Determination of the Metabolic Faecal Nitrogen and the Endogenous Urinary Nitrogen on Mink

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Determination of the biological value (BV) of proteins for maintenance and growth, according to Thomas (1909) and Mitchell (1924), has found use to a great extent with rats and other species of domestic animals. However the equation for calculation of BV depends on values for metabolic faecal nitrogen (metabolic N) and endogenous urinary nitrogen (endogenous N), which cannot be determined directly in a single experiment. Several experiments have been carried out to investigate the amount of nitrogen excreted as respectively metabolic N and endogenous N. Many investigators consider the amount of metabolic N to be a direct function of the amount of dry matter consumed. A critical evaluation of the factors, which could have some influence on the metabolic N in rats was made by Njaa (1963). He found that besides the influence of the consumed amount of nitrogen and dry matter, metabolic N was dependent on the body weight of the animal and the amount of protein consumed. However, this will not have very much influence on the BV. This can easily be seen from the classic equation for calculation of BV:

$$BV = \frac{\text{N intake} - (\text{faecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})}{\text{N intake} - (\text{faecal N} - \text{metabolic N})} \times 100$$

in which metabolic N and endogenous N serve as correction factors.

The metabolic N appears in the equation both in the numerator as well as in the denominator. This results in only small differences in the BV caused by changing the metabolic N within certain limits.

On the other hand, the amount of endogenous N influences the BV a great deal. Thus it is very important to determine the endogenous N as accurately as possible. It has been usual to calculate endogenous N as a function of the body weight, the body weight taken to some power less than unity or the ebody surface, assumed to be the (body weight)$^{0.67}$ (Allison et al., 1946; Bricher & Mitchell, 1947; Nasset, 1957; Forbes et al., 1958; Brody, 1945; Goyco & Asenjo, 1947; Barnes et al., 1946; Mitchell, 1955).

Njaa (1963) investigated the influence of different factors on the endogenous N. He found the weight increment to have a greater influence on the endogenous N than has the body weight. The correlation between endogenous N and weight increment was found to be negative. Rakowska et al. (1970) has investigated the effect of age of rats on the net protein utilisation (NPU). With a protein content of 4%, they found no difference between rats of different ages, but by increasing the amount of protein to 10 or 20% they found decreasing NPU by increasing age.

The technique in the modified Thomas–Mitchell method, described by Eggum & Mercer (1964), using rats, includes elimination of differences in body weight and age of the animals. This is not possible in similar experiments with mink, which only have kits once a year. An adaptation of the Thomas–Mitchell method to mink therefore includes investigation of the influence of body weight and age on the metabolic- and endogenous-N, besides the factors N-intake, weight gain, and intake of dry matter, which also must be taken into consideration in experiments with rats.

Material and Methods

The cages used for the experiments were constructed so as to facilitate separate collection of faeces and urine. A detailed description of the cages is presented by Jørgensen & Glem Hansen (1972).

Table 1. *Composition of test diets*

Test diet	A	B	C	D
Casein[a]	20.67	14.53	11.41	8.30
Vitamin mixture[b]	1.00	1.00	1.00	1.00
Mineral mixture[c]	5.00	5.00	5.00	5.00
Dextrose	42.50	46.50	49.00	51.50
Cellulose	5.00	5.00	5.00	5.00
Soyoil	12.50	13.97	14.09	14.70
Lard	13.33	14.00	14.50	14.50
Protein percentage in dry matter	18.15	12.69	9.93	9.19
K calories in 100 g dry matter	544	557	555	553

[a] Casein enriched with 0.33% L-arginine, 0.16% DL-methionine and 0.16% L-cystine.
[b] Vitamin mixture containing pr. kg: 24 000 I.U. vitamin A; 2 400 I.U. vitamin D_3; 800 mg vitamin E; 2 000 mg vitamin C; 200 mg thiamine; 400 mg riboflavin; 200 mg pyridoxine; 10 mg biotin; 5 000 mg inositol; 10 000 mg para-amino-benzoic acid; 500 mg menadione; 40 mg folic acid; 0.8 mg vitamin B_{12}; 800 mg niacin and 300 mg Ca-pantothenate.
[c] Mineral mixture with the following composition: 8.19 calcium carbonate, 33.25% calcium phosphate, 13.43% sodium chloride, 29.35% potassium bicarbonate, 12.73% magnesium sulphate, 2.25% ferrous sulphate, 0.35% manganese sulphate, 0.07% sodium iodate, 0.15% cupric sulphate, 0.20% zink sulphate, 0.02% cobalt sulphate, 0.001% sodium selenite and 0.003 sodium molybdate.

