milk production and the AV of the main energy supplying nutrients for oxidation in the mammary gland, i.e. acetate, glucose and BHB (Bickerstaffe *et al.*, 1974; Cant *et al.*, 1993), see figure 1.

When acetate was infused not only milk protein but also fat yield increased compared to control treatment. This was probably an effect of the increasing mammary supply and uptake of acetate, indicated by the increase in arterial concentration and arterio-venous difference (AV), see table 2.

Table 1. Treatment effects on milk production and milk composition in dairy goats jugularly infused with: saline (C) infusion, acetate(A), and EAA with (+T) and without Trp (-T).

| | Treatments ¹ | | | | SEM | Contrast ¹ | | | |
|-----------------|-------------------------|-----|------|------|-----|-----------------------|--------|------|------|
| | C | A | +T | -T | | Ac | Energy | Prot | Trp. |
| DM intake (g/d) | 857 | 776 | 813 | 773 | 64 | 0.08 | 0.06 | 0.66 | 0.36 |
| Milk (g/d) | 940 | 998 | 1021 | 1019 | 223 | 0.17 | 0.05 | 0.52 | 0.96 |
| Protein (g/d) | 43 | 46 | 47 | 47 | 7 | 0.08 | 0.02 | 0.57 | 0.84 |
| Fat (g/d) | 44 | 55 | 50 | 44 | 5 | 0.02 | 0.10 | 0.05 | 0.16 |
| Lactose (g/d) | 43 | 46 | 46 | 46 | 10 | 0.18 | 0.08 | 0.77 | 0.95 |

¹ Ac = C vs. A, Energy = C vs. A, +T & -T, Prot. = A vs. +T & -T, and Trp = +T vs. -T

In agreement, the mammary RQ was found to be positively affected by the acetate infusion, as the high mammary RQ well above 1 indicates an increased *de novo* fat synthesis, because the oxygen-rich acetate is converted to oxygen-poor lipids and the liberated oxygen substitute oxygen supplied from blood. The close relationship between mammary RQ-values and AV of acetate further supports that *de novo* fat synthesis was increased when acetate was infused, see figure 2. The infusion treatments did not have any effect on arterial glucose and BHB concentration, but glucose AV was significantly higher when Trp was included in the EAA infusion, and BHB AV was negatively affected by acetate infusion compared to the amino acid infusions. The difference in glucose AV was surprising, as no differences in the production parameters were observed;

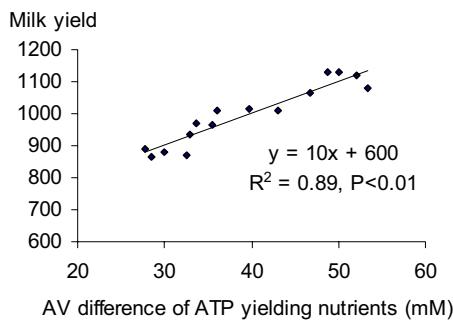


Figure 1. Milk production (corrected for effect of animal and period) in response to arterio-venous difference of energy from acetate, glucose and BHB measured in theoretical ATP yield.

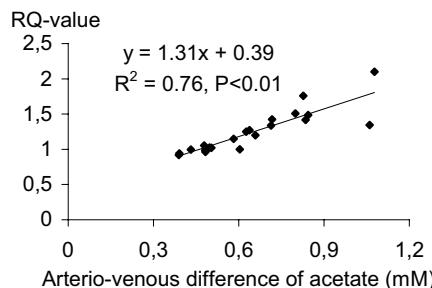


Figure 2. Mammary RQ-value (corrected for effect of animal and period) in response to arterio-venous difference of acetate.

Table 2. Arterial concentrations (A) and mammary arterio-venous differences (AV) of acetate, glucose, BHB and energy (ATP), and mammary RQ-values in dairy goats.

| | Treatments | | | | SEM | Contrast | | | |
|-----------------------|------------|------|------|------|------|----------|--------|-------|------|
| | C | A | +T | -T | | Ac | Energy | Prot | Trp. |
| Acetate | | | | | | | | | |
| A (mM) | 0.76 | 1.04 | 0.81 | 0.84 | 0.13 | <0.01 | <0.01 | <0.01 | 0.27 |
| AV (mM) | 0.60 | 0.77 | 0.63 | 0.65 | 0.09 | <0.01 | <0.01 | <0.1 | 0.47 |
| Glucose | | | | | | | | | |
| A (mM) | 3.18 | 3.15 | 3.12 | 3.17 | 0.14 | 0.61 | 0.48 | 0.91 | 0.38 |
| AV (mM) | 0.68 | 0.71 | 0.90 | 0.70 | 0.08 | 0.72 | 0.15 | 0.16 | 0.02 |
| BHB | | | | | | | | | |
| A (mM) | 0.34 | 0.33 | 0.39 | 0.34 | 0.13 | 0.78 | 0.40 | 0.09 | 0.07 |
| AV (mM) | 0.25 | 0.22 | 0.26 | 0.28 | 0.08 | 0.26 | 0.62 | 0.01 | 0.42 |
| RQ-value ¹ | 1.21 | 1.68 | 1.16 | 1.12 | 0.15 | <0.01 | 0.19 | <0.1 | 0.69 |

¹ RQ-value for the mammary glands (CO_2 AV-difference / O_2 AV-difference)

therefore no plausible explanations could be given for this difference. The effect on BHB AV could be due to a negative correlation between BHB and acetate uptake, as these two nutrients are utilized by the same metabolic pathways, i.e. increasing acetate uptake could have a sparing effect on BHB utilization and thereby uptake in the mammary gland.

In conclusion, mammary supply of energy yielding substrates (A as well as EAA's) can limit milk and milk protein synthesis in mid to late lactating dairy goats fed grass hay based diet. At the present feeding level and lactation stage EAA including Trp does not seem to be the only limiting factor in milk and milk protein synthesis. Additional acetate supply increases the arterial concentration and thereby mammary supply and uptake, which affects not only milk protein synthesis but also milk fat production, probably through increased de novo fat synthesis, as indicated by the increased mammary RQ.

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Mammary uptake of amino acids in response to supplementation of rumen protected lysine and methionine in early and late lactating dairy goats

T.G. Madsen, L. Nielsen & M.O. Nielsen

Department of Anatomy and Physiology, The Royal Veterinary and Agricultural University, Grønnegaardsvej 7, DK-1870 Frederiksberg C, Denmark

Summary

Goats in early and late lactation were fed a basal diet designed to be low in lysine (Lys) and methionine (Met). The diets were either not supplemented (C), or supplemented with rumen protected Lys (L), Met (M) or both Lys and Met (LM). In order to estimate mammary supply and uptake, arterial and milk vein blood samples were withdrawn and mammary blood flow (MBF) was measured. The supplementation of both Lys and Met increased milk yield and protein production in early but not late lactation. Arterial Lys and Met concentrations were increased significantly when the amino acids were added to the feed, however, the increased concentrations did not affect mammary arterio-venous differences. The analysis of uptake in response to supply showed that the mammary gland seemed less sensitive to changes in supply in early compared to late lactation. It was concluded that it is possible to increase arterial concentration by supplementation of rumen protected Lys and Met. But supplementation to ruminants fed according to their energy and protein requirements can not be expected to have any major effects on milk production, because the mammary gland seems to possess mechanisms enabling it to adjust the extraction of Lys and Met according to its needs.

Keywords: ruminants, milk protein, lysine, methionine

Introduction

Dietary amino acid (AA) composition has in ruminants traditionally not been taken into consideration because of the microbial protein synthesis. However, supply of rumen bypass Lys and Met have in some, but not all, studies increased milk protein production. The limited knowledge about the actual post absorptive distribution of AA's makes it problematic to foresee the effect additional supply of single AA have on milk synthesis.

The objectives of the present experiment were therefore to study how supplementation of Lys and Met to a diet low in these two AA's would affect 1) the arterial concentrations and thereby mammary supply and uptake of these two AA's, and 2) the milk and milk protein synthesis in early and late lactation.

Material and methods

Four goats were fed restrictedly according to energy and protein requirements with a basal ration designed to be low in absorbable Lys and Met from the small intestine. The goats were randomly assigned to one of four treatments in a balanced 4 x 4 Latin square design performed in late (280 days in milk) and the following early lactation (35 days in milk). The four treatments were either no supplementation of the basal diet (C), or supplementation with rumen protected Lys (L), Met (M), or both Lys and Met (LM). The protected amino acids (SmartamineTM M and ML obtained from Aventis Animal Nutrition, Rhône-Poulenc, Antony Cedex, France) were given per orally twice a day when the goats were fed.

By the end of each treatment period (14 days) the goats were fitted with temporary catheters in one of the exteriorized carotid artery and in both exteriorized milk veins. Arterial and venous blood samples were then withdrawn simultaneously six times during the next 24 hours. Mammary blood flow (MBF) recordings were performed twice daily, applying the ultra sound Doppler technique (Christensen *et al.*, 1989).

Deproteinized plasma samples were analyzed for free Lys and Met by precolumn derivatization (6-N-aminoquinolyl-N-hydroxysuccinimidyl carbamate) chromatography on a cation-exchange resin column (Cohen & Michaud, 1993).

Data from early and late lactation were analysed separately by the mixed model procedure of SAS version V8 (SAS Institute Inc., Cary, NC, USA). Goat and period were included as random effects and treatment as fixed effects. Results were expressed as least squares means (LS-means) with standard error of mean (SEM). Statistical significance was declared at P<0.05.

Results and discussion

In early lactation the combined supplementation of Lys and Met had a positive effect on milk and milk protein yield, whereas no effect was observed in late lactation. These results indicate that Lys and Met were co-limiting synthesis of milk protein in early but not late lactation, where other factors like mammary synthetic capacity must have become the limiting factor for production. Supply and uptake by the mammary gland varied mainly according to changes in arterial and arterio-venous difference (AVD), respectively, as MPF, in agreement with findings by Mabjeesh *et al.* (2002), was not affected by supplementation of Lys and Met (table 2). Arterial concentrations

Table 1. Feed characteristics and intake, and milk yield and contents in early and late lactating dairy goats fed a diet supplemented with rumen protected lysine and methionine¹.

| | Treatment | | | | SEM | P-value ² | | |
|----------------------------|-----------|------|------|------|------|----------------------|------|-------|
| | C | L | M | LM | | L | M | L*M |
| early lactation | | | | | | | | |
| DM intake (g/d) | 2170 | 2190 | 2197 | 2227 | 93 | 0.07 | 0.06 | 0.23 |
| AAT (g/kg DM) ³ | 98 | 98 | 98 | 99 | | | | |
| Lys (% of AAT) | 6.36 | 7.18 | 6.33 | 7.16 | | | | |
| Met (% of AAT) | 2.10 | 2.44 | 2.77 | 2.77 | | | | |
| Milk yield (g/d) | 3179 | 3124 | 3037 | 3307 | 119 | 0.04 | 0.65 | <0.01 |
| Protein (%) | 3.09 | 3.06 | 3.09 | 3.10 | 0.08 | 0.76 | 0.26 | 0.31 |
| (g/d) | 98 | 96 | 94 | 103 | 5 | 0.04 | 0.31 | <0.01 |
| late lactation | | | | | | | | |
| DM intake (g/d) | 1215 | 1244 | 1229 | 1225 | 44 | 0.29 | 0.85 | 0.19 |
| AAT (g/kg DM) | 83 | 84 | 84 | 85 | | | | |
| Lys (% of AAT) | 6.23 | 7.12 | 6.18 | 7.09 | | | | |
| Met (% of AAT) | 2.11 | 2.45 | 2.86 | 2.88 | | | | |
| Milk yield (g/d) | 675 | 673 | 577 | 692 | 186 | 0.17 | 0.32 | 0.15 |
| Protein (%) | 4.22 | 4.05 | 4.20 | 4.13 | 0.18 | 0.08 | 0.63 | 0.41 |
| (g/d) | 27 | 24 | 30 | 25 | 7 | 0.42 | 0.31 | 0.28 |

¹ The treatments are diets with low lysine & low methionine (C), high lysine & low methionine (L), low lysine & high methionine (M), and high lysine & high methionine (LM).

² Possibilities for effect of lysine (L), methionine (M) or interaction of lysine and methionine (L*M)

³ Amino acids absorbed in the intestine (AAT) pr. kg of dry matter (DM)

Table 2. Mammary plasma flow (MPF), arterial concentrations, arterio-venous differences (AVD), and mammary uptake:output ratios (O:U) of lysine and methionine in dairy goats fed a diet supplemented with rumen protected lysine and methionine in early and late lactation¹

| | Treatment | | | | SEM | P-value ² | | |
|------------------------|-----------|-------|-------|-------|------|----------------------|-------|-------|
| | C | L | M | LM | | L | M | L*M |
| early lactation | | | | | | | | |
| MPF (L/h) | 38 | 38 | 41 | 38 | 8 | 0.785 | 0.807 | 0.731 |
| Arterial Lys (µM) | 169.7 | 207.5 | 168.4 | 192.5 | 20.2 | 0.002 | 0.211 | 0.282 |
| Arterial Met (µM) | 48.4 | 50.8 | 53.8 | 55.1 | 2.0 | 0.381 | 0.049 | 0.777 |
| Lys AVD (µM) | 83.5 | 86.4 | 82.9 | 83.3 | 6.6 | 0.835 | 0.751 | 0.817 |
| Met AVD (µM) | 25.2 | 25.6 | 23.6 | 25.7 | 2.1 | 0.633 | 0.636 | 0.770 |
| O:U of Lys | 1.26 | 1.23 | 1.32 | 1.23 | 0.20 | 0.752 | 0.885 | 0.881 |
| O:U of Met | 1.27 | 1.29 | 1.30 | 1.30 | 0.19 | 0.947 | 0.899 | 0.950 |
| late lactation | | | | | | | | |
| MPF (L/h) | 28 | 27 | 24 | 31 | 5 | 0.398 | 0.992 | 0.265 |
| Arterial Lys (µM) | 145.8 | 149.7 | 142.8 | 156.4 | 6.2 | 0.108 | 0.706 | 0.332 |
| Arterial Met (µM) | 48.8 | 48.6 | 51.7 | 53.2 | 3.2 | 0.585 | 0.014 | 0.458 |
| Lys AVD (µM) | 41.0 | 51.2 | 39.5 | 49.2 | 11.6 | 0.186 | 0.803 | 0.967 |
| Met AVD (µM) | 14.3 | 13.3 | 13.0 | 16.7 | 5.4 | 0.643 | 0.729 | 0.426 |
| O:U of Lys | 1.53 | 1.96 | 1.40 | 2.65 | 0.57 | 0.185 | 0.631 | 0.490 |
| O:U of Met | 1.95 | 2.05 | 1.97 | 2.85 | 0.71 | 0.442 | 0.521 | 0.539 |

¹ See comment for table 1

of lysine and methionine were relatively high compared to previously reported levels for dairy goats (Mabjeesh *et al.*, 2002). One could therefore question how severe the lysine and methionine limitations actually were on the basal ration fed in early lactation.

