LIVESTOCK PRODUCTION SCIENCE

Research paper

The combined use of triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative to nutritional antibiotics in piglet nutrition

II. In vivo release of MCFAs in gastric cannulated and slaughtered piglets by endogenous and exogenous lipases; effects on the luminal gut flora and growth performance[☆]

N.A. Dierick[a],[*], J.A. Decuypere[a], K. Molly[b], E. Van Beek[c], E. Vanderbeke[d]

[a]*Department of Animal Production, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Proefhoevestraat, 10,
B-9090 Melle, Belgium*
[b]*Vitamex N.V., Drongen, Belgium*
[c]*Kemin Europa N.V., Herentals, Belgium*
[d]*Aveve N.V., Merksem, Belgium*

Abstract

In a first experiment with gastric cannulated piglets, the simultaneous addition of 5% triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs: coconut oil, MCTAG1 oil, butter oil) and two lipolytic enzymes (L2, L5: 1000 ppm) to piglet diets resulted in a physiological environment in the stomach which regulates and stabilizes the gastro-intestinal flora. It is striking that the amount of MCFAs released in the stomach parallels the degree of suppression ($P < 0.05$) of the bacterial load (total anaerobic count, *Lactobacilli, Streptococci, E. coli*) in the stomach. The most pronounced reduction in bacterial load in the stomach occurred with 1 g FFAs (free fatty acids) per 100 g fresh content (= 60% fat hydrolysis) or 0.6 g MCFAs per 100 g with MCTAG1 oil + L5, followed by coconut oil + L5 with 0.8 g FFAs or 0.3 g MCFAs per 100 g and butter oil + L5 with 0.8 g FFAs or 0.06 g MCFAs per 100 g gastric content. In a second experiment under practical field conditions, four diets (A (control), 2.5% soybean oil; B, 2.5% MCTAG2 oil; C, 2.5% MCTAG2 oil + 1000 ppm lipase L5; D, 2.5% soybean oil + 1.5% organic acids), containing no antibiotics, were used in a zootechnical experiment lasting 21 days with 244 early weaned piglets, divided into four groups. Out of each treatment group, five piglets were sacrificed on day 18 after weaning and the contents of the proximal gut were sampled for bacteriological analysis and the analysis of the hydrolysis of the fat. Piglets performed better (overall daily weight gain, $+10\%$ ($P < 0.10$); feed conversion, -3%) on the diets containing MCTAG2 oil or MCTAG2 oil + lipase compared with the control (A) and the acid-supplemented diet (D). The results from the slaughter experiment indicated that with diet C, there was a correlation between the amount of released

---

[*]Corresponding author. Tel.: +32-9-264-9010; fax: +32-9-264-9099.
E-mail address: noel.dierick@rug.ac.be (N.A. Dierick).

MCFAs (0.45 g per 100 g fresh content) in the stomach and duodenum and the inhibitory effect on the gastric and duodenal luminal flora (total count, *Lactobacilli, E. coli*), which decreased about 10-fold. These results are in line with previous in vitro experiments concerning the enzymatic release of MCFAs from specific TAGs and their antibacterial effects, showing that a minimal concentration of 0.35 g MCFAs per 100 g or 0.025 M in the medium (stomach, proximal gut) was necessary to obtain a significant (> 10-fold) suppression of the luminal flora. This strong in vitro and vivo suppressive and stabilizing (non-caloric) effect of MCFAs on the pig proximal gut flora is essential to obtain a growth promotion comparable to that obtained with classic nutritional antibacterials, without the risks of the latter.

1. Introduction

Weaning at 3–4 weeks of age exposes piglets to nutritional and environmental stresses often resulting in reduced feed intake and weight gain and sometimes diarrhoea and death. Indeed, morbidity and mortality data indicate that weaning presents a considerable challenge for the young pig. This post-weaning lag-phase results from insufficient digestive and absorptive capacity due to sudden changes in feed composition and intake (Aumaître et al., 1995; Thacker, 1999). In order to prevent diarrhoea or poor performance, antimicrobial feed additives are usually applied in weaner and grower diets. In recent years, public concerns about development of cross-resistance in human pathogens and residues in animal products and the environment, has caused a pressure to search for consumer friendly alternatives.

The most important and commonly accepted explanations of the mode of action of growth promoting antibiotic feed additives are based on a control of the gastro-intestinal microflora. Indeed, pig productivity looks to be negatively related to the qualitative and quantitative microbial load in the gut and the environment. The growth promoting effect of antibacterial feed additives can occur in several ways, resulting grosso modo in 1° more available nutrients for the host and 2° less metabolic demands for maintaining the absorptive and immunological function of the gastro-intestinal tract (Vervaeke et al., 1979; Dierick et al., 1986a,b; Jensen, 1993; Chesson, 1994; Thomke and Elwinger, 1998; Anderson et al., 1999).

In order to be a real alternative to growth promoting antibiotics, the proposed products must show a similar mode of action. This is claimed for some probiotics and prebiotics, herbal extracts and organic (and inorganic) acids. The most promising in pig production seem to be the short-chain organic acids (Eidelsburger, 1998; Piva, 1998; Roth and Kirchgessner, 1998; Partanen and Mroz, 1999). However the zootechnical improvements with these common organic acids are not always consistent and appear to be smaller than those obtained with antibiotics. Feeding of fermented liquid diets post-weaning could further be an alternative (Geary and Brooks, 1998). However, diet acidification seems not the complete answer to the post-weaning growth check in piglets.

The unique nutritional, physiological and antimicrobial properties of various short to medium chain fatty acids (C4:0 to C12:0) and monoacyl-glycerols compared with those of long chain triacyl-glycerols (LCTAGs) have been investigated for many years (Kabara et al., 1972; Meeus, 1994; Decuypere and Meeus, 1995; Willis and Marangoni, 1999).

In a previous publication, an in vitro screening of several lipases and MCFAs containing TAGs for lipolysis at different pH values, simulating pig's gastric conditions, was described (Dierick et al., 2002). These studies revealed the possibility of a controlled production of significant amounts of MCFAs in gastric simulated conditions. In other follow up in vitro experiments, the optimal combination of different concentrations of MCFAs containing TAGs with different doses of selected lipases was looked for. From these studies it was concluded that a minimal concentration of 0.35 g MCFAs per 100 g or 0.025 M in the medium (stomach, proximal gut) was necessary to obtain a significant (> 10-fold) suppression of the luminal flora. Therefore the concept was set up to combine the use of triacyl-

glycerols containing medium chain fatty acids and exogenous lipolytic enzymes as an alternative to nutritional growth promoting antibiotics in piglet nutrition.

The first experiment in vivo in the present investigation was set up to check if the above mentioned concept and results obtained in the vitro studies, could also be obtained in situ, in gastric cannulated piglets by the use of selected sources of MCFAs containing fats and lipases.

The second experiment was carried out to see if the concept is applicable and suitable in practical commercial conditions and when a growth promotion was obtained, this was comparable to the growth promotion in early weaned piglets with a combination of organic acids, of which the effects are well established (Thomke and Elwinger, 1998; Anderson et al., 1999).

2. Materials and methods

2.1. Experiment I

Four (three and one reserve) weaned female piglets (Belgian Landrace, stress negative) with an initial weight of 8.5 kg were prepared with a gastric cannula, essentially as described by Decuypere et al. (1977). The cannulae were placed midway in the curvatura major in the fundic area. The experimental protocols used were approved by the Ghent University Experimental Animal Ethics Committee.

The following three fat sources were used: coconut oil (Sigma, C-1758, Sigma–Aldrich, St Louis, USA); MCTAG1 (Aldo MCTAGS Kosher Food Grade, fractionated coconut oil ester; Lot# G6410798; Lonza Inc., Fair Lawn, NJ, USA); butter oil (Aveve Zuivel, Merksem, Belgium). The fat sources used contained 65.2, 97.2 and 12.6% bound MCFAs. The individual MCFA composition (g/100 g fat) is presented in Table 1. Two lipases L2 (microbial, 9362 U/g) and L5 (microbial, 6563 U/g), both from Kemin Europa N.V. (Belgium) were selected for the experiment. Lipolytic activity is expressed as the amount of enzyme (1 U) producing 1 μmol free acid per min and per g from tricaprylin at pH 5.5 and 40 °C (Kemin Europa, N.V., Belgium).

