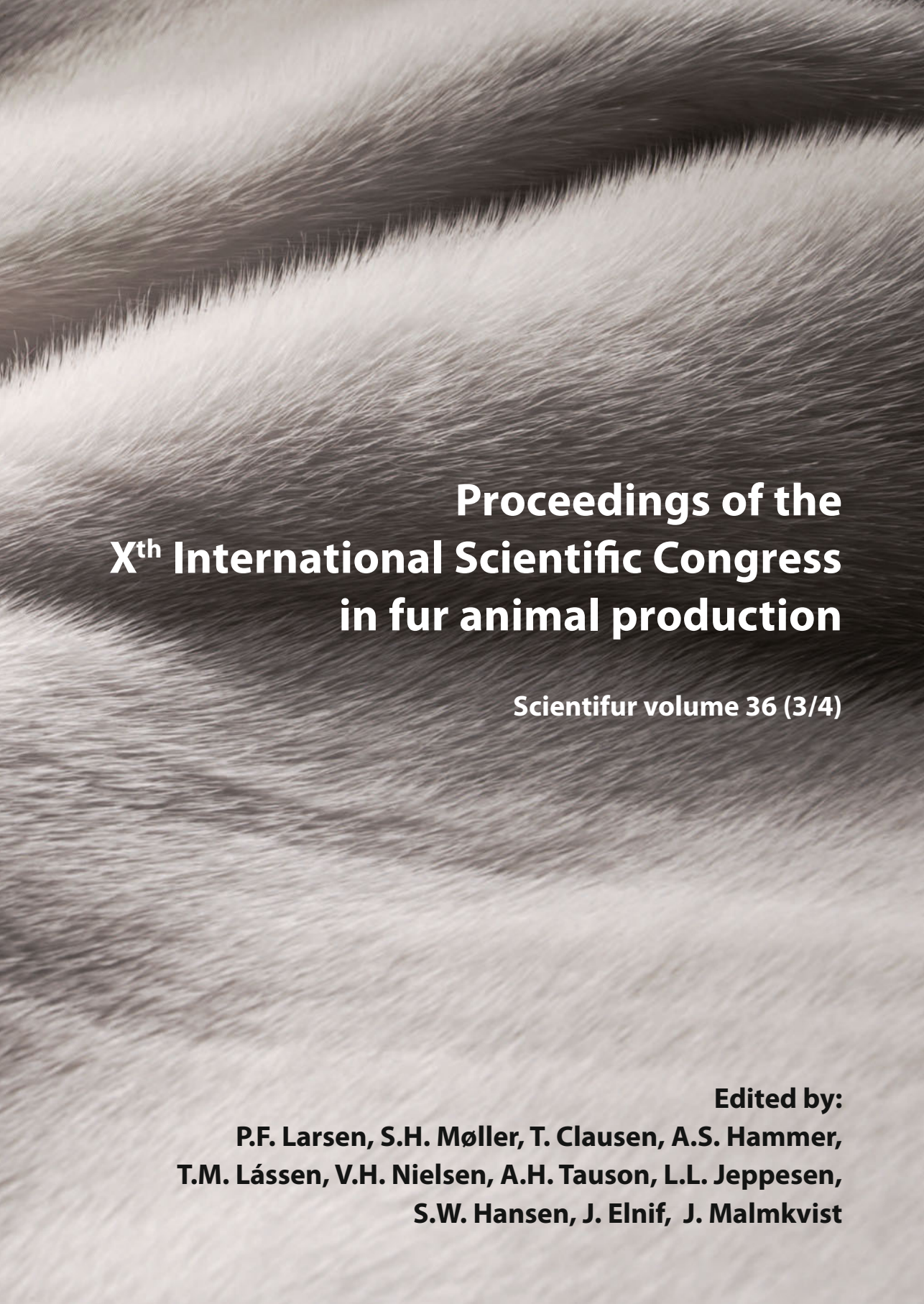

Proceedings of the Xth International Scientific Congress in fur animal production

Scientifur volume 36 (3/4)

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Table of contents

Preface 15
Peter Foged Larsen, Steen H. Møller, Tove Clausen, Anne Sofie Hammer, Tor Mikael Lássen, Vivi Hunnicke Nielsen, Anne-Helene Tauson, Leif Lau Jeppesen, Steffen W. Hansen, Jan Elnif and Jens Malmkvist

Part 1. Nutrition, feeding and management 17

Protein digestibility of some traditional and new feed ingredients for mink 19
Ø. Ahlstrøm, M. Tjernsbekk and A-H. Tauson

Effects of low-protein, *DL*-methionine and lysine-supplemented diets on growth performance of blue foxes (*Alopex lagopus*) during the growing-furring period 25
H.H. Zhang, F.H. Yang, Q.K. Jiang, Z.G. Yue, X.M. Xing, W.L. Sun, G.Y. Li

Effects of dietary protein level on growth, health and physiological parameters in growing-furring mink 32
B.M. Damgaard, P.F. Larsen and T.N. Clausen

Foetal life protein deprivation of mink dams affects the growth and metabolism of the F_2-generation 40
C.F. Matthiesen and A.-H. Tauson

Effect of reduced protein in the growth and furring period in 2010 and 2011 in mink 47
T.N. Clausen, T.M. Lassén and P.F. Larsen

Validation of the ^{13}C-bicarbonate tracer technique for estimation of CO_2 production and energy expenditure in mink (*Neovison vison*) 54
C. Larsson and A.-H. Tauson

Body condition and reproduction success in farmed mink males (*Neovison vison*) 63
B.K. Hansen, K. Gautason, M. Sønderup and H. Bækgaard

Maternal preconceptional nutrition leads to variable fat deposition of adult offspring mice (C57BL/6JBom) 69
E.L. Krogh Mortensen, T. Wang, H. Malte, D. Raubenheimer and D. Mayntz

Influence of nest box environment on kit survival 78
V.H. Lund and J. Malmkvist

The influence of succinic acid on the quality of fur-bearing animals skin 84
A.E. Kokorina, O.Yu. Bespyatykh and T.V. Tebenkova

Impact of metal amino acid complexes on arctic fox reproductive performance 87
D.J. Tomlinson, N. Koskinen and T.L. Ward

Yttrium oxide as an inert marker for nutrient digestibility in ferrets (*Mustela putoris furo*), mink (*Neovison vison*) and cats (*Felis catus*) 91
L. Sundling, Ø. Ahlstrøm and A.-H. Tauson

High storage stability of amino acids in mink feed 97
M. Schulin-Zeuthen and P.F. Larsen

Mortality in pair- and group-housed mink after weaning 103
J. De Rond and F.C. Kleyn Van Willigen

Life cycle assessment of mink and fox pelts produced in Finland 106
F. Silvenius, N. Koskinen, S. Kurppa, T. Rekilä, J. Sepponen and H. Hyvärinen

Comparative digestibility of nutrients and energy in ferrets (*Mustela putorius furo*), mink (*Neovison vison*) and cats (*Felis catus*) 112
L. Sundling, Ø. Ahlstrøm and A-H. Tauson

Evaluation of the choline status in mink fed different levels and sources of choline 121
M.S. Hedemann, B.M. Damgaard, T.N. Clausen and P.F. Larsen

Part 2. Health and disease 129

Evaluating DNA extraction kits for PCR detection of Aleutian mink disease virus 131
S.K. Cross, A.H. Farid and I. Hussain