The decrease in milk production from early to late lactation was obviously associated with a decreased nutrient utilization by the mammary gland. The lower mammary uptake of Lys and Met was induced by a decrease in both MPF and AVD. However, the decrease in Lys and Met uptake from early to late lactation did not equal the decrease in output as shown by the increase in uptake:output ratio of Lys and Met. As a consequence, intracellular distribution of Lys and Met, between milk protein synthesis and other metabolic pathways, must have changed from early to late lactation.

A higher oxidation of amino acids in mammary tissue with advancing lactation could easily lead to the assumption that the gland becomes less sensitive to changes in arterial supply. It was therefore surprising that AVD in the present study seemed more sensitive to changes in arterial concentration in late compared to early lactation, see figure 1. These results indicate that the mammary gland in early lactation to some extent is capable of extracting Met and Lys according to requirements independently of arterial supply. The changes in Lys and Met affinity indicate a change in K_m -value for the transporters from early to late lactation. Actually it has been shown that cationic amino acids in mouse are not only transported by the y^+ transporter system, but also by a broader specific system with a lower K_m (Sharma & Kansal, 2000), and the distribution between these two types of transporters have been shown to be influenced by the lactogenic hormones (Sharma & Kansal, 2000) and thereby stage of lactation. However, this is an area which needs further investigation.

In conclusion, it is possible to increase arterial concentration by supplementation of rumen protected Lys and Met. But supplementation to ruminants fed according to their energy and protein

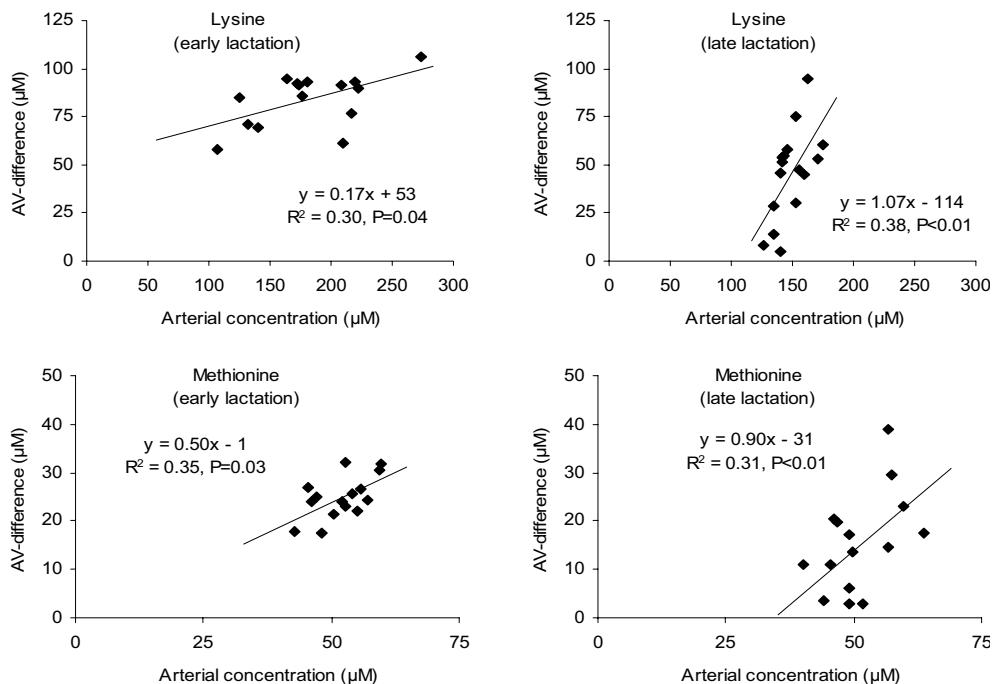


Figure 1. Arterio-venous differences for lysine and methionine in response to arterial concentration of lysine and methionine, respectively, in early and late lactation.

requirements can not be expected to have any major effects on milk production, because the mammary gland seems to possess mechanisms enabling it to adjust the extraction of Lys and Met according to its needs. However, in early lactation when production peaks and thereby also mammary demand for nutrients, low Lys and Met supplies can become limiting for milk production.

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Arterio-venous differences in amino acids and acid-base parameters in the mammary gland of sows around parturition

Z. Mroz¹, W. Krasucki² & S.J. Koopmans¹

¹ Institute of Animal Science and Health, Edelhertweg 15, 8200 AB Lelystad, the Netherlands

² Agricultural University of Lublin, Department of Animal Nutrition, Akademicka 13, 20-950 Lublin, Poland

Summary

Six multiparous sows at late pregnancy were fitted with three blood catheters (*A. epigastrica cranialis superficialis*, *A. iliaca externa*, *V. epigastrica cranialis superficialis*) to study 1) arterio-venous differences (AVD), extraction rate (ER) and uptake of essential amino acids in the mammary gland, and 2) blood acid-base characteristics as affected by two factors 1) sampling time (pre-prandial versus post-prandial); 2) phase of the reproductive cycle (pre- and post-farrowing). We found that this multi-catheterisation technique can be useful in a qualitative and quantitative evaluation of the relative importance among essential amino acids for colostrum or milk synthesis, and mammary extraction rates of amino acids reflect their supply and mammary utilisation. In consequence, a new factorial approach for estimating amino acid dietary requirement estimates to mammary gland physiology may be elaborated.

Keywords: sow, mammary gland, amino acid uptake

Introduction

In contrast to dairy cows, a precise quantification of metabolic processes in the mammary gland of reproductive sows around parturition and lactation is complicated since they are able to mobilise own body reserves of nutrients for maintaining colostrum or milk production, as manifested by their body weight loss. Due to a very limited data available, the mathematical approaches are proposed to estimate the amino acid requirements of sows for milk production, assuming that these levels will also maximise subsequent reproductive performance (NRC, 1998). As the body reserves used for colostrums/milk production appear to vary widely, a better accuracy in optimising the pre- and post-farrowing requirements of sows for dietary amino acids and acid-base balance may be achieved when knowing more about the relative importance among essential amino acids for colostrum or milk synthesis, and mammary extraction rates of amino acids reflecting their supply and mammary utilisation. So far, plasma amino acid uptake by the mammary gland of sows has been calculated from the arterio-venous differences and plasma flow rates in the postcolostral phase of sows (Trottier et al., 1995, 1997), and not in the colostral phase or before parturition. An adequate cannulation technique of the mammary arterio-venous system is a pre-requisite for studying uptake and kinetics of amino acids or acid-base changes in this organ.

The objectives of this study were 1) to examine a stressless, permanent cannulation of arterio-venous vessels for continuous monitoring of metabolic functions in the mammary gland of reproductive sows; 2) to measure post-prandial arterio-venous differences (AVD) in plasma amino acids, and changes in blood acid-base characteristics across the mammary gland of sows in the pre- and post-farrowing periods.

Material and methods

Six multiparous sows were assigned for this experiment comprising a pre-farrowing phase (14 days) and post-farrowing phase (10 days). After mating, they were kept in individual pens (2×3 m) at a thermoneutral zone ($22-25^\circ\text{C}$; relative humidity of 55%) and fed a cereal-soybean-meal based diet (12.5% CP, 0.5% lysine in the pregnancy, and 17% CP, 0.85% lysine in the lactation). On day 100 of the pregnancy, three blood catheters (i.d.=1.02 mm, o.d.=1.78 mm) were fitted in *A. epigastrica cranialis superficialis* (AECS), *A. iliaca externa* (AIE) and *V. epigastrica cranialis superficialis* (VECS). After a recovery period of seven days, the sows were used for blood sampling 3 times, i.e., on day 108 of pregnancy (pre-farrowing); day 3 of lactation (colostral phase), and day 10 of lactation (postcolostral phase). To achieve equal litters ($n=10$), newly born piglets were cross-fostered after birth. The daily rations on the pre-, and post-farrowing phases were 3.2 and 6.2 kg/sow, respectively. Arterio-venous blood sampling (10 mL) in each phase was performed in the pre-prandial (0600 h), prandial (0700 h), and post-prandial (0800, 0900 and 1000 h) intervals to obtain in total five arterial and venous samples per sow on each sampling day. The same schedule was maintained for colostrum/milk sampling (5 mL per gland on one side of the mammary gland and pooled) after a transcatheter injection of oxytocin (1 IU). To calculate amino acid supply and consumption across the mammary gland as arterio-venous differences (AVD), an indicator-dilution technique with *para*-aminohippuric acid (PAH) was used (Ten Have et al., 1995). This marker was infused through *A. iliaca externa* at a rate of 30 mL/h after a bolus of 5 mL PAH solution on the morning of blood sampling (0500 h) to obtain a steady state PAH concentration. The plasma flow (PF) through the mammary gland was calculated with the following formula: $\text{PF}(\text{mL/kg BW/min}) = \text{PAH infusion rate} \times [\text{PAH}]_{\text{infused}} / [\text{PAH}]_{\text{AECS}} - [\text{PAH}]_{\text{VECS}}$. To estimate colostrum/milk production (\mathbf{P}) in the measurement periods (from 0600 to 1000 h), the weigh-suckle-weigh technique was used. Amino acid uptake ($\text{AA}_{\text{uptake}}$) was calculated as follows: $\text{AA}_{\text{uptake}} = [\text{AA}] \text{ in colostrum or milk} \times \mathbf{P} / [\text{AA}]_{\text{AVD}} \times \text{PF}$. The extraction rate (ER) was calculated as $\text{ER} (\%) = [\text{AA}]_{\text{AVD}} \times 100 / [\text{AA}]_{\text{A}}$. Characteristics of acid-base balance (pH, pCO_2 , pO_2 , HCO_3 , O_2 saturation) were measured in each blood sample using a blood gas analyser (ABL-3), and haematocrit values were determined by centrifugation in a micro centrifuge. The effects of sampling time and reproduction or lactation phases were subjected to the analysis of variance for repeated measurements using the GLM procedure of SAS (1990), and the level of significance of differences among the means was tested using t-test.

Results

The venous drainage of the mammary complex is via a deep and a superficial network (*V. epigastrica cranialis superficialis*) similar to the arterial networks.

Over the whole 24-day period of this study no incidental removals, dislocations or occlusions of the blood catheters were encountered. A post-mortem examination revealed no signs of catheter-

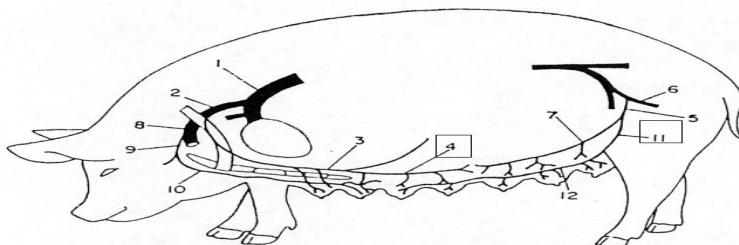


Figure 1. Sow arterial blood cannulation in the mammary glands: [4] *A. epigastrica cranialis superficialis*, [11] *A. iliaca externa*.

related infections (presumably due to a preventive use of gentamycin-moistened tampons at their exit from the body). Numbers of alive born piglets per litter were from 9 to 13, and piglet's birth body mass was 1.54 kg. Arterio-venous differences (AVD) for the essential amino acids in the pre-farrowing period were partly affected by the sampling time (Table 1).

Table 1. Amino acid arterio-venous differences ($\mu\text{mol/L}$) in the pre-farrowing phase (day 108 of pregnancy), as affected by the sampling time.

| Amino acid | Sampling time (h) | | | | | | Pooled SEM |
|---------------------|--------------------|---------------------|--------------------|-------------------|-------------------|-------------------|------------|
| | 0600 | 0700 ¹⁾ | 0800 | 0900 | 1000 | 1100 | |
| Lysine (Lys) | -22.3 ^a | -19.1 ^a | -15.2 ^a | 3.7 ^{ab} | 4.1 ^{ab} | 6.9 ^b | 7.8 |
| Methionine (Met) | -1.6 | 1.9 | 2.2 | 2.0 | 2.5 | 2.0 | 1.1 |
| Tryptophan (Try) | -4.2 | -3.1 | -2.5 | -1.1 | 1.0 | 0.3 | 2.4 |
| Arginine (Arg) | -9.7 | -15.9 | -3.6 | 0.1 | 2.4 | 1.2 | 6.9 |
| Histidine (His) | -5.9 ^a | -3.1 ^a | 4.8 ^{ab} | 7.4 ^{ab} | 8.8 ^b | 8.1 ^b | 4.0 |
| Isoleucine (Ile) | -7.8 | -6.4 | 0.5 | 1.3 | 1.5 | 2.0 | 4.6 |
| Leucine (Leu) | -12.1 | -13.4 | 4.5 | 6.4 | 7.2 | 4.7 | 8.1 |
| Phenylalanine (Phe) | -10.5 ^a | -11.4 ^a | 2.0 ^{ab} | 2.4 ^{ab} | 7.1 ^b | 4.5 ^b | 5.6 |
| Threonine (Thr) | -15.4 ^a | -10.5 ^a | -1.0 ^{ab} | 2.4 ^{ab} | 3.5 ^b | 2.8 ^b | 6.8 |
| Valine (Val) | -25.4 ^a | -18.6 ^{ab} | -4.0 ^{bc} | 4.0 ^c | 8.0 ^c | 10.0 ^c | 8.7 |

¹⁾Feeding time.

^{abc}Means within a row with a different superscript are different at $P < 0.05$.

Despite the AVD in lactating sows were lower ($P < 0.05$) in the pre-prandial time compared to the post-prandial time, the composition of colostrum and milk from particular sows did not vary meaningfully ($P > 0.05$) over the sampling time, whereas a between-animal variation was greater ($P < 0.05$). On average, the contents of dry matter and protein ($N \times 6.38$) were greater in colostrum (23.7 and 15.7%, respectively) than in milk (17.5 and 5.9%, respectively). Amounts of essential amino acids (as % of protein) in the colostrum/milk were as follows: Lys (7.3/7.2), Met (1.9/1.5), Try (1.5/1.3), Arg (5.7/5.0), His (2.0/2.3), Ile (2.8/3.4), Leu (9.1/8.5), Phe (4.9/4.1), Thr (5.9/4.2), Val (5.1/4.1).

The pre- and post-farrowing changes in the acid-base characteristics (pH, pCO_2 , pO_2 , HCO_3 , O_2 saturation) of arterial and venous blood were only numerically ($P > 0.05$) affected by the sampling time. The effects of sampling time on the mammary extraction rate (%) of amino acids were of minor magnitude ($P > 0.05$), whereas the day of lactation (colostral versus milk phase) exerted a clear impact ($P < 0.001$) on the quantitative estimates of AVD, ER, and amino acid uptake (Table 2).