Nine feeds (D1–D9), free of antibiotics or other growth promoting supplements, were prepared using

Table 1

Medium chain fatty acid profile of the fat sources used in Experiments I and II[a]

Fat source	Medium chain acid				
	C4:0	C6:0	C8:0	C10:0	C12:0
Coconut oil[b]	0.00	0.74	8.63	6.35	49.47
MCTAG1 oil[c]	0.00	2.70	67.20	26.90	0.40
MCTAG2 oil[d]	0.00	0.16	53.69	39.50	0.29
Butter oil[e]	2.92	2.01	1.44	2.99	3.56

[a] g fatty acid/100 g fat.
[b] Sigma, C-1758, Sigma–Aldrich, St Louis, MO, USA.
[c] Aldo MCTAGS Kosher Food Grade, fractionated coconut oil ester; Lonza Inc., Fair Lawn, USA.
[d] Stabilox 860; Loders Crocklaan B.V., Wormerveer, The Netherlands.
[e] Aveve Zuivel, Merksem, Belgium.

a basal piglet diet (Table 2) supplemented with 5% of the fat (coconut oil: D1, D2, D3; MCTAG1 oil: D4, D5, D6; butter oil: D7, D8, D9) and supplemented with lipases L2 (D2, D5, D7) or L5 (D3, D6, D9) (1000 ppm on fresh feed basis). The fats (eventually liquid) were simply poured on the meal and thoroughly mixed in a horizontal feed mixer. The experimental diets were further supplemented with 2% Celite (545, Fluka, CH) and 0.02% $C_{36}H_{74}$ (Sigma, USA) as inert markers for measuring digestibilities of fat and fatty acids in the stomach as indicated in Table 3, in which the chemical analysis and some physico-chemical characteristics of the diets are also presented. The feed was given dry, in

Table 2

Ingredients and calculated composition of the formulated basal diet (% as fed) (Experiment I)

Barley	17.50	Crude protein	18.00
Manioc	6.20	Ether extract	3.33
Soybean meal	16.15	Lactose	6.53
Corn	40.00	Ash	7.04
Herring meal	5.00	Crude fibre	2.38
Acid whey powder	10.00	Starch + sugar	46.95
Soya oil	0.72	Ca	1.00
Phosphate	1.34	P	0.71
Limestone	1.13	NEv (kJ/kg)[b]	9660
L-LYS HCl	0.47		
DL-METH	0.22		
L-THRE	0.22		
L-TRYPT	0.06		
Premix[a]	1.00		

[a] Antibiotic free vit./min. premix (Roche, Belgium).
[b] Net energy for pigs (CVB Table, Centraal Veevoederbureau, Lelystad, The Netherlands).

Table 3
Chemical analysis and physico-chemical characteristics of the basal and the nine experimental feeds (% as fed) (Experiment I)

	Basal diet; no fat	D1, D2, D3; coconut oil	D4, D5, D6; MCTAG1 oil	D7, D8, D9; butter oil
DM	89.1	90.6	90.7	90.8
Crude ash	7.0	7.8	7.9	8.5
Crude protein	16.9	15.1	15.4	14.8
Crude fat[a]	3.8	8.5	8.3	8.3
MCFAs				
C4:0	0.00	0.00	0.00	0.11
C6:0	0.00	0.00	0.06	0.09
C8:0	0.00	0.40	2.92	0.15
C10:0	0.00	0.26	1.19	0.11
C12:0	0.00	2.06	0.00	0.13
4 N HCl[b] insoluble ash	0.51	2.07	2.08	2.17
$C_{36}H_{74}$[c]	0.000	0.016	0.016	0.012
pH[c]		5.4	5.4	5.4
Buffering capacity	(Bc-3)[d]	506	530	486
Microbial count $\log_{10}$	CFU/g[e]	0	0	0

[a] Without HCl pre-hydrolysis.
[b] Including added Celite as inert marker (545, Fluka, CH).
[c] pH after soaking a 10% slurry for 20 min.
[d] Buffering capacity in mequiv. HCl/kg feed (amount of acid used to reduce pH to 3).
[e] Total microbial load: colony forming units: $\log_{10}$ CFU/g feed (detection limit).

three equal meals (9, 13 and 17 h) at 85% of the ad libitum intake of comparable pigs. The experiment had a 3×3 Latin square design: one oil source (sequence of application: oil; oil + L2; oil + L5) per pig and per week, consisting of 2 days of adaptation and 5 days of collection.

Sampling of the gastric contents for the chemical analysis was done on days 3–7, twice a day, 30 and 90 min after the first (9 h) and second meal (13 h). pH of the contents was measured immediately; afterwards, samples were kept frozen ($-25\,^{\circ}$C) until chemical analysis. Bacteriological sampling was done on days 3, 5 and 7, 90 min after the first (9 h) and second meal (13 h).

2.2. Experiment II

Two hundred and forty-four newly weaned piglets (Seghers Hybrid F1, Belgium), initial weight 5.8 ± 0.2 kg, were alloted depending on litter, sex and weight to four treatments with: A: 68, B: 61, C: 60 and D: 55 piglets. The experiment was done in commercial settings in temperature-controlled

facilities. The feed was prepared by a commercial company using a spray equipment for fats and liquid supplements. Ingredients and approximate calculated composition of the four diets are presented in Table 4. The experiment lasted 4 weeks. As the experimental feeds were already offered 1 week before weaning, grouping was already done at litter level at that time. At weaning time (21 days of age) each group was further divided into four pens of about 15 piglets each. Piglets were fed dry, ad libitum, while water was continuously available via nipples. The piglets were weighed individually 1 week before weaning, at weaning and weekly thereafter. Feed intake was recorded during 1 week before weaning and daily per two pens thereafter because there was one joint feed hopper for two pens. The visual 'feces + health' condition of the piglets was checked daily and coded per pen on a scale ranging from 0 (extremely poor condition, long hair, diarrhoea and high mortality) to 10 (normal feces, normal hair, excellent condition).

On day 18 after weaning, five barrows from each experimental group were sacrificed, without any previous fasting. The experimental protocol was

Table 4
Feed ingredients of the diets and calculated composition (% as fed) (Experiment II)

	A	B	C	D
Barley	37.0	37.0	37.0	37.0
Wheat	12.0	12.0	11.9	10.5
Corn flakes	5.0	5.0	5.0	5.0
Corn extruded	10.0	10.0	10.0	10.0
Soya Danex	10.0	10.0	10.0	10.0
Soyabean meal	5.0	5.0	5.0	5.0
Soyabean oil	2.5	–	–	2.5
MCTAG2[a]	–	2.5	2.5	–
Acid mixture[b]	–	–	–	1.5
Lipase L5[c]	–	–	0.1	–
Herring meal	6.0	6.0	6.0	6.0
Start meal	12.5	12.5	12.5	12.5
NEv97 (MJ/kg)[d]	10.4	10.4	10.3	10.4
Moisture	11.2	11.2	11.1	11.0
Crude protein	18.9	18.9	18.8	18.7
Starch + sugar	43.7	43.7	43.4	42.8
Crude fat	6.9	6.9	6.9	6.9
Ash	5.2	5.2	5.1	5.3
Crude fibre	3.7	3.7	3.7	3.6
Lactose	5.2	5.2	5.2	5.2
dLYS[e]	1.07	1.07	1.07	1.07
dMETH + CYS	0.65	0.65	0.64	0.64
dTHRE	0.66	0.66	0.65	0.65
dTRYPT	0.19	0.19	0.18	0.18

[a] Loders Croklaan, The Netherlands.

[b] 0.25% citric acid, 0.75% fumaric acid, 0.50% Ca-formate.

[c] Microbial source (6563 U/g) (lipolytic activity is expressed as the amount of enzyme (1 U) producing 1 μmol free acid per min and per g from tricaprylin at pH 5.5 and 40 °C) (Kemin Europa N.V., Belgium).

[d] Net energy for pigs (CVB Table, 1997, Centraal Veevoeder-bureau, Lelystad, The Netherlands).

[e] d(amino acid) = ileal digestible amino acid.

approved by the Ghent University Experimental Animal Ethics Committee. The gastro-intestinal tract (GIT) was immediately removed and dissected; samples were taken from the contents of stomach, duodenum and ileum for bacteriological and chemical analyses.

