Protein and Amino Acids in the Nutrition of the Growing-Furring Blue Fox

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Academic dissertation

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ABSTRACT

This thesis evaluates the protein and amino acid requirements of the modern grow-ing-furring blue fox (*Alopex lagopus*). The effects of dietary protein and the supple-mented amino acids, methionine and lysine, on nitrogen (N) retention, nutrient di-gestibility, growth performance, health, and fur characteristics were estimated in digestibility and N balance trials and production experiments. As well as visual grad-ing of the skin, objective physical determinations of the leather were conducted to provide a complete picture of both the fur and the leather properties. In addition, the ideal pattern and the limiting order of some essential amino acids were determined.

An insufficient dietary content of methionine impaired the digestibility of the diet. Supplementing low-protein diets with methionine may therefore increase the metabolisable energy (ME) value of the diet. The DL-form of methionine was effec-tively utilised by the growing-furring blue fox. In this respect, blue foxes, like dogs, differ from mink. A level of 21–22% protein of ME with 0.40 g DigMet (digestible methionine)/MJ ME (0.50 g of digestible methionine and cystine, SAA) was found to satisfy the requirement for growth, normal hair priming and production of high-quality skins with firm and elastic leather. Even 15–16% protein of ME (with 0.40 g DigMet/MJ ME) may be adequate for the blue fox during the later phases of the growing-furring period. Methionine supplementation improved guard hair quality. The effects of lysine supplementation in low-protein diets, 15% or 22% protein of ME, were inconsistent. At 15% protein of ME, and about 0.60 g digestible lysine/MJ ME, supplementation of lysine did not improve performance of the growing-furring blue fox.

The dietary deletion of SAA in the N balance trial resulted in severe responses of young blue foxes, exceeding those to the deletion of any other amino acid (lysine, threonine, histidine, tryptophan). The results corroborated those of the production experiments. On typical diets containing slaughter by-products and fish, the first limiting amino acids for the growing-furring blue fox are thus SAA, then histidine followed by threonine and tryptophan (not necessarily in that order) and, finally, lysine.

The ideal pattern of amino acids was: lysine = 100, SAA 77, threonine 64, histidine 55, and tryptophan 22. As this pattern is based on measurements conducted on young weaned blue foxes, the relative SAA requirement of blue foxes is likely to be higher later in the growing-furring period (from September onwards) due to the develop-ment of winter fur.

Neither disturbances in clinical health nor mortality related to experimental diets were found. At the lowest level, 15% protein of ME, the relative weight of liver was increased. This could be prevented by methionine supplementation up to 0.40 g DigMet/MJ ME. The blue fox seems able to adapt to changes in dietary protein and amino acid supply to a greater extent than mink, which is a strict carnivore. Blue foxes use protein primarily for tissue synthesis, not as an energy source.

Nitrogen excretion can be declined by 2–3 g per blue fox per day by reducing the dietary protein level from about 25–30% to 22% of ME. The practical application of low-protein diets, with supplemental methionine when required, is recommended. This would be beneficial in terms of reduced feed expenses and lower N emissions to the environment, without compromising the welfare and performance of the growing-furring blue fox.

ACKNOWLEDGEMENTS

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications referred to in the text by their Roman numerals:

I   Dahlman, T., Kiiskinen, T., Mäkelä, J., Niemelä, P., Syrjälä-Qvist, L., Valaja, J. and Jalava, T. 2002. Digestibility and nitrogen utilisation of diets containing protein at different levels and supplemented with DL-methionine, L-methionine and L-lysine in blue fox (*Alopex lagopus*). Anim. Feed Sci. Technol. 98: 219–235 (© Elsevier).

II   Dahlman, T., Valaja, J., Jalava, T. and Skrede, A. 2003. Growth and fur characteristics of blue foxes (*Alopex lagopus)* fed diets with different protein levels and with or without DL-methionine supplementation in the growing-furring period. Accepted for publication in Can. J. Anim. Sci. – June 2003 issue. In press.

III   Dahlman, T., Valaja, J., Niemelä, P. and Jalava, T. 2002. Influence of protein level and supplementary L-methionine and lysine on growth performance and fur quality of blue fox (*Alopex lagopus*). Acta Agric. Scand. 52: 174–182 (© Taylor & Francis).

IV   Dahlman, T., Mäntysalo, M., Rasmussen, P.V. and Skovløkke, L.L. 2002. Influence of dietary protein level and the amino acids methionine and lysine on leather properties of blue fox (*Alopex lagopus*) pelts. Arch. Anim. Nutr. 56: 443–454 (© Taylor & Francis, reference to the journal's web-site: http://www.tandf.co.uk).

V   Dahlman, T., Valaja. J., Venäläinen, E., Jalava, T. and Pölönen, I. 2003. Optimum dietary amino acid pattern and limiting order of some essential amino acids for growing-furring blue foxes (*Alopex lagopus*). Submitted to Anim. Sci.

ABBREVIATIONS

AD	apparent total tract digestibility
BRL	breaking load
CC	crude carbohydrates
CF	crude fibre
CCK	cholecystokinin
CP	crude protein
DigMet	digestible methionine
DM	dry matter
DigSAA	digestible methionine and cystine
EE	ether extract
L	lysine
M	methionine
ME	metabolisable energy
N	nitrogen
OM	organic matter
P	dietary protein level
PEB	relative elongation at break
SAA	sulphur-containing amino acids (methionine and cystine)
SAM	S-adenosylmethionine
TEN	tensile strength
THB	thickness

1. INTRODUCTION

Finland is the world's leading producer of farm-raised foxes, accounting for about 60% of global production. During the last ten years Finland's production has averaged 2.1 million fox skins per year, with a total annual value of about EUR 200 million (in selling season 2001/2002, Finnish Fur Breeders Association, 2002a). Today, the majority (over 90%) of the fox skins produced are from the blue fox. The domesticated blue fox, a colour mutation of the wild polar fox (*Alopex lagopus*), is a carnivorous mammal of the Canidae family. The diet of blue foxes thus contains a relatively high proportion of animal protein.

Feed constitutes the greatest individual item of cost, amounting to 50% of the total production cost in fox farming (Hernesniemi, 2000). As protein is the most expensive dietary nutrient, any reduction in protein level contributes to a saving in production costs and, moreover, to a reduction in nitrogen (N) emissions. The directives of the Ministry of the Environment concerning the protection of waters against pollution (Ministry of the Environment, 1998) require the amount of N originating from fur farms to be cut by 55% from the 1993 level by 2005. The goal is ambitious but achievable. Over 70% of total annual feed consumption, and, thus, of the respective N emissions occur during the four months of the growing-furring season (August – November, Finnish Fur Breeders Association, 2002a). Lowering the dietary protein level during this period would therefore be very important but first we need better knowledge of the amino acid requirements of the growing-furring blue fox. The availability of fish, traditionally the main feed ingredient, is likely to decline globally, in addition to which domestic fishing is strictly regulated by the European Union. The annual quotas for Baltic herring (*Clupea harengus L.*) catches have been consistently reduced in recent years. Indeed, the composition of slaughter by-products as a whole has become less favourable as the nutritionally best parts are no longer included in the mass used for fur animals. What is more, the use of by-products originating from fur animal carcasses in the feed of fur animals is likely to be restricted. At the same time as the supply of ingredients has changed, the number of animals has remained more or less the same, thus presenting modern fox feed manufacturers with a major challenge. The increased use of alternative sources of protein with a less favourable amino acid composition for growing-furring blue foxes may require dietary supplementation with the limiting amino acid.