Discussion

Lactation efficiency represents a major economic factor in pig production, and further progress requires more basic understanding of mammary gland uptake and kinetics of colostrum or milk precursors using adequate arterio-venous cannulation techniques. In this study we proved that the multiple catheterisation of *A. epigastrica cranialis superficialis* (AECS), *A. iliaca externa* (AIE) and *V. epigastrica cranialis superficialis* (VECS) in pregnant sows enabled simultaneous, stressless measurements of the arterio-venous differences across the anterior mammary glands. The advantage of this model is the possibility of studying various dynamic physiological processes and effects of dietary/pharmacological treatments around parturition.

Table 2. Amino acid arterio-venous differences (AVD), extraction rate (ER) and amino acid uptake in the mammary gland of sows as affected by the post-farrowing phase.

| Lactation day: | AVD μ mol/L | | | ER (%) | | | Uptake (g/4 h) | | |
|---------------------|-----------------|------|---------|--------|------|---------|----------------|-----|---------|
| | 3 | 10 | P-value | 3 | 10 | P-value | 3 | 10 | P-value |
| Lysine (Lys) | 97.5 | 34.8 | <0.001 | 78.9 | 43.8 | <0.001 | 13.0 | 4.8 | <0.001 |
| Methionine (Met) | 25.5 | 11.0 | <0.001 | 65.9 | 28.9 | <0.001 | 3.2 | 1.2 | <0.001 |
| Tryptophan (Try) | 26.2 | 8.3 | <0.001 | 39.9 | 19.7 | <0.001 | 4.6 | 1.7 | <0.001 |
| Arginine (Arg) | 106.9 | 41.6 | <0.001 | 78.6 | 35.1 | <0.001 | 15.0 | 5.4 | <0.001 |
| Histidine (His) | 30.8 | 10.8 | <0.001 | 45.0 | 16.9 | <0.001 | 3.2 | 1.3 | <0.001 |
| Isoleucine (Ile) | 81.5 | 33.0 | <0.001 | 67.8 | 44.1 | <0.001 | 9.1 | 3.3 | <0.001 |
| Leucine (Leu) | 161.5 | 57.9 | <0.001 | 59.0 | 37.9 | <0.001 | 17.1 | 6.6 | <0.001 |
| Phenylalanine (Phe) | 55.7 | 21.8 | <0.001 | 65.4 | 28.0 | <0.001 | 7.0 | 2.6 | <0.001 |
| Threonine (Thr) | 82.0 | 32.2 | <0.001 | 54.8 | 24.7 | <0.001 | 8.0 | 2.7 | <0.001 |
| Valine (Val) | 113.6 | 39.9 | <0.001 | 45.3 | 25.6 | <0.001 | 10.2 | 3.5 | <0.001 |

^{abc}Means within a row with a different superscript are different at $P < 0.05$.

Until now, the needs of the mammary system in the particular phases of the reproductive cycle in sows are not so well defined yet to be implemented for optimising dietary formulations. Some results have been presented by Trottier et al. (1995, 1997) with respect to lactating sows. Our new quantitative data on the plasma arterio-venous difference and the uptake of essential amino acids across the porcine mammary gland document clearly that free amino acids in the blood of sows constitute the major precursors of colostrum or milk proteins. Moreover, we confirmed that such criteria as amino acid extraction, amino acid uptake and mammary amino acid retention in sows around parturition and during lactation could be used in a novel, factorial approach for estimating amino acid dietary requirement estimates to mammary gland physiology.

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The nutritional value of legumes in a balanced diet depends on the antagonism between protein metabolism in digestive tissues and in muscles

T. Pirman¹, E. Combe², J.M.A. Stekar¹, P. Patureau Mirand², A. Oresnik¹, M.C. Ribeyre² & J. Prugnaud²

¹ University of Ljubljana, Biotechnical Faculty, Zootechnical Department, Chair of Nutrition, Groblje 3, 1230 Domzale, Slovenia

² INRA Clermont-Ferrand - Theix, Nutrition and Protein Metabolism Unit, 63122 Saint Genès Champelanelle, France

Summary

Legume seeds have nutritional and worldwide commercial importance because they are easy to store and are rich in protein, fibre and minerals. Cooked beans and cooked lentils were used as a unique source of protein in experimental balanced diets for young rats. The legume diets resulted in high N faecal excretion and consequently in low digestibility. Protein synthesis rates in the intestinal tissues were increased by legume feeding. The effect was more pronounced by bean feeding and in large intestine. On the contrary, legume feeding significantly decreased protein synthesis rates in skeletal muscles.

Keywords: *legumes, growing rats, protein metabolism*

Introduction

Cooked legumes are eaten in several countries as the main source of protein. Insofar as their amino acids pattern fits amino acid requirements, the nutritional value of legume proteins depends firstly on nitrogen digestive losses because legumes are known to increase nitrogen fecal losses (Godinez *et al.* 1992). These losses result from dietary protein digestion, microflora activity and metabolism in the intestinal tissues. Our aim was to determine the impact of cooked legume feeding on protein digestion and on intestinal and muscle protein metabolism.

Material and methods

Two experiments were performed in young growing rats. In both experiments, diets were prepared with only one source of protein supplemented with sulphur amino acids. It was either Slovenian bean Cesnjevec (Semenarna) either French lentil Lentille verte du Puy (Cilverpuy), or casein for the control diet. Diets were designed to meet all the nutritional requirements of growing rats and to be isocaloric (18 MJ gross energy) and isonitrogenous (180 g of crude protein in the first experiment and 200 g in the second). A high protein level was used to emphasise specific effects of legume feeding. Beans and lentils were cooked using normal cooking methods. They were lyophilised and mixed with the other ingredients of the experimental diets. The lentil and bean diets were fed *ad lib.* to young growing rats for 20 days and compared with pair fed casein control diets. The first experiment included two successive balance trials in which beans and lentils were respectively compared with casein. In both trials, food intake and growth rate were registered. Nitrogen digestibility and balance were measured between day 9 and day 14. On the last day, rats were anaesthetized and sacrificed. Viscosity, pH and volatile fatty acids (VFA) concentrations were measured in the small and large intestine contents; protein, RNA and DNA contents were determined in the tissues.

In the second experiment, food intake and growth rate were also measured. Protein and nucleic acids contents as well as protein synthesis rates were determined in vivo, in intestinal tissues and muscles by the flooding dose method, with ^{13}C valine.

Data are presented as means \pm standard deviation. The significance of differences between means was assessed by Student's t test (experiment 1) or anova (experiment 2).

Results and discussion

In the first experiment (Table 1), growth rates and final muscle masses (not shown) were not different in the rats fed the legume diets and in the rats fed the control diets. However legume proteins had a lower apparent digestibility than casein and nitrogen balances were significantly lower in the legume-fed rats than in their pair fed controls. This is in agreement with data in literature for cooked beans (*e.g.* Godinez *et al.*, 1992) and cooked lentils (*e.g.* Combe and Cvirn, 1995). The marked effect of legume feeding on nitrogen balance measured during 5 days had only minor consequential effects on the overall growth rate measured over 20 days and on final masses of *m. gastrocnemius* and *m. soleus* and their protein contents (not shown). This suggests that legume protein intake was not the limiting factor for growth or that differences in legume feeding effects could depend on age or on length of time they are fed.

The masses of small intestine contents were higher in the legume-fed rats than in the casein pair-fed controls. This was in keeping with literature (Key *et al.*, 1996, Combe and Cvirn, 1995). Viscosity of these contents was the highest in the cooked bean fed rats but it was not different in the lentil fed and in the casein fed rats (Table 2). The differences in intestinal content viscosity were in agreement with viscosity differences measured in water extracts of cooked seeds and of the experimental diets and also with the differences in the seed content in soluble fibre (Pirman, 2000).

These larger amounts of undigested substrates promoted microbial fermentation in the distal parts of the digestive tract as shown by the low pH and high VFA concentrations of caecum contents in the legume fed rats, mostly in the cooked bean group (Table 2).

These differences in protein digestibility and in the composition of digestive contents between legume fed rats and their pair-fed controls were associated with differences in protein metabolism in the intestinal tissues (Table 3). Masses of small and large intestine tissues and their protein and nucleic acid contents were higher in the legume fed rats than in their controls, mostly in the large intestine and with beans.

Table 1. Growth rate, food intake, nitrogen digestibility and balance (Experiment 1)

| | Trial 1 | | Trial 2 | |
|--|------------------|-----------------|-------------------|-----------------|
| | Cooked bean (7) | Casein (5) | Cooked lentil (7) | Casein (5) |
| <i>Growth study</i> | | | | |
| Average growth rate (g day $^{-1}$) | 4.80 \pm 0.94 | 4.84 \pm 0.41 | 4.62 \pm 0.30 | 4.82 \pm 0.50 |
| Protein efficiency (g gain g protein intake $^{-1}$) | 1.90 \pm 0.23 | 1.95 \pm 0.06 | 1.86 \pm 0.16 | 1.91 \pm 0.23 |
| <i>Nitrogen balance study</i> | | | | |
| N in faeces (mg day $^{-1}$) | 119 \pm 25** | 25 \pm 8 | 99 \pm 13** | 27 \pm 3 |
| N in urine (mg day $^{-1}$) | 55 \pm 4** | 68 \pm 5 | 128 \pm 6** | 80 \pm 7 |
| Apparent digestibility (%) | 68.1 \pm 2.5** | 93.2 \pm 1.7 | 74.7 \pm 3.6** | 93.7 \pm 0.6 |
| Nitrogen balance (mg day $^{-1}$) | 194 \pm 28* | 269 \pm 20 | 167 \pm 17** | 313 \pm 19 |

Within each trial, significantly different from casein control: * P \leq 0.05; ** P \leq 0.01, respectively.

Table 2. Small and large intestine fresh contents (Experiment 1)

| | Trial 1 | | Trial 2 | |
|---|-----------------|--------------|-------------------|--------------|
| | Cooked bean (7) | Casein (5) | Cooked lentil (7) | Casein (5) |
| <i>Small intestine</i> | | | | |
| Content mass (g) | 2.33 ± 0.43** | 0.47 ± 0.14 | 2.21 ± 0.24** | 0.99 ± 0.29 |
| Viscosity (mPa s ⁻¹) | 41.50 ± 23.38** | 0.97 ± 0.31 | 1.17 ± 0.33 | 1.05 ± 0.11 |
| <i>Caecum</i> | | | | |
| pH | 6.0 ± 0.3** | 7.1 ± 0.1 | 6.7 ± 0.2* | 7.0 ± 0.2 |
| Acetic acid (mmol kg ⁻¹) | 33.57 ± 5.32* | 27.01 ± 5.26 | 30.68 ± 4.33* | 24.18 ± 5.89 |
| Propionic acid (mmol kg ⁻¹) | 10.95 ± 2.17** | 8.02 ± 1.59 | 7.85 ± 0.68 | 6.18 ± 2.07 |
| n-Butyric acid (mmol kg ⁻¹) | 18.12 ± 11.41** | 5.04 ± 2.18 | 5.03 ± 1.82 | 4.66 ± 1.38 |

Within each trial, significantly different from casein control: * P ≤ 0.05; ** P ≤ 0.01, respectively.

Table 3. Small and large intestine tissues (Experiment 1)

| | Trial 1 | | Trial 2 | |
|---------------------------------------|-----------------|--------------|-------------------|--------------|
| | Cooked bean (7) | Casein (5) | Cooked lentil (7) | Casein (5) |
| <i>Small intestine</i> | | | | |
| Protein (mg 100 g BM ⁻¹ §) | 356.0 ± 55.1* | 274.0 ± 28.0 | 365.5 ± 33.8* | 300.6 ± 23.8 |
| RNA (mg 100 g BM ⁻¹) | 17.85 ± 4.40* | 11.38 ± 1.64 | 17.95 ± 2.02* | 12.73 ± 2.42 |
| DNA (mg 100 g BM ⁻¹) | 9.57 ± 1.67* | 5.38 ± 0.82 | 9.31 ± 1.72* | 7.46 ± 0.47 |
| <i>Large intestine</i> | | | | |
| Protein (mg 100 g BM ⁻¹) | 158.0 ± 22.6* | 79.4 ± 8.4 | 127.3 ± 13.6* | 85.3 ± 16.1 |
| RNA (mg 100 g BM ⁻¹) | 6.22 ± 0.54* | 2.82 ± 0.32 | 5.60 ± 1.28* | 3.08 ± 0.37 |
| DNA (mg 100 g BM ⁻¹) | 4.04 ± 1.06* | 1.91 ± 0.09 | 3.82 ± 0.79* | 2.18 ± 0.35 |

§ BM = final body mass.

Within each trial, significantly different from casein control: * P ≤ 0.05; ** P ≤ 0.01, respectively.

In the second experiment (Table 4), performed in rats slightly younger than in experiment 1, and fed higher amounts of legume, legume feeding had a depressive effect on rat growth rates, and on the masses and the protein and nucleic acid contents of *m. gastrocnemius* and *m. soleus* (not shown). The lower muscle protein content could be explained by significantly lower protein synthesis rates. In this experiment, like in the first experiment, legume feeding resulted in higher protein and RNA contents in small and large intestine than casein feeding and the effects were more pronounced (Table 5).

In addition, protein synthesis rates were significantly higher. These rates were significantly correlated with RNA contents ($r= 0.89$ and 0.96 for small and large intestine, respectively). This indicates that legume feeding had stimulated intestinal protein during the first experiment too, but to a lower extent than during the second experiment, presumably because of the differences in the legume intakes.

In conclusion, legume feeding activates the anabolic drive in intestinal tissues. Its depressive effect on muscle protein anabolism seems to depend on the level of stimulation of protein synthesis in intestinal tissues. Thus, nutritive value of legumes, which is mainly reflected in muscle protein anabolism, can be modulated by their impact on intestinal protein metabolism.