In vivo MALDI-TOF markers for early detection of Aleutian disease (AD) among the AD virus infected mink 134
A. Cepica, A. Zargar, B. Mohamed and I. Iwamoto

Preliminary results of field and experimental investigations of pre-weaning diarrhea in mink 146
A.S. Hammer, K. Ullman, T.H. Jensen, K.O. Hedlund, G. Czifra, H.H. Dietz and C. Baule

Outbreaks of influenza A virus in farmed mink (*Neovison vison*) in Denmark: molecular characterization of the viruses 153
L.E. Larsen, S.Ø. Breum, R. Trebbien, K. Bradstad, L.P. Nielsen, M. Chriél, T.H. Jensen, C.K. Hjulsager, K. Handberg, P.H. Jørgensen, J. Le Févre Harslund, L. Rangstrup-Christensen, B. Pedersen and A.S. Hammer

Lessons learned from typing *Pseudomonas aeruginosa* isolates from hemorrhagic pneumonia in mink using pulsed field gel electrophoresis 157
C.M. Salomonsen, N. Høiby, S. Molin and A.S. Hammer

Antimicrobials in mink – consumption and resistance patterns 165
M. Chriél, J.F. Harslund, T.H. Jensen, E. Jakobsen, A.S. Hammer and A.M. Lassen-Nielsen

The influence of succinic acid in the formation of immunity against salmonellosis in foxes 172
O.Yu. Bespyatykh, A.E. Kokorina, T.V. Tebenkova, I.A. Domskiy and Z.N. Beltyukova

Cage housed marmot's diseases 177
I.A. Plotnikov

Influenza on Dutch mink farms in 2011 180
F.C. Kleyn van Willigen and R. Dijkman

Consequences of outbreaks of influenza A virus in farmed mink (*Neovison vison*) in Denmark in 2009 and 2010 186
M. Chriél, T.H. Jensen, C. Hjulsager, L.E. Larsen, P.H. Jørgensen, J. Le Févre Harslund, L. Rangstrup-Christensen, B. Petersen and A.S. Hammer

Application of Ultrasonography for *in vivo* diagnosis of fatty liver disease in mink (*Neovison vison*) 190
A.S. Hammer and T. Clausen

The ixodic tick borreliosis monitoring 194
D. Zhuravlev, M. Perevozchikova, I. Domsky and Y. Berezina

Field evaluation of CIEP and PCR detection/removal control methods of Aleutian mink disease (AD) in Canada 196
A. Cepica and T. Iwamoto

Part 3. Breeding, genetics and reproduction 207

Sequencing of the mink genome: plans and perspectives 209
R. Anistoroaei, K. Christensen and B. Benkel

Identifying QTL for fur quality traits in mink (*Neovison vison*) 216
J.P. Thirstrup, R.M. Anistoroaei, B. Guldbrandtsen, R.S. Labouriau, K. Christensen, M. Fredholm and V.H. Nielsen

Resolving the enigma of embryonic diapause, a forty-year scientific journey 223
B.D. Murphy

Application of sequence-characterized amplified regions for detection of self-biting in the blue fox 229
E. Ren, Z. Liu, S. Wang, X. Xing and F. Yang

Nucleotide sequence polymorphism of the growth hormone gene in American mink (*Neovison vison*) 236
J.J. Skorupski and M. Kmieć

Evidence for genetic variation in bite marks in group housed mink 240
S.W. Alemu, P. Bijma and P. Berg

The regulation role of behavior in coat color formation in mink (*Neovison vison*) 246
O.V. Trapezov

Genetic parameters of eye infection in Finnish blue fox (*Alopex lagopus*) 253
R. Kempe, T. Köykkä, N. Koskinen and I. Strandén

The size and constitution of the body of dark-brown mink under different breeding schemes 261
L.V. Gerasimova

Reproductive efficiency of scanbrown and mahogany mink females, selected for litter size 267
M. Brzozowski, B. Maćkowiak-Kondrak and R. Głogowski

Investigation of placental scars in mink females 270
L. Tinggaard, T. N. Clausen and P.F. Larsen

Comparative analysis of morphometrics of wild and farm foxes (*Vulpes vulpes* L.) – preliminary results 275
M. Zatoń-Dobrowolska, M. Moska, H., Wierzbicki, P. Przysiecki and A. Mucha

Benefit of high number of active mammary nipples in mink 280
J. De Rond and F.C. Kleyn van Willigen

Similarity of coat color aberrations in northern fur seal (*Callorhinus ursinus*), mink (*Neovison vison*), and sable (*Martes zibellina*) 286
O.V. Trapezov, S.V. Fomin and N.S. Fomina

Coat color mutations and the monoamine content in the brain of the farm-bred mink (*Neovison vison*) 294
O.V. Trapezov, L.I. Trapezova, T.A. Alekhina, D.V. Klochkov and Yu.N. Ivanov

A single nucleotide polymorphism in the promoter region of the mink tumor necrosis factor-alpha gene 300
N.S. Yudin, R.B. Aitnazarov, I.V. Kulikov, E.V. Ignatieva and O.V. Trapezov

Detection of SNP markers and practical applications in mink (*Neovison vison*) 305
P.F. Larsen, D. Demontis, V.H. Nielsen, J.P. Thirstrup and C. Pertoldi

Part 4. Behaviour and welfare 313

Stress influencing production and welfare in farmed mink 315
J. Malmkvist

The effects of climbing cages on behaviour of female mink during the lactation period 328
L. Lidfors, H. Axelsson, J. Loberg and S.W. Hansen

Impact of weaning age on kit performance 336
T.N. Clausen and P.F. Larsen

High need for drinking water in young mink kits between 30 and 50 days of age 341
J. De Rond and F.C. Kleyn van Willigen

Mink's adaptation to group housing in practice 350
S.W. Hansen and S.H. Møller

The effect of handling and training on measures of the affective state of farmed mink (*Neovison vison*) 360
P.M. Svendsen

Discrimination between the domesticated and the feral American mink in Poland based on the left track of a forelimb 368
M. Sulik, A. Radek and P. Bilski

Welfare consequences of digging substrates in blue foxes 376
H. T. Korhonen, P. Eskeli and H. Huuki

Effect of slurry channels to collect urine and faeces in mink farming 381
K. Meldgaard and P.F. Larsen

Black spots in subcutis of mink pelts are no bite marks 386
F.C. Kleyn van Willigen, N.M. Meertens, J. De Rond and L. Boekhorst

Coat color mutations and defensive reaction towards man in farm-bred minks and sables 396
O.V. Trapezov, L.I. Trapezova and E.G. Sergeev

Diversity of selected features of integumentary system in mink 404
M. Piórkowska and A. Natanek

Part 5. Theme on 'Welfur for mink and foxes' 409

WelFur – mink: development of on-farm welfare assessment protocols for mink 411
S.H. Møller, S.W. Hansen, T. Rousing and J. Malmkvist

WelFur-mink: on-farm welfare assessment of mink (*Neovision vision*) – effect of sample size on animal based measures 420
T. Rousing, S.H. Møller and S.W. Hansen

Abnormal behaviour in Swedish farm mink during winter 426
L. Olofsson and L. Lidfors

WelFur – foxes: development of the on-farm welfare assessment scheme for foxes 433
J. Mononen, T. Koistinen, A.L. Hovland, H. Huuki and L. Ahola