Experimental data and scientific information on the protein requirement of the blue fox are scarce. Some studies on protein in the diets of silver foxes were conducted in North America in the 1940s (Harris et al., 1951). At that time, fox diets normally contained 35% to 45% protein on a dry matter (DM) basis. In the light of findings in growing-furring silver foxes, Harris et al. (1951) concluded that 28% of protein in DM was sufficient to produce growth as rapid as that at higher protein levels. In Norway, a series of production studies on dietary protein was carried out in blue foxes from the 1950s to 1970s. A summary of these was presented at the first International Scientific Congress in Fur Animal Production in Helsinki (Rimeslåtten, 1976). A reduction in protein levels, from 45% to 22% of metabolisable energy (ME), was reported not to affect body weight but a protein level below 28–30% of

ME reduced body length at pelting significantly. As no updated information is available, the established protein recommendations are still based on the results of older experiments (NRC, 1982; Hansen et al., 1991).

Since then, however, the blue fox stock has undergone an important change in that the size of the animal has increased markedly, notably during the past decade (Finnish Fur Sales, 1990; 1999). In the 1970s, average body weight at pelting was 6–7 kg and skin length 90–95 cm (Rimeslåtten, 1976). Today the pelting weight of a blue fox in Finland can be double that reported by Rimeslåtten and the corresponding average skin length has increased by 30–40% (Finnish Fur Breeders Association, 2002b; Finnish Fur Sales, 1999).

The established blue fox nutrition requirements do not contain any data on amino acids. In mink, another carnivorous fur-bearing animal, research on dietary protein and amino acids has been intensive (e.g. Skrede, 1978; Glem-Hansen, 1980 and 1982; Børsting and Clausen, 1996; Dahlman et al., 1996; Damgaard, 1997; Kerminen-Hakkio et al., 2000). These studies have found methionine to be the first limiting amino acid for hair growth and thus for fur quality. Research on young pigs and poultry has found lysine to be the first limiting amino acid for growth (e.g. ARC, 1981; Boisen et al., 2000; Roth et al., 2001). Very few research data are, however, available on the blue fox's requirement for dietary amino acids. In fact, to my knowledge, only one paper has been published on the effects of amino acids on the performance of growing-furring blue foxes (Työppönen et al., 1987).

Another subject not studied is the balance between essential amino acids in blue foxes. Lysine, methionine, threonine and tryptophan are generally considered to be the first limiting essential amino acids for growing animals. In addition, a low content of dietary histidine has been demonstrated to cause severe reductions in performance and N retention in growing chicks (Roth et al., 2001). A number of studies have estimated the ideal amino acid patterns for pigs and poultry (Fuller et al., 1989; Wang and Fuller, 1989 and 1990; Gruber et al., 2000; Roth et al., 2001) and also for growing-furring mink (Børsting and Clausen, 1996). The ideal protein pattern can be defined as the optimal ratio of the essential amino acids required for maintenance and production (Boisen et al., 2000). It enables the requirements of all essential amino acids to be calculated once the requirement of a single individual amino acid has been established. In practice, optimal low-protein diets can be formulated only when the amino acid requirements are accurately known.

Another important parameter in fur animal skins besides pelt size and fur properties is the quality of leather, but knowledge of quality criteria and the effect of feed on leather characteristics is scarce. Rouvinen and Mäntysalo (1989) studied the effects of dietary fat on the physical properties of mink and blue fox leather. Before the present research, however, no studies had been published on the effects of dietary protein and amino acids on blue fox leather properties.

2. OBJECTIVES OF THE THESIS

The overall objective of the thesis was to gain knowledge about the protein and amino acid requirements of modern growing-furring blue foxes and thus to establish a more precise basis for diet composition in feed formulation. Recent changes in the supply of feed ingredients may lead to a shortage of dietary lysine and methionine. Attention was therefore paid to these amino acids. More specifically, experiments were conducted to assess:

- the digestibility of dietary components and amino acids at varying protein, methionine and lysine levels (**I**). The determined digestibility in turn formed the basis of dietary calculations in the subsequent feeding experiments;
- the effects of protein level and supplementary methionine and lysine on N retention and excretion (**I**);
- the influence of different levels of protein, lysine and methionine on the performance of the growing-furring blue fox (**II** and **III**). The two issues of special interest were: the protein, lysine and methionine requirements of blue foxes during the period of fast growth after weaning, with the emphasis on lysine's effects on body growth; and the requirement of protein, methionine and lysine for winter fur development, with the emphasis on methionine's effects on skin quality;
- the effects of dietary protein and the amino acids lysine and methionine on leather properties (**IV**);
- the ideal pattern and limiting order of some essential amino acids in the growing-furring blue fox (**V**). This experiment was conducted on lysine, the sulphur-containing amino acids methionine and cystine (SAA), threonine, tryptophan and histidine.

3. MATERIALS AND METHODS

The experimental arrangements including the animals, skins and diets, and all the analyses conducted, are described in detail in the original papers (**I-V**). A general description of the research is presented briefly as follows.

3.1. Animals, diets and experimental procedures

Two consecutive production experiments were conducted on 125 and 175 male cubs, respectively. In the first experiment (**II**), the animals were allocated to five treatments: P30 (P = planned protein content % of ME), P22.5, P22.5M (M = DL-methionine supplementation), P15 and P15M. The first experiment started on 14 August (average age of cubs 79 days) and ended on 30 November. In the second experiment (**III**), the animals were randomly allocated to seven feeding groups: P30, P22.5, P22.5M (M = L-methionine supplementation), P22.5L (L = L-lysine supplementation), P15, P15M and P15L. The second experiment started on 13 July (average age of cubs 51 days) and ended on 14 December. The diets were designed to provide high-quality protein at the recommended level or slightly below, or clearly below, the Finnish recommendations. All diets comprised equal proportions of crude protein from fish meal, fresh fish and slaughterhouse by-products with low bone content. The animals were housed individually in standard cages. Feed consumption (on a group basis) and weight gain were recorded in both experiments. Body length was measured at pelting. Skin length, skin weight and skin thickness were measured and fur properties evaluated on dried skins. Overall quality, fur density and guard hair quality were visually assessed on a scale ranging from 1 to 10 (best). Colour intensity was measured by the optic technique of the Finnish Fur Sales. The incidence of fur defect woolliness, and, in the second experiment, also bite marks, was recorded. In the second experiment, the weight of raw skins with (unfleshed skins) and without (fleshed skins) subcutaneous fat was recorded.

Digestibility and N retention trials (**I**) were conducted during the two production experiments on six approximately 21-week-old animals with an average weight of 9.3 kg (in the first trial) and 9.5 kg (in the second trial) (initial age and weights). The diets were the same as in the production experiments discussed in **II** and **III**. The animals were kept in individual cages with separate quantitative collection of faeces and urine. The trial period comprised three days of adjustment and four days of faecal and urine collection.

Ten (the first investigation) and seven (the second investigation) pelts from the two production experiments were used in physical investigations to study the effects of dietary protein and the supplemented amino acids methionine and lysine on leather characteristics in blue foxes (**IV**). Straining of skins was calculated from the difference between the length of the dried raw skin and body length. The pelts were dressed with aluminium by standard chemical and mechanical processing methods. After the dressing, the length of the pelt was measured and the relative shrinkage (the difference between dried raw and dressed skin in percentage of dried raw skin) calculated. In dressed blue fox pelts, the following characteristics of the leather were

measured: thickness (THB, mm), breaking load (BRL, N = newton), tensile strength (TEN, MPa = megapascal) and relative elongation at break (PEB, %).