Table 4. Growth rates and protein, nucleic acid contents and protein synthesis rates in gastrocnemius muscle (Experiment 2).

| | Cooked bean (8) | Cooked lentil (8) | Casein (8) |
|--|---------------------------|--------------------------|---------------------------|
| Growth rate (g day ⁻¹) | 1.79 ± 0.25 ^a | 2.09 ± 0.16 ^a | 5.09 ± 0.36 ^b |
| <i>Gastrocnemius muscle</i> | | | |
| Protein (mg) | 173.5 ± 16.1 ^a | 186.5 ± 9.7 ^a | 255.8 ± 16.2 ^b |
| RNA (mg) | 1.11 ± 0.11 ^a | 1.21 ± 0.04 ^b | 2.08 ± 0.13 ^c |
| Protein synthesis (mg 100 g BM ⁻¹) | 15.0 ± 2.2 ^a | 19.3 ± 2.2 ^b | 27.1 ± 2.9 ^c |

Values with the same subscript are not significantly different: P ≤ 0.05

Table 5. Protein metabolism in small and large intestine (Experiment 2)

| | Cooked bean (8) | Cooked lentil (8) | Casein (8) |
|--|--------------------------|--------------------------|--------------------------|
| <i>Small intestine</i> | | | |
| Protein content (mg 100 g BM ⁻¹) | 464 ± 44 ^a | 414 ± 21 ^b | 313 ± 22 ^c |
| RNA (mg 100 g BM ⁻¹) | 24.2 ± 1.7 ^a | 22.0 ± 1.8 ^b | 16.0 ± 0.9 ^c |
| Protein synthesis (mg 100 g BM ⁻¹) | 569 ± 111 ^a | 513 ± 105 ^a | 256 ± 35 ^b |
| <i>Large intestine</i> | | | |
| Protein content (mg 100 g BM ⁻¹) | 282 ± 19 ^a | 207 ± 38 ^b | 76 ± 6 ^c |
| RNA (mg 100 g BM ⁻¹) | 6.72 ± 0.59 ^a | 4.99 ± 0.85 ^b | 1.62 ± 0.27 ^c |
| Protein synthesis (mg 100 g BM ⁻¹) | 162 ± 18 ^a | 131 ± 31 ^b | 35 ± 3 ^c |

Values with the same subscript are not significantly different: P ≤ 0.05

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Whole-body protein turnover in Iberian and Landrace pigs fed adequate or amino acid deficient diets

M.G. Rivera-Ferre, R. Nieto & J.F. Aguilera

Unidad de Nutrición Animal, Estación Experimental del Zaidín (CSIC), Camino del Jueves s/n, 18100 Armilla, Granada, Spain.

Summary

Whole-body protein turnover (WBPTO) was measured on forty nine Iberian (Ib) and Landrace (Ld) gilts fit with urinary bladder catheters and fed on either adequate amino acid profile diets containing 120 (A12) or 160 (A16) g crude protein (CP)/kg as fed, or diets deficient in lysine (DLys) or threonine (DThr), given at 120 g CP/kg for Ib and 160 g CP/ kg for Ld pigs, respectively. Diets were approximately isoenergetic and supplied on average 13.5 kJ ME/g dry matter. Mean body weights (BM) were 26.5 kg and 30.7 kg for Ld and Ib pigs, respectively. Cumulative ^{15}N excretion in urinary urea and ammonia up to 60h after receiving a ^{15}N -glycine oral dose was used to calculate N flux (Q) using the mean value of both end products. On average, Ld pigs grew faster than Ib ($P<0.001$) increasing their BM by 2.31% and 1.81% daily, respectively (612 vs. 559 g/d, respectively). NR was lower in Ib pigs (1.20 vs. 1.35 g N/kg $\text{BM}^{0.75}$ d; $P<0.05$) and increased significantly with diet A16 only for the Ld breed ($P<0.01$). Q did not differ significantly between breeds, while protein synthesis (S) and degradation (D) were lower for the Ib pigs (6.21 vs. 7.42 and 5.01 vs. 6.05 g N/kg $\text{BM}^{0.75}$ d, respectively; $P<0.05$). Increasing the level of protein (PL) did not enhance significantly Q, S or D in Ib pigs, but significantly increased Q and S in the Ld breed ($P<0.05$). Fractional rates of protein growth (FGR), synthesis (FSR) and degradation (FBR) did not differ between breeds. For Ld pigs, Q decreased by 32% and S and D by 42 and 38%, respectively, when the DLys diet was offered. In the Ib breed the effect of DLys diet was less marked. No significant effects were detected for DThr diets when compared to adequate amino acid profile diets.

Keywords: whole- body protein turnover, Iberian pig, lysine deficiency

Introduction

Extensive pig production systems receive today increased attention. They stimulate the production of native pig breeds and the use of local feed resources, which helps maintaining genetic diversity and facilitates the provision of niche products to the market. The production of the Iberian pig complies with these prospects. It is strongly linked to the Mediterranean forest ('dehesa') in which takes place its final fattening period based on acorn and pasture.

Previous work has demonstrated that in the growing Iberian pig the capacity for protein deposition is considerably lower than that obtained in animals from selected breeds (Nieto *et al.*, 2002). It is known that changes in body protein mass are the result of the simultaneous synthesis and degradation of proteins (Reeds & Fuller, 1983). The aim of this work was to investigate the causes of the low protein deposition in the Iberian pig at the whole body level, with the ultimate objective of amplifying the protein synthetic or degradative responses to changes in nutrients supply in order to obtain an animal with an optimum muscle conformation at the early stages of growth, before the fattening period outdoors. As dietary AA imbalance is a frequent situation during this final fattening phase, it was also decided to study its effects on WBPTO.

Materials and methods

The experiment was performed with 23 Iberian (Ib) and 26 Landrace (Ld) gilts fit with urinary bladder catheters and fed on either adequate amino acid profile diets containing 120 (A12) or 160 (A16) g crude protein (CP)/kg diet as fed, or diets deficient in lysine (DLys) or threonine (DThr), all based on wheat and gluten feed. Amino acid deficient diets were given at 120 g CP/kg and 160 g CP/kg for the Iberian and Landrace breed, respectively, and provided approximately 35% and 75 % of recommended lysine and threonine, according the ideal protein concept. Each dietary treatment was assayed in at least 5 animals. All diets were approximately isoenergetic and supplied 13.2-13.9 kJ ME/g dry matter (DM). Mean body weights were 26.5 ± 0.68 kg and 30.7 ± 0.63 kg for the Landrace and Iberian groups, respectively. During 10 days animals were fed four times daily at 90% *ad libitum*, allocated in metabolic cages at $21 \pm 1^\circ\text{C}$ room temperature with free access to water. WBPTO was determined by the end product method (Waterlow *et al.*, 1978). On day 11th they were given a single oral dose of ^{15}N -glycine (99 at%, 7.5 mg/kg body weight) and total urine and faeces collection were performed during the following 5 days. Total urinary output was collected every 12 h for the first 72 h and then, every 24 h for the following 48 h. Cumulative ^{15}N excretion in urinary urea and ammonia up to 60h after receiving the ^{15}N -glycine dose was used to calculate N flux, using the mean value of both end products. The ^{15}N analysis was carried out by a continuous flow mass spectrometer (GIRMS) on the two fractions resulting from the separation of urinary ammonia and urea. N retention was determined by classic balance methods. Corresponding analysis of DM, total nitrogen (N) and gross energy (GE) in faecal samples and diets, and total N and GE in urine, were performed.

Data were analysed by two factorials, the first corresponding to a 2 (protein level (PL): 120 (A12) or 160 (A16) g CP/kg) \times 2 (breed (B): Ib vs Ld) and the second, to a 3 (amino acid profile (AAP): adequate (A), lysine deficient (DLys) or threonine deficient (DThr)) \times 2 (B: Ib vs Ld).

Results and discussion

Ld pigs grew faster than Ib pigs ($P<0.001$; Table 1) increasing their BM by 2.31% and 1.81% daily (612 vs. 559 g/d, respectively). Correspondingly, N retention (NR, g/kg BM $^{0.75}/\text{d}$) was greater in the Ld than in the Ib breed ($P<0.05$) but differences were statistically significant only when comparing A16 diet values. Raising PL lead to an increase on NR ($P<0.001$; Table 1) of 27% in the Ld breed ($P<0.01$) and 11.5% in the Ib breed, although the latter change was not statistically significant. PL had no significant effect on the efficiency of N retention (NR:NI). NR:NI was also significantly greater for Ld pigs ($P<0.001$). The results obtained in the Ib breed support previous observations in growing (15 - 50 kg BM) animals of this breed in which the maximum protein deposition (74 g/d) was achieved with a diet supplying 129 g of ideal protein/kg DM (Nieto *et al.*, 2002). Energy metabolizability (m(E)) was higher in the Ld than in the Ib breed ($P<0.01$) with no effect of PL on this parameter.

Nitrogen flux (Q), protein synthesis (S) and degradation (D) were significantly affected by dietary PL ($P<0.001$ for Q; $P<0.05$ for S and D, respectively; Table 2). This effect was more apparent in the Ld breed with increases of 20% in Q (7.73 vs 9.28; $P<0.05$) and 18.3% in S (6.76 vs 8.00; $P<0.05$) as PL increased. Protein degradation also increased in this breed (15.9%) but not significantly. In the Iberian pigs, the parameters of protein turnover studied were not significantly affected by this factor despite the increases observed. S and D were affected by breed (6.21 vs. 7.42 and 5.01 vs. 6.05 g N/kg BM $^{0.75}/\text{d}$, for S and D in Ld and Ib pigs, respectively; $P<0.05$). Differences between breeds reached statistical significance only for A16 diets, with both S and D being 23% greater (6.52 vs 8.00; 5.26 vs 6.47, respectively; $P<0.05$) for the Ld breed. These results suggest a greater N retention in pigs of the Ld breed with the higher PL as a result of increased N flux and protein synthesis along with a non significant enhance in degradation. Under the

Table 1. Energy metabolizability ($m(E)$), nitrogen retention (NR; g/kg $BM^{0.75} d$), efficiency of retention of ingested N (NR/NI) and average daily gain (ADG; %/d) in pigs of Iberian (Ib) and Landrace (Ld) breeds (B) fed on diets adequate in their AA profile but differing in their protein level (PL): 120 (A12) or 160 (A16) g CP/kg.

| | | A12 | A16 | PL | B | PLxB |
|-------|----|---------------|---------------|-----|-----|------|
| m(E) | Ib | 0.840 ± 0.010 | 0.838 ± 0.009 | ns | ** | ns |
| | Ld | 0.866 ± 0.005 | 0.863 ± 0.005 | | | |
| NR | Ib | 1.13 ± 0.06 | 1.26 ± 0.07 | *** | * | ns |
| | Ld | 1.18 ± 0.06 | 1.50 ± 0.06 | | | |
| NR/NI | Ib | 0.407 ± 0.022 | 0.360 ± 0.020 | ns | *** | ns |
| | Ld | 0.477 ± 0.021 | 0.480 ± 0.018 | | | |
| ADG | Ib | 1.78 ± 0.08 | 1.83 ± 0.11 | ns | *** | ns |
| | Ld | 2.28 ± 0.06 | 2.33 ± 0.08 | | | |

Table 2. Nitrogen flux (Q), protein synthesis (S) and degradation (D) (g N/kg $BM^{0.75} d$) in pigs of Iberian (Ib) and Landrace (Ld) breeds (B) fed on diets adequate in their AA profile but differing in their protein level (PL): 120 (A12) or 160 (A16) g CP/kg.

| | | A12 | A16 | PL | B | PLxB |
|---|----|-------------|-------------|-----|----|------|
| Q | Ib | 6.99 ± 0.18 | 8.29 ± 0.59 | *** | ns | ns |
| | Ld | 7.73 ± 0.35 | 9.28 ± 0.45 | | | |
| S | Ib | 5.84 ± 0.24 | 6.52 ± 0.54 | * | * | ns |
| | Ld | 6.76 ± 0.36 | 8.00 ± 0.47 | | | |
| D | Ib | 4.71 ± 0.22 | 5.26 ± 0.53 | * | * | ns |
| | Ld | 5.58 ± 0.33 | 6.47 ± 0.43 | | | |

recommended protein supply, the overall rate of protein turnover was between 33 - 37% greater in the Ld than in the Ib breed.

No differences were found between breeds in fractional rates of protein growth (FGR), synthesis (FSR) or degradation (FBR), *i.e.*, both breeds were mobilizing a similar quantity of protein when expressed in relation to total body protein mass (2.18 vs 2.22 %; 11.27 vs 12.05 % and 9.09 vs 9.83% for Ib and Ld breeds, respectively). FSR was affected by PL ($P<0.01$), achieving an increase of 19% in the Ld group ($P<0.05$) meanwhile a non significant increment (11%) was observed for the Ib breed. FBR was not significantly affected by PL in any of the breeds studied. The quality of dietary protein (AAP) had a highly significant effect on Q, S and D ($P<0.001$; Table 3). A significant interaction was observed between B and AAP ($P<0.05$) for Q and S, due to the greater effect of the DLys diet on the Ld breed. In the Ib breed, Q, S and D were not significantly altered by lysine deficiency. For the Ld breed, however, the decreases were 31% for Q, 42% for S and 38% for D ($P<0.001$).

The decreases in S and D found with the DLys diets confirm the results obtained by other authors (Salter *et al.*, 1990; Roy *et al.*, 2000) who observed that the addition of lysine to a lysine deficient diet lead to a greater protein deposition by increases in both S and D. However, Fuller *et al.* (1987) did not find differences in these parameters when lysine deficient diets were supplemented with this amino acid. No significant effects on protein metabolism were found for DThr diets when compared to adequate AAP diets.

Table 3. N flux (Q), protein synthesis (S) and protein degradation (D) (g N/kg BM^{0.75} d) in pigs of Iberian and Landrace breeds (B) fed on diets containing the recommended concentration of protein for each breed, with either an adequate (A), lysine deficient (DLys) or threonine deficient (DThr) amino acid profile (AAP).

| | DLys | A | DThr | AAP | B | AAPxB |
|---|------|-------------|-------------|--------------|-----|-------|
| Q | Ib | 6.08 ± 0.34 | 6.99 ± 0.18 | 7.26 ± 0.54 | *** | *** |
| | Ld | 6.36 ± 0.31 | 9.28 ± 0.45 | 10.08 ± 0.70 | | * |
| S | Ib | 4.48 ± 0.31 | 5.83 ± 0.23 | 6.04 ± 0.57 | *** | *** |
| | Ld | 4.66 ± 0.28 | 8.00 ± 0.47 | 8.72 ± 0.71 | | * |
| D | Ib | 3.81 ± 0.32 | 4.71 ± 0.22 | 4.93 ± 0.55 | *** | *** |
| | Ld | 4.04 ± 0.29 | 6.47 ± 0.43 | 7.34 ± 0.64 | | ns |

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Protein synthesis in muscle and visceral tissues of Iberian and Landrace pigs fed adequate or lysine deficient diets.