WelFur – foxes: the inter-observer reliability of the WelFur health measures, and the prevalence of health disorders on fox farms during the growth period 441
L.K. Ahola, H. Huuki, A.L. Hovland, T. Koistinen and J. Mononen

WelFur – foxes: do feeding test, temperament test and a measure of stereotypic behaviour differentiate between farms? 448
T. Koistinen, H. Huuki, A.L. Hovland, J. Mononen and L. Ahola

Electrophysiological indices in mink (*Neovison vison*) during CO and CO_2 euthanasia 455
H.T. Korhonen, S. Cizinauskas, J. Jesernics and P. Eskeli

Applying Welfare Quality® strategy to design a welfare assessment tool for foxes and mink farms 460
R. Botreau, M. Gaborit and I. Veissier

WelFur – mink: inter-observer reliability of on-farm welfare assessment in the growth season 469
S.H. Møller, T. Rousing and S.W. Hansen

Part 6. Short communications 477

Lessons learned from not fattening juvenile mink kept as future breeders 479
S.H. Møller

Effect of dietary protein level during preparative mating period in female minks 480
Q.K. Jiang, Z.Q. Zhang, X.H. Gao, X.M. Xing and F.H. Yang

Effect of dietary protein level during gestation period in female minks 481
Q.K. Jiang, Z.Q. Zhang, X.H. Gao, X.M. Xing and F.H. Yang

Effect of dietary protein level during lactation period in female minks 482
Q.K. Jiang, Z.Q. Zhang, X.H. Gao, X.M. Xing and F.H. Yang

Effects of dietary protein levels and implanted melatonin on growth performance, serum biochemical parameters and nutrient digestibility of minks (*Mustela vison*) 483
X. Song, H. Xue, H. Wei, G. Li and F. Yang

Effect of different diet protein levels on the digestibility and metabolism of nutritions and growth performance in young male mink 484
T.T. Zhang and X.H. Gao

Preliminary determination and analysis of 12 serum biochemical parameters in mink (*Mustela vison*) 485
X. Song, H. Wei, H. Xue, X. Chen and Y. Yang

Detection of the Aleutian mink disease virus DNA and anti-viral antibodies in experimentally infected mink 10 days post-inoculation 486
A.H. Farid, I. Hussain and P.P. Rupasinghe

Aleutian mink disease virus in furbearing mammals in Nova Scotia, Canada 487
A.H. Farid

A real-time polymerase chain reaction assay for the quantification of the Aleutian mink disease virus 488
A.H. Farid and P.P. Rupasinghe

Status of Aleutian mink disease virus in Denmark 489
J. Østergaard and T.H. Jensen

The assembly of the mink blood transcriptoms generated by the next generation sequencing using the dog genome 490
A.H. Farid, S.S. Moore and U. Basu

Simple year-round cage enrichments for mink 491
G.J. Mason, K. Bahlmann, L. Dawson, J. Ahloy Dallaire, R.K. Meagher, S. Bowyer, M. Diez-Leon, and D. Campbell

Elevated resting bunks for nursing mink dams 492
G.J. Mason, D. Haley and L. Dawson

Keyword index 493

Preface

Dear reader,

Scientifur Volume 36, No. 3/4 is the proceedings from the IFASA 2012 congress held in Copenhagen, Denmark in 21-24 August 2012 and hosted by Kopenhagen Fur. As editors we were pleased to have received 88 abstracts for IFASA 2012 congress. We were even more thrilled that the majority of scientists wanted to give an oral presentation of their work to their colleagues, even though this challenged the group of editors with the difficult task of disappointing some. We have therefore made an effort to give ample time and good location for the many poster presentations, in order to make room for thorough reading and discussion of results presented in this way. These proceedings contain the revised papers within the topics: Nutrition, feeding and management, Health and disease, Breeding, genetics and reproduction, Behaviour and welfare, and the theme on 'WelFur for mink and foxes'. Finally, some contributions in the form of short communications are presented in Part 6. The reviews have focused on making results clear and easy to understand. The content is the responsibility of the author and has not been part of Scientifur's paper review process.

We would like to thank all of the people who have made the Xth International Scientific Congress in Fur Animal Production possible in Copenhagen, Denmark. We hope you will enjoy the congress as well as the proceedings!

The editors
Peter Foged Larsen
Steen H. Møller
Tove Clausen
Anne Sofie Hammer
Tor Mikael Lássen
Vivi Hunnicke Nielsen
Anne-Helene Tauson
Leif Lau Jeppesen
Steffen W. Hansen
Jan Elnif
Jens Malmkvist

Part 1. Nutrition, feeding and management

Protein digestibility of some traditional and new feed ingredients for mink

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Abstract

The study comprised 19 protein sources, four meals made from salmon by-products, three enzyme-hydrolyzed protein concentrated products made from salmon by-products, two fish meals, three krill meals, two poultry meals, one lamb meal, raw poultry by-products, raw cod scraps, raw eggs and cooked eggs. Protein digestibility was measured in adult male mink. Protein digestibility was significantly different among the sources, it was highest for one of the hydrolyzed salmon protein concentrates, 91.4% (SD 0.5), and lowest for the lamb meal, 64.9% (SD 1.2). Within the different categories of protein meals (salmon by-products meals, the hydrolyzed by-products, krill meals) there were also significantly differences in protein digestibility. Some of these products revealed similar chemical content and protein digestibility as high quality fish meal, but some did not. Raw egg white has been shown to reduce protein digestibility in young dogs. No difference in protein digestibility between raw and cooked eggs was observed in mink. Generally, the raw protein sources revealed digestibility coefficients between 85 and 90%, while most of the meals revealed values between 75 and 85%. The study showed that animal derived meals most often have protein digestibility around 80%, but variation can be large and digestibility studies are needed to have exact information on each protein ingredient.

Keywords: protein sources, salmon by-products, krill meal, raw egg

Introduction

Numerous raw materials with various origin, conservation and storage are involved in fur animal feeding. Animal by-product ingredients have been the basis for the feed production because of the high value nutritional value and palatability for foxes and mink. These ingredients include fresh, frozen stored, acid preserved and dry meals made of raw materials of animal origin. Some of these raw materials have no other alternative use but destruction. The flexibility in choice of ingredients is high in fur animal production because the commercial product is none-food and concerns regarding product quality as in human food productions are not considered. Moreover, wet feed production allows for utilization of both wet and dry ingredients and the rapid turnover of the feed opens for short time changes in the ingredient composition if necessary. The feed industry will always search for new and cheaper feed raw materials and the market price will be a main decisive factor for which raw materials to be used. Nutritional quality is the important factor to take into consideration before new ingredients are incorporated into the feed. Protein supply, which is a main nutritional focus in fur animal production, is dependent on amino acid level, amino acid composition as well as digestibility. Digestibility values will directly affect the amino

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acids and energy supplied by the protein source. Protein digestibility is therefore an important factor in the evaluation of protein quality of feed ingredients.

Our study aimed at making screening of protein content and protein digestibility in some traditional and some new potential ingredients in fur animal feed. The inclusion of the traditional ingredients in study was done to make good a set of comparisons with the new ingredients.