The experiments were carried out in a well ventilated house where the temperature was kept between 16 and 22°C.

Fourty-eight Standard male mink were used throughout the study. One hundred and thirteen nitrogen-balances were carried out on mink aged from 10–24 weeks. Four different diets were used in the experiment. The sole source of protein was casein enriched with a supplement of amino acids, fed at 4 different levels (see Table 1). The feed was mixed with water and made to a soft mass before it was weighed and frozen until the day of feeding. Left-over feed was collected, frozen and determined for content of dry matter at the end of the experiment. The diet was fed restricted, but it was aimed to adjust the daily ration to the requirement of the animals.

The experimental period was 7 days with a 4-day collection period after 3 days of feeding the test diets.

Urine was collected from the animals individually. As a preservative for the urine, 10 ml of a 5% sulphuric acid was placed in each collection flask at the beginning of the collection period. Once daily the screen for faeces collection and the funnel which leads the urine to the flask were washed down with a 5% solution of citric acid in distilled water. At the end of the experiment, the urine was stored in a freezer at −20°C until analysis.

Faeces were collected once daily and kept at −20°C during the experimental period. Before analysis they were freeze-dried, ground and sifted for separation of hair in the faeces.

The animals were weighed at the start and the end of the collection period.

The feed was analysed for the content of dry matter, nitrogen and gross calories, the urine for nitrogen, and the faeces for dry matter, nitrogen and gross calories. Nitrogen determination was carried out by the Kjeldahl method and determination of gross calories by the bomb calorimetric method.

The analyses were carried out at the Department of Animal Physiology and Chemistry, Copenhagen.

Results and Discussion

Metabolic N

Calculations showed positive correlation between faecal nitrogen and nitrogen intake, body weight, weight increment, age, and intake of dry matter (Table 2). This is not surprising, because several investigators have shown a positive correlation between faecal nitrogen and nitrogen intake and all the other measurements are positively correlated to the nitrogen intake. However a multiple regression calculation showed an influence of these factors to be independent of the nitrogen intake. As the regression between nitrogen intake and faecal nitrogen is clearly linear, (Fig. 1) the metabolic nitrogen will not only, as generally assumed, be related to the intake of dry matter, but also to the body weight, the weight increment, and the age of the animal. As mentioned earlier in this report, Njaa (1963) also found metabolic nitrogen in rats to be dependent on body weight

Table 2. *Coefficients of correlation between faecal N and respectively N-intake, body weight, weight gain, age and intake of dry matter*

	N in take	Body weight	Weight gain	Age	Dry matter intake
Coefficient of correlation	0.76	0.37	0.39	0.26	0.49