M.G. Rivera-Ferre, R. Nieto & J.F. Aguilera

Unidad de Nutrición Animal, Estación Experimental del Zaidín (CSIC), Camino del Jueves s/n, 18100 Armilla, Granada, Spain

Summary

Protein synthesis rates in tissues have been determined in thirty gilts from Iberian (Ib) and Landrace (Ld) breeds, surgically prepared with indwelling catheters in the external jugular veins, and fed on either adequate aminoacid profile (AAP) diets containing 120 or 160 g crude protein (CP)/kg diet as fed, or diets deficient in lysine (DLys) given at 120 g CP/kg for the Ib and 160 g CP/kg for the Ld breed. Diets were approximately isoenergetic and supplied on average 13.5 kJ ME/g dry matter. Mean body mass (BM) was 21.7 and 24.9 kg for Ib and Ld pigs, respectively. A flooding dose of d₅-phenylalanine was used to obtain fractional protein synthesis rates (k_s , %/d) in muscle, liver and duodenum. Ib pigs had higher muscle k_s values when fed on diets of adequate AAP. Mean k_s values were 7.9 vs 6.3; 8.3 vs 6.3 and 7.7 vs 6.4%/d for *longissimus dorsi*, *biceps femoris* and *semimembranosus* muscles, for the Ib and Ld breeds, respectively ($P<0.01$). When expressed as a proportion of total body mass, muscles were between 20-32% smaller in the Ib breed ($P<0.01$). Dietary protein level (PL) did not affect either muscle k_s or size irrespective of the breed. Lysine deficiency dramatically reduced muscle k_s in both breeds ($P<0.001$). Only *longissimus dorsi* size was significantly ($P<0.05$) decreased by the DLys diet. No significant differences in k_s for liver and duodenum were encountered between breeds (46.8 vs 43.9 %/d for liver and 64.3 vs 66.2 %/d for duodenum, in Ib and Ld pigs, respectively). Contribution of visceral tissues to total BM was higher for the Ib gilts, with liver representing on average 3.35 and 2.85 % and gastrointestinal tract 6.84 and 5.66% of total BM, for Ib and Ld breeds, respectively ($P<0.001$). No significant effects of lysine deficiency were detected for k_s or relative proportions of total BM for visceral tissues. Probably, higher protein degradation rates in muscle of the Ib pig are related to the low protein deposition observed in this native breed.

Keywords: tissue protein turnover, Iberian pig, lysine deficiency

Introduction

Previous work demonstrated the limited capacity of the growing Iberian pig for protein deposition (Nieto *et al.*, 2002). Studies performed at the whole-body level have shown that at approximately 28 kg body mass (BM) protein turnover is between 25-27% lower in this breed in comparison with Landrace pigs (Rivera Ferre *et al.*, 2003). The aim of the present work was to further investigate differences in protein synthesis rates, at the tissue level, between the Landrace and this native breed. The tissues chosen were muscle, liver and duodenum; the first as the main protein reservoir of the organism and the other two for their highly active metabolic rate which can influence nutrient availability to peripheral tissues. This information will presumably enable the manipulation of protein synthetic or degradative responses to changes in nutrients supply at the tissue level, in order to obtain animals with an optimum muscle conformation at the early stages of growth, before they are grazed outdoors. The effect of lysine deficiency (a frequent situation during this final fattening phase) on tissue protein turnover was also studied.

Material and methods

The experiment was performed with 15 Iberian (Ib) and 15 Landrace (Ld) gilts fed on either adequate amino acid profile (AAP) diets containing 120 (A12) or 160 (A16) g crude protein (CP)/kg diet as fed, or diets deficient in lysine (DLys), all based on wheat and gluten feed. Lysine deficient diets were given at 120 g CP/kg and 160 g CP/kg for the Iberian and Landrace gilts, respectively, and provided approximately 35% of recommended lysine according to the ideal protein concept. All diets were approximately isoenergetic and supplied 13.3-13.9 kJ ME/g dry matter. Each dietary treatment was assayed in 5 animals which were fed four times daily at 90% *ad libitum*. At the beginning of the experimental period, animals were surgically implanted with indwelling catheters in each of the exterior jugular veins. After surgery they were placed in mobile cages (1.0 x 1.8 m) at 21 ± 1°C under rigorous cleaning conditions. Once recovered, the animals remained in the conditions previously described for at least 8 days. The average BM during the experiment was 21.7 ± 0.3 kg for the Ib and 24.9 ± 0.5 kg for the Ld pigs, respectively. After this period, the animals were infused during 10 min with a flooding dose of phenylalanine (5-10 times the animal free Phe pool of which approximately 15% was d₅-Phe) to determine fractional protein synthesis rates in tissues. A series of blood samples was taken at 12, 15, 20, 25, 30 and 40 min after the start of the infusion. After that, the animals were slaughtered by injection of a lethal dose of sodium thiopental. The tissues and organs sampled for k_s determination were: muscle (*longissimus dorsi* (*l.d.*)), *biceps femoris* (*b.f.*) and *semimembranosus* (*sm.*)); liver and duodenum (50 cm distal to the pyloric sphincter). Other components of the gastrointestinal tract (stomach, rest of the small intestine and large intestine) were dissected and weighted. To determine the isotope enrichment in the free Phe pool and that incorporated into tissue proteins, the methodology of Calder *et al.* (1992), with slight modifications, was followed.

Data were analysed by two factorials, the first was: 2 (protein level (PL): 120 (A12) or 160 (A16) g CP/kg) x 2 (breed (B): Ib vs Ld) and the second: 2 (amino acid profile (AAP): adequate (A) or lysine deficient (DLys)) x 2 (B: Ib vs Ld).

Results and discussion

Fractional protein synthesis rates (k_s %/d) in muscular tissue were between 25-30 % greater for the Ib breed (P<0.01; Table 1). Mean k_s values for the muscles studied were 7.89 vs 6.32% for *l.d.* (P<0.01), 8.28 vs 6.29% for the *b.f.* (P<0.001) and 7.74 vs 6.36% for the *sm* (P<0.001) for the Ib and Ld breeds, respectively. The protein level (PL) of the diet had no effect upon this parameter. Conversely, muscle weights were larger for the Ld breed. When expressed as a proportion of total BM (%) the average values found were: 2.70 vs 3.36% for the *l.d.* (P<0.01), 1.00 vs 1.47% for the *b.f.* (P<0.001) and 1.17 vs 1.70 for the *sm*. (P<0.001) in Ib and Ld gilts, respectively. Dietary

Table 1. Fractional protein synthesis rate in muscles (k_s %/d) of Iberian (Ib) and Landrace (Ld) pig breeds (B) fed on diets of adequate amino acid profile but differing in their protein level (PL): 120 (A12) or 160 (A16) g CP/kg.

| | | A12 | A16 | PL | B | PLxB |
|--------------------------|----|-------------|-------------|----|-----|------|
| <i>Longissimus dorsi</i> | Ib | 7.77 ± 0.19 | 8.00 ± 0.68 | ns | ** | ns |
| | Ld | 6.23 ± 0.42 | 6.42 ± 0.43 | | | |
| <i>Biceps femoris</i> | Ib | 8.15 ± 0.67 | 8.40 ± 0.37 | ns | *** | ns |
| | Ld | 6.27 ± 0.42 | 6.31 ± 0.14 | | | |
| <i>Semimembranosus</i> | Ib | 7.61 ± 0.31 | 7.86 ± 0.36 | ns | ** | ns |
| | Ld | 6.09 ± 0.58 | 6.64 ± 0.46 | | | |

PL had no effect on muscle size irrespective of the breed. Thus, despite each day the Ld pig synthesised a smaller proportion of its muscle protein, they had a greater muscle protein pool, resulting in greater absolute protein synthesis values. Probably, higher protein degradation rates in the Ib breed, among other mechanisms, are involved in the lower muscle protein pool and protein deposition observed for this breed. The greater muscle k_s values found for the Ib pig might be related to the higher abundance and diameter of type I fibers described for this breed (Serra *et al.*, 1998). These fibers, of eminently oxidative metabolism, show k_s values greater than those of type II (Garlick *et al.*, 1989) and are related to improved meat quality.

Lysine deficient diets reduced severely muscle k_s in both breeds with decreases of 43-47% in Ib and 48-50% in Ld pigs, respectively (Table 2; $P<0.001$). In both type of pigs, the muscle most affected by the lysine deficiency was the *longissimus dorsi* which reduced not only its absolute weight but also its proportion of total BM ($P<0.05$), meanwhile the proportions of the other two muscles remained constant.

Liver and duodenum did not show significant differences in k_s between breeds when animals were fed on adequate AAP diets. Dietary PL had also no effect upon this parameter. The average values obtained in pigs of the Ib and Ld breeds were 46.8 and 44.3%/d for the liver and 64.3 and 66.1%/d for the duodenum, respectively. When comparing k_s values in these organs, a significant interaction between both factors (AAP and B) was observed due to the opposite tendencies found in the two breeds (Table 3; $P<0.05$). In the Ib pigs fed on the DLys diet, k_s tended to increase or remained constant meanwhile for the Ld pigs offered the DLys diet the trend was a decrease of k_s . Nevertheless, the effects were rather moderate compared to those on muscle.

The relative weights of visceral organs were higher in the Iberian gilts, except for the small intestine (Table 4; $P<0.001$). The liver and total gastrointestinal tract were 18 and 21% higher, respectively, and the proportions for the stomach and large intestine between 38-39% larger

Table 2. Fractional protein synthesis rates in muscles (k_s , %/d) of Iberian (Ib) and Landrace (Ld) pig breeds (B) fed on diets containing the recommended concentration of protein for each breed, with either an adequate (A) or lysine deficient (DLys) amino acid profile (AAP).

| | | DLys | A | AAP | B | AAPxB |
|--------------------------|----|-------------|-------------|-----|----|-------|
| <i>Longissimus dorsi</i> | Ib | 4.26 ± 0.48 | 7.77 ± 0.19 | *** | ** | ns |
| | Ld | 3.23 ± 0.22 | 6.42 ± 0.44 | | | |
| <i>Biceps femoris</i> | Ib | 4.28 ± 0.51 | 8.15 ± 0.67 | *** | ** | ns |
| | Ld | 3.35 ± 0.41 | 6.30 ± 0.15 | | | |
| <i>Semimembranosus</i> | Ib | 4.36 ± 0.26 | 7.61 ± 0.31 | *** | * | ns |
| | Ld | 3.49 ± 0.29 | 6.63 ± 0.46 | | | |

Table 3. Fractional protein synthesis rates in viscera (k_s , %/d) of Iberian (Ib) and Landrace (Ld) pig breeds (B) fed on diets containing the recommended concentration of protein for each breed, with either an adequate (A) or lysine deficient (DLys) amino acid profile (AAP).

| | | DLys | A | AAP | B | AAPxB |
|-----------------|----|------------|------------|-----|----|-------|
| <i>Liver</i> | Ib | 50.7 ± 4.9 | 46.6 ± 2.2 | ns | * | * |
| | Ld | 36.0 ± 3.4 | 46.0 ± 1.6 | | | |
| <i>Duodenum</i> | Ib | 75.8 ± 5.7 | 60.5 ± 5.5 | ns | ns | * |
| | Ld | 54.7 ± 5.1 | 65.7 ± 5.2 | | | |

Table 4. Contribution of viscera to total BM (%) in Iberian (Ib) and Landrace (Ld) pig breeds (B) fed on diets of adequate amino acid profile but differing in their protein level (PL): 120 (A12) or 160 (A16) g CP/kg.

| | | A12 | A16 | PL | B | PLxB |
|------------------------|----|-------------|-------------|----|-----|------|
| Liver | Ib | 3.22 ± 0.14 | 3.48 ± 0.11 | ns | *** | ns |
| | Ld | 2.86 ± 0.11 | 2.84 ± 0.10 | | | |
| Small Intestine | Ib | 3.33 ± 0.10 | 3.37 ± 0.19 | ns | ns | ns |
| | Ld | 3.21 ± 0.18 | 3.07 ± 0.20 | | | |
| Gastrointestinal tract | Ib | 6.92 ± 0.18 | 6.75 ± 0.37 | ns | *** | ns |
| | Ld | 5.73 ± 0.18 | 5.58 ± 0.30 | | | |

(P<0.001; data not shown). These relative proportions were not affected either by dietary PL or amino acid deficiency.

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Protein accretion in pigs as affected by previous feeding a high fibre diet up to 50 or 80 kg body weight

G. Skiba, H. Fandrejewski, S. Raj & D. Weremko

*The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences,
05-110 Jabłonna, Poland*

Summary

The study was conducted to investigate protein accretion in pigs during temporary feeding a high fibre diet as well as during following realimentation. Study was carried out on 54 pigs fed *ad libitum*. Control pigs (the C) were continuously fed the diet B (13.1 MJ EM, 38.2 g crude fibre) from 25 to 105 kg. Restricted feeding lasted from 25 kg to 50 (GM₅₀ pigs) or from 25 to 80 kg (GM₈₀ pigs) and was imposed by feeding a high fibre diet (the GM, 11.6 MJ ME, 76.6 g crude fibre), which was formulated by dilution the B diet with 20 % of grass meal. During realimentation period up to 105 kg all pigs were fed the diet B. Comparative slaughter technique was used to establish an accretion of protein in the body. During restriction period pigs fed a high fibre diet grew slower and as a consequence deposited less ($P<0.05$) but better utilised protein. During realimentation the GM₅₀ pigs grew 72 g/day faster ($P<0.01$) and deposited 17 g more protein ($P<0.01$), but only up to 80 kg, as compared to the C pigs. The GM₈₀ pigs grew at similar rate but tended to deposit more protein (16 g/day) than the C animals. The compensatory growth of the GM₅₀ pigs was observed, as during whole growth period they grew at similar rate and deposited similar amount of protein as the C pigs.

Keywords: pig, protein deposition, compensatory growth

Introduction

In some modified production systems sustaining welfare of animals (e.g. out-door) young pigs have temporary restricted access to concentrate diet but unrestricted access to roughage feeds characterised by high fibre content. Afterwards they are given a concentrate *ad libitum* again. It is well known that, after change from restricted to unrestricted feeding, pigs grow with higher rate (Fandrejewski 1994; Skiba et al., 1997) named as a compensatory growth, which is usually imposed by previous restriction of daily allowances or nutritive value of feeds (e.g. decrease of protein content). However, there is a lack of information on compensatory response of pigs previously fed high fibre diet. It is supposed that they should differ in response imposed by decreasing daily allowances or protein intake. This study was conducted to investigate a protein gain of pigs during temporary feeding (up to 50 or 80 kg) a high fibre diet as well as during following realimentation period up to 105 kg.

Material and methods

Experiment was carried out on 54 crossbreed pigs (Landrace × White Large × Duroc) fed *ad libitum*. For the sake of technical possibilities of pig housing roughage feed used in out door system was replaced with grass meal. Basal diet (B) contained, per kg fresh weight (calculated values), 13.1 MJ ME and 8.2 g digestible lysine, 38.2 g crude fibre. Experimental diet (GM) was obtained by dilution the diet B with 20 % of grass meal and contained, per kg fresh weight (calculated values), 11.6 MJ ME and 6.5 g digestible lysine, 76.6 g crude fibre. Control pigs (C)

were fed from 25 to 105 kg the diet B. The GM₅₀ pigs were restricted from 25 up to 50 kg, whereas the GM₈₀ from 25 to 80 kg by feeding them the diet GM. During realimentation period from 50 (the GM₅₀ group) or 80 (the GM₈₀ group) up to 105 kg pigs were fed the diet B. Comparative slaughter technique was used to establish an accretion of protein in the body. Protein content in the body was determined according to AOAC (1994) method. Animals were slaughtered at 25 (n=6), 50 (n=12; 6 from the C, 3 from GM₅₀ and 3 from GM₈₀), 80 (n=18; 6 from each group) 105 kg (n=18; 6 from each group). Accretion of protein in the pig body was calculated by difference between final and initial content. Statistical analyses were performed by ANOVA analysis of variance using Statgraphics version 6.0 Plus software.