Material and methods

Animals and diets

The digestibility study comprised 19 protein ingredients; four meals from salmon by-products (Marine Harvest, Bergen Norway), three enzyme hydrolyzed protein concentrate meals made of salmon by-products (Marine Bioproducts, Storbø, Norway), two fish meals (Nordsildmel, Bergen, Norway), three krill meals (Aker Biomarine, Oslo, Norway), one lamb meal (Norsk Protein, Ingeberg, Norway), two poultry meals (Meal A, low ash, Gepro, Diepholz, Germany, Meal B, Norsk Protein, Ingeberg, Norway), raw poultry by-products (deboned chicken meat, Prior, Rakkestad, Norway), raw cod scraps (Fryserienes Fôromsetning, Tromsø, Norway) and raw eggs and cooked eggs. The protein ingredients were added as the only protein source in the experimental diets. The other ingredients were soybean oil, precooked corn starch, cellulose powder, vitamin/mineral mixture and water. Protein, fat and carbohydrate accounted for approximately 32, 35 and 33% of dietary dry matter content in the experimental diets, respectively.

A group of 24 adult males of the black genotype (>6 months) comprised the experimental animals at the laboratory. Body weights (BW) of the males were between 2.0 and 2.5 kg. Four males were given each of diets and digestibility was calculated as the mean of the four replicates. Daily feed allowances were planned to approximately meet the maintenance metabolizable energy (ME) requirement. Daily rations were approximately 150 g per day corresponding to 60-65 g dry matter. The mink was kept in standard cages equipped for quantitative collection of faeces and separation of urine. The animals were fed once daily and had free access to drinking water. The experiment lasted for seven days of which the three first was an adaptation period. During the four last days the feed intake were measured precisely and faeces were collected daily. Faeces were kept frozen stored during the experiment and freeze dried before analysis.

Apparent protein digestibility was calculated quantitatively using this formula:

Digestibility (%) = ((Protein consumed – Protein excreted in faeces)/(Protein consumed) ×100

Chemical analyses

Samples of protein sources and faeces were analysed for dry matter (by heating at l05 °C for 16-18 h), ash (by combustion at 550 °C to constant weight), crude protein as nitrogen×6.25 (by the semi-micro-Kjeldahl method, Kjeltec-Auto System, Tecator, Sweden), fat (by diethylether extraction in a Fosstec analyzer (Tecator, Sweden) after HCl-hydrolysis.

Statistics

Differences in protein digestibility were tested by analyses of variance (GLM procedure by SAS) and difference between sources were tested with Students T test. Significant differences between products were set at $P<0.05$.

Results and discussion

All the digestibility experiments were carried out without any problems and the feed intakes were close to 100% for all diets. Thus, palatability of all the protein sources was satisfactory. The standard deviations in protein digestibility were minor among animals given each diet, mainly equal or less than 1.0 (Table 1).

Chemical content and digestibility

The four salmon by-products meals was produced from partly viscera and partly scraps. According to the producer the mixture of viscera and scraps were random and not identical

*Table 1. Chemical content (g/kg), apparent crude protein (N*6.25) digestibility (%). Standard deviation in parentheses.*

Product	Dry matter	Ash	Protein	Fat	Protein digestibility[1]
Salmon meal A	900	82	528	237	81.8 (0.6) f
Salmon meal B	918	118	644	100	74.7 (0.8) g
Salmon meal C	929	126	576	182	80.0 (1.4) f
Salmon meal D	923	115	622	161	84.4 (0.6) e
Hydrolyzate A	928	115	646	161	84.7 (1.2) de
Hydrolyzate B	597	64	519	12	91.4 (0.5) a
Hydrolyzate C	934	108	823	6	89.9 (0.9) ab
Norse LT 94	928	130	699	92	87.9 (0.5) bc
Norseamink	908	145	695	76	81.5 (0.6) f
Krill meal A	900	82	528	237	81.7 (1.0) f
Krill meal B	918	118	644	100	85.1 (0.6) de
Krill meal C	943	117	663	80	76.1 (1.0) g
Poultry meal A	938	110	658	139	80.9 (1.7) f
Poultry meal B	952	127	601	167	67.4 (1.8) i
Lamb meal	933	205	563	120	64.9 (1.2) h
Raw cod scraps	279	58	202	8	85.2 (0.4) de
Raw poultry by.	403	21	153	205	88.2 (0.9) bc
Raw eggs	352	11	100	77	86.8 (0.6) cde
Raw boiled eggs	362	11	101	81	87.4 (0.5) cd

[1] Different letters with protein digestibility values indicate significant difference ($P<0.05$).

among the batches of meal. Viscera contain more fat and less protein and ash compared with scraps. The chemical content analyses among the four meals confirmed that mixture of the two ingredients varied. Protein content varied from 528 to 622 g/kg and fat content from 100 to 237 g/kg and ash from 82 to 126 g/kg (Table 1). Compared with the guaranteed chemical content of Norse LT 94 and Norseamink qualities of fish meal, the protein levels of the salmon meals were lower. The minimum guaranteed protein content of Norse LT 94 and Norseamink is 680 g/kg and the typical content is even higher, 710 g/kg. Regarding the fat and ash content, Norse LT 94 and Norseamink have maximum guaranteed fat content of 115 g and 130 g/ kg, respectively, and a maximum ash content of 140 g/kg. Only Salmon meal B obtained such low fat content, while all four salmon meals had lower ash content than 140 g/kg. The protein digestibility varied among the salmon meals (Table 1). High ash content is known to be an indicator of low protein digestibility because of increased proportion of protein from structural proteins such as collagen from connective tissue and bone (Skrede, 1979a). No such relation was observed with the four salmon meals, but the ash content was generally low for all the salmon meals in the present study compared with other type by-product meals made of cod scraps (Ahlstrøm *et al.,* 2004). The variation in chemical content and protein digestibility for the salmon meals suggests that the quality of these meals was not as high as for Norse LT 94, but comparable to that of Norseamink.

The enzyme-hydrolyzed protein concentrated products were made of similar by-products from salmon as the meals described above. Two of them were meals and one was a liquid, hydrolyzed protein B. (Table 1). The hydrolyzed proteins are mainly used in pet-foods to provide higher protein digestibility in special purpose foods. The chemical compositions for the three products were in accordance with the product declarations given by the producer. Previous tests carried out by the producer have showed protein digestibility values of more than 90% in mink and roosters. However, our study revealed somewhat lower digestibility values, but still the highest among the products in the present study.

Norse LT 94 and Norseamink are produced from raw, whole fish that is cooked, pressed, dried and milled. The raw material is gently dried to avoid any heat damage that may reduce protein digestibility. Norse LT 94 have a minimum biological protein digestibility of 90% (true digestibility in mink), while the Norseamink quality meals have somewhat lower digestibility (Ahlstrøm *et al.,* 2004). The batches of Norse LT 94 and Norseamink meal that were included in the present experiment revealed declared values for chemical content and protein digestibility, except for slightly higher ash content than maximum level for the Norseamink fishmeal. The protein digestibility value given for the Norse LT fishmeal is the apparent value (Table 1). If true digestibility is estimated by applying endogenous protein factors determined by Skrede (1979b), digestibility will be higher than 90% in accordance with the declaration.

