Amino acid availability of protein meals of different quality for adult and growing mink (*Neovison vison*)

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Abbreviations

AA - amino acid, ATTD - apparent total tract digestibility, BV- biological value, BW - body weight, CHO - carbohydrate, CP - crude protein, EAA - essential amino acid, EE - ether extract, FM - fishmeal, LM - lamb meal, NEAA - nonessential amino acid, ME - metabolizable energy, PER - protein efficiency ratio, PM - poultry meal, RNDN - retained N of digested N, SEM - standard error of the mean.

Abstract

Protein and amino acid (AA) availability of three protein meals of expected different quality were evaluated in young growing and adult mink. Lamb meal (LM), poultry meal (PM) or fishmeal (FM) were used as main protein sources in three extruded diets investigated by determining apparent total tract digestibility (ATTD) and nitrogen balance in 12 growing mink males aged 8-11 weeks in a Latin square design. In adult mink, ATTD of the diets was determined. The diets had lower protein content than recommended for growing mink, protein contributing 23 % of total metabolizable energy (ME), to ensure differences in growth response. The LM diet with expected low protein quality revealed lower content of essential AA than the PM and FM diets. The ATTD of major nutrients and essential AA was significantly affected by diet, with the poorest values for LM, intermediate for PM and the highest values for FM. Mink kits revealed lower ATTD values than adults for protein, AA and especially fat, resulting in lower dietary ME content for kits than for adults. The mean difference was greatest for the LM diet with lowest ATTD and smallest for the FM diet with the highest ATTD. Nitrogen retention differed significantly among diets and was 0.66 (LM), 1.04 (PM) and 1.18 (FM) g /$BW^{0.75}$/d, and the growth rate was 8.2 (LM), 26.8 (PM) and 35.3 (FM) g/d, respectively. Different dietary essential AA content and ATTD were the main factors to explain the difference in growth response. Generally, plasma essential AA concentrations did not clearly reflect the different dietary supply and the different growth response. Methionine is the most limiting AA for mink. The LM, PM and FM diets supplied 0.17, 0.26 and 0.33 g ATTD methionine /MJ ME, respectively, and the methionine provision was therefore probably the main reason for the

observed difference in N retention and growth response. The study shows that recommended level of 0.31 g ATTD Met/MJ ME covers the minimum requirement with a safety margin. To obtain optimal growth, the lower digestive capacity in young mink kits than in adults should be considered when choosing feed ingredients.

Keywords: mink, digestibility, protein efficiency ratio, growth

1. Introduction

Mink (*Neovison vison*) is a strict carnivore with a high dietary protein requirement (NRC 1982; Lassén et al. 2012), mainly provided from animal sources. Typical ingredients in commercial mink diets are unprocessed wet by-products from slaughterhouses and fish industry, but also heat-treated ingredients such as meat-and-bone meal, poultry meal or fishmeal. These meals may have variable protein quality due to different raw material composition, mainly bone content, and temperature during drying. Thus, to obtain diet formulations that will meet the protein requirement, reliable information on amino acid (AA) content, composition, apparent total tract digestibility (ATTD) and bioavailability is crucial. AA bioavailability is a term defined as the proportion absorbed in a form utilizable by the animal (Batterham 1992). Nitrogen (N) balance studies combined with determination of ATTD of AA, provide the most complete information on AA availability as digestibility, urinary N excretion and N retention are determined, and biological value (BV) of the diets can be calculated. In growing animals, protein efficiency ratio (PER) (g growth/g protein ingested) is a useful measure that sums up the AA availability factors and enables ranking of ingredients regarding protein quality and bioavailability. The objectives of this study were to investigate AA bioavailability of three protein meals of different quality in young growing mink to provide more information on the AA supply from these meals in relation to the AA requirement. However, table values on ATTD of major nutrients and AA are based on values found in experiments with adult animals. The few available data on effects of age on ATTD of major nutrients suggest that young kits have poorer digestive capacity than adults (Elnif et al. 1988; Hedemann et al. 2011), but to our knowledge, no systematic comparison on AA level exists. Therefore, ATTD values of the three protein meals were investigated in young growing and adult mink to reveal differences that could have importance for the optimal use of these meals and for correct feed formulation for different life stages.

The protein meals examined in the study were ranked according to expected protein quality; lamb meal (poor), poultry meal (good) and fishmeal (superior) and it was hypothesized that

1) ATTD of protein, AA, and other main nutrients will be higher in adults than in young kits.

2) The respective protein meals will facilitate different N retention and growth response in mink kits according to the expected protein quality.

Data on growth parameters in the present study have been partly published earlier (Tjernsbekk et al. 2016), but not the effects on nutrient digestibility in adult and growing mink, plasma AA concentrations and comparisons to current knowledge on AA requirement and guidelines for dietary AA supply to young growing mink.