Results

During restriction feed intake did not differ between groups and amounted on average 1.90 kg (from 25 to 50 kg) and 2.56 kg (from 50 to 80 kg), (Table 1). However, intake of digestible protein through pigs consuming a grass meal diet was lower ($P<0.01$) during growth from 25 to 50 kg by 57 (on average) and by 80 g/day in the GM₈₀ pigs restricted fed from 50 to 80 kg as compared to the C pigs. As a consequence during considerable period of restriction restricted pigs grew at slower rate ($P<0.01$) by 97 (on average for GM₅₀ and GM₈₀ pigs) and by 74 g/day, respectively. Daily protein deposition was lower respectively by 9 g ($P<0.01$) (on average for GM₅₀ and GM₈₀ pigs) and by 6 g (difference insignificant) as compared to the C pigs. Pigs fed the GM diet utilised protein by 7 % better ($P<0.05$) during both restriction

Table 1. Performance of pigs and protein deposition in the body during particular growth period.

| Item | Group | Growth period | | | |
|---|------------------|--------------------|--------------------|-----------|------------------|
| | | 25-50 kg | 50-80 kg | 80-105 kg | 25-105 kg |
| Feed intake (kg/day) | C | 1.89 | 2.53 ^a | 3.05 | 2.45 |
| | GM ₅₀ | 1.94 | 2.78 ^b | 3.09 | 2.64 |
| | GM ₈₀ | 1.87 | 2.60 ^a | 3.10 | 2.47 |
| | s.e. | 0.016 | 0.036 | 0.060 | 0.034 |
| Digestible protein intake (g/day) | C | 286 ^B | 394 ^B | 472 | 378 ^B |
| | GM ₅₀ | 232 ^A | 431 ^B | 478 | 383 ^B |
| | GM ₈₀ | 225 ^A | 315 ^A | 480 | 328 ^A |
| | s.e. | 4.13 | 7.92 | 9.24 | 6.59 |
| Average gain (g/day) | C | 892 ^B | 951 ^B | 1002 | 946 ^B |
| | GM ₅₀ | 806 ^A | 1023 ^C | 958 | 938 ^B |
| | GM ₈₀ | 783 ^A | 877 ^A | 947 | 846 ^A |
| | s.e. | 0.012 | 0.015 | 0.030 | 0.017 |
| Protein deposition (g/day) | C | 129 ^b | 132 ^A | 134 | 135 |
| | GM ₅₀ | 122 ^a | 149 ^B | 131 | 136 |
| | GM ₈₀ | 121 ^a | 126 ^A | 150 | 129 |
| | s.e. | 2.06 | 2.70 | 4.09 | 1.88 |
| Protein utilisation, deposited/consumed | C | 0.450 ^a | 0.336 ^a | 0.285 | 0.358 |
| | GM ₅₀ | 0.530 ^b | 0.346 ^a | 0.275 | 0.354 |
| | GM ₈₀ | 0.541 ^b | 0.408 ^b | 0.323 | 0.394 |
| | s.e. | 0.006 | 0.009 | 0.10 | 0.007 |

A, B, C $P<0.01$

a, b, c $P<0.05$

period. During realimentation GM₅₀ pigs (compensating from 50 kg) consumed more ($P<0.01$) feed (2.78 vs 2.53 kg) and consequently also more digestible protein (431 vs 394 g/day) as well as grew at faster rate (1023 vs 951 g/day) and deposited more protein in the body (149 vs 132 g/day) but similarly utilised a protein (34.6 vs 33.6 %), (Table 1) during growth from 50 to 80 kg bw. Pigs compensating from 80 kg (the GM₈₀) had similar feed (3.10 vs 3.05 kg daily) and protein (480 vs 472 g/day) intake, grew insignificantly slower (947 vs 1002 g/day) but utilised protein slightly better (32.3 vs 28.45 %) and tended to deposit more protein in the body (150 vs 134 g/day) as compared to the C pigs. At this period of growth a performance and protein deposition and utilisation of the GM₅₀ pigs did not differ from the C pigs.

Discussion

Supplementing a basal diet with grass meal did not influence a voluntary feed intake of pigs but decreased concentration of energy and nutrients. During restriction both underfed groups of pigs consumed nearly 20 % less protein but their growth rate was only by 12 % and protein deposition in the body by 7 % lower compared to the normal fed counterparts. Lower difference between growth rate (protein accretion) and protein consumption could be explained by significantly better protein utilisation in underfed pigs regardless of the duration of restriction as both underfed groups utilised protein 7 % better than control pigs. In earlier work on compensatory growth a response of pigs to restriction for protein (e.g. de Greef, 1992) as well as feed intake (e.g. Bikker, 1994) was also lower protein deposition strongly correlated to the intensity (duration) of the restriction. It could be supposed that decrease in protein deposition during this period of growth resulted from highly decreased in both protein synthesis and degradation that had been shown in earlier study on rats restricted by diluting a basal diet with cellulose powder (Schadereit et al., 1995). In our study a compensatory growth (body gain) was found only in the pigs previously fed a grass meal diet up to 50 kg, which grew over 7 % faster than control pigs. This higher growth rate lasted approximately four weeks after change from restriction to realimentation (up to c.a. 80 kg) during later time of realimentation compensatory response gradually disappeared. Compensatory growth was closely connected with higher feed intake as these pigs consumed daily 10 % more feed what caused greater (by 13 %) protein accretion as compared to adequate fed pigs. Prolonged duration of previous restriction up to 80 kg bw did not cause higher growth rate of pigs, although the GM₈₀ pigs tended to deposit more protein. So, this group of pigs was able to show compensatory protein gain without higher feed intake. It proved that compensatory protein gain does not have to be closely connected with increased appetite of realimented pigs. Higher protein deposition found in previously restricted animals are in agreement with other studies carried out on pigs previously restricted for protein (Tulis et al., 1986, Fandrejewski 1994) as well as feed intake (Skiba and Fandrejewski 1997). All the factors influencing compensatory protein gain phenomenon are still unknown. Our data indicate that it is not connected with better protein utilisation as both previously underfed groups of pigs during realimentation utilised protein similarly as the control pigs. Also better protein digestibility seems not to play a crucial role because it was similar between realimented and control animals (data not showed). Literature data suggest that compensatory response could be partly explained by change in rate of protein turn-over, as in other studies realimented pigs has shown increase in both protein synthesis and degradation (Schadereit et al., 1995). Increase of protein in the muscle depends on satellite cells proliferation, which fusing with the muscle fibres increase their possibility for protein synthesis. Data given by Oksbjerg et al., (2002) proved that DNA content in the muscles of realimented pigs is higher. Those authors suggested that it could be caused by increased satellite cell proliferation, which can contribute to the compensatory response. Data given by Therkildsen et al., (2002) also suggested that muscle protein synthesis predominate protein degradation following change from restriction to realimentation.

Conclusion

Based on the results it could be concluded that pigs, previously temporary fed a high fibre diet, are able to show a compensatory growth during later growth stage. Compensatory response involves not only body mass gain but also amount of protein deposited in daily body gain.

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Utilisation of essential milk amino acids for body gain in mink kits

A-H. Tauson, R. Fink, A. Chwalibog & N.E. Hansen

Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark

Summary

Utilisation of essential amino acids (EAA) in mink milk was estimated by use of 36 mink dams and their litters. Milk and EAA intake of individual kits, and kit body composition including accretion of EAA were measured during lactation weeks 1 through 4 (9 dams and litters each week). Utilisation of EAA was high during the first two weeks of life (close to 60% for all EAA) but declined during weeks 3 and 4, the utilisation of all EAA being below 40% during week 4. Lysine was determined to be most rate limiting for growth, and it was also most efficiently utilised.

Keywords: growth, retention, efficiency

Introduction

The mink is a strict carnivore, and a polytocous species giving birth to altricial young. Neonatal mink kits weigh about 10 g, are almost devoid of mobilisable body energy reserves, almost hairless and lack thermoregulatory capacity. Milk is the only source of nutrients until an age of approximately 24–25 days when the kits start to eat solid food in addition to milk. Mink kits have capacity for rapid growth, the average relative growth rate being 12% per day during the first 21 days (Tauson, 1994). This implies a substantial protein accretion, for which milk is the single supply of amino acids (AA). The milk AA must sustain the requirements both for protein accretion in the body and for maintenance, requirements that are distinctly different (Fuller *et al.*, 1989) and not constant, maintenance requirements making up only a minor part in the early neonatal period and then increasing successively. There is paucity of data on the efficiency of utilisation of milk AA for body accretion and therefore, the objective of this investigation was to establish how efficiently mink kits use milk AA for accretion in tissue protein during the course of the four first weeks of life.

Materials and methods

A total of 36 2-year-old mink dams of the standard brown genotype were used. Each dam and litter were measured once either in lactation week 1 (day 3–5), 2 (day 10–12), 3 (day 17–19) or 4 (day 24–26), hence 9 dams each week. The animals were fed *ad libitum* with a conventional wet lactation period mink diet. In each measurement period milk intake of individual kits was determined by means of deuterium dilution technique as described by Fink *et al.* (2001). At the end of each measurement period milk samples were collected from the dams under anaesthesia, and by complete emptying of the udder by use of a laboratory vacuum pump. At the same occasions one or two kits per litter were killed for analyses of body composition. Chemical analyses procedures on milk and bodies were as described by Fink *et al.* (2001) and Hansen (1982). Amino acids were analysed according to EC (1998). Deuterium in plasma was analysed by means of isotope ratio mass spectroscopy.

The contents of individual AA in mink milk and kit bodies were calculated as mg of each individual AA per g of total AA in the sample. Amino acid ingestion was calculated based on daily milk intake of individual kits, and the AA contents of milk from the respective dams. Calculations of daily gain, body AA composition and accretion in kit bodies were based on live

weights of individual kits and average litter chemical / AA composition under the assumption that they remained constant during the 48 h measurement periods. The efficiency of utilisation of individual AA was calculated as retained AA divided by the amount of ingested AA. Response variables were tested for the fixed effects of lactation week / kit age by the GLM-procedure in SAS (SAS Institute Inc., 1990).

Results

Milk intake increased from 13.0 g per kit and day during week 1 to 32.8 g in week 4 ($P<0.001$). The intake of milk per g body gain was lowest (3.5 g) during the first week of life and then increased successively to 5.3 g during week 4 ($P<0.001$). Kit live weights increased from 22.0 g in week 1 to 130.5 g in week 4 ($P<0.001$), and simultaneously the recorded daily gains were increased from 3.7 g to 6.4 g ($P<0.001$). Similarly, the accretion of protein, fat and energy in kit bodies increased significantly throughout the first 3 weeks of life, whereas only a moderate increase in protein accretion and decreases in daily fat and energy accretion occurred during week 4 (data not shown).

The most abundant AA in milk were glutamate, leucine and aspartate making up about 40% of total AA. The branched chained AA made up slightly more than 20% and sulphur containing AA accounted for less than 5% of total milk AA. Only the proportions of isoleucine and arginine remained stable throughout the measurement period. Leucine and valine showed increasing concentrations whereas the concentration of all other EAA declined significantly as lactation progressed, hence resulting in the sum of all EAA being significantly lower in weeks 3 and 4 than during weeks 1 and 2 (data not shown).

The sum of glutamate, aspartate and leucine made up about 32% of body AA, branched chain AA about 16% and sulphur containing AA about 4%. Kit body AA composition was strongly affected by kit age, the exception being phenylalanine which showed a non-significant increase. The proportion of arginine increased clearly whereas histidine had a numerically small but significant increase. The patterns of methionine and threonine showed significantly lower concentrations in weeks 2 and 3 than during weeks 1 and 4. Concentrations of all other EAA declined as lactation progressed, resulting in a successive decrease in total EAA (data not shown).

The ratio between AA in body and milk can be used as a measure of how limiting the individual AA are for body growth: a ratio above 1 would suggest this EAA to be rate limiting for growth. The only EAA that had a ratio above 1 throughout the measurement period was lysine, whereas the ratios of phenylalanine and histidine increased for each week, from values below 1 in week 1 to above 1.1 in week 4. For arginine the ratio increased slightly and reached values over 1 in weeks 3 and 4, whereas valine decreased from slightly over to clearly below 1 during weeks 1 through 4. Threonine, isoleucine, methionine and leucine never showed values over 1, leucine having the lowest ratio of around 0.6 (Figure 1).

The efficiency of utilisation of most EAA for accretion in body protein was strongly affected by kit age. Generally, milk EAA were efficiently utilised during the first week of life with values ranging from 74.7% (lysine) to 42.1% (leucine), and an overall efficiency of utilisation of EAA of 58.4%. There were non-significant tendencies for improved utilisation of lysine (74.7 to 78.2%), phenylalanine (61.0 to 70.0%), histidine (62.4 to 68.8%), arginine (61.3 to 70.4%) and all EAA (58.4 to 60.2%) from week 1 to week 2.

During weeks 3 and 4 the efficiency of EAA utilisation declined, and for all EAA the average utilisation was 38.1% during week 4. Exceptions from the generally impaired utilisation were phenylalanine and methionine the utilisation of which only declined non-significantly ($P=0.08$). Amino acids which showed the relatively largest declines were valine, leucine, isoleucine, arginine and lysine (Table 1).

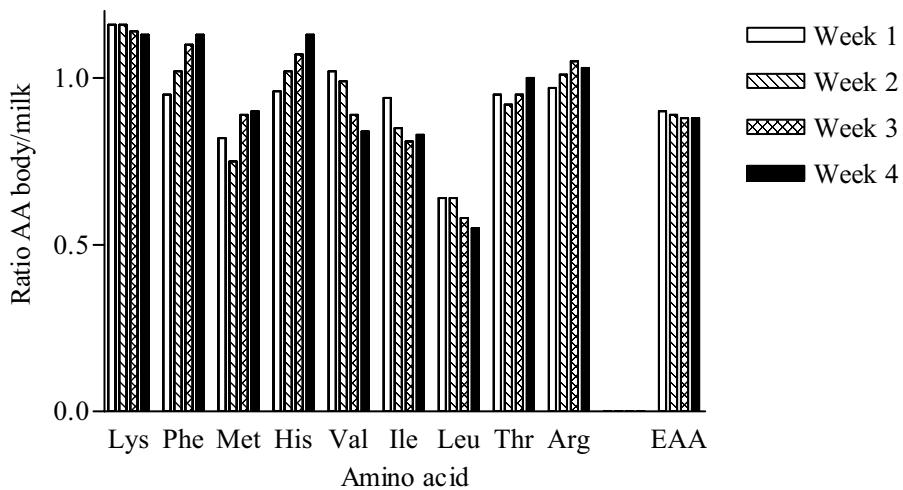


Figure 1. Ratio between EAA in kit bodies and milk.