Utilization of Antartic krill (*Euphasia superba*) has increased during the last decade because higher demand for marine protein and fat sources. The three krill meals included in our study was produced at sea on the vessel. Two of the meals were defatted (B,C) and therefore contained more protein and less fat than the crude meal (A). Krill meal A revealed fairly similar chemical content as found in a recent published study by Øvrum Hansen *et al.* (2010). Protein digestibility varied significantly among the meals in our study, from 76.1 to 85.1%. The reason for this variation is not clear, but it could be caused by different content of krill shells that have been shown to reduce

protein digestibility in Atlantic salmon (Øvrum Hansen *et al.,* 2010). When comparing the krill meals with the other protein sources in this study, they had comparable chemical content and protein digestibility as the salmon meals.

The chemical composition of the poultry meals A and B was similar, but the protein digestibility was much lower for poultry meal B. The most likely reason for the difference was the temperature during heat treatment. Poultry meal B was heat treated at 133°C, while the drying temperature for poultry meal A was not known, but it was probably considerably lower than that of poultry meal B. Similar to poultry meal B, the lamb meal had gone through the same harsh heat treatment and came out with even lower protein digestibility. The chemical content and protein digestibility of poultry meal B and the lamb meal is typical for meat-and bone meal (Ahlstrøm *et al.,* 2004; Cramer *et al.,* 2007). These two products revealed the poorest protein digestibility in the present study. Moreover, the amino acid composition and digestibility (not presented) revealed that these ingredients cannot be applied as the only protein sources in fur animal feed.

The chemical composition and digestibility of the raw cod scraps were typical and similar to results from other reports (Skrede, 1979; Ahlstrøm *et al.,* 2004). The low fat content and high protein content make this ingredient very useful for regulation of protein level of fur animal feed. In contrast, the raw poultry by-products contained more fat than protein and is therefore primarily a fat source. Both these ingredients showed very high protein digestibility in comparison with the different types of meals.

Protein digestibility of raw and cooked eggs was carried because previous studies in young dogs have indicated poor protein digestibility of raw eggs because of an anti-trypsin factors present in raw egg white, but not in cooked (Lineweaver *et al.,* 1947). Since the use of raw eggs in mink nutrition only has been an issue due to the content of avidin that can destroy biotin, we wanted to examine whether protein digestibility also was affected. The results showed no difference in protein digestibility between raw and cooked eggs in mink.

Conclusions

Protein sources such as salmon meals, enzyme-hydrolyzed protein concentrates and krill meals reveal high protein digestibility in mink. The study confirmed that most animal derived meals have protein digestibility around 80%, but variation can be large and digestibility studies are needed to have exact information on each protein ingredient.

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Effects of low-protein, *DL*-methionine and lysine-supplemented diets on growth performance of blue foxes (*Alopex lagopus*) during the growing-furring period

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Abstract

An experiment was carried out to examine the effects of low-protein diets supplemented with different levels of *DL*-methionine (Met) and Lysine (Lys) on growth performance of growing-furring blue foxes in order to find the optimal dietary supplementation levels of Met and Lys. For two protein levels, conventional 27% (P27) and low 19% (P19) on dry matter basis, respectively, and the low-protein diets were supplemented with Met (0.3%, 0.5%, 0.7%) and Lys (0.4%, 0.6%, 0.8%). An entirely random experimental design was adopted with two factors (3×3) and totally 10 groups (P27, L1M1, L1M2, L1M3, L2M1, L2M2, L2M3, L3M1, L3M2 and L3M3). From mid-September to pelting, based on the average daily gain, daily N retention, N retention ratio and the performance of blue foxes in different groups, 0.6% Met supplementation in low-protein diet was optimum; based on the daily N retention, N biological value and the quality of the fur, 0.3% and 0.5% Lys supplementation were optimum; based on the N apparent digestibility and daily N output, 0.3% Lys supplementation was optimum. In this experiment, the performance of blue foxes in L1M2 0.3% Lys×0.6% Met group was better than that in the other groups, which indicates that low-protein diets supplemented with *DL*-methionine and lysine for blue foxes can be beneficial to reduce feed expenses and nitrogen emission to the environment.

Keywords: low-protein; growth performance; fur characteristic; blue fox (*Alopex lagopus*)

Introduction

Blue fox (*Alopex lagopus*) is by nature a carnivorous species with a high protein requirement, and diets used in commercial blue fox production characteristically contain high levels of animal protein. Feed constitutes the greatest individual item of cost, amounting to 50% of the total production costs 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 emission. In the light of findings in growing-furring silver foxes, Harris *et al.* (1951) concluded that 28% of protein in dry matter (DM) was sufficient for growth as that in higher protein levels. A reduction in protein levels, from 45% to 22% of metabolic energy (ME), was reported not to affect body weight. However, a protein level below 28%-30% of ME significantly reduced body length at pelting (Rimeslåtten, 1976). The established blue fox nutrition requirements do not contain any data on amino acids. In mink, another carnivorous fur-bearing animal, researches on dietary protein and amino acids are intensive (Kerminen-Hakkio *et al.*, 2000). These studies displayed that methionine (Met) was the first limiting amino acid for hair growth and thus for fur quality, and Lys was the second limiting amino acid. There were some

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experiments which confirmed that animals fed diets of low-protein with some essential amino acid supplementations had no significant differences in their growth performance compared with those fed conventional diets (Kerr *et al.*, 1995). Earlier feeding experiments showed that it was possible to decrease the recommended protein level during the growing-furring season of blue foxes (Dahlman *et al.,* 2002; Koskinen *et al.*, 2005). The crucial point of this study is to evaluate the growth performance, N balance and fur characteristics of blue foxes fed low-protein diets supplemented with Met and Lys during the growing-furring period, and to quantify the optimal supplementation levels of amino acids in the low-protein diets.

Materials and methods

The experiment was carried out at the Fur Animals Experiment Station of Institute of Special Animals and Plants Sciences of the Chinese Academy of Agricultural Sciences (44° N, 126° E) in Northeast China during the period from September 27 to December 13.

Diet

Foxes were fed two protein levels: conventional 27% (Control) and low 19% (Basal experimental diet) on dry matter basis (Table 1), and the low-protein diets were supplemented with Met (M1 0.3%, M2 0.5%, M3 0.7%) and Lys (L1 0.4%, L2 0.6%, L3 0.8%).

Animals and experimental design

One hundred and twenty male blue foxes were selected with similar weight (5.34±0.57 kg) from different litters in an attempt to minimize genetic variation. The animals were allotted to 10 treatments of 12 animals each and fed twice a day at 08:00 and 16:00. Water and experimental diets were provided for *ad libitum* consumption throughout the trial. Before the formal experiment, the animals were accustomed to the experimental feed for two weeks. The N-balance experiments were carried out with eight 21-week old male blue foxes from each treatment group. The feces and urine collection period lasted for 3 days.

Chemical analyses

The chemical compositions of feed and feces were analyzed by standard methods. Dry matter (DM), ash, crude protein (CP), calcium, and phosphorus contents were analyzed according to AOAC procedures. The calculation of ME content and the proportional composition of ME were based on the digestibility coefficients achieved and the following ME values: protein 18.8 MJ/kg, fat 39.8 MJ/kg and carbohydrate 17.6 MJ/kg.

Calculations and statistical analyses

Statistical analyses of experimental data were performed with the general linear model (GLM) procedure of the SAS statistical package.

Table 1. Composition nutrient levels of the diets (DM basis) %.