To study the ideal protein balance of lysine, SAA, threonine, histidine and tryptophan, a 5-week balance trial was carried out on 24 newly weaned blue fox male cubs, average age 9 weeks (initial age) (**V**). The method used in this experiment was based on the fact that N retention is determined primarily by the intake of the first limiting amino acid. As long as the supply of the first limiting amino acid is insufficient, N retention responds linearly to an increase in the dietary content of the amino acid in question until the requirement is met. Further supplementation with this amino acid has no effect on N retention. The protein levels in the positive and negative control diets (167 and 96 g/kg DM, respectively) were based on the results of the previous studies (**I**). Only high-quality N sources (casein and synthetic free amino acids) were used. In the positive control diet, part of the casein was replaced with a mixture of free amino acids, and in the experimental diets, one of the experimental amino acids was sequentially deleted. The experimental design was an 8×5 cyclic change-over with blue fox cubs randomly allotted to three blocks of eight animals each. The average initial weight of the cubs was 3.94 kg. The animals were housed in single metabolism cages permitting the quantitative collection of faeces and urine. Each period comprised four days of adjustment and three days of collection. The animals were weighed at the start of every new period and again at the termination of the experiment.

3.2. Laboratory analyses of diets, faeces and urine

The chemical compositions of feed and faeces were analysed by standard procedures. The DM, ash and crude protein (CP) contents were determined according to NMKL (1995) (**I**–**III**) or AOAC (1990) (**V**). Ether extract (EE) was determined after acid hydrolysis, and crude fibre (CF) in accordance with the methods of AOAC (1990). Crude carbohydrates (CC) were calculated by subtracting ash, CP and EE from the DM content. The amino acid composition of the diets was analysed with a Biochrom 20 Amino Acid Analyser (Pharmacia Biotech Ltd., Cambridge, England) according to Directive 98/64/EC (European Commission, 1998). Urea N in urine was determined with a Gilford 3 000 Auto Analyser.

3.3. Calculations and statistical analyses

The apparent total tract digestibility (AD) coefficient of nutrients and amino acids was calculated as follows: AD = (a–b) / a, where a = intake of nutrient from feed, and b = nutrient in faeces. Statistical analyses of experimental data were performed with the general linear model (GLM) procedure of the SAS statistical package (SAS Institute Inc., 1989). Experimental data were subjected to analysis of variance using a model that included the effect of diet (**I**–**V**), the effects of sire and of dam within sire (**II**), and the effects of animal and period (**V**). The physical characteristics of leather were assessed by analysis of variance for repeated measurements using a model that included the cutting angle (**IV**).

4. RESULTS AND GENERAL DISCUSSION

4.1. Effects of dietary protein level

4.1.1. Digestibility and nitrogen utilisation

The apparent digestibility of the main nutrients, as well as of amino acids, declined along with a decrease in dietary protein level (**I** and **V**). Data on the influence of the dietary protein level on the apparent digestibility of fat and carbohydrates seem to be scarce. The influence of protein level on the apparent digestibility of protein has been studied more. Research on ileal-fistulated blue foxes by Szymeczko and Skrede (1991) showed a slight reduction in the apparent digestibility of protein and amino acids at lower protein levels. The finding was attributed to an increasing proportion of endogenous protein with a decrease in protein level. Similar results were obtained by Skrede (1979), working with mink. The amount of metabolic faecal N excretion has been evaluated at 233 mg N per 100 g dietary DM in adult blue foxes fed protein-free diets (Skrede et al., 1980). Application of this value to estimate true protein digestibility in the present research does not totally eliminate the differences between the dietary protein levels. Also Szymeczko and Skrede (1991) reported that the total amount of endogenous protein was rather limited, and had only a minor effect on apparent digestibility in blue foxes fed normal diets.

The influence of protein level on the apparent digestibility of protein has been under intensive research in other animals, pigs in particular. The results on pigs tend to be conflicting (Batterham, 1994). A number of studies suggest that reductions in dietary protein lead to increases in the relative amount of endogenous N secretion, which, in turn, reduce the apparent digestibility of protein and amino acids (Low, 1980; Baker, 1989). In contrast, in studies by Li et al. (1993) the apparent digestibility of protein and amino acids was not affected by the dietary level of protein. Fan et al. (1994) showed that the influence of dietary protein, ranging from 4% to 24%, on an as-fed basis, and various amino acid levels on the respective apparent ileal digestibilities in pigs varied, depending on their contents. Initially, the apparent ileal amino acid digestibility increased sharply, gradually reaching a plateau, after which there was no further increase and the digestibility became independent of the dietary amino acid level. The current results indicate that, for blue foxes, the plateau for the effect of dietary protein level on protein digestibility may be close to P22.5 and, accordingly, further increases in dietary protein are not likely to improve the apparent digestibility of protein.

Nitrogen retention in 9-week-old blue foxes (**V**) was significantly ($P < 0.05$) dependent on dietary protein content. In 21-week-old blue foxes, N retention (g/d) did not differ in relation to the dietary protein level (**I**) and, thus, even the lowest level (P15) was sufficient to satisfy the animals' requirement for protein. The protein requirement of blue foxes at this age is likely to be determined mainly by hair growth, suggested by studies of Blomstedt (1998a; 1998b) and also by recent studies in mink (Rasmussen and Børsting, 2001). No data are available on the blue fox's maintenance requirement or on the division of the total protein requirement between body growth and hair growth. As this issue is of great importance, it is tempting to make some estimations, although direct measurements were not included in this research. In young animals, the proportion of protein required for maintenance is in general

lower than in older animals. In fast-growing pigs, the maintenance requirement can account for about 10% or more of the total requirement, whereas in pigs that have passed the peak in body protein deposition it can be 20-30% (Moughan, 1995). Like-wise, in young blue foxes most of the protein requirement is likely to be due to body growth, and our results (**V**) seem to corroborate those presented by Moughan (1995). In 9-week-old blue foxes, the measured daily N retention (about 2 g) corresponds well with body growth (about 58 g) (maintenance requirement being 10% of the total and the protein content of the weight increase 20%). In 21-week-old blue foxes, daily N retention was on average 2.5 g per animal (**I**). An assumed maintenance requirement of 25% will leave 75% for protein accretion, which, according to our results, would be about 1.9 g N/d. The average daily weight gain of the blue foxes at that time was 60 g, of which 10–15% can be estimated to comprise of protein (unpublished data from **II** and **III**). On the basis of these assumptions, 1.2 g N/d (on average, corresponding to about 60% of the total requirement for protein accretion) was required for body weight gain. This would leave 0.7 g N/d for hair growth, which, in turn, corresponds with the growth rate of winter fur in blue foxes at this age (Blomstedt, 1998a and 1998b; unpub-lished data from **II** and **III**). However, there may be some overestimation in the abso-lute amount of the retained N owing to possible inaccuracies (N evaporation) in the experiment. These are only rough estimates and should be interpreted with caution.