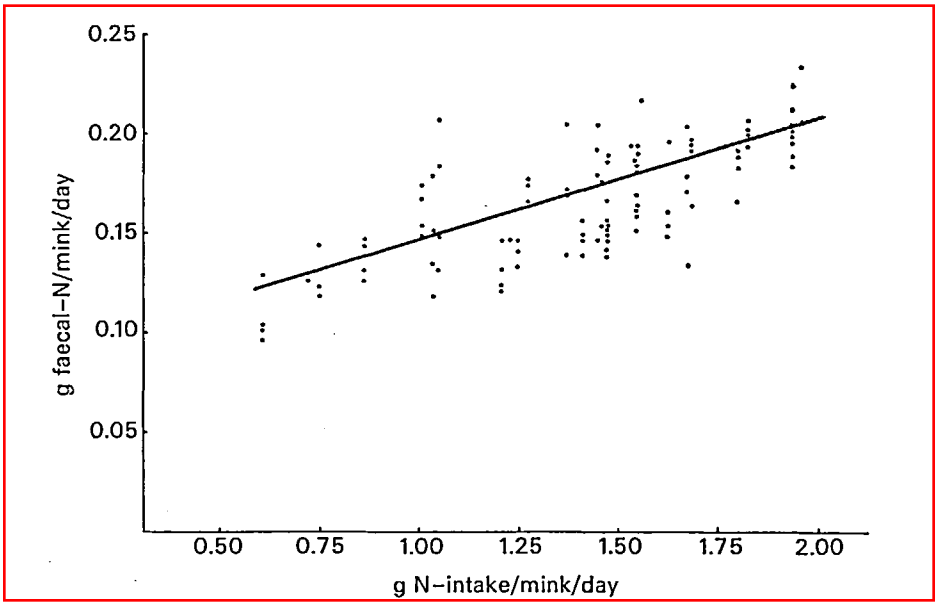


Fig. 1. The regression of faecal nitrogen to the nitrogen intake in 113 N-balances.

and weight gain. However, differences in body weight and age can be eliminated in experiments with rats, but if this were done with mink it would limit the number of experiments to very few per year. The average amount of metabolic nitrogen in the 113 nitrogen balances was calculated to 0.34 g N per mink in 4 days or 0.085 g N per day per mink with an average body weight of 1.2 kg. The multiple regression equation for calculation of the metabolic N in the 4-day collection period is:

Metabolic N = 0.261 − (kg body weight × 0.075) − (g weight gain × 0.00025) + (age in weeks × 0.004) + (g dry matter consumed × 0.0004).

The multiple correlation coefficient was 0.77.

Endogenous N

Higher degree of correlation were found, using the logarithm to urinary N and the measured values of nitrogen intake, body weight, weight gain, age and dry matter intake than between the direct measured amount of urinary N and these values (Tables 3 and 4 and Figs. 2 and 3).

A calculation of the multiple regression with log urinary N as the dependent variable and the other measured values as independent variables, justified consideration of body weight as well as weight gain, age, and dry matter intake when endogenous N is to be evaluated. Njaa (1963) also found endogenous N to depend on weight gain in addition to, as generally assumed, the body weight. The content of dry matter in this experiment was kept constant and so was the energy content. Therefore, an effect of dry matter on the urinary N excretion could just as well be an effect of energy. Miller & Payne (1961) concluded that under conditions of caloric restriction, the protein value of a diet will depend upon the energy avaible for protein anabolism rather than on the

Table 3. *Coefficients of correlation between urinary-N and respectively N-intake, body weight, weight gain, age and intake of dry matter*

	N intake	Body weight	Weight gain	Age	Dry matter intake
Coefficient of correlation	0.87	0.54	0.34	0.41	0.38

Table 4. *Coefficients of correlation between log urinary-N and respectively N-intake, body weight, weight gain, age and intake of dry matter*

	N intake	Body weight	Weight gain	Age	Dry matter intake
Coefficient of correlation	0.89	0.56	0.35	0.42	0.44