2.3. Analyses

Bacteriological counts were carried out using the ring-plate technique of Van Der Heyde and Henderickx (1963) and different all or not selective media as described before (Dierick et al., 2002). Ten-fold serial dilutions were made from 1-g aliquots of gastric, duodenal and ileal contents, using a sterilized peptone solution (1 g peptone + 0.4 g agar + 8.5 g NaCl in 1 liter distilled water). Determination of lipid classes, free and total fatty acids (TFAs) were done as described previously (Dierick et al., 2002). No freeze drying was applied to avoid evaporative losses of MCFAs; 4 N HCl insoluble ash (Celite) was measured according to the method of McCarthy et al. (1974). The *n*-alkane marker $C_{36}H_{74}$ was analysed according to a modification of the techniques of Vulich et al. (1991) and Duncan et al. (1999).

3. Calculations and statistical analysis

Mean values between treatments were compared using ANOVA followed by a LSD test. The statistics were done using the SPSS 7.5 program for Windows (SPSS Inc., Chicago, IL, USA). Due to the construction of the pens (two pens/feed hopper) statistics were done only for weight measurements. Means of bacterial counts were calculated as decimal values and presented as $\log_{10}$ CFU (colony forming units)/g fresh content. A non-parametric test (Mann–Whitney–Wilcoxon) was used to compare the counts of the different flora components.

4. Results and discussion

4.1. Experiment I

No differences were noted for the pH or buffering capacity of the diets (Table 4), with values in line with those of normally used compound piglet feeds. The pH of the stomach contents measured 30 min and 1 h 30 min after feeding did not differ between treatments and ranged between 4.2 and 5.0 (data not shown). This is within the optimal range for the lipolytic activity of lipases L2 and L5, as was found in earlier work (Dierick et al., 2002). The results of the microbial counts are presented in Table 5. The most important results are: with coconut oil, both L2 and L5 reduced > 10-fold ($P < 0.05$) the total count and the number of *Lactobacilli*; with MCTAG1 oil, both enzymes had a very pronounced (mostly $P < 0.01$) effect and reduced the total count and the

Table 5
Bacterial counts in gastric contents of piglets ($\log_{10}$ CFU/g fresh content (Experiment I)

	Total count	Lactobacilli	Streptococci	E. coli
Coconut oil diet				
D1	7.1[a]	6.6[a]	5.1[a]	2.8
D2 (+ 1000 ppm L2)	5.3[b]	5.1[b]	4.5[a]	3.0
D3 (+ 1000 ppm L5)	5.6[b]	5.3[a]	3.6[b]	3.9
S.E.M.	0.20	0.18	0.31	0.37
MCTAG1 oil diet				
D4	6.1[a]	5.9[a]	5.3[a]	3.8[a]
D5 (+ 1000 ppm L2)	4.4[b]	3.9[b]	0.0[b]	2.6[b]
D6 (+ 1000 ppm L5)	4.3[b]	3.4[b]	2.1[b]	2.0[b]
S.E.M.	0.38	0.36	0.59	0.42
Butter oil diet				
D7	6.5[a]	6.1	5.3[a]	4.3[a]
D8 (+ 1000 ppm L2)	6.0[b]	5.6	4.4[b]	3.4[b]
D9 (+ 1000 ppm L5)	6.0[b]	5.7	4.4[b]	5.3[a]
S.E.M.	0.19	0.15	0.34	0.30

a,b within column and per fat source, means with a different superscript differ significantly at least $P < 0.05$.

Lactobacilli by a factor of 100–500; Streptococci were mostly reduced to non-detectable levels, while E. coli numbers were halved; with butter oil, there was a five- to 10-fold reduction ($P < 0.05$) of the total count and the number of Streptococci. The most pronounced effects were noted with the combination of MCTAG1oil + L5, which nearly eliminated the Streptococci and E. coli. The eventual effect of the supplementation of the basal diet with the three fat sources, without exogenous enzymes, on the flora in the stomach, could not be examined in this experimental set-up, as no diet without MCTAGs containing fat was included in the experimental set-up. However from Table 5, it is clear that with the MCTAG1 oil source compared with coconut oil, total flora and Lactobacilli were lowered by a factor 10 and seven, respectively. This, most probably, was due to the release of MCFAs by endogenous lipase activity originating from the pig stomach and/or the feed ingredients, as will be further discussed. The effects of butter oil on the E. coli number however are less clear, probably because butter oil contains only 4.5% C8 + C10 (Table 1), being the most effective constituents for reducing the flora. This fact left not much room for endogenous lipase sources for releasing MCFAs (diet D7).

Few in vivo studies have attempted to correlate changes in the gastrointestinal ecosystem in response to diet acidification. From earlier in vitro research (Dierick et al., 2002) it was found that with a minimal concentration of 0.35 g MCFAs per 100 g medium (or 0.025 M in the medium: stomach, proximal gut) a significant (> 10-fold) and persistent suppression of the indigenous luminal flora occurred. The effects of the commonly used organic acids such as citric, fumaric, propionic and formic acid on the gut flora on the other hand seem rather inconsistent (Jensen, 1998) while the G + flora is rarely influenced by these acids (Partanen and Mroz, 1999).

Figs. 1–4, show the lipid classes in the gastric contents of the piglets on the basal (samples taken during the pre-experimental period) and three selected experimental diets (D3, D6, D9), taken 1 h after feeding and illustrate that the major product of (microbial and/or pre-duodenal) lipolysis are FFAs. Indeed, the main products of the action of the preduodenal lipases on fats are short and medium chain free fatty acids released with preference from the sn-3 position of the TAGs together with diacylglycerols and small amounts of MAGs. The activity of preduodenal lipase also diminishes at the DAGs, which seem to have no antimicrobial effects at all (Kabara et al., 1972). Also, in general, lipases
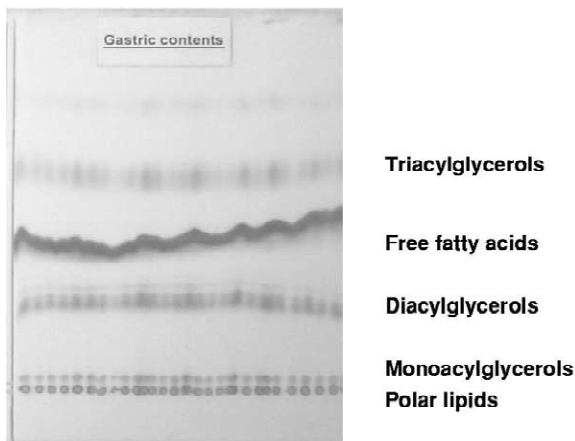
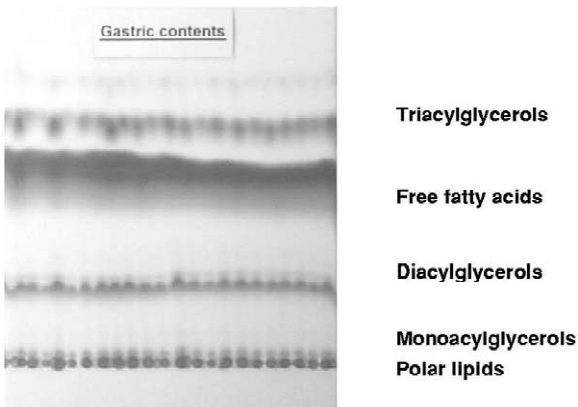Fig. 1. Thin layer chromatogram of lipid classes found in gastric contents from piglets fed the basal diet.

Fig. 2. Thin layer chromatogram of lipid classes found in gastric contents from piglets fed diet D3: coconut oil + lipase L5.
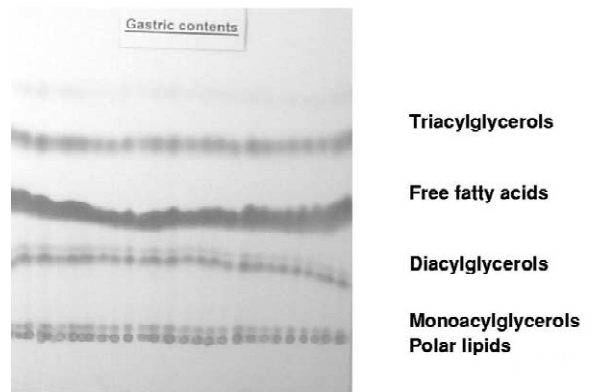

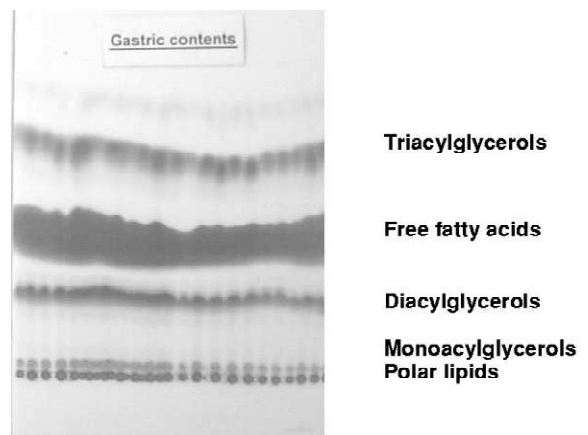Fig. 3. Thin layer chromatogram of lipid classes found in gastric contents from piglets fed diet D6: MCTAG1 oil + lipase L5.