Table 1. Efficiency of utilisation of essential milk amino acids for body accretion in mink kits, (%).

| Amino acid | Lactation week | | | | RMSE | <i>P</i> , week |
|---------------|--------------------|-------------------|--------------------|--------------------|------|-----------------|
| | 1 | 2 | 3 | 4 | | |
| Lysine | 74.7 ^{ac} | 78.2 ^a | 61.6 ^{bc} | 49.5 ^b | 15.8 | <0.001 |
| Phenylalanine | 61.0 ^{ab} | 70.0 ^a | 59.9 ^{ab} | 49.8 ^b | 17.6 | 0.08 |
| Methionine | 55.0 | 49.1 | 49.1 | 39.9 | 12.4 | 0.08 |
| Histidine | 62.4 ^{ab} | 68.8 ^a | 58.3 ^{ab} | 49.6 ^b | 15.4 | 0.04 |
| Valine | 66.2 ^a | 66.6 ^a | 48.0 ^b | 36.7 ^b | 13.5 | <0.001 |
| Isoleucine | 61.0 ^a | 57.8 ^a | 44.0 ^b | 35.8 ^b | 13.2 | <0.001 |
| Leucine | 42.1 ^a | 41.8 ^a | 31.3 ^b | 23.8 ^c | 8.7 | <0.001 |
| Threonine | 63.1 ^a | 61.6 ^a | 52.1 ^{ac} | 43.8 ^{bc} | 13.6 | 0.008 |
| Arginine | 61.3 ^{ac} | 70.4 ^a | 55.6 ^{bc} | 44.0 ^b | 14.5 | 0.001 |
| All essential | 58.4 ^a | 60.2 ^a | 47.6 ^b | 38.1 ^b | 12.6 | <0.001 |

RMSE: Root mean square error.

a,b,c Values that share no common superscript differ significantly (*P*<0.05).

Discussion

The ratio between EAA in the body and the milk can be used to express which EAA that are the most rate limiting for growth, and simultaneously it can be expected that the most limiting EAA are those that are most efficiently utilised. In this study only lysine constantly had a higher concentration in the body than in milk and therefore could be considered as most rate limiting for growth. Phenylalanine, histidine, valine and arginine had ratios higher than 1 in part of the measurement period but all other EAA had ratios below or even far below 1, indicating that they were provided in excess and therefore were used for maintenance and as energy source. These results are to some extent conflicting with corresponding data for rat pups (Davis *et al.*, 1993), for which all EAA except threonine and possibly leucine would be rate limiting for growth. Similar to our data the highest ratios were found for lysine, phenylalanine and histidine, but contrary to our

results also methionine had a high ratio. Using the ratio of body/milk EAA as an indicator of EAA utilisation thus would suggest that the mink kits utilised lysine the best, being followed by phenylalanine, histidine, valine and arginine. This was indeed the case, and leucine which had the lowest ratio body/milk had consequently the lowest value for utilisation. Our values for utilisation are generally lower than those for rat pups (Davis *et al.*, 1993) but show the same general trend with the highest rate of utilisation of the most limiting EAA and the lowest for those with the highest provision by milk. Because our ratios between body and milk EAA generally were lower than those of Davis *et al.* (1993) the lower values for milk EAA utilisation were expected and allowing for a greater part of the EAA being used for maintenance and as energy source.

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Food absorption and conversion monitored in cannulated rainbow trout

G.J. Vianen¹, G.E.E.J.M. van den Thillart¹, M. Markwat¹, M.F. Onderwater¹, M.P. de Zeeuw¹ & S.J. Kaushik²

¹ *Institute of Biology, Department of Integrative Zoology, Kaiserstraat 63, P.O. Box 9516, 2300 RA Leiden, The Netherlands*

² *Unité Mixte INRA-IFREMER, Fish Nutrition Research Laboratory, 64310, Saint Péé sur Nivelle, France*

Summary

Rainbow trout were fed either a fish meal (FM) diet or a plant protein (PP100) diet. After 4-6 weeks of adaptation the fish were fitted with a permanent cannula both in the stomach and dorsal aorta. Via the gastric cannula each animal received either a bolus of food (0.5% BW) corresponding to the diet to which the fish was adapted or a bolus of saline (15 ml kg⁻¹) as control. Following the artificial feeding the ammonia excretion was monitored at timed intervals over 48 hours. Simultaneously blood samples were withdrawn via the dorsal aorta cannula in order to measure the transient changes in amino acid profiles and glucose. Both a bolus FM or PP100 diet resulted in a 2-3 fold increase of the ammonia production within 30 minutes. The ammonia excretion remained elevated around these levels until 9.5 hours post-feeding followed by a gradual decrease, reaching basal levels after 24 hours. Some amino acid levels in whole blood showed a significant increase post-feeding indicating the appearance of dietary amino acids in the systemic circulation. Plasma glucose levels increased in both diet groups likely due to gluconeogenesis. Monitoring the transient changes of amino acid and glucose levels in combination with ammonia excretion provides important information on the digestibility of the food and the capacity of the liver to transaminate and deaminate dietary free amino acids.

Keywords: *food conversion, continuous monitoring, rainbow trout*

Introduction

In aquaculture fish meal replacement by plant protein sources has received increasing attention during recent years (Lee *et al.*, 2002). However, little is known regarding the dynamics with which the nutrients - especially amino acids - from different dietary composition are digested, absorbed and converted mainly by the liver. It is hypothesized that a diet of plant protein origin has an imbalanced amino acid composition (with respect to the essential amino acids) such that other amino acids will be in excess. This amino acid surplus will be deaminated and metabolized (gluconeogenesis) and/or converted into lipid.

In order to obtain the required essential/non-essential amino acid profile the insufficient essential amino acids have to be supplemented to the plant protein diet. Consequently, it is expected that the absorption of these added amino acids will be accelerated resulting in an earlier appearance of these amino acids in the circulation (Schuhmacher *et al.*, 1997).

In this study we aim to monitor the digestion of dietary protein and the capacity of the liver to metabolise the absorbed amino acids in cannulated rainbow trout.

Materials and methods

Rainbow trout were fed either a fish meal (FM) diet or a plant protein (PP100) diet (Table 1). After 4-6 weeks of adaptation the fish were captured and placed in the experimental setup. After

Table 1. Experimental diets¹.

| Ingredients (g/kg) | FM | PP100 | Analytical composition | FM | PP100 |
|-----------------------------|--------|--------|--------------------------|-------|-------|
| Fish Meal | 637.99 | 0 | Energy (kJ/g DM) | 22.71 | 23.63 |
| Corn gluten meal | 0 | 232.36 | Phosphorus (% DM) | 1.89 | 1.34 |
| Wheat gluten | 0 | 200.00 | Ash (% DM) | 7.63 | 5.86 |
| Extruded Peas (Aquatex) | 0 | 163.26 | Amino acids (%DM) | | |
| Rapeseed meal (PrimorOO) | 0 | 100.00 | ARG | 3.59 | 3.32 |
| Extruded whole wheat | 203.37 | 0 | LYS | 4.03 | 3.51 |
| Fish oil | 128.65 | 158.67 | HIS | 0.92 | 1.26 |
| Binder (Na alginate) | 10.00 | 10.00 | ILE | 1.85 | 2.22 |
| Mineral premix ¹ | 10.00 | 10.00 | LEU | 3.39 | 4.20 |
| Vitamin premix ¹ | 10.00 | 10.00 | VAL | 2.18 | 2.86 |
| CaHPO4.2H2O (18%P) | 0 | 40.00 | MET | 1.21 | 0.99 |
| L-Arg | 0 | 12.47 | PHE | 1.68 | 2.04 |
| L-His | 0 | 3.96 | THR | 2.41 | 2.32 |
| L-Lys | 0 | 27.62 | TYR | 1.40 | 1.57 |
| DL-Met | 0 | 4.11 | Asp | 4.27 | 2.10 |
| L-Trp | 0 | 2.73 | Glu | 7.46 | 12.89 |
| L-Thr | 0 | 8.29 | Ser | 2.13 | 2.11 |
| L-Ile | 0 | 7.14 | Pro | 2.11 | 3.31 |
| L-Val | 0 | 9.37 | Gly | 3.12 | 1.41 |
| Analytical composition | | | Ala | 2.98 | 2.04 |
| Dry matter % | 94.42 | 91.61 | Sum AA ² | 44.73 | 48.15 |
| Protein (%DM) | 51.53 | 48.58 | Sum EAA ² | 22.66 | 24.29 |
| Lipid (%DM) | 19.73 | 19.21 | Sum NEAA ² | 22.07 | 23.86 |
| Starch (%DM) | 13.79 | 10.51 | EAA/NEAA | 1.03 | 1.02 |

¹Data of experimental diets obtained from INRA-IFREMER (Saint Peé sur Nivelle, France)

²AA: amino acids; EAA: essential amino acids (in upper case letters); NEAA: non essential amino acids

three days the animals were fitted with a permanent cannula both in the stomach and dorsal aorta followed by 36-48 hours recovery. Via the gastric cannula each animal received either a bolus of food (0.5% BW) corresponding to the diet to which the fish was adapted or a bolus of saline (15 ml kg⁻¹) as control. In this way there were four groups: 1) a fish meal adapted/fish meal bolus group (FM-FM; n=7); 2) a fish meal adapted/saline bolus group (FM-saline; n=6); 3) a PP100 adapted/PP100 bolus group (PP100-PP100; n=7) and 4) a PP100 adapted/saline bolus group (PP100-saline; n=5).

Following the artificial feeding the ammonia excretion was monitored individually by taking water samples at timed intervals over 48 hours. Simultaneously blood samples were withdrawn via the dorsal aorta cannula in order to measure the transient changes in amino acid profiles and plasma glucose.

Ammonia was measured using the enzymatic kit of Roche B.V. (Almere, The Netherlands) and plasma glucose was measured with the enzymatic kit of Instruchemie (Hilversum, The Netherlands). Amino acids were analyzed with an HPLC-system according to the method of Gratzfeld-Huesgen (1998).

Table 2. Resting amino acid levels (at t=0h) in trout whole blood.

| EAA | FM | | PP100 | | NEAA | FM | | PP100 | |
|-----|-------|-------|-------|-------------------|------------------|-------|-------|-------|-------------------|
| | Mean | Std | Mean | Std | | Mean | Std | Mean | Std |
| His | 702.2 | 161.8 | 659.3 | 160.7 | Ala | 448.7 | 114.5 | 407.8 | 83.1 |
| Ile | 308.8 | 77.0 | 280.6 | 44.6 | Arg ¹ | - | - | - | - |
| Leu | 162.5 | 41.3 | 165.4 | 78.4 | Asp | 67.8 | 30.5 | 73.1 | 25.4 |
| Lys | 307.9 | 34.8 | 368.9 | 233.2 | Asn | 74.9 | 9.4 | 52.0 | 15.1 [#] |
| Met | 96.3 | 28.6 | 90.8 | 28.8 | Cys | 359.4 | 74.0 | 341.5 | 53.8 |
| Phe | 222.7 | 61.8 | 190.8 | 8.6 | Glu | 248.2 | 55.0 | 210.8 | 46.3 |
| Thr | 265.6 | 51.3 | 252.3 | 88.4 | Gln | 320.4 | 45.3 | 318.0 | 95.7 |
| Trp | 391.4 | 109.0 | 463.1 | 63.1 [#] | Gly | 488.4 | 85.2 | 487.0 | 59.5 |
| Tyr | 72.7 | 13.9 | 76.4 | 14.5 | Pro | 46.1 | 40.0 | 41.0 | 41.5 |
| Val | 76.0 | 13.3 | 69.4 | 17.7 | Ser | 68.1 | 22.8 | 57.8 | 7.9 |

[#]: P<0.05 between FM and PP100 group

¹Arginine could not be detected in whole blood

Results and discussion

This study is the first where food digestion and conversion in fish can be followed without handling.

Both a bolus FM or PP100 diet resulted in a 2-3 fold increase of the ammonia production within 30 minutes (figure 1A). The ammonia excretion remained elevated around these levels until 9.5 hours post-feeding followed by a gradual decrease, reaching basal levels after 24 hours. These results indicate that deamination of amino acids by the liver in the PP100 fish did not differ from the FM animals.

Plasma glucose levels increased in both diet-fed groups (figure 1B) likely due to gluconeogenesis from amino acids (Suarez & Mommsen, 1987).

Resting amino acid levels (at t=0h) did not differ between the FM and PP100 group, except for significantly lower levels of Asn and elevated levels of Trp in the PP100 group (Table 2).

Most amino acids i.e. the essential amino acids His, Leu, Lys, Thr, Trp and Tyr and the non-essential amino acids Ala, Asp, Asn, Glu and Gly did not change in response to the food bolus.

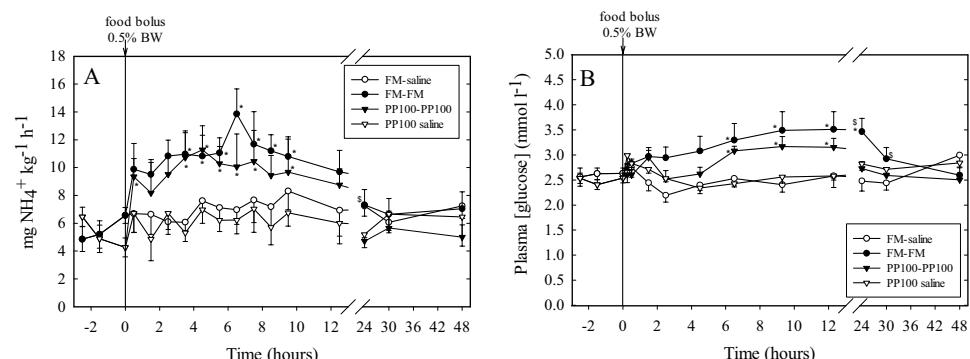


Figure 1. Ammonia production (1A) and plasma glucose (1B) in rainbow trout before and after a food bolus (0.5% BW). Values are expressed as mean ± SEM. *: P<0.05 versus the initial value (t=0h). \$: P<0.05 between the FM-FM and PP100-PP100 group.

These results indicate a very high capacity of the liver to metabolize these amino acids, which is supported by the rapid stepwise increase of the ammonia excretion in both the FM-FM and PP100-PP100 diet group.

Marked changes in amino acid levels after a bolus FM or PP100 were observed regarding the essential amino acids Val (figure 2A), Met (figure 2B), Phe and Ile and the non-essential amino acids Gln (figure 3A), Pro (figure 3B), Cys and Ser. These observations point to a lower capacity of the liver to metabolize these dietary amino acids.