From an environmental point of view, a substantial reduction in N emissions can be achieved by decreasing the dietary protein level. According to the present research, N excretion in urine declines significantly ($P < 0.05$) when the protein level in the diet is lowered from P30 to P22.5 (Figure 1). The results are consistent with earlier findings that N excretion declined noticeably along with a reduction in dietary pro-tein in pigs (e.g. Jongbloed and Lenis, 1992; Valaja et al., 1993). According to Valaja et al. (1993), a reduction in dietary protein from 180 to 120 (g/kg) resulted in a 38.3% decrease in urinary N excretion in growing pigs. This finding is corroborated by the present research on blue foxes showing a 36.8% reduction in N excretion when the protein level declined from about 320 to 250 (g/kg DM) (**I**).
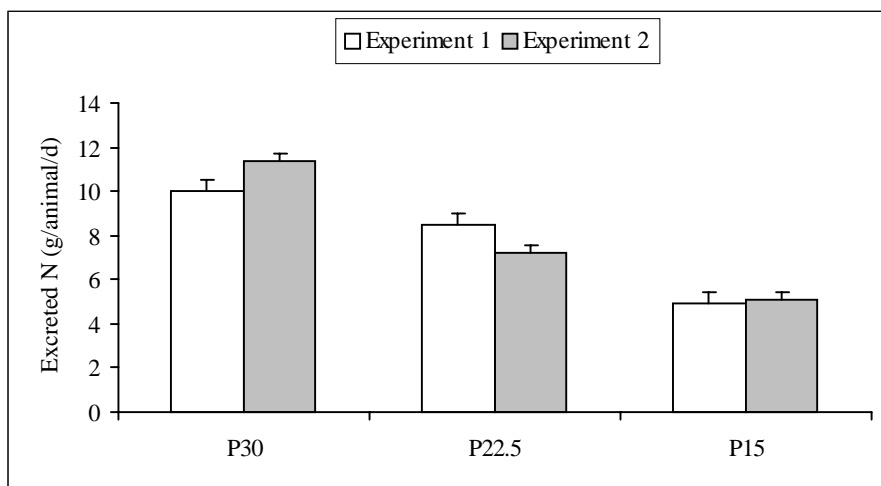

Figure 1. Influence of dietary protein level (P) on the amount of excreted nitrogen (N), (g/animal/day), means and SEM ($P < 0.05$). (Based on **I**).

4.1.2. Growth

As pointed out earlier, the blue fox stock has undergone changes during the past decades. Fresh light can be shed on how greater body size may have affected protein requirement by comparing the data from our experiments (**II** and **III**) with those from the 1960s (Rimeslåtten, 1976). The body weight of blue foxes at pelting has increased by about 70%. In contrast, body length at pelting has increased by only about 10% and skin length by about 30%. Skin length is affected also by factors not related to diet, such as pelting techniques, especially the blocking of skins on drying boards. Body length at pelting reflects the requirement of protein for actual body growth (development of muscles and skeleton) in a more valid way than does the corresponding skin length or body weight.

The requirement for protein (g/d/kg live weight or g/kg dietary DM) is higher during the early than the late phases of growth, as demonstrated earlier in blue foxes (Rimeslåtten, 1976) and in mink, too (Skrede, 1978; Damgaard, 1997). In our study, a lower protein level linearly reduced the early growth of blue foxes, that is, from weaning to mid-September (with the exception of P15M). Muscular and skeletal development seemed to have come to an end by the age of 14 weeks, as indicated by the high correlation between body weight in early September and final body length (r = 0.57, $P < 0.001$; **II**). These results imply that the growth rate of the modern blue fox is more rapid than that of the earlier blue fox (Rimeslåtten, 1976). When the average feed intake (ME) of blue foxes from the present experiments is compared with that of blue foxes from the 1960s (data from Rimeslåtten, 1976), we see that it has increased by about 65%. This figure, in turn, corresponds well with the increase in body weight at pelting. The increase in body weight (blue foxes today vs. earlier) comprises prominently of fat. Thus, the modern blue fox's protein requirement for weight gain during the late phases of growth is likely to be satisfied by lower dietary protein levels (of ME) than earlier.

According to our results, there was a positive and increasing correlation between body weights during the late growth period, from mid-September to pelting, and the weight of unfleshed skin (= including subcutaneous fat), the correlation being highest at the final body weight (r = 0.70, $P < 0.001$; **III**). The average weight of the subcutaneous fat removed from the skin by fleshing was 1.7–1.9 kg per animal in different feeding groups. This equals 32–40% of the weight increase during the late growth period (14–15% of total body weight at pelting). Dietary protein level did not affect the weight of unfleshed skin but the weight of fleshed skin decreased linearly ($P < 0.001$) with a decline in protein level. Thus, the proportion of subcutaneous fat was higher in P15 than in P30. This may be due to the fact that at insufficient protein levels blue foxes use the available energy to accrete fat.

In animals fed *ad libitum*, sub-optimal increase in body weight during the early growth period can be compensated for during the late growth period, as shown by us in the late growth of the animals in the P15 and P15L groups (**III**). Due to this compensation, no differences could be found in final body weights (Figure 2). As pointed out earlier, dietary protein content substantially lower than that previously recommended (Rimeslåtten, 1976; Hansen et al., 1991) may be sufficient to satisfy the protein requirement of blue foxes for weight gain during this period. This level may be close to 15% of ME as even the content of essential amino acids in P15 appeared to be adequate

for weight increase during the late growth period (**II** and **III**). This figure is in good agreement with earlier results for growing dogs fed purified amino acid diets in which an increase in dietary protein content from 14% to 28% suppressed rather than improved growth rate (Milner, 1981). As the growth rate of dogs, however, is slower than that of blue foxes, the absolute values for dietary protein are not comparable as such.
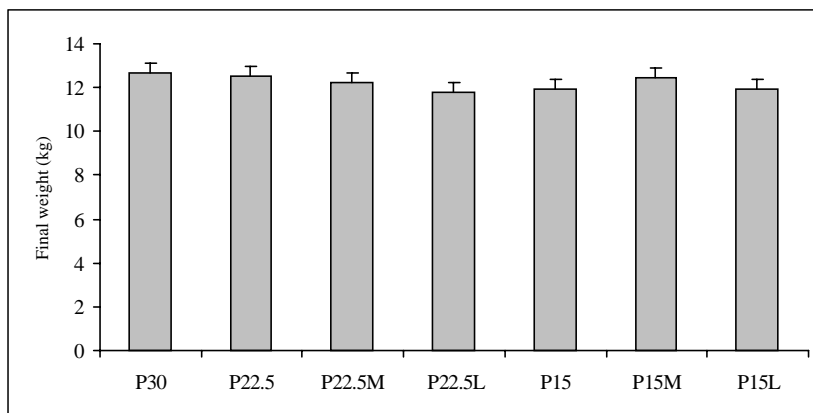
Figure 2. Influence of dietary protein level (P), and of supplemented methionine (M) and lysine (L) on final body weights of blue foxes (ns), means and SEM. (Based on **III**).

Nevertheless, it would seem clear that the blue fox's metabolism is well adapted to much lower protein levels than is that of mink. The animals in all feeding groups appeared to be in normal clinical health, and health disturbances related to the experimental diets were not found. The liver weight of blue foxes in relation to body weight was, however, significantly higher ($P < 0.05$) in animals fed diets with the lowest protein level (P15) than in those fed the higher protein levels (unpublished data from **III**; Figure 3). Mortality related to the experimental diets was not found,
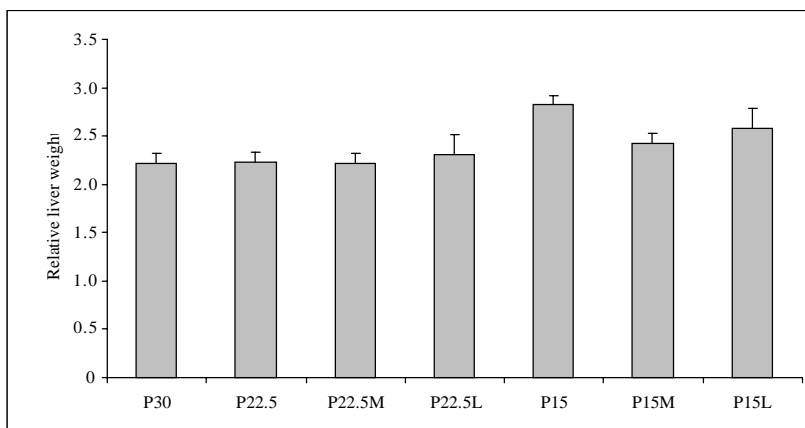

Figure 3. Effect of dietary protein level (P), and of supplemented methionine (M) and lysine (L) on relative liver weight (% of body weight), means and SEM. Liver weight was higher in group P15 than in the groups at the higher protein levels ($P < 0.05$). (Based on determinations on 49 animals sampled from the production experiment in **III**, unpublished data).

not even at the lowest protein level (P15). Our results thus agree with earlier findings of Työppönen et al. (1987) that a reduction in protein level, from 35% of ME (30% from September onwards) to 22% (18%), did not have any effect on the mortality of growing-furring blue foxes.