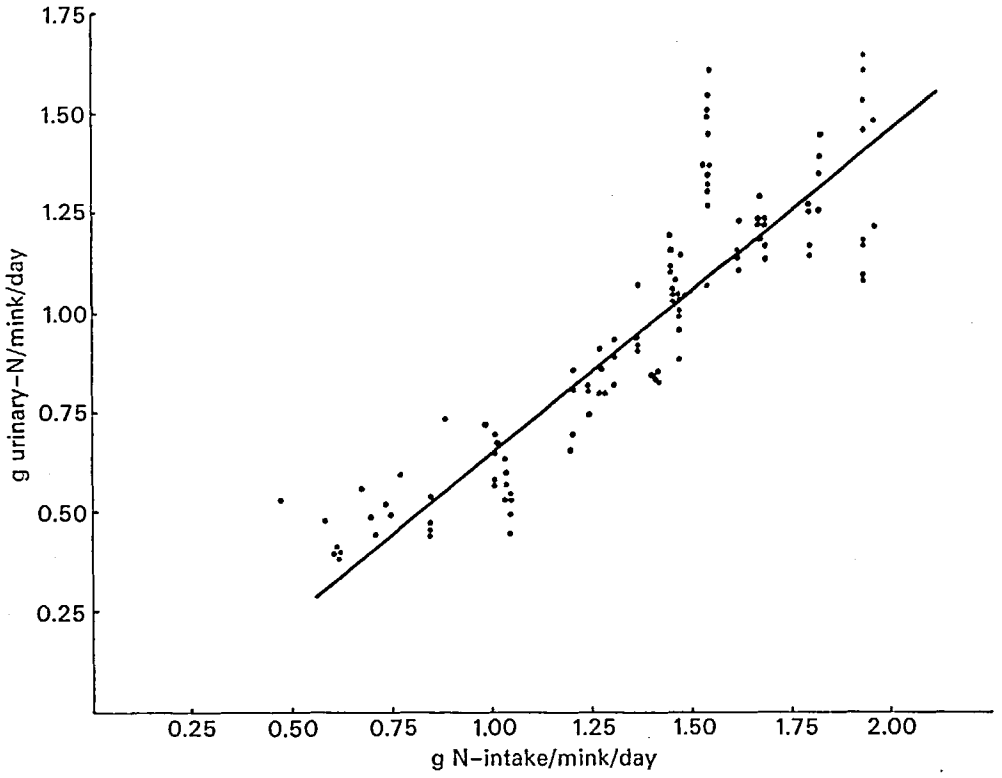
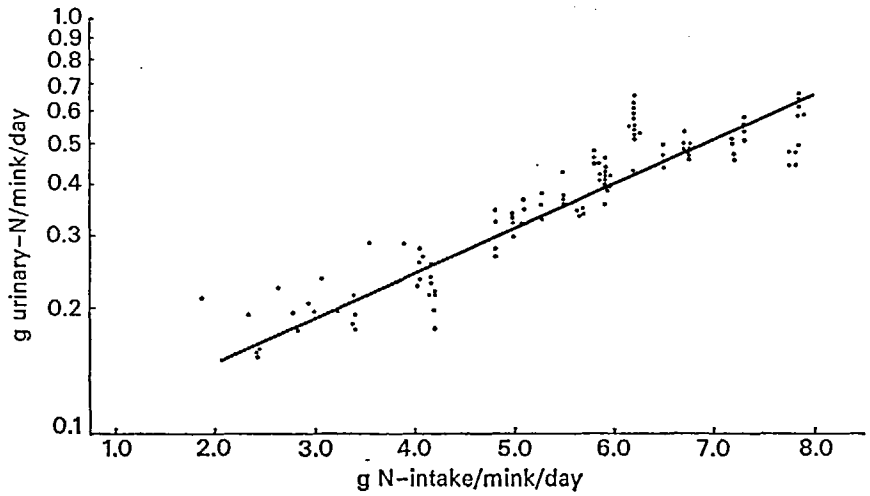


Fig. 2. The regression of urinary nitrogen to nitrogen intake in 113 N-balances.

Fig. 3. The regression of log. urinary nitrogen to nitrogen intake in 113 N-balances. The ordinate is in a logarithmic scale.

concentration and the nature of the protein it contains. Thus, for any diet, one might expect a range of food intake with a constant protein content proportional to the energy content, below which the biological value will fall with decreasing intake of calories.

The multiple regression equation also shows the



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urinary N and therefore the endogenous N to depend on the age of the animals. As mentioned Rakowska et al. (1970) found decreasing NPU with increasing age, using a relatively high level of protein in the diets. They used the carcass nitrogen method where the retained nitrogen is measured directly. Thus, in this case the decrease in NPU is real and not caused by a change in the amount of endogenous N, but is rather a consequence of decreasing protein requirement with increasing age.

The amount of endogenous N in the present study is calculated to 0.245 g N per day per mink with an average body weight of 1.2 kg. The multiple regression equation for calculation of endogenous N in the 4 days collection period was:

Endogeneous N = 0.02 + (kg body weight × 0.083) − (gram weight gain × 0.00054) + (age in weeks × 0.00377) − (gram dry matter × 0.00085).

The multiple correlation coefficient was 0.93.

It is not directly obvious that endogenous N should depend on the amounts of calories consumed and age of the animals. Therefore correction of the endogenous N on the basis of these factors could rather be considered as corrections for the unavoidable lack of uniformity of the experimental animals than a real difference in the amount of endogenous N proportional to these factors.

Summary

One hundred and thirteen N-balances were carried out to determine the influence of N-intake, body weight, weight increment, age and dry matter intake on the amount of metabolic N and endogenous-N in mink. Multiple regression analyses showed that all the above-mentioned parameters statistical significantly influenced the metabolic-N as well as the endogenous-N. Multiple regression equations for calculation of metabolic-N and endogenous-N are shown.

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