In conclusion: Insertion of a permanent cannula in the stomach in combination with dorsal aorta cannulation provides a powerful tool to follow food absorption and conversion in fish without handling. Low stress levels and continuous monitoring will likely provide new insights in the dynamics of food absorption and conversion.

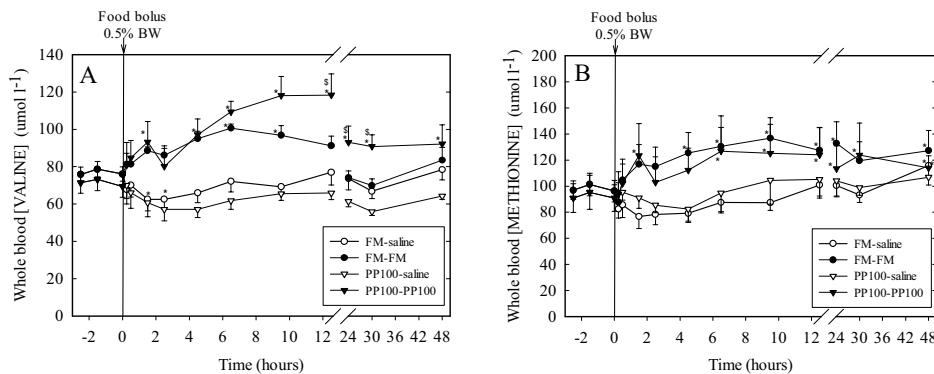


Figure 2. Whole blood concentration of the essential amino acids valine (2A) and methionine (2B) in rainbow trout before and after a food bolus. For other details see figure 1.

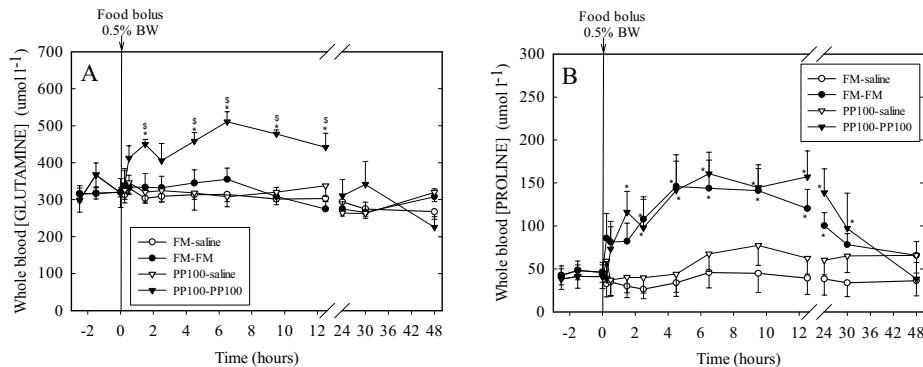


Figure 3. Whole blood concentration of the non-essential amino acids glutamine (3A) and proline (3B) in rainbow trout before and after a food bolus. For other details see figure 1.

Acknowledgement

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Effects of breed, calving pattern and stage of lactation on protein concentration of cow's milk from three Australian dairy regions

C.L. White

CSIRO Livestock Industries, Private Bag 5, PO Wembley Australia 6913

Summary

An analysis of test records from herds in three dairy areas within Australia was undertaken to describe regional and breed differences in protein concentration of milk and to gain insight into factors responsible. Two breeds (Holstein/Friesian (H/F) and Jersey) and three regions (The Riverina, North Queensland and South-Western Australia) were chosen for analysis. The two breeds represent the main breeds in Australia, and the climatic regions represented temperate low rainfall (450 mm) with irrigation, tropical with 8% of area irrigated and mediterranean with 30% of area irrigated, respectively.

The results showed clear main effects and interactions between breed, calving month and region for each month of sampling. Protein concentration in Jersey milk was 5 g/L higher than from H/F milk in most months and stages of lactation. For main effects in H/F cows, calendar month of sampling accounted for 2 g/L difference in protein concentration, calving month accounted for 0.5 g/L and stage of lactation 5.9 g/L. For H/F cows at month two of lactation, calving month accounted for $\pm 5\%$ variation in protein concentration, or about 3 g/L. This range was thought to mainly represent the effect of nutrition.

The results were modelled and highlighted possible strategies for managing low milk protein in nutritionally extreme environments. The work also highlighted the need for further information about the components of diet that control milk protein concentration under practical grazing conditions.

Keywords: protein, concentration, milk, calving, breed, region

Introduction

Dairy companies in tropical and mediterranean climatic regions in Australia report a seasonal problem with cheese manufacture, and they attribute this to low milk protein concentrations. Dairy farmers in these regions face price penalties for delivering low protein milk, and so there is an incentive for them to remedy this. In contrast, dairy companies in most temperate zones report no such problems, and although there is a price discount for water volume, no specific penalty thresholds apply for low protein milk.

To understand the reasons for the seasonal decline in protein concentration a desktop analysis of herd test data was undertaken to firstly describe and map regional and seasonal differences in milk protein concentration across Australia's dairy regions, and secondly to assess the contribution of breed, calving pattern and environment to these differences. In this paper, results from three regions and two main breeds are presented and an attempt is made to identify some of the key factors involved. The regions represent three extremely different climatic zones (temperate, tropical and mediterranean) and the breeds are the major dairy breeds in Australia.

Methods

Herd test data from over 1 million individual cows and 10,000 herds held by the Australian Dairy Herd Improvement Scheme (ADHIS) was analysed for effects of breed, age, calving month and

herd geographical region on milk protein concentration and milk yield. Although the data varied by year, the trends were similar and data for 2000 is presented here.

Cows were classified as Holstein/Friesian (H/F) or Jerseys based on ADHIS criterion. A cow needed to have a sire and dam history in order to be classified. Herds were assigned to regions on the basis of shire boundaries. While 41 regions were analysed, only three are presented here: The Riverina District (representing a temperate low rainfall (450 mm) region), North Queensland (a tropical region of 1500-3000 mm rainfall) and a specific area of South Western Australia (a mediterranean region of 800 mm rainfall). Almost all dairy farms in the Riverina and the irrigation region of S-W Australia use some form of irrigation during the dry season, and about 8% of area in N Queensland is irrigated during the dry cooler months. In all areas cows obtain most of their diet from grazing pasture. Concentrate use ranges from less than 1 tonne per head per year to over 2 tonnes.

Milk sampling and protein analyses were undertaken by commercial herd test companies. For most herds, milk yield was measured using flow meters on individual cows once every month or every second month. True protein and fat were measured on a milk sub sample using near infrared reflectance spectroscopy. Quality control within laboratories was based on standard milk samples of known true protein content.

Data analysis was carried out on aggregated means using ANOVA (Systat software, SPSS Inc, Chicago). Cow age, while having a statistically significant effect on milk protein concentration, was of minor practical importance and so results were pooled across ages. For convenience of handling the large data set, analysis was performed on weighted (cow numbers) and aggregated mean values within a herd on the basis of breed, age, calving month and sampling month. Contour maps of milk protein by month of calving and lactation were derived for H/F cows using cosine series bivariate least squares analysis.

Results

Milk from the temperate region contained a higher protein concentration than milk from other areas in all months for both H/F and J breeds, although there was a significant interaction in terms of relative differences (Figure 1; $P < 0.001$). The protein concentration of Jersey milk was approximately 5 g/L higher than H/F milk, regardless of time of year ($P < 0.001$).

Breed composition of herds varied between regions, with respective percentage values for Holstein and Jerseys being 60 and 8 for temperate herds, 72 and 3 for tropical herds and 65 and 0.3 for mediterranean herds, with the remainder classed as "other" breeds including unknowns. "Other" breeds in the mediterranean region consisted mainly of H/F with unknown parentage, and their milk characteristics closely paralleled those of H/F. While these breed differences would partly contribute to the higher milk protein in the temperate region, an analysis of milk from H/F cows showed that there were independent regional effects. Values are not shown, but within the H/F breed, region accounted for a maximum difference of approximately 2 g/L, stage of lactation accounted for 5.9 g/L and calving month about 0.5 g/L. Mean annual per head milk yield for H/F cows for the respective regions (temperate, tropical and mediterranean) were 23.1, 19.2 and 23.6 L/day, with differences between regions significant at $P < 0.001$. Respective protein yields were 0.75, 0.59 and 0.74 kg/day, differences being significant at $P < 0.001$. Thus, low protein concentrations were not necessarily associated with low or high protein yields.

Calving patterns in 2000 for the three regions were relatively uniform, and ranged from 6 to 10% of the herd calving in each month for each region. Cow age effects on milk protein were significant at $P < 0.001$, but numerically were small as a main effect (< 0.5 g/L) and so were not analysed separately.

Lactation curves for protein concentration were different for different regions and calving months (Figure 2). They were derived for H/F cows by fitting a cosine series bivariate equation using least squares analysis. The respective r^2 values were 0.44, 0.44 and 0.52 for the temperate, tropical and

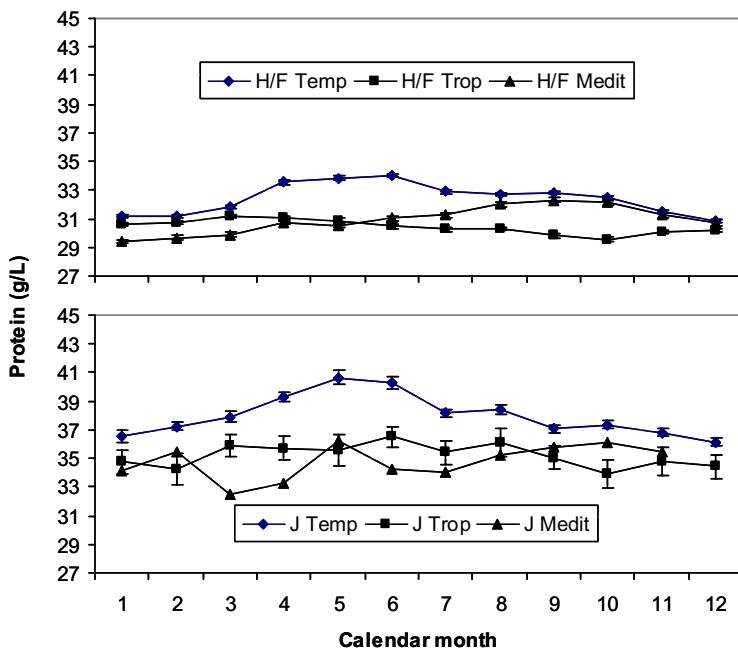


Figure 1. Effect of geographical region (temperate, tropical and mediterranean) and breed (H/F and Jersey) on the concentration of protein in milk (mean \pm s.e.m.). For the H/F breed, standard error bars are smaller than the symbol.

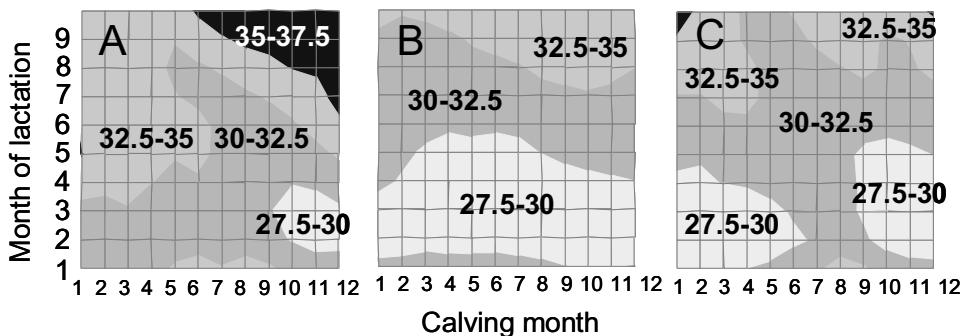


Figure 2. Contour maps of protein concentration showing effects of stage of lactation and calving month. Cows are H/F from temperate (A), tropical (B) and mediterranean (C) regions. Values within shaded areas are ranges for protein concentration (g/L) for the contour area.

mediterranean regions ($P < 0.001$).

The effect of season and region on milk constituents independent of breed or stage of lactation is shown in figure 3 for H/F cows in month 2 of lactation. It shows that relative changes in protein concentration do not mirror those for fat concentration or yield of protein or fat. Milk yield (not shown) mirrored protein yield almost exactly.

Discussion

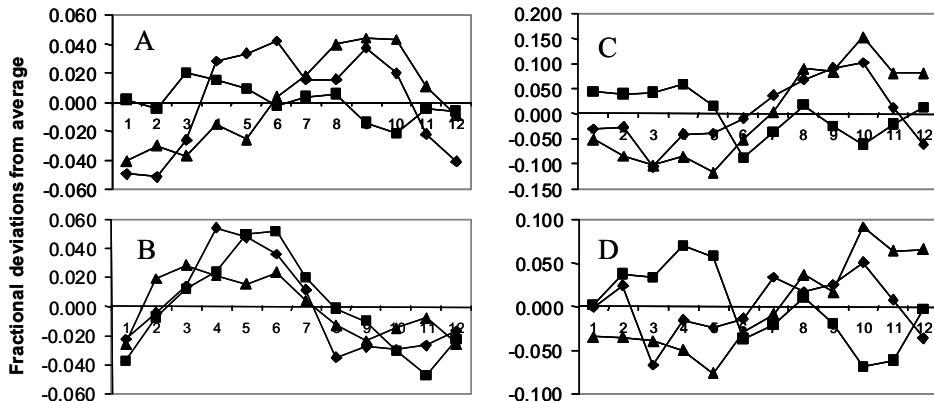


Figure 3. Fractional deviations from the mean for annual protein and fat concentration (A, B), and protein and fat yield (C and D), respectively for H/F in lactation month 2 at the month of sampling.

The results show that major differences exist in protein concentration between dairy regions in Australia, and that the differences were independent of those caused by calving pattern and breed. While the contour maps show that changing calving pattern can offer a solution to the low protein problem in Mediterranean regions, it is likely to be less effective in the tropics.

Where breed and stage of lactation were held constant (Fig 3), protein concentration varied in milk from early lactation H/F cows by approximately $\pm 5\%$, or about 3 g/L. Since pasture and feeding systems differ greatly between regions and between seasons, differences in milk composition are likely to be due mainly to differences in the quality and quantity of feed on offer. High temperature per se is not thought to contribute greatly to the seasonal differences because protein yields did not reflect protein concentration, as might be expected if heat was depressing intake. This is further supported by the fact that deviations in fat concentration differed markedly from those of protein.

The results provide benchmarks for milk yield and composition, and suggest some possible management strategies for farmers to manage milk protein yield and composition in order to meet specifications for drinking milk or milk for manufacturing. Further details of the role of nutrition in terms of pasture and supplement quality and quantity are required in order to provide farmers with additional tools to properly manage milk protein concentration.

Acknowledgement

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