4.1.3. Skin quality

4.1.3.1. Fur quality

The overall quality of fur was clearly poorer in the skins of blue foxes fed the lowest protein level, P15, than in those from foxes fed the highest protein level, P30 (**II, III**). In contrast, P22.5 was adequate to support normal fur quality and skin length, with no statistical differences from P30. The results are in agreement with those of an earlier blue fox study (Työppönen et al., 1987). The fur quality and skin length of blue foxes fed diets containing 22% protein of ME (18% from September onwards) did not differ significantly from those of blue foxes fed a conventional high-protein diet (35/30% protein of ME) (Työppönen et al., 1987). In mink, the protein require-ment seems to be higher: 25% of ME from protein was found to support normal skin length and fur quality in the studies of Børsting and Clausen (1996). The current results with blue foxes suggest that the requirement of protein in respect of fur qual-ity can be fully satisfied by a dietary protein level of about 21% to 22% of ME, and, thus, there seems to be potential for reducing the protein level previously recom-mended (Hansen et al., 1991).

4.1.3.2. Leather quality

Our research demonstrated, for the first time in blue foxes, that low dietary protein (15% of ME) impairs leather resistance to mechanical load, indicated by reduced BRL (**IV**). This implies damage in collagen functioning, as collagen chains provide the major scaffold for cell attachment and anchorage of macromolecules, and permit the shape and form of tissues to be maintained (Stryer, 1998). Our results seem to corroborate those of Marjoniemi et al. (1991), who found that damage to the fibre structure of collagen was reflected in reduced BRL in blue foxes. Dietary factors that can damage the fibre structure of leather are most likely to damage the hair follicles, too. This, in turn, may have adverse effects on fur quality. The lowest dietary protein level (15% of ME), which resulted in reduced BRL, also impaired fur quality (**II–IV**). The present results thus confirm the findings of earlier studies (Marjoniemi et al., 1991; Rouvinen et al., 1991) that a decrease in BRL due to hard skin straining is connected to a significant deterioration in fur quality in blue foxes. At protein levels below 21–22% of ME, leather quality remains sub-optimal.

4.2. Effects of dietary amino acids

4.2.1. Digestibility and nitrogen utilisation

Methionine supplementation in low-protein diets significantly improved ($P < 0.05$) the digestibility of EE, CC and DM in older (21-week) blue foxes (**I**) (Figure 4). Similarly, SAA improved the digestibility of organic matter (OM) in young (9-

week) blue foxes (**V**) (Figure 5). The lower the dietary protein content, the more pronounced was the effect. Methionine and cystine were the only amino acids among those investigated that affected OM digestibility (**V**). Furthermore, even if all other amino acids were supplied to meet the level of 167 g protein/kg DM, but the supplementary SAA were lacking, the digestibility of the diet was lower than that of the negative control with a very low protein content (96 g/kg DM).

Figure 4. Digestibility of fat ($P < 0.001$) and carbohydrates ($P < 0.05$) in respect of dietary methionine supply. CV = Coefficient of variation. (Based on results of the second trial in **I**).
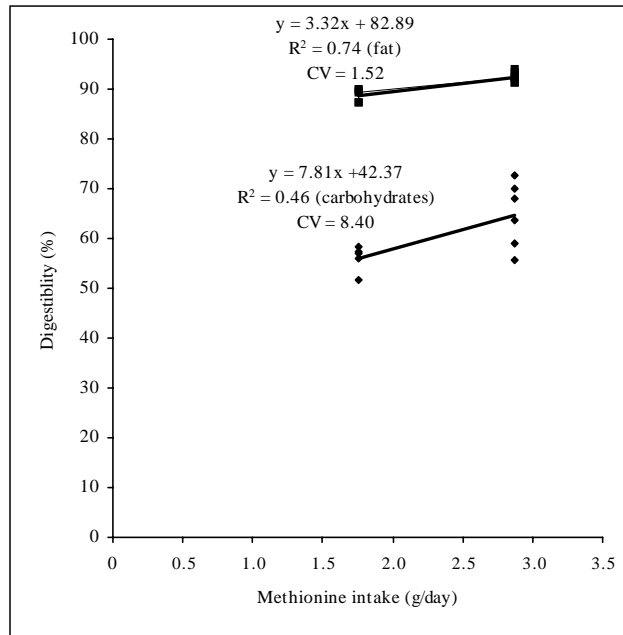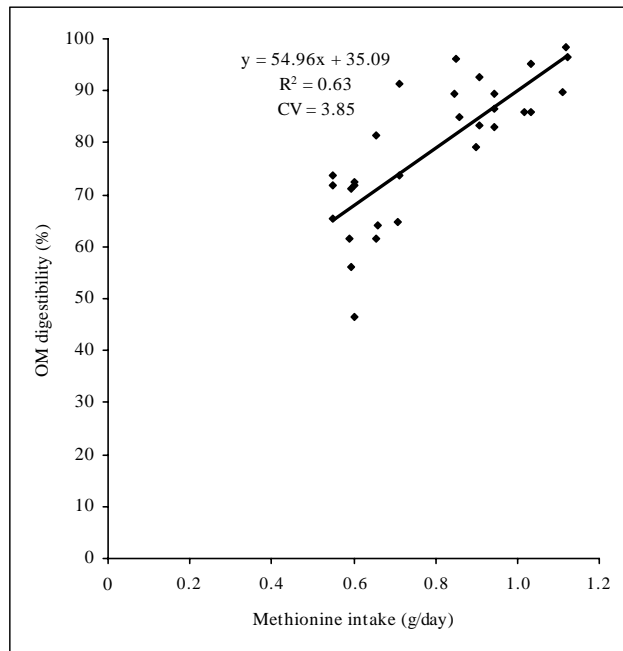
Figure 5. Digestibility of organic matter (OM) ($P < 0.05$) in 9-week-old blue foxes in respect of dietary methionine supply. CV = Coefficient of variation. (Based on results in **V**).