Fig. 4. Thin layer chromatogram of lipid classes found in gastric contents from piglets fed diet D9: butter oil + lipase L5.

from microbial origin (L5) show a very broad or a sn-1,3 specificity with no pronounced fatty acid specificity (Dierick et al., 2002). Therefore, other fat digesta products (diacylglycerols (DAGs), monoacylglycerols (MAGs)) were not further analysed for their fatty acid content.

The results concerning the total fatty acid content and the release of individual MCFAs in gastric content are presented in Table 6. As no significant differences were found for the sampling time (30 and 90 min), within the each diet, for the contents of FFAs and TFAs, samples were treated together. The degree of fat hydrolysis was assessed by analysing the FFAs content in free and bound form (ratio of total free fatty acids to total present fatty acids). In the groups without exogenous lipase, endogenous gastric lipase was responsible for the hydrolysis of $\pm 17\%$ of the fat, corresponding to 0.20–0.30 g free fatty acids per 100 g in the content, without significant differences between the fats used. However the profile of the MCFAs released in the stomach differed significantly ($P < 0.05$) between the three fat sources used: with diet D4, 0.12% C8:0 + C10:0 were present, while with diet D1 and D7, the concentration of C8:0 + C10:0 (most important antimicrobial compounds) was 0.03 and 0.00%, respectively. This difference most probably is responsible for the lower bacterial counts in the stomach of the

Table 6
Content of free and total fatty acids (g/100 g fresh content) and overall degree of hydrolysis (= free FAs: total FAs × 100) in gastric contents as influenced by dietary treatments (Experiment I)

Diet	D1 Coconut	D2	D3	D4 MCTAG1	D5	D6	D7 MCTAG2	D8	D9	S.E.M.
Oil										
Enzyme	0	L2	L5	0	L2	L5	0	L2	L5	
Free MCFAs (g/100 g fresh content)										
C4:0							0.00a	0.01b	0.01b	0.001
C6:0	0.00a	0.00a	0.01bc	0.00a	0.02e	0.02e	0.00ab	0.01c	0.01d	0.001
C8:0	0.02ab	0.05b	0.05b	0.09c	0.43d	0.47e	0.00a	0.01a	0.01a	0.015
C10:0	0.01a	0.03c	0.03c	0.03bc	0.16d	0.17e	0.00a	0.01ab	0.01ab	0.005
C12:0	0.05b	0.19c	0.21c	0.00a	0.01a	0.01a	0.00a	0.01a	0.02a	0.007
SUM of free MCFAs (g/100 g fresh content)										
	0.07ab	0.28c	0.30c	0.12b	0.61d	0.68e	0.02a	0.01ab	0.06ab	0.019
SUM of total free FAs (g/100 g fresh content)										
	0.32a	0.79bc	0.82bc	0.29a	0.91cd	1.01d	0.20a	0.68b	0.73b	0.024
Total FAs (g/100 g fresh content)										
	1.93e	1.83cd	1.83cd	1.54bc	1.56a	1.66bcd	1.21a	1.46ab	1.60bc	0.038
DH = (free FAs/total FAs) × 100										
	16.6a	42.8b	44.5b	18.9a	56.6c	61.0d	16.7a	46.1b	45.9b	1.22

a–e: within the same row, means with different superscript differ significantly at least $P < 0.05$.

piglets on diet D4 (Table 5). This could be due to the fact that MCFAs with a lower MW more easily penetrate into the bacterial cells and have a bactericidal effect, as can be deduced from earlier studies (Dierick et al., 2002). Addition of L2 and L5 to the diets increased the lipolysis about threefold to 45–60% ($P < 0.05$), corresponding to 0.70–1.00 g FFAs per 100 g contents (50–75 mM in the contents), with no significant difference between the two lipases, except for diets D5 and D6. Of those about 70% will be undissociated at pH 4.5, according to the Henderson–Hasselbach equation.

Earlier work in which the in vitro release of FFAs in gastric simulating conditions (synthetic diet, 3-h incubation, pH 5, 37 °C, 10 000 ppm lipase on fat) was studied, revealed that some lipases could release about 25% FFAs from the same fat sources (Dierick et al., 2002). Perhaps some synergistic effects between endogenous (mammalian) and exogenous (microbial) added lipases could have occurred. The origin of gastric lipolysis seems not to be due to pancreatic lipases, as a yellow colour by antiperistalsis of bile was never observed in the collected gastric contents and because pancreatic lipase only has optimal activity at about pH 6 and in the presence of co-lipase and bile acids. Therefore, the action of a gastric lipase looks very likely. Indeed, in most mammals there is a more or less pronounced preduodenal lipase activity, originating from lingual or gastric secretions, active in a broad pH range with a preference for MCFAs in milk fat (Nelson et al., 1977; Moreau et al., 1988). Endogenous gastric lipase (optimal pH range 5–9 (Höller, 1970) located in the cardiac region of the pig stomach, is thought to play a significant role in the hydrolysis of fats in the stomach but its quantitative contribution to total fat digestion still remains to be elucidated. Furthermore the enzyme seems to be resistant to acid and pepsin and functions without bile acids or co-factors. The results (Tables 1 and 6) further indicate that the lipases used did not show any specificity regarding the released free fatty acids; indeed, the release was proportional to their content in the TAGs.

The degree of fat hydrolysis (17% FFAs in total fat) noted in the stomach of piglets after feeding diets with no added lipases, is in accordance with the scarce literature data (Newport and Howarth, 1985; Chiang et al., 1989). Jensen et al. (1997) followed the development of lipases in pigs and noted a decreased pancreatic and an increased stomach lipase activity in piglets at weaning. Postweaning activity of pancreatic lipase activity decreased, while

stomach lipase activity (only 0.2% of pancreatic activity) remained constant. Höller (1970) estimated the gastric lipase activity to be only 5% of the pancreatic lipase activity, while Newport and Howarth (1985) estimated that the total lipase activity in stomach tissue was only about 3% of that found in the pancreas; however, the fact that digesta, especially the fat fraction, are retained for a longer time in the stomach (Mean Retention Time, MRT of non-fat food particle: 3.8 h) than in the small intestine (MRT of non-fat food particle: 3.0–3.4 h), may explain why gastric lipase, despite its low activity, may hydrolyze a considerable part of the fat in the diet (Furuya and Takahashi, 1975; Furuya et al., 1978). Furthermore, gastric lipase may still remain active in the duodenum, working in synergy with pancreatic lipase (Edwards-Webb and Thompson, 1977). Another possible source of endogenous lipase activity could have originated from the ingredients used in the compound piglet feed (Elstner, 1997; O'Connor et al., 1992). As indicated in Table 3, no contaminating bacteria were detected in the prepared feeds, so lipolytic activity from microbial origin seems unlikely. However some endogenous lipase activity originating from the other feedstuffs (cereals, legumes, etc.) used in this study cannot be excluded because the endogenous lipolytic activity of such feedstuffs may differ widely depending on cultivar, treatment, storage time and conditions, etc. (Dierick et al., 2002, in preparation). The release of small amounts of MCFAs during storage of feed is not disadvantageous per se because it can cause a protection by reducing the growth of microbial contaminants in the feed prior to ingestion; high amounts of free MCFAs however, may produce an adverse odour.

Finally from Table 6 and from previous in vitro work (Dierick et al., 2002), it appears that the endogenous lipase activity in the stomach of the piglets in present experiment is too low for producing enough MCFAs able to inhibit significantly the gut flora. This could be due to the lower (4–5) than optimal pH (5–9) for pig gastric lipase activity. Therefore, the addition of exogenous lipolytic activity through lipase supplementation with an optimal pH in the acid range (4–5) to the diet seems to be essential. In previous in vitro research, in which gastric conditions were simulated, a minimal concentration of 0.35 g MCFAs per 100 g of the medium at pH 5 was necessary to obtain a significant suppression of the flora, while from 0.60 g per 100 g upwards, all counted bacteria including *E. coli*, were totally eliminated (Dierick et al., 2002). Also, in the present experiment, the bacterial suppression was positively correlated with the amount of MCFAs generated. The most pronounced reduction of the bacterial count occurred with MCTAG1 oil + L5 by which 1 g FFAs per 100 g (= 60% fat hydrolysis) or 0.6 g MCFAs per 100 g were found, followed by coconut oil + L5 with 0.8 g FFAs per 100 g or 0.3 g MCFAs per 100 g and butter oil + L5 with 0.8 g FFAs per 100 g or 0.06 g MCFAs per 100 g gastric content.