Ingredients	Control diet	Basal experimental diet
Extruded soybean	18.0	20.0
Extruded corn	34.0	42.0
Soybean meal	9.0	5.0
Bone and meat meal	10.0	6.0
Corn germ meal	7.5	10.0
Fish meal	16.0	12.0
Lysine	0.4	
DL-methionine	0.6	
Soy oil	3.5	4.0
Premix[1]	1.0	1.0
Total	100.0	100.0
Crude protein	27.12	19.01
Fat	10.22	10.59
Carbohydrates	45.65	52.28
ME/(MJ/kg)	17.20	16.99
Lys	1.38	1.22
Met	0.63	0.59
Cys	0.43	0.40

[1] The premix provided the following per kg of diet: Ca(as $CaHPO_4 \cdot 2H_2O$), 6.4mg; P(as $Ca(H_2PO_4)_2 \cdot H_2O$), 4.4mg; Mg(MgO), 1.6mg; Na (NaCl), 24mg;Fe (as $FeSO_4 \cdot H_2O$), 16 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 4.0 mg; Zn (as $ZnSO_4 \cdot H_2O$), 10 mg; Mn (as $MnSO_4 \cdot H_2O$), 12mg; VA, 300 IU; VB_1, 0.15 mg; VB_2, 0.40 mg; VB_6, 0.30 mg; folic acid, 0.30 mg; nicotinic acid, 1.60 mg, *D*-pantothenic acid, 1.3 mg.

Results

Feed intake and growth performance

The ADFI values were affected by Lys levels ($P<0.01$), and were higher in L1 and L2 than in L3. Final body weight, average daily gain (ADG), body length and dry fur length were similar among the groups ($P>0.05$, Table 2). Different Met and Lys levels did not affect the ADG ($P>0.05$), L1M2 was the highest one. There was no Met effect or Met-Lys interaction on the ADFI and ADG ($P>0.05$).

N-balance

Daily N intake, urine nitrogen (UN) and N retention were significantly affected by dietary protein levels with amino acid supplements ($P<0.01$, Table 3). N intake was affected by different levels of Lys ($P<0.01$), and L2 group was the highest one. Fecal nitrogen (FN) of the control group was higher than most low-protein groups, except for L2M2, L2M3 and L3M1 ($P<0.05$). Different

Table 2. Effects of low-protein, Lys and Met-supplemented diets on performance of blue foxes.

Treatment[1]		Final weight (kg)	ADG (g/d)	ADFI (g/d)	Body length (cm)	Dry fur length (cm)
Control		8.69±0.67	55.67±6.71	334.88±5.37^{CD}	67.12±2.70	107.14±2.97
L1M1		8.57±0.44	53.61±5.33	350.48±29.04^{BC}	64.88±1.81	105.29±2.75
L1M2		8.79±0.84	57.51±5.27	364.93±14.17^{AB}	68.44±2.72	106.67±3.20
L1M3		8.71±0.81	56.90±8.21	355.73±17.33^{BC}	67.38±2.50	105.83±2.04
L2M1		8.77±0.49	57.17±7.62	350.92±14.01^{BC}	67.25±2.43	107.43±4.03
L2M2		8.65±0.83	55.17±4.93	382.02±19.68^{A}	67.81±2.70	105.86±4.10
L2M3		8.43±0.46	51.33±4.12	359.76±16.02^{B}	68.62±2.67	105.57±2.30
L3M1		8.52±0.84	53.00±4.74	335.80±20.31^{CD}	67.62±1.41	102.14±4.85
L3M2		8.64±0.48	54.83±5.17	327.11±13.80^{D}	67.00±1.51	106.57±2.82
L3M3		8.44±0.49	51.71±10.80	323.15±24.49^{D}	66.38±2.00	104.43±2.37
L1		8.69±0.69	55.78±6.27	356.99±20.18^{A}	67.90±2.34	105.90±2.66
L2		8.61±0.59	54.56±5.55	356.31±16.57^{A}	67.90±2.60	106.29±3.47
L3		8.53±0.60	53.11±6.90	328.83±19.46^{B}	67.00±1.64	104.38±3.34
M1		8.69±0.59	55.83±5.89	341.20±20.12	67.75±1.88	104.95±3.87
M2		8.62±0.71	54.67±5.12	351.00±15.83	67.58±2.31	106.35±3.37
M3		8.53±0.58	52.94±7.71	346.21±19.21	67.46±2.39	105.25±2.23
P-value	C & T	0.1524	0.3959	0.0001	0.0001	0.8981
	Lys	0.8341	0.5886	0.0004	0.0010	0.7133
	Met	0.6165	0.8514	0.2125	0.2685	0.9031
	Lys×Met	0.2613	0.8433	0.2303	0.3311	0.5597

[1] The values are the mean±SD, and each of the estimated requirement levels is based on 8 blue foxes. C & T means the control group compared with the other groups. In the same column of the same stage, values with different capital letter superscripts mean significant difference (P<0.01).

levels of Met and the interaction between Lys and Met had no effects on N intake, N retention, FN and UN of different groups (P>0.05). Different Lys levels had significant effects on UN of each group (P<0.05), and L1 and L2 groups were the highest and the lowest, respectively. N retention was the highest in the M2 level.

Discussion

Feed intake and growth performance

Feed intake can be affected by many factors, including animal growth period, feed energy, the balance of amino acids and other nutrients, palatability, ion concentrations in intestines, and feedstuff digestibility and so on. Feed intake of some animals would be reduced if protein in diets was absent (Han and Kong, 2007), and this may induce the synthesis of digestive enzyme being decreased and the body protein being broken down. Chickens and rats can slightly improve their

Table 3. Effects of low-protein, Lys and Met-supplemented diets on N-balance of blue foxes.

Treatment[1]		N intake (g/d)	FN (g/d)	UN (g/d)	N retention (g/d)	N apparent digestibility%
Control		13.55±0.22^{A}	4.68±0.40^{abc}	5.16±0.59^{A}	3.74±0.24^{A}	67.31±3.28^{A}
L1M1		11.16±0.89^{BC}	4.07±0.89^{c}	3.75±0.65^{BC}	3.34±0.37^{A}	63.64±6.28^{AB}
L1M2		10.88±0.49^{CD}	4.20±0.85^{c}	3.58±0.28^{BCD}	3.10±0.71^{AB}	61.44±7.31^{BC}
L1M3		10.69±0.52^{CDE}	4.22±0.66^{c}	3.46±0.75^{BCD}	3.19±0.52^{A}	60.58±5.39^{BCD}
L2M1		11.13±0.44^{BC}	4.38±0.41^{bc}	3.82±0.28^{B}	3.24±0.52^{A}	60.73±2.36^{BCD}
L2M2		11.87±0.59^{B}	5.19±0.65^{a}	3.41±0.59^{BCD}	3.35±0.66^{A}	56.29±2.58^{CDE}
L2M3		11.32±0.50^{BC}	5.09±0.81^{ab}	3.46±0.67^{BCD}	3.08±0.90^{AB}	55.12±6.32^{DE}
L3M1		10.30±0.45^{DE}	4.78±0.53^{abc}	3.30±0.31^{BCD}	2.22±0.66^{C}	53.61±4.44^{E}
L3M2		10.21±0.49^{DE}	4.16±0.38^{c}	2.99±0.83^{D}	2.98±1.28^{ABC}	57.70±2.10^{CDE}
L3M3		9.96±0.81^{E}	4.47±0.46^{abc}	3.05±0.67^{CD}	2.35±0.50^{BC}	55.07±2.75^{DE}
L1		10.91±0.63^{A}	4.16±0.80^{A}	3.58±0.56^{a}	3.21±0.53^{A}	61.40±6.32^{A}
L2		11.45±0.51^{A}	4.91±0.62^{B}	3.46±0.51^{ab}	3.22±0.69^{A}	56.92±3.75^{B}
L3		10.16±0.58^{B}	4.47±0.45^{AB}	3.1±0.60^{b}	2.50±0.81^{B}	55.48±3.09^{B}
M1		10.88±0.59	4.40±0.61	3.53±0.41	2.95±0.51	59.09±4.36
M2		11.02±0.52	4.53±0.62	3.32±0.56	3.15±0.88	58.51±3.99
M3		10.68±0.61	4.60±0.64	3.31±0.69	2.90±0.64	56.56±4.82
P-value	C & T	0.0001	0.0357	0.0001	0.0043	0.8318
	Lys	0.0001	0.0014	0.0308	0.0024	0.2880
	Met	0.2569	0.6453	0.4615	0.5277	0.9035
	Lys×Met	0.3375	0.0802	0.9639	0.4467	0.4931