2. Material and methods

2.1 Protein meals and diets

The selected protein meals were lamb meal (LM) (Norsk Protein AS, Ingeberg, Norway), poultry meal (PM) (Low Ash, GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG, Diepholz, Germany) and fishmeal (FM) (Norse-LT 94, Norsildmel AS, Bergen, Norway) (Table 1). The meals were main protein sources in extruded diets produced at Centre for Feed Technology, Norwegian University of Life Sciences, Ås, Norway. The main reasons for the expected quality difference among the meals were the composition of the raw materials and the temperature during processing of the meals. The LM and PM were meat-and-bone meals made from by-products from the rendering industry. The LM had much higher ash content than the PM, indicating more bony raw material and poorer AA composition. Both LM and PM had undergone a harsh heat treatment at 133 °C and 3 bars for 20 min in accordance with EU regulations. The FM was made from whole fish containing more muscle protein than the LM and PM raw materials. The thermal conditions during drying of the FM were not known, but normally the temperature during drying is about 100° C. The AA profiles of the meals were in line with table values for AA composition of feed ingredients for fur animals (NRC 1982). More details on the dietary composition and processing are given elsewhere (Tjernsbekk et al. 2014).

(TABLE 1 HERE)

The diets were composed to have similar crude protein (CP) content, CP contributing to 23-24 % of total metabolizable energy (ME). This level would most likely cover the requirement in adult mink. For 8-10 weeks old kits the practical recommendation for CP contribution is as high as 45 % of ME (Lassén et al. 2012). However, the recommended CP level includes a safety margin to ensure adequate AA intake as the requirement for essential AA (EAA) is not well known in young kits. Thus, for 8-11 weeks old mink kits in the present study, the CP level contributing to 23-24 % of ME was low enough to be expected to result in a different growth response caused by different AA composition and ATTD among the three diets. Nutrient composition of the diets is presented in Table 2. (TABLE 2 HERE)

2.2 Animals and experimental design

In growing mink, an N balance study was performed at University of Copenhagen, Fur animal laboratory, Rørrendegård, Taastrup, Denmark. Bioavailability of CP and AA was measured as ATTD, N balance, PER and BV, during three balance periods of seven days in 12 male kits. The kits were three brothers from four litters that were allocated into three groups. Each group consisted of four animals, one male from each litter. The balance periods were initiated when the kits were 8, 9, and 10 weeks of age and are denoted as period 1, 2, and 3, respectively. Mean body weights (BW) were similar for each group at the start of the study, 0.89 ±0.08 kg. Each group of kits was given all three diets *ad libitum* in a 3 x 3 Latin square design. The dry feed was mixed with water to obtain a feed: water ratio of 1:2 and blended to a porridge before fed. The three seven-day balance periods included a three-day adaptation period followed by four days with quantitative collection of faeces and urine and accurate registrations of feed intake. BW was registered at the start at the end of the four-day collection period. Blood samples were collected at the last day of each balance period by punctuation of *V. cephalic antebrachi*. The last feeding of the animals was the day before sampling. Mink are intermittent eaters, consuming many small meals per day. Feed not consumed in the late afternoon was available during the night, but it was not recorded when last feed intake occurred. Feed was removed two hours prior to the start of sampling. The animals were therefore expected to be in post-absorptive state at the time of collection. Blood was drawn into heparinized tubes, which were centrifuged for collection of plasma for examination of AA. Samples of blood plasma were stored frozen at -20°C pending analyses.

The study in adult mink was performed at the research farm at the Norwegian University of Life Sciences, Ås, Norway with the same diets as for young growing mink. To determine nutrient ATTD, four two-year-old males with a BW of 2.10 ± 0.2 kg received each diet for seven days following the same procedure as with the young growing mink. Daily feed allowance was 70 g food mixed with water in a feed: water ratio of 1:2. The daily feed allowance was adjusted to cover the daily maintenance energy requirement of 0.53 MJ/kg BW $^{0.75}$ (Chwalibog et al. 1980).

2.3 Chemical analyses

The extruded feeds, and faeces were analysed for dry matter (DM), ash, N, ether extract, starch and AA according to standard methods (Tjernsbekk et al. 2016). Carbohydrates (CHO) was calculated by difference:

CHO = DM – (CP + EE + ash).

For determination of free AA in plasma, samples of plasma (100 µl) were initially deproteinized by mixing with 10 µl of 35 % sulfosalicylic acid solution. The mixture was incubated at 4 °C for 20 min and centrifuged at 16 000 ***g*** for 15 min (Biofuge Fresco, Heraeus Instruments, Kendro Laboratory Products GmbH, Hanau, Germany). Of the supernatants, 80 µl were diluted with 80 µl 0.2 mol l^{-1} lithium citrate loading buffer, pH 2.2 (Biochrom Ltd) and micro-filtrated (0.2 µm) Spartan membrane filter, Schleicher & Schuell, Dassel, Germany) prior to injection (40 µl). S-2-aminoethyl-1-cysteine was used as an internal standard. The concentrations of free AA in plasma samples were analysed by ion exchange chromatography on a lithium high performance column

(Biochrom Ltd, Cambridge, UK) in an automated AA analyser (Biochrom 30, Biochrom Ltd), using lithium-