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A reduction in the dietary protein content was found to reduce pancreatic secretions of trypsin, chymotrypsin, amylase and lipase in pigs (Ozimek et al., 1985, ref. De Lange et al., 1989). Although not reported, the reason for this may have been a reduction in methionine content. In growing dogs, methionine supplementation was found to increase energy digestibility, the highest methionine level in the diet (0.74%) thus resulting in the best digestibility of energy (Blaza et al., 1982). This phenomenon is most likely associated with the composition or activation of the digestive enzymes involved. Methionine is the key amino acid required to start enzyme synthesis; thus, in the absence of methionine, enzyme synthesis does not proceed (Stryer, 1998). Sulphur-containing amino acids are essentially involved in the synthesis of bile salts, such as glycocholate and taurocholate, and so play an important role in the digestion of lipids (Stryer, 1998). Digestive secreta contain substantial levels of SAA, e.g. cysteine in glutathione (Figure 6), and they are also abundant components of several digestive enzymes (Dahm and Jones, 1994; Stryer, 1998). A positive correlation exists between lipolytic activity and lipid digestibility, and between proteolytic activity and protein digestion, indicating that conditions triggering enzyme secretion or delaying enzyme inactivation also increase lipid and protein digestibilities, as found in fish by Nordrum et al. (2000). Their study demonstrated improved fat and starch digestibilities with dietary SAA supplementations, implying increased secretion of lipase and amylase. In the present research on blue fox, with the exception of the content of methionine, the content and composition of fat and all other nutrients were identical within the protein level (P15 vs. P15M, and P22.5 vs. P22.M).
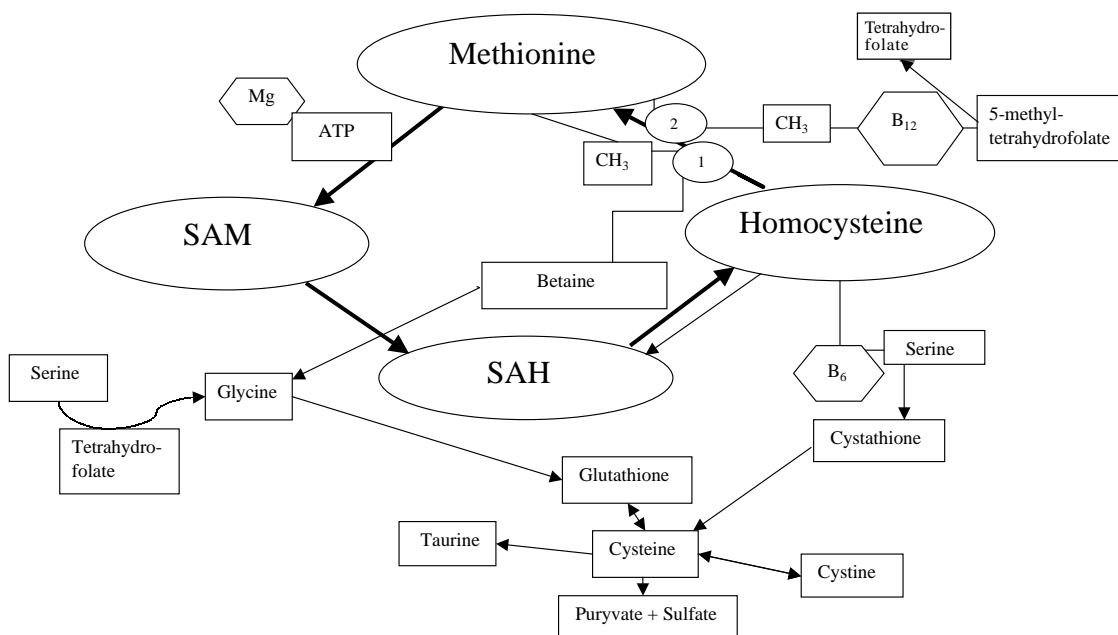

Figure 6. Methionine metabolism. Numbers refer to the following enzymes: 1 = betaine homocysteine methyltransferase, 2 = 5-methyltetrahydrofolate homocysteine methyltransferase. SAH = S-adenosylhomocysteine, SAM = S-adenosylmethionine.

Here, methionine as such or as a precursor of other amino acids, e.g. cysteine, cystine and taurine (Figure 6), appeared to be a vital factor in the synthesis or function of the enzymes essentially associated with the degradation processes of the nutrients. Methionine may also be related to the stimulation of cholecystokinin (CCK). Cholecystokinin contains methionine and is found in abundance in the alimentary tract. It has major effects on gastrointestinal smooth muscle motility, such as gallbladder contraction, and stimulation of small intestinal and colonic motility (Mutt, 1994). Further, it stimulates the release of pancreatic enzymes into the duodenum, as shown in studies on humans (Boyd et al., 1986): outputs of lipase, amylase, trypsin and chymotrypsin responded to increases in CCK. The exact mechanism of the increase in digestibility as a response to added methionine remains, however, to be clarified in future studies on blue foxes.

The two forms of methionine, DL-methionine and L-methionine, were studied in digestibility and N balance trials (**I** and **V**). Methionine improved the digestibility of OM, that of EE and CC in particular. In this respect, no difference at all could be found between the two forms of methionine. As, however, the two methionine forms were studied in different experiments, the results of these studies should be interpreted and conclusions drawn with caution. We must also bear in mind that the DL-form is composed of 50% L-methionine. Nevertheless, the result regarding utilisation of DL-methionine was confirmed by a recent study comparing simultaneously the effects of DL-methionine and L-methionine. In this parallel trial, there was no difference in their effects on N retention and growth in blue foxes (Figure 7, unpublished data from Dahlman and Valaja). Hence our results imply that blue foxes are able to utilise the D-form in DL-methionine effectively. This finding contradicts earlier results for mink (e.g. Glem-Hansen, 1982; Børsting and Clausen, 1996) but is fully consistent with those for growing dogs. In the dog, another Canidae species, both D-methionine and DL-methionine have been shown to be effective in satisfying the L-methionine requirement (Burns and Milner, 1981).
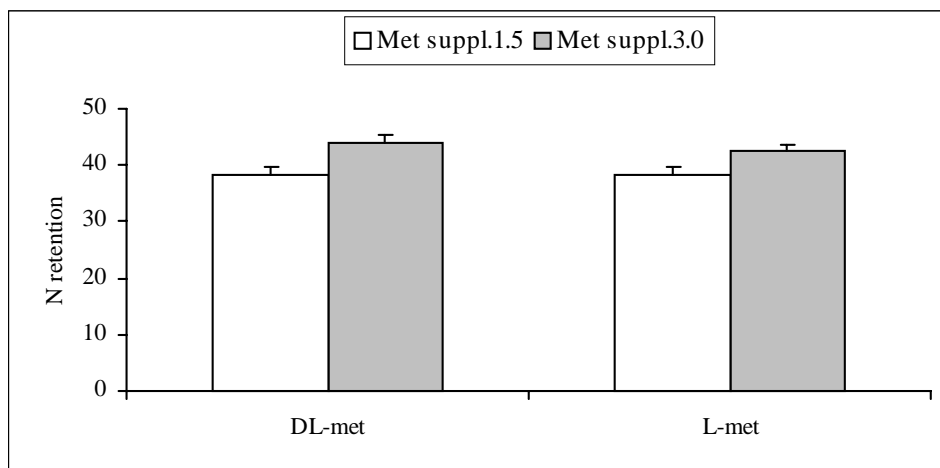

Figure 7. Comparison between DL-methionine (DL-met) and L-methionine (L-met) in nitrogen (N) retention (% of absorbed) in growing-furring blue foxes (ns). Methionine was supplemented at two levels: 1.5 and 3.0 g/kg dietary dry matter. (Based on unpublished data of Dahlman and Valaja).

A reduction in dietary protein content with the simultaneous supplementation of the limiting amino acids permits substantial savings in terms of environmental N emissions, as earlier evidenced in pigs (e.g. Valaja et al., 1993; Valaja and Siljander-Rasi, 1998). In the present research, a lowering of the protein level from 320 to 240 (g/kg DM) and supplementation with methionine significantly reduced N excretion ($P < 0.05$), by 34.1% (**I**). A decline of 32% in urinary N excretion was found in pigs when their dietary protein content was lowered from 180 to 140 (g/kg) and supplemented with lysine, methionine and threonine (Valaja and Siljander-Rasi, 1998).

4.2.2. Growth

As discussed earlier, the composition of growth varies during the different phases of the growing-furring period, which, in turn, affects the respective amino acid requirements of the blue fox. Here, the supplemented methionine affected early growth (until mid-September) and late growth (from mid-September onwards) very differently: methionine supplementation increased the early growth of the animals fed the lowest protein level (P15M), bringing it up to the same level as in P22.5 (**II**) and even in P30 (**III**), but did not increase the late growth (**II** and **III**). Methionine, unlike any other indispensable amino acid, is the key required for the start of protein synthesis. Also earlier studies with growing dogs have enlightened the role of methionine for growth (Blaza et al., 1982). According to these results, dogs failed to grow at a satisfactory rate until the diet was supplemented to 0.57% or 0.74% methionine. In the study of Työppönen et al. (1987), in which a low-protein diet was supplemented with methionine or methionine and lysine, neither of the amino acids affected growth. Unfortunately, only the final body weights were given by Työppönen et al. (1987). In contrast to findings in growing pigs and chickens (Boisen et al., 2000; Roth et al., 2001), methionine is the first limiting amino acid in growing-furring blue foxes on diets based on fish and slaughter by-products.

In respect of actual muscular growth (early growth), the effects of lysine were minor and rather suppressive than promoting (**III**). Moreover, contradicting the established data on other species (e.g. ARC, 1981; Boisen et al., 2000; Roth et al., 2001), lysine turned out to be the least limiting amino acid in growing-furring blue foxes (**III**; **V**). This finding indicates that the dietary amount of lysine (% of total amino acids) is relatively high in terms of the requirement of the blue fox for this amino acid, as seen when both feed composed of practical ingredients (the production experiment) and feed based on casein were used (the balance trial). The relatively low lysine requirement of the blue fox in relation to SAA may explain the lack of a positive response in blue foxes fed lysine-supplemented diets (**III**). Similarly, according to Milner (1981), the lysine requirement of growing immature dogs is lower than that reported for growing cats or pigs. The growth of dogs fed a purified diet containing 17.3 g lysine per kg feed was significantly lower than that of dogs fed diets containing 5.77 to 8.07 g lysine. Dietary lysine below these levels also suppressed growth. The methionine content was 8.2 g per kg feed in all diets. Our results show that levels of 8.5 g digestible lysine and 7.2 g digestible methionine (DigMet) per kg dietary DM support normal growth of blue foxes, even during the early growth period, provided that the amino acid sources are highly digestible.

Blue foxes were clinically healthy in all experimental groups. Methionine supplementation at the low protein level (P15M) reduced relative liver weight by 10% and by 14% compared to the respective non-supplemented diet (unpublished data from **II** and **III**, respectively). These findings are most likely associated with differences in the metabolic state of liver, as shown in mink by Damgaard (1997). She showed that low-protein diets increased the hepatic fatty infiltration associated with high liver weight. The results of the present investigation suggest that supplemental methionine may prevent weight increase of liver in blue foxes fed low-protein diets. This finding may be related to the synthesis or function of lipoproteins in which methionine appears to play a vital role due, among other things, to the numerous metabolic reactions mediated via S-adenosylmethionine (SAM) and to the role of methionine as cysteine precursor (Stryer, 1998; Overton and Piepenbrink, 1999) (Figure 6). Mortality of blue foxes was negligible and not found to be related to dietary level of protein or amino acids. According to Damgaard et al. (1998), mortality in growing-furring mink is related to the dietary protein level, and increases at low protein levels. When low protein (16% of ME) was supplemented with essential amino acids, mortality fell to the level of high protein (31% of ME) whereas no influence of amino acid supplementation was shown at 20% protein of ME (Damgaard et al., 1998). The authors concluded that a high protein level is required to ensure good health and a low mortality rate. The apparent differences between mink and blue foxes may be due to differences in protein or amino acid requirements (NRC, 1982; Hansen et al., 1991) or diet composition, such as protein quality or the fat: carbohydrate ratio. Furthermore, mink, unlike blue foxes, is a strict carnivore. Our results indicate that the enzymes (e.g. aminotransferases) in blue fox liver differ from those of strict carnivores, which have high activity of N catabolism and high obligatory N losses in metabolism. As discussed earlier, it would seem that the blue fox has a substantially greater capacity to adapt to changes in its dietary protein supply.

4.2.3. Skin quality

4.2.3.1. Fur quality

Methionine supplementation improved the overall fur quality (**II**; **III**) and skin length (**III**) of blue foxes on the lowest protein diet, bringing them up to the level of animals on the highest protein diet. The effect of methionine on guard hair quality was especially clear, a characteristic that plays an important role in skin quality evaluation (Figure 8). The effects of supplemented lysine were not clear. In the blue fox study of Työppönen et al. (1987), supplementation of methionine or lysine, or both, in low-protein diets did not produce significant differences in fur quality compared to the high-protein control group. The present results further corroborate those obtained in a large-scale field trial conducted on a total of 400 blue foxes from mid-September to pelting, in which two protein levels, 25% and 30% of ME with equal methionine (and SAA) contents, resulted in similar fur quality traits (Dahlman et al., 2001). The results of another recent field experiment are in very good agreement with those obtained here. In that experiment, a feed planned specifically for mink (protein 33% of ME) was compared with a completely fish-free feed (19% protein, supplemented with methionine to a level of 0.40 g DigMet/MJ ME) fed to growing-furring blue foxes from September to pelting (Nenonen et al., 2003).
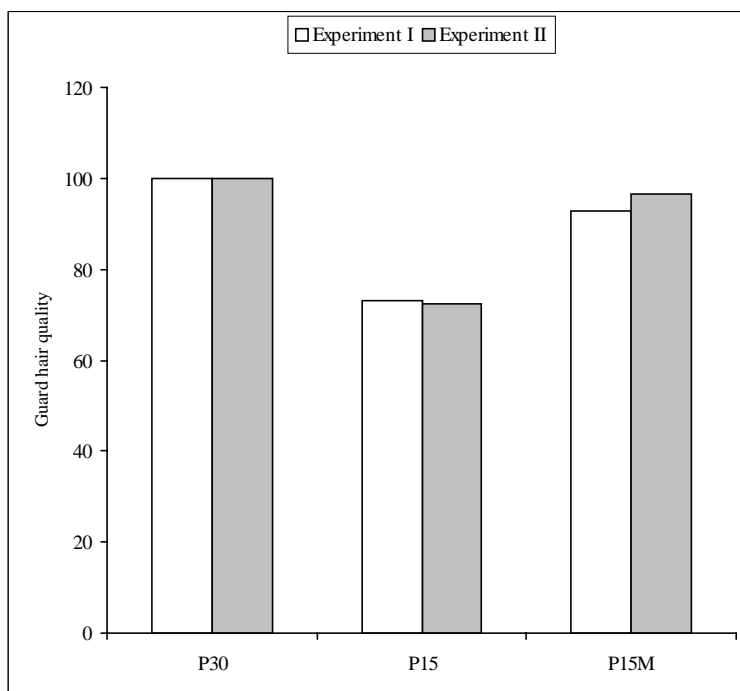
Figure 8. Influence on guard hair quality of methionine (M) supplementation at the lowest protein level (P) (15% of ME); comparison with the respective non-supplemented diet ($P < 0.05$) and the 30% level (ns). Relative values, the highest protein level P30 = 100. (Based on results in **II** and **III**).

The development of hair and the quality of fur appear to be crucially affected by dietary factors during the latter part of the growing-furring period as shown in recent studies in mink (Rasmussen and Børsting, 2001). Furthermore, methionine supplementation seems to be capable of compensating for the delay in hair priming caused by reduced dietary protein (from 30% to 15% of ME) (Dahlman and Blomstedt, 2000). On the basis of these findings, the results of **II** and **III**, and the results of the field experiments, a dietary content of DigMet at 0.40 or of digestible methionine and cystine (DigSAA) at 0.50 g/MJ ME would appear to be adequate for normal hair development and skin quality in growing-furring blue foxes. In respect of fur quality, the level of protein may be reduced, even down to about 15% (of ME, corresponding to 174 to 177 g/kg DM), provided that the supply of DigMet (Dig SAA) is sufficient, in the diets of growing-furring blue foxes from September to pelting.

4.2.3.2. Leather quality

Supplemental lysine elicited no clear response in leather properties; its content at any rate was therefore adequate in the diets. Methionine supplementation in low-protein diets, however, appeared to improve leather quality, as shown by increased TEN and PEB. This finding is supported by biochemical measurements of dried skins: methionine supplementation increased the collagen content of leather in blue foxes fed the

lowest protein diet (15% of ME), raising it, at least, to the level obtained in leather of blue foxes fed the highest protein diet (30% of ME) (Figure 9; Dahlman and Riis, 1999). The increased collagen content was related to the higher value for TEN and to improved fur, especially guard hair, quality (**II** and **IV**). The high PEB value reflects the elasticity of leather. High elasticity, at least within reasonable limits, is desirable in manufacturing processes. According to Marjoniemi et al. (1991), leather with a higher PEB is more stretchable, which makes for smoother quality for furriers' treatment.
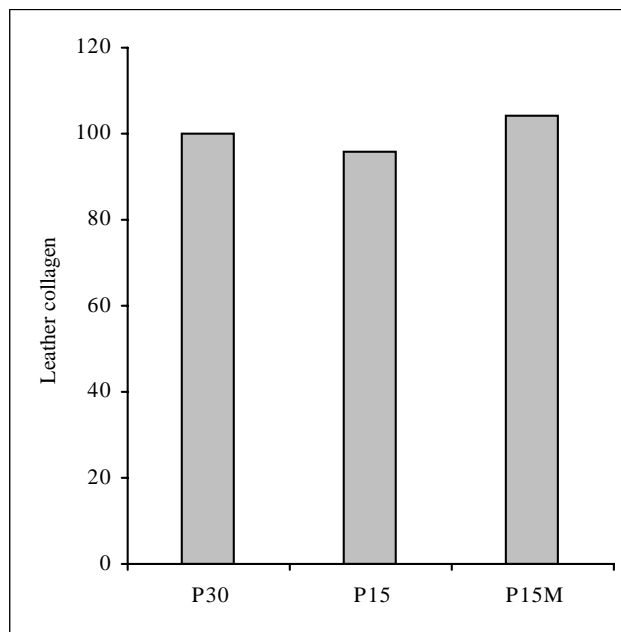

Figure 9. Influence on leather collagen content of methionine (M) supplementation at the lowest protein level (P) (15% of ME); comparison with the respective non-supplemented diet ($P < 0.05$) and the 30% level (ns). Relative values, the highest protein level P30 = 100. (Calculated from data in Dahlman and Riis, 1999).

4.3. Optimum dietary pattern of amino acids

Based on the present results and expressed relative to lysine (=100), the ideal pattern for 9–14-week-old blue foxes is: methionine + cystine 77, threonine 64, histidine 55, and tryptophan 22 (**V**). The responses of blue foxes to the deletion of SAA from the diet were severe and exceeded those due to deletion of any other amino acid. This shows how great is the impact of these SAA on the nutrition of the young blue fox. Prior to the present research there were no data on the optimum amino acid pattern for blue foxes. In mink, another fur-bearing animal, the ideal pattern is the following: lysine = 100, SAA 80, threonine 62, histidine 58, and tryptophan 18 (calculated from the results of Børsting and Clausen, 1996). Our results are thus in very good

agreement with those for mink. Relative to SAA, the lysine requirement of the blue fox seems to be slightly higher than that of mink. This may be due to the fact that our results are for young blue foxes whereas those for mink are from the whole growing-furring period. The relative requirement of blue foxes for SAA, methionine in particular, may be higher during the latter part of the growing-furring period due to winter hair growth. Our results are in accordance with previous findings in poultry. In relation to lysine (=100), SAA vary from 71 to 96 in recommendations for young broilers (Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere, 1999). In respect of their SAA requirement, blue foxes, then, are close to broilers owing to the high requirements for hair/feather growth. With growing pigs, SAA in the ideal amino acid pattern are lower (lysine = 100, SAA=63) (Wang and Fuller, 1989) due to the relatively lower requirements for hair growth in pigs.

In practical fox feeding, in which autumn diets can contain up to 50%, or even slightly more, fresh and dried slaughter by-products, there seems to be a major lack of SAA relative to lysine, especially during the growing-furring period. After SAA, the next limiting amino acid in relation to lysine appears to be histidine. In slaughter by-products the relative shortage (lysine = 100) of SAA fluctuates widely, possibly approaching even 50%, whilst that of histidine ranges from 20% to 40% (Finnish Fur Breeders Association, 2002c; Tuori et al., 2002). According to the ideal pattern of amino acids assessed in the present study, the content of threonine and tryptophan in slaughter by-products is probably close to, or only slightly under, the optimum. As the lysine content in common blue fox diets is relatively high, its shortage is unlikely under practical conditions.

5. CONCLUDING REMARKS AND PRACTICAL APPLICATIONS OF THE RESULTS

1. At reduced dietary protein levels the apparent faecal digestibility of the diet decreases. Supplementary methionine improves the digestibility of low-protein diets for blue foxes. Thus, supplementing low-protein diets with methionine may increase the ME value of the diet; at a level of 15% protein of ME, a 5–6% increase in the dietary ME content due to methionine supplementation was achieved.

2. With respect to health and growth, a protein level of 21–22% of ME seems adequate for growing-furring blue foxes. Even 15% protein of ME, together with methionine supplementation up to about 0.40 g DigMet (0.50 g DigSAA) per MJ ME, may ensure normal hair priming and provide the requirement for the production of high-quality pelts with good guard hair quality. Thus, blue foxes differ from mink. Fur quality appears to be closely related to the dietary methionine (SAA) content. The earlier recommendations for protein in blue fox diets (NRC, 1982; Hansen et al, 1991) therefore seem to be unnecessarily high and could well be lowered as long as the methionine requirement is met.

3. Methionine and cystine are the first limiting amino acids for growing-furring blue foxes. The next limiting amino acid appears to be histidine, and after that threonine and tryptophan, not necessarily in that order, depending on the amino acid composition and digestibility of the diet. Especially with low-fish diets, methionine deficiency is very likely, and supplementation of the practical diet formulation with this amino acid should be recommended. The ideal pattern for 9–14-week-old blue foxes is: lysine = 100, methionine + cystine 77, threonine 64, histidine 55, and tryptophan 22. Further studies are needed to determine the optimum pattern of all essential amino acids.

4. The lower the protein level in the diet the better is the utilisation of N and the smaller the proportion excreted. Reducing the percentage of CP in dietary DM by one unit led, on average, to a 1.2 percentage unit decline in the daily amount of N excreted in urine. In absolute amounts, an approximately 3 g decline in N excretion per blue fox per day can be achieved by reducing the dietary protein level from about 30% to 22% of ME. Applied to the whole Finnish blue fox stock, the daily excretion of N in urine could be reduced by about 5000-6000 kg during the growing-furring period.

5. To conclude, the present results show that the dietary protein level during the latter part of the growing-furring season can be reduced without compromising the health or performance of blue foxes. Thus, considerable potential exists for lowering N emission from fur farms and achieving substantial savings in feed costs. With high protein quality ingredients, a level of 22% of ME from protein is recommended in diets of the blue fox from the age of about 14 weeks, that is, from early September until pelting, provided that the methionine content is sufficient.

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