[1] The values are the mean±SD, and each of the estimated requirement levels is based on 8 blue foxes. C & T means the control group compared with the other groups. In the same column of the same stage, values with different small letter superscripts mean significant difference ($P<0.05$), with different capital letter superscripts mean significant difference ($P<0.01$).

intake to compensate the shortage of amino acids when fed the diets with inadequate amino acids (Yan, 2009). The ADFI of this study was affected by different diets and may be influenced more by different dietary Lys levels. Our results suggested that supplemental Met could improve the biological value of low protein diets for blue foxes, which shows good agreement with Dahlman's results (Dahlman *et al.*, 2002). Our results were also consistent with the results reported in swine and poultry that free amino acids are used as efficiently as protein-bound ones, and it is therefore possible to obtain the same performance with low-protein, amino acid-supplemented diets as with high-protein controls (Gruber *et al.*, 2000; Roth *et al.*, 2001).

The development of hair and the quality of fur were crucially affected by dietary factors in later growing-furring period of mink in recent studies (Rasmussen and Borsting, 2001). In our study, there were no significant differences observed in body length and dry fur length. Body length of L1M2 and L2M2 (68.44 and 68.62 cm, respectively) was higher compared with other groups. In

an earlier study of blue foxes, low-protein diets added with Met, Lys, or both, did not affect fur quality compared with high-protein control group (Työppönen *et al.*, 1987). These results were consistent with previous studies that Met supplementation improved the overall fur quality of blue foxes fed the lowest protein diet (Dahlman *et al.*, 2002), and brought them up to the level of animals in the highest protein diet group.

N-balance

N intake was decreased with dietary protein level being reduced. It may be related to the increased dietary content of extruded corn. The results were consistent with earlier findings (Jongbloed and Lenis, 1992; Zhang *et al.*, 2008). N excretion in urine declined significantly ($P<0.01$) when the protein level in the diet was lowered from 27% to 19% (Table 3). The results were consistent with the earlier findings that N excretion declined noticeably along with a reduction in dietary protein in pigs (Valaja *et al.*, 1993). N retention and N retention ratio were not significantly different among most groups. Our results showed very good agreement with the view that the lower the protein level in the diet, the better the utilization of N and the smaller the proportion excreted. A previous study showed that in 21-week-old blue foxes, N retention did not differ in relation to dietary protein level and even 15% of protein was sufficient to satisfy the animals' requirement (Dahlman *et al.,* 2002b). The protein requirement of blue foxes at this age is likely to be determined mainly by hair growth, suggested by studies of Blomstedt (1998) and also by recent studies in mink (Rasmussen *et al.*, 2001). Our results showed that when the dietary protein level decreased from 27% to 19% with Met and Lys supplements, the performance of blue foxes was not affected, but the N excretion was decreased. This discovery was confirmed by the present research on blue foxes which showed that there was a 36.8% reduction in N excretion when the protein level declined from about 320 to 250 g/kg (DM basis) (Dahlman *et al.*, 2002).

Fur parameters

The development of hair and the quality of fur were crucially affected by dietary factors in later growing-furring period of mink in recent studies (Rasmussen and Børsting, 2001). In our study, there were no significant differences observed in body length and dry fur length. Body length of L1M2 and L2M2 (68.44 and 68.62 cm, respectively) was higher compared with other groups. In an earlier study of blue foxes, low-protein diets added with Met, Lys, or both, did not affect fur quality compared with high-protein control group (Työppönen *et al.*, 1987). These results were consistent with previous studies that Met supplementation improved the overall fur quality of blue foxes fed the lowest protein diet (Dahlman *et al.*, 2002), and brought them up to the level of animals in the highest protein diet group.

Conclusions

Reducing the percentage of CP in dietary DM by 1 unit, on average, led to a 4.1 percentage unit decline in the daily amount of N excreted in urine. In absolute amounts, an approximately 1.72 g decline in N excretion per blue fox per day can be achieved by reducing the dietary protein level from about 27% to 19% of DM. The optimal dietary Lys and Met levels to attain the best growth performance and fur characters were 1,035 and 2,070 mg/d, respectively, during growing-furring period.

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Effects of dietary protein level on growth, health and physiological parameters in growing-furring mink

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Abstract

The aim of the study was to investigate the effects of the dietary protein level and the feeding strategy on growth, health and physiological blood and liver parameters in growing-furring male mink. Effects of dietary protein levels ranging from 22% of metabolizable energy (MEp) to 32% of MEp were included in the study. The dietary protein levels were unchanged during the experimental period or reduced from a higher to a lower level in August or September. The results indicated negative effects on health and signs on immunosuppression in mink fed low dietary protein levels throughout the growing-furring period. The liver content of fat and fatty acids was higher in mink fed low dietary protein levels than in mink fed high dietary protein levels. The mortality rate was influenced of the dietary protein level in October.

Keywords: fatty liver, mortality rate, erythrocytes, leucocytes

Introduction

The mink is a carnivorous animal and, consequently, its natural diet contains a high proportion of energy from protein, a variable amount from fat, and a low amount from carbohydrate. Until recently, protein has contributed with 30% to 35% of metabolizable energy (ME) in Danish mink feed in the growing-furring period.

Since protein constitutes the most expensive part of the mink feed, possibilities to decrease the level of dietary protein in order to provide inexpensive feed and still support animal performance and health have, therefore, been the subject of several investigations for several years (e.g. Skrede, 1978; Børsting and Clausen, 1996; Sandbøl *et al.*, 2004).

Furthermore, there is a general wish to minimize the emission of nitrogen via urine and faeces to the environment and the Danish mink farmers are required to reduce the emission of nitrogen from the mink production with 15% before 2015.

Hepatic fatty infiltration or hepatic lipidosis is a common pathological finding in carnivores such as the mink, where fat accumulation in the liver often occurs due to metabolic or nutritional causes (Rouvinen-Watt *et al.*, 2010). In mink, hepatic fatty infiltration may be caused by several factors, including amino acid or fatty acid imbalances, excess dietary carbohydrate intake, cholin and vitamin B deficiency, poor feed quality, restricted or food deprivation (Damgaard *et al.*, 1994; Mustonen *et al.*, 2005). In earlier investigations a deficient protein supply caused hepatic

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fatty infiltration, and the degree of hepatic fatty infiltration increased during the growth period (Damgaard *et al.*, 1998). Furthermore, the primary cause of death at low protein feeding could be related to fatty infiltration of the liver. Damgaard *et al.* (1998) found that a dietary supplement of essential amino acids to a low protein diet did not improve the health status of the mink, but had a positive effect on growth performance compared with un-supplemented low protein diets. The plasma activity of alanine-aminotransferase (ALAT) was found to be a useful tool in the estimation of hepatic fatty infiltration, and clinical blood parameters were helpful in determining the level of protein necessary to sustain animal health and support normal growth performance (Damgaard *et al.*, 1998).