Compared with the commonly used organic acids, which act primarily on G − micro-organisms (*E. coli*) and *Streptococci*, MCFAs and acids used for food preservation (e.g. sorbic, benzoic acid) have a broader antimicrobial spectrum, acting on both G − and G + micro-organisms.

Previous (Dierick et al., 2002) and present results allow to conclude that the combined use of TAGs containing MCFAs and lipases in the feed is able to reduce considerably and persistently (factor 100–500) the total bacterial count and the dominant flora in vitro and in situ in the stomach. The antibacterial effects of the commonly used organic acids on the other hand are rather inconsistent and much less pronounced as reviewed by Jensen (1998); most probably this is related to the fact that their effects are much more dependent on type of diet used (buffering capacity, complexity).

The fate of the MCFAs in the stomach and upper gastro-intestinal tract was also studied. The use of gastric cannulated piglets was preferred because it is very difficult to cannulate pigs before the entrance of the bile duct to exclude the interference of bile and pancreatic lipase. The apparent digestibility coefficient (DC) or 'disappearance' from the stomach was determined using two markers, 4 N HCl insoluble ash and an alkane $C_{36}H_{74}$. Disappearance of MCFAs (C8, C10, C12) from the stomach ranged from 0 to + 20.9% with coconut oil and MCTAG1 as source of fat, without preference for the different MCFAs, present in the fat. Evidence of direct absorption of short chain and/or MCFAs across the stomach wall is given by Clark et al. (1969) and Aw and Grigor

(1980) in rats, by Hamosh et al. (1989) and by Borum (1992) in preterm neonates and by Argenzio and Eisemann (1996) for pigs. The absorption rate depends greatly on the pK_a of the acid and the luminal pH. When the luminal pH is below the pK_a of the stomach, MCFAs may be absorbed. It looks further that more than 80% of the MCFAs will be transported to the upper small intestine, where they could further exert their antibacterial activity. Also the MAGs formed (Figs. 1–4), containing MCFAs, but not measured here, could join the pool of antimicrobial fat products, as MAGs could be even more effective than the free acids, as demonstrated by Kabara et al. (1972).

It is commonly accepted that the most important effect of antibacterial growth promoters is the reduction of the microbial load in the small intestine. This influences positively the performances of the animals. There are several explanations for this growth promotion. However it is outside the scope of this report to go to into detail about this. Several excellent reviews can be consulted (Jensen, 1993; Chesson, 1994; Thomke and Elwinger, 1998; Anderson et al., 1999). The role of MCFAs in inhibiting growth, proliferation and (or) colonization of non-indigenous microflora, including pathogenic bacteria (*E. coli, Salmonella, Serpulina, Clostridium,* etc.) needs further research. Present known (potentially) pathogenic bacteria that are inactivated by MCFAs or their monoglycerides include *Pseudomonas, Campylobacter, Vibrio cholerae, Salmonella typhi, Shigella sonnei, Hemophilus influenza, Listeria monocytogenes, Staphylococcus aureus, Streptococcus agalactiae, Helicobacter pylori* and enterotoxigenic *E. coli* as indicated by Isaacs et al. (1992) and Petschow et al.

(1998). The effect of MCFAs noted in this study on *E. coli*, being by far the dominant type of the coliform bacteria in the gut, can be assumed to be representative for most enteric bacteria, such as *Salmonella*, *Shigella* and *Enterobacter* (Överland et al., 2000).

4.2. Experiment II

In the slaughter experiment, the pH of the gastric, duodenal and ileal contents was 3.5, 5.7 and 7.0, respectively, with no differences between treatments. The total anaerobic bacterial count and the counts of *Lactobacilli*, *Streptococci* and *E. coli* are given in Table 7. The results indicate that with feed C (MCTAG2 oil + L5) there was a significant (10-fold; $P < 0.05$) suppression of the total bacterial load, both in the stomach and duodenal chyme. The same was true for the *Lactobacilli*, although the differences did not reach significance. High and, in our experience, abnormal counts were noted for the *Streptococci* in the stomach and duodenum for treatment C, for which no explanation can be given. Diet C (MCTAG2 oil + L5) also reduced significantly ($P < 0.05$) the *E. coli* count in gastric and duodenal contents. With diet D, containing the acid mixture, total microbial load in the stomach and duodenum, was not different from that with the control diet; only the number of *E. coli* was significantly reduced at both levels. Compared with the experiments with gastric cannulated piglets (Experiment I), the suppressive effect of MCTAG2 oil + L5 on the gastro-intestinal flora was somewhat lower. This is most likely because of the lower fat level used (2.5 vs. 5.0% in previous experiment) and

Table 7
Microbial counts in gastric and duodenal contents of slaughtered piglets (2 weeks after weaning) as influenced by the treatments ($\log_{10}$ CFU/g fresh content) (Experiment II)

Treatment	Stomach				Duodenum			
	Total	*Lactobacilli*	*Streptococci*	*E. coli*	Total	*Lactobacilli*	*Streptococci*	*E. coli*
A	7.0[a]	7.2[ac]	4.2[a]	4.6[a]	6.4[a]	6.9	1.6[a]	4.9[a]
B	7.0[ac]	7.6[a]	0.6[b]	0.8[bc]	6.1[a]	6.8	0.0[a]	4.8[a]
C	5.9[b]	6.6[bc]	5.3[a]	2.0[b]	5.6[b]	5.9	4.7[b]	1.8[b]
D	6.9[ac]	7.3[a]	5.1[a]	0.0[c]	5.9[a]	6.4	4.7[b]	1.8[b]
S.E.M.	0.13	0.13	0.48	0.48	0.13	0.19	0.54	0.51

a,b,c: different superscripts in the same column denote significant differences at least $P < 0.05$.

subsequently the lower release of MCFAs within the stomach. Nevertheless, Experiment II confirms the results concerning the antibacterial activity of MCFAs in gastric cannulated piglets from Experiment I. In contrast with present results, the described effects of classical organic acid supplementation on the quantitative composition of the gastro-intestinal flora are not always consistent. One reason for that could be that samples are often taken at the end of a growth trial. By this time, the digestive system of the weanling pig may be fully developed (Gabert and Sauer, 1994). Another reason could be that the samples for bacterial counts were sometimes frozen or freeze-dried before plating. It is well known that some *Lactobacilli* do not survive well in these circumstances.

The release of MCFAs in the stomach and upper small intestinal lumen is presented in Table 8 and confirms the results from Experiment I. In the diets without added lipase, the initial FFAs content as percentage of the TFAs in the stomach amounted to 25–30%. The MCFAs content in gastric digesta was 0.20 and 0.42 g per 100 g fresh content in diets B and C, respectively. In the stomach of the pigs on the other diets (A,D) no MCFAs were found. In Experiment I with gastric cannulated piglets, the ratio FFAs/TFAs in gastric contents amounted to 17%. The reason for this discrepancy in degree of hydrolysis most likely is not caused by regurgitated pancreatic lipases, because in the present experiment, a yellow colour, indicative for a contamination with bile, was never observed in the gastric contents of the slaughtered piglets. Another reason is that pancreatic lipase only works optimally at about pH 6 and in the presence of co-lipase and bile acids. A more plausible explanation could be that there was a higher preduodenal lipase activity in the stomach and/or a higher endogenous lipase activity in the feedstuffs used for the formulation of the present diets. When exogenous lipase L5 was added to the diet, the degree of hydrolysis increased significantly ($P < 0.05$) from 35 to 70% in stomach content. Also the amount of free MCFAs increased significantly ($P < 0.05$) from 0.22 to 0.45 g per 100 g contents. In the duodenal digesta, hydrolysis amounted to 70–80% without large difference between diets, while in ileal digesta, 80–95% of the remaining fat was in the free form. About 0.04 g MCFAs/100 g fresh materi-

Table 8

Content of free and total fatty acids (g/100 g fresh content) and overall degree of hydrolysis (= free FAs: total FAs $\times$ 100) in gastric contents as influenced by dietary treatments (Experiment II)

	Diet				S.E.M.
	A	B	C	D	
Oil	Soyabean	MCTAG2	MCTAG2	Soyabean	
Lipase	0	0	L5	0	
Organic acids	0	0	0	Mixture[a]	
Free MCFAs (g/100 g fresh content)					
C6:0	0.00	0.00	0.00	0.00	0.000
C8:0	0.00a	0.13b	0.26c	0.00a	0.026
C10:0	0.01a	0.07b	0.17c	0.00a	0.016
C12:0	0.01	0.01	0.02	0.01	0.002
SUM of free MCFAs (g/100 g fresh content)					
	0.02a	0.22b	0.45c	0.01a	0.043
SUM of total free FAs (g/100 g fresh content)					
	0.28a	0.44a	0.95b	0.31a	0.074
Total FAs (g/100 g fresh content)					
	1.05a	1.25ab	1.35b	1.07a	0.051
DH = (free FAs/total FAs) $\times$ 100					
	26.7a	35.2a	70.4b	28.9a	4.82

a,b,c: within the same row, means with different superscript differ significantly at least $P < 0.05$.

[a] 1.5% of a mixture containing 25% citric acid, 75% fumaric acid, 50% Ca-formate.

al were found in the duodenum of the piglets on the diets containing the MCTAG oil source; it must be stressed however that duodenal chyme contained only 7%, while gastric contents held about 25% DM.

From Tables 7 and 8, it follows that the concentration of MCFAs in the stomach is bactericidal: 0.44 and 0.95 g FFAs per 100 g contents and 0.22 and 0.45 g MCFAs per 100 g contents are present in gastric contents from treatment B and C, respectively. A 100-fold decrease in the total bacterial count was noted in Experiment I when the MCFAs concentration reached 0.61–0.68 g per 100 g gastric contents. Again it is clear that endogenous plant lipase activity of the feed and endogenous gastric lipase activity in the pig's stomach are not high enough to release appropriate amounts of MCFAs from MCTAGs oil sources for inhibiting the flora (total count, *Lactobacilli*). Calculations based on individual measurements (sum of free MCFAs or individual MCFAs vs. total anaerobic count in each sample) from both experiments further indicate that there is a correlation between the amount of released MCFAs in the stomach and the inhibitory effect on the flora ($r = 0.85$). Also the MCFAs profile (carbon chain length) within the TAGs had an important effect on this correlation: the higher the C8:0 and C10:0 and the lower the C12:0 content in the MCFAs profile, the higher the correlation.

Digestibility coefficients in the stomach using the unabsorbable marker $C_{36}H_{74}$ amounted, depending on the fat source, to 14–35% for C8:0 and 3–16% for C10:0. In the present experiment, the amount of the marker $C_{36}H_{74}$ in the feed was increased to 0.1%, while in Experiment I, only 0.025% was added, resulting in more analytical errors and unrealistic DC values. Both experiments are the first to indicate that MCFAs could be absorbed from the pig stomach. In the duodenum, the DC values for C8:0 and C10:0, using the marker $C_{36}H_{74}$ amounted to 85 and 68%, respectively, while at the ileal level digestibility amounted to 98%, without marked differences between the diets.

During the performance trial the visual 'feces + health' scores ranged between 4 and 9 on treatment A; for the other treatments, the range was between 8 and 9 without marked differences.

The daily growth rate (Table 9) did not differ between treatment A and D and between B and C.

Table 9
Feed intake, growth rate and feed conversion ratio (FCR) of the piglets (Experiment II)

	Day − 7–0	Day 0–7	Day 7–14	Day 14–21	Day 0–21	Day 0–21 (relative A = 100)
Feed intake (g/day)						
A		156	365	472	331	100
B		191	376	536	368	111
C		180	391	533	361	110
D		189	355	469	338	102
Growth rate (g/day)						
A	187a	127a	127a	300a	185ab	100
B	162a	164b	160b	301a	208a	112
C	157a	165b	161b	297a	207a	111
D	168a	141ab	123a	280a	181b	98
P-value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.10	
FCR (kg feed/kg LWG)						
A		1.23	2.88	1.57	1.79	100
B		1.16	2.35	1.78	1.77	99
C		1.09	2.43	1.79	1.74	97
D		1.34	2.89	1.68	1.87	104

Mean Live Weight at weaning age (3 weeks) (day 0) = 5.81 ± 0.18 kg (S.D.).

The most pronounced differences were obtained in the first 2 weeks after weaning at 21 days, during which the greatest increase in the performance (+ 30%) over the control was obtained with treatments B and C. The better growth was mainly due to increased feed intake. The best feed conversion ratio (FCR) was obtained with diet MCTAG2 oil + L5. The improved performance with MCTAG2 oil + L5 is comparable with improvements noted with in feed antibiotics in piglets of comparable weight (Meeus, 1994; Decuypere and Meeus, 1995). Overall, pigs on diets containing MCTAG oil performed better (+ 15%, $P < 0.10$) and had a better FCR (+ 7%) compared with those on diet D, containing classical organic acids. Compared with soybean oil (diet A), the addition of MCTAG2 oil (diet B) alone already resulted in a significant improvement of the growth combined with a significant reduction of *Streptococci* and *E. coli* in gastric contents. This probably was the result of the relatively high concentration of MCFAs (0.22 g per 100 g fresh matter, Table 8) in the gastric contents, although no exogenous lipase was added. A higher than normal preduodenal lipase activity or a high endogenous plant lipase activity in the feedstuffs used could have been the reason for the rather high level of total FFAs in the piglet stomach on treatments A, B and D, as already mentioned. However the preferential release of MCFAs (C8:0 + C10:0) in the stomach of the piglets on diet B is more typical for preduodenal lipase activity. Also in Experiment I, more MCFAs (0.12 g per 100 g) were released in the stomach of the piglets on the diet containing MCTAG oil without exogenous lipase (diet D4) compared with the amounts (0.02–0.07 g per 100 g) found in piglet's stomach on diets D1 and D7, containing oils, less rich in MCFAs (Table 6).

Several authors studied the caloric effect of free MCFAs or MCFAs containing fat (MCTAGs, coconut oil) on the performance of neonatal, weaned or growing pigs in comparison to commonly used fats (butter fat, tallow, lard, corn oil). The effects of feeding MCTAGs to neonatal and weaned piglets are rather controversial however (Allee et al., 1972; Newport et al., 1979; Mahan, 1991; Fakler et al., 1992; Cera et al., 1989a; De Rodas and Maxwell, 1990). In growing pigs (30–90 kg), Glaps (1970) did not find any difference in performance with the feeding of 2 ml/day per kg weight of MCTAGs or LCTAGs. This is in agreement with the results of Takada et al. (1992), feeding 8% MCTAGs in comparison to 8% LCTAGs. Feeding of free MCFAs to weaned piglets also resulted in variable results (Cera et al., 1989b; Dove, 1993). With growing pigs, Rys et al. (1969/70) fed 5% pure synthetic free MCFAs (C5–C12) and obtained an increase in growth rate of 6% compared to an iso-energetic control diet. The discrepancies between the caloric effects of MCTAGs on pig and piglet performances described in the literature and the results obtained in the present study (caloric and non-caloric effects), may be related to the absence or very low levels of endogenous gastric or plant lipases or to the fact that no exogenous lipases were added to the diets, with the result that inappropriate levels of MCFAs were obtained in the stomach and in the duodenum to be suppressive for the gut flora. The lack of positive influences of free MCFAs in young growing pigs, on the other hand, may be related to the fatty acid level and profile of the fat sources used. Another possibility is that the MCFAs caused a reduction in feed intake, due to the unpleasant odor of C8:0 and C10:0 fatty acids.

Feeding intact MCTAGs sources in combination with appropriate levels of exogenous lipase could counteract the negative effects of pure free MCFAs, while enhancing the MCFAs action on the gut flora, as found in the present experiment.

The concept of using triacylglycerols containing medium chain fatty acids, in appropriate amounts, combined with exogenous lipolytic enzymes as an alternative to nutritional antibiotics is initially targeted for piglet and grower nutrition. Applying higher doses of MCTAGs, alone or in combination with lipases, in pig feeding needs some attention however. Feeding low levels of MCTAGs (3% or less) or MCFAs containing TAGs to pigs showed that little MCFAs are deposited as such in the carcass, confirming their rapid catabolism to CO_2 and ketone bodies (Rys et al., 1969/70; Newport et al., 1979; Février et al., 1994). However, MCFA enriched diets (4%) selectively increase fat firmness by MCFA chain elongation in pigs although without greatly undesired side-effects in other traits of product quality (Jaturasitha et al., 1996; Kreuzer et al., 1997). In order to avoid increases in degree of saturation of carcass fat in slaughter pigs and be-

cause the major changes in fatty acid composition due to diet will occur within 4–5 weeks (Koch et al., 1968; Wiseman and Garnsworthy, 1997; Wiseman and Agunbiade, 1998), high levels of MCTAGs (4% or more) are not recommended in the finishing phase of pigs.

The present experiments fully validate the concept of the combined use of MCFAs containing fats with an external added lipase activity.

The present study illustrates the similar strong action of MCFAs and commonly used therapeuticals on the gut flora (total flora, G +, G −, potential pathogens) especially in the foregut. This means that these naturally occurring antimicrobial agents, that possess little or no human or animal toxicity, can be a promising alternative to in feed antibiotics used for growth promotion and even for the preventive and curative treatment of gastro-intestinal diseases of bacterial origin. This strong in vivo suppressive and stabilizing, non-caloric effect of MCFAs on the flora is essential to obtain a growth promotion comparable to that of classic antibacterials, without the negative effects of the latter however.

However, the practical application of the concept may be hampered by the relatively high cost of the available MCTG fats. The MCTG fat sources used in our experiments are prepared industrially by esterification of glycerol with octanoic and decanoic acids, obtained from coconut and palm kernel oils. However other naturally occurring fats of seeds rich in MCTAGs are available. Also lipolytic enzymes are widely distributed among animals, plants and microorganisms and are not expected to be cost-limiting; however, only appropriate lipases should be used when applying this concept (Decuypere and Dierick, 2000). Furthermore, endogenous lipase activity in raw materials and mixed feeds may have implications for choosing the appropriate dose of exogenous lipases to be added to the feed for applying the concept, in order to obtain optimal lipolysis in vivo.

5. Conclusions

The addition of TAGs containing MCFAs (coconut oil, MCTAG1 oil, butter oil) to piglet diets in combination with lipolytic enzymes (Experiment I) results in a physiological environment in the stomach which regulates and stabilizes the gastrointestinal flora. It is striking that the controlled release of MCFAs in the stomach parallels the degree of suppression of the bacterial load in the stomach. The most pronounced reduction in bacterial load in the stomach occurred with 1 g FFAs (free fatty acids) per 100 g fresh content (= 60% fat hydrolysis) or 0.6 g MCFAs per 100 g with MCTAG1 oil + L5, followed by coconut oil + L5 with 0.8 g FFAs or 0.3 g MCFAs per 100 g and butter oil + L5 with 0.8 g FFAs or 0.06 g MCFAs per 100 g gastric. Present experiments allow for the conclusion that a minimal concentration of 0.35 g MCFAs per 100 g or 0.025 M in the medium (stomach, proximal gut) is necessary to obtain a significant (> 10-fold) suppression of the luminal flora, in line with previous research (Dierick et al., 2002).

Previous findings were validated in commercial settings and compared with the effects of a combination of organic acids with a known and well established growth promotion in early weaned piglets in Experiment II. From the slaughter experiment, it can be concluded that there is a correlation between the amount of enzyme released free MCFAs and the strong inhibitory effect on the gastric and duodenal luminal flora (total count, *Lactobacilli, E. coli*). Furthermore, manipulation of the gut ecosystem by the in situ enzyme released MCFAs in the stomach and duodenum resulted in improved performances of the piglets (daily gain + 10% (*P* < 0.10); feed conversion − 3%) on the diets containing MCTAG2 oil or MCTAG2 oil + lipase, exceeding the diets based on soybean oil, with or without supplemented organic acids.

Applying the concept in weaning piglet nutrition results in persistent and significant effects on the gut luminal flora combined with improved performances, which make it a valuable alternative to in feed nutritional antibiotics. A patent has been applied for by the authors and funding partners for the protection of the concept (Decuypere and Dierick, 2000).

References

Allee, G., Romsos, D., Leveille, G., Baker, D., 1972. Metabolic consequences of dietary medium-chain triglycerides in the pig. Proc. Soc. Exp. Biol. Med. 139, 422–427.

Anderson, D., McCracken, V., Aminov, R., Simpson, J., Mackie, R., Verstegen, M., Gaskins, H., 1999. Gut microbiology and growth-promoting antibiotics in swine. Pig News and Information 20, 115N–122N.

Argenzio, R., Eisemann, J., 1996. Mechanisms of acid injury in porcine gastroesophageal mucosa. Am. J. Vet. Res. 57, 564–573.

Aumaître, A., Peiniau, J., Madec, E., 1995. Digestive adaptation after weaning and nutritional consequences in the piglet. Pig News and Information 16, 73N–79N.

Aw, T., Grigor, M., 1980. Digestion and absorption of milk triacylglycerols in 14-day-old suckling rats. J. Nutr. 110, 2133–2140.

Borum, P., 1992. Medium-chain triglycerides in formula preterm neonates: implications for hepatic and extrahepatic metabolism. J. Pediatr. 120, S139–S145.

Cera, K., Mahan, D., Reinhart, G., 1989a. Apparent fat digestibilities and performances responses of postweaning swine fed diets supplemented with coconut oil, corn oil or tallow. J. Anim. Sci. 67, 2040–2047.

Cera, K., Mahan, D., Reinhart, G., 1989b. Postweaning swine performances and serum profile responses to supplemental medium-chain free fatty acids and tallow. J. Anim. Sci. 67, 2048–2055.

Chesson, A., 1994. Probiotics and other intestinal mediators. In: Cole, D. (Ed.), Principles of Pig Science. Nottingham University Press, Loughborough, pp. 197–214.

Chiang, S., Pettigrew, J., Clarke, S., Cornelius, S., 1989. Digestion and absorption of fish oil by neonatal piglets. J. Nutr. 199, 1741–1743.

Clark, S., Brause, B., Holt, P., 1969. Lipolysis and absorption of fat in the rat stomach. Gastroenterology 56, 214–222.

Decuypere, J., Dierick, N., 2000. The combined use of triglycerides containing medium chain fatty acids and selected lipases as feed supplements as an alternative for in feed antibiotics in early weaned pigs. European Patent Publication EP 1059041 (13 December 2000); PCT Publication WO 00/74497 (14 December 2000).

Decuypere, J., Meeus, J., 1995. Aspects nouveaux de la mode d'action des acides dans l'alimentation porcine, Journée d'étude KEMIN Europa, Rennes, 27 June, 1995, 22 pp.

Decuypere, J., Vervaeke, I., Henderickx, H., Dierick, N., 1977. Gastro-intestinal cannulation in pigs: a simple technique allowing multiple replacements. J. Anim. Sci. 46, 463–468.

De Rodas, B., Maxwell, C., 1990. The effect of fat source and medium-chain triglyceride level on performance of the early-weaned pig. Okla. Agric. Sta. Res. Rep., pp. 278–287.

Dierick, N., Vervaeke, I., Decuypere, J., Henderickx, H., 1986a. Influence of the gut flora and of some growth-promoting feed additives in nitrogen metabolism in pigs. I. Studies in vitro. Livest. Prod. Sci. 14, 161–176.

Dierick, N., Vervaeke, I., Decuypere, J., Henderickx, H., 1986b. Influence of the gut flora of some growth-promoting feed additives in nitrogen metabolism in pigs. II. Studies in vivo. Livest. Prod. Sci. 14, 177–193.

Dierick, N., Decuypere, J., Molly, K., Van Beek, E., Vanderbeke, E., 2002. The combined use of triacylglycerols (TAGs) containing medium chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative for nutritional antibiotics in piglet nutrition. I. In vitro screening of the release of MCFAs from selected fat sources by selected exogenous lipolytic enzymes in simulated pig gastric conditions and their effects on the gut flora of piglets. Livest. Prod. Sci., in press.

Dove, C., 1993. The effect of adding copper and various fat sources to the diets of weanling swine on growth performance and serum fatty acid profiles. J. Anim. Sci. 71, 2187–2192.

Duncan, A., Mayes, R., Lamb, C., Young, S., Castillo, I., 1999. The use of naturally occurring and artificially applied n-alkanes as markers for estimation of short-term diet composition and intake in sheep. J. Agric. Sci. Cambridge 132, 233–246.

Edwards-Webb, J., Thompson, S., 1977. Studies on lipid digestion in the preruminant calf. 2. A comparison of the lipolysis of milk fat by salivary and pancreatic lipases in vitro. Br. J. Nutr. 37, 431–440.

Eidelsburger, U., 1998. Feeding short-chain organic acids to pigs. In: Garnsworthy, P. (Ed.), Recent Advances in Animal Nutrition. Nottingham University Press, Nottingham, pp. 93–106.

Elstner, F., 1997. Expander zur Verarbeitung von Ölsaaten. Fett/Lipid 99, 147–152.

Fakler, T., Sohn, K., Maxwell, C., 1992. Effect of protein and fat source on performance in early weaned pigs. Okla. Agric. Exp. Sta. Rep., pp. 366–372.

Février, C., Mourot, J., Jaguelin, Y., Mounier, A., Lebreton, Y., 1994. Compared nutritive values of cows' and goat's milks assessed through pig digestibility and body fat composition. In: Proc. 45th EAAP Meeting, Edinburgh, 5–8 September, p. 11.

Furuya, S., Sakamoto, K., Asano, T., Takahashi, S., Kameoka, K., 1978. Effects of added dietary sodium acrylate on passage rate of markers and apparent digestibility by growing pigs. J. Anim. Sci. 47, 159–165.

Furuya, S., Takahashi, S., 1975. Rate of passage of chromic oxide and polyethylene glycol and digestibility in the digestive tract of pigs. Jpn. J. Zoot. Sci. 46, 630–641.

Gabert, V., Sauer, W., 1994. The effect of supplementing diets for weanling pigs with organic acids—a review. J. Anim. Feed Sci. 3, 73–87.

Geary, T., Brooks, P., 1998. The effect of weaning weight and age

on the post-weaning growth performance of piglets fed fermented liquid diets. Pig J. 42, 10–23.

Glaps, J., 1970. Omowiennie wstepnych wynikow bedan prrzeprowadzonych w Instytucie Zooteniki nad stosowasiem suszu ziemniaczanego do zywienia zwierzat. Rockzniki Nauk Rolniczych B93–94, 549.

Hamosh, M., Bitman, J., Liao, T., Mehta, N., Buczek, R., Wood, D., Grylack, L., Hamosh, P., 1989. Gastric lipolysis and fat absorption in preterm infants: effect of medium-chain triglyceride or long chain triglyceride containing formulas. Pediatrics 83, 86–92.

Höller, H., 1970. Untersuchungen über Sekret und Sekretion der Cardiadrüsenzone im Magen des Schweines. Zbl. Vet. Med. A17, 685–711.

Isaacs, C., Litov, R., Marie, P., Thormar, H., 1992. Addition of lipases to infant formulas produces antiviral and antibacterial activity. J. Nutr. Biochem. 3, 304–308.

Jaturasitha, S., Kreuzer, M., Lange, M., Köhler, P., 1996. Quality of subcutaneous, intermuscular and intramuscular fat tissue using elevated quantities of medium chain fatty acids in pig fattening. Fett/Lipid 98, 149–156.

Jensen, B., 1993. The possibility of manipulating the microbial activity in the digestive tract of monogastric animals. In: Proc. 44th Annual Meeting of the European Association for Animal Production, Aarhus (DK), 16–19 August, p. 20.

Jensen, B., 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. Anim. Feed Sci. 7, 45–64.

Jensen, M., Jensen, S., Jakobsen, K., 1997. Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. J. Anim. Sci. 75, 437–445.

Kabara, J., Swieczkowski, D., Conley, A., Truant, J., 1972. Fatty acids and derivates as antimicrobial agents. Antimicrob. Agents Chemother. 2, 23–28.

Koch, D., Pearson, A., Magee, N., Hoefer, J., Schweigert, B., 1968. Effect of diet on the fatty acid composition of pork fat. J. Anim. Sci. 27, 360–365.

Kreuzer, M., Gerdemann, M., Reyer, W., 1997. Selective improvement of porcine firmness by a diet enriched in medium-chain fatty acids. Fett/Lipid 99, 436–442.

Mahan, D., 1991. Efficacy of initial postweaning diet and supplemental coconut oil or soybean oil for weanling swine. J. Anim. Sci. 69, 1397–1402.

McCarthy, J., Aherne, F., Okai, D., 1974. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54, 107–109.

Meeus, J., 1994. De antimicrobiële activiteit van organische zuren en pH op de darmflora bij het varken. Thesis Landbouwkundig Ir., University of Ghent, Faculty Agricultural and Applied Biological Sciences, 110 pp.

Moreau, H., Gargouri, Y., Lecat, D., Junien, J.-L., Verger, R., 1988. Screening of preduodenal lipases in several mammals. Biochim. Biophys. Acta 959, 247–252.

Nelson, J., Jensen, R., Pitas, R., 1977. Pregastric esterase and oral lipases—a review. J. Dairy Sci. 60, 327–362.

Newport, M., Howarth, G., 1985. Contribution to the digestion of fat in the neonatal pig. In: Proc. 3rd Int. Seminar on Digestive Physiology in the Pig, Copenhagen, 16–18 May, pp. 143–145.

Newport, M., Storry, J., Tuckley, B., 1979. Artificial rearing of pigs. 7. Medium chain triglycerides as a dietary source of energy and their effect on live-weight gain, feed:gain ratio, carcass composition and blood lipids. Br. J. Nutr. 41, 85–93.

O'Connor, J., Perry, H., Harwood, J., 1992. A comparison of lipase activity in various cereal grains. J. Cereal Sci. 16, 153–163.

Överland, M., Granli, T., Kjos, N., Fjetland, O., Steien, S., Stokstad, M., 2000. Effect of dietary formates on growth performance, carcass traits, sensory quality, intestinal microflora and stomach alterations in growing-finishing pigs. J. Anim. Sci. 78, 1875–1884.

Partanen, K., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12, 117–145.

Petschow, B., Batema, R., Talbott, R., Ford, L., 1998. Impact of medium-chain monoglycerides on intestinal colonisation by *Vibrio cholerae* or enterotoxigenic *Escherichia coli*. J. Med. Microbiol. 47, 383–389.

Piva, A., 1998. Non-conventional feed additives. J. Anim. Feed Sci. 7, 143–154.

Roth, F., Kirchgessner, M., 1998. Organic acids as feed additives for young pigs: nutritional and gastrointestinal effects. J. Anim. Feed Sci. 7, 25–33.

Rys, R., Urbanczyk, J., Gawlik, J., 1969/70. Anwendung synthetischer Fettsäuren in der Schweinefütterung. Jahrbüch für Tierernährung und Fütterung 7, 224–231.

Takada, R., Shidara, O., Saitoh, M., Mori, T., 1992. Effects of feeding medium chain triglycerides on growth performance, digestibility, backfat thickness and fatty acids composition in finishing pigs. Jpn. J. Swine Sci. 29, 32–40.

Thacker, P., 1999. Nutritional requirements of early weaned pigs: a review. Pig News and Information 20, 13N–24N.

Thomke, S., Elwinger, K., 1998. Growth promotants in feeding pigs and poultry. II. Mode of action of antibiotic growth promotants. Ann. Zootechn. 47, 153–167.

Van Der Heyde, H., Henderickx, H., 1963. Zur Vereinfachung der quantitativen und qualitativen Bestimmung der Bakterien unter Verwendung von 'Ringplatten'. Zentralblatt Bakteriol. I. Orig. 189, 224–228.

Vervaeke, I., Decuypere, J., Dierick, N., Henderickx, H., 1979. Quantitative in vitro evaluation of the energy metabolism influenced by Virginiamycin and Spiramycin used as growth promoters in pig nutrition. J. Anim. Sci. 49, 846–856.

Vulich, S., O'Riordan, E., Hanrahan, J., 1991. Use of n-alkanes for the estimation of herbage intake in sheep: accuracy and precision of the estimates. J. Agric. Sci. 116, 319–323.

Willis, W., Marangoni, A., 1999. Biotechnological strategies for the modification of food lipids. Biotechnol. Genet. Rev. 16, 141–175.

Wiseman, J., Garnsworthy, P., 1997. The role of lipids in animal nutrition. In: Gunstone, F., Padley, F. (Eds.), Lipid Technologies and Applications. Marcel Dekker, New York, pp. 557–577.

Wiseman, J., Agunbiade, J., 1998. The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. Livest. Prod. Sci. 54, 217–227.