It can be hypothesized that the dietary protein content can be reduced in relation to the usual protein level in Danish mink feed and still support performance and health. Furthermore, that the protein requirement in mink kits is decreasing during the growing-furring period. The aim of the present investigation was to investigate the effects of the dietary protein level and the feeding strategy on growth, health and physiological blood and liver parameters in growing-furring male mink. Growth response and effects on pelt quality of the experimental diets are reported in a companion paper (Clausen *et al.*, 2012).

Material and methods

Animals

The experiment was carried out at Kopenhagen Farm in Holstebro, Denmark. A total of 1,380 mink kits of the colour type Scanbrown were included in the experiment. The experimental period covered the period from primo July to pelting in mid November. The animals were weighed at the start of the experiment in July, in August and September and at pelting in November. Dead animals were recorded and subjected to autopsy with particular focus on macroscopic signs of fat infiltration in the liver.

The animals were housed in pairs (male and female) in standard mink cages (L:90 cm, W: 30 cm, H: 45 cm) with access to a wooden nest box (L:28 cm, W: 30 cm, H 24 cm) embedded and covered with straw.

Diets

The mink kits were randomly distributed on 5 experimental groups which were allocated different dietary protein contents and fed according to different feeding strategies: (1) 32% metabolizable energy from protein (MEp) during the whole experimental period (32MEp); (2) 32% MEp in July/August and 25% MEp from September (32/25MEp); (3) 28% MEp in July and 24% MEp from August (28/24MEp); (4) 22% MEp during the whole experimental period (22MEp); (5) 32% MEp in July and 22% MEp from August (32/22MEp). The composition of the diets is shown in Table 1. The mink kits were fed *ad libitum* and had free access to drinking water.

Table 1. Dry matter and ash content, calculated content of metabolizable energy (ME) and distribution of ME on protein, fat and carbohydrates in the experimental diets.

	32MEp		32/25MEp		28/24MEp		22MEp	32/22MEp	
	July-Sept.	Oct.-Nov.	July-Sept.	Oct.-Nov.	July	Aug.-Nov.	July-Nov.	July	Aug.-Nov.
Dry matter (DM), %	41.8	40.7	42.2	41.7	40.9	42.4	42.9	41.8	42.9
Ash, % of DM	2.3	2.4	2.3	1.6	2.3	2.0	1.9	2.3	1.9
ME, MJ/ kg	8.3	7.7	8.2	8.1	8.0	8.1	8.6	8.2	8.6
ME, MJ/kg DM	19.9	19.0	19.4	19.5	19.7	19.1	20.1	19.6	20.0
Distribution of ME[1] on:									
Protein, %	30	31	30	25	28	24	21	30	21
Fat, %	54	50	52	53	54	52	56	52	56
Carbohydrates, %	16	19	18	22	18	24	23	18	23

[1] The contents of ME and the quantitative proportional composition of ME were calculated on the basis of the analyzed amounts of nutrients, estimated digestibility coefficients of the nutrients, and the following values of ME in kJ per g: protein 18.8; fat 39.8; carbohydrate 17.6 (Hansen *et al.*, 1991).

Blood samples

Blood samples were collected from 20 male kits per experimental group in early October and at pelting in November. The blood samples were collected between 08:00 and 12:00 after a fasting period of 16 hours. The blood samples were taken by venipuncture of *vena cephalica antibrachii* in October and by heart-puncture at pelting. Whole blood for cell counting was collected into K-EDTA and stored at room temperature until analysis within 4 h as described by Damgaard *et al.* (2012).

Whole blood for clinical-chemical analysis was collected in plastic tubes added Na-heparin, stored on ice and centrifuged within 4 h at 2,000×*g* for 20 min at 4 °C. The plasma was separated from the blood cells and stored at -20 °C until clinical-chemical analysis according to the methods described by Damgaard *et al.* (2012).

Liver tissue samples

After blood sampling at pelting the animals were killed by an overdose pentobarbital sodium. The liver was immediately excised, emptied for blood by slight pressure between paper towels and weighed. Liver tissue samples for chemical analysis were taken, immediately frozen in liquid nitrogen and stored at -80 °C until analyzed according to the methods described by Damgaard *et al.* (2012).

Statistical methods

All variables were subjected to analysis of variance using the Restricted Maximum Likelihood method in the mixed model procedure with multiple error terms in the statistical package, SAS (SAS Institute Inc., 1996). The model used for blood parameters included experimental group (32MEp, 32/25MEp, 28/24MEp, 22MEp, 32/22MEp) and month (October, November), and interaction between these effects as general fixed effects. Mink kits were treated as random factor. The residual variance was assumed to be different for each month. This was modelled using repeated statement of PROC mixed with month as a GROUP factor. Due to not totally balanced data the number of degrees of freedom (df) was estimated by using Kenward-Roger's (KR) approximation. When necessary, log transformations were performed to normalize data distribution. The model used for liver parameters included only experimental group as general fixed effect.

All analyses were performed as two-tailed tests. Results of the analysis performed by the mixed model procedure are given in least squares means±SEM. Significance is reported for $P<0.05$.

Results

There were no differences in body weight at pelting among the experimental groups (Table 2). Furthermore, there were no effects of the experimental diets and the feeding strategies on the liver weight and on the liver weight relatively to body weight (Table 2). The liver content of triacylglycerol (TAG) ($P=0.01$) and non-esterified fatty acids (NEFA) ($P=0.01$) were higher for the 22MEp group than for the groups fed a higher dietary protein content throughout the experimental period (Table 2). The liver content of phospholipids showed tendency to differ among the experimental groups ($P=0.07$). The liver content of glucose (79.0±4.5 μmol/g) and glycogen (43.0±8.1 μmol/g) were not different among the experimental groups.

Table 2. Body weight (BW), liver weight, liver weight relative to BW and liver content of TAG, NEFA and phospholipids in mink males in the experimental groups. Values are least squares means and SEM.

Parameter	32MEp	32/25MEp	28/24MEp	22MEp	32/22MEp	*P*-value[1] (effect of group)
BW, g	3121±89	3190±92	3085±87	3114±92	3058±92	0.88
Liver, g	71.0±2.5	73.1±2.5	73.3±2.4	69.9±2.5	67.1±2.5	0.39
Liver, % af BW	2.28±0.072	2.31±0.074	2.40±0.070	2.25±0.074	2.20±0.074	0.36
TAG, μmol/g	115±12 b	134±12 ab	111±12 b	167±12 a	143±12 ab	0.01
NEFA, μEq/g	294±27 b	342±28 ab	285±26 b	411±28 a	357±28 ab	0.01
Phospholip., μmol/g	21.4±0.51	20.5±0.52	19.9±0.50	21.0±0.52	21.8±0.52	0.07

[1] Within rows, values with different letters are significant different ($P<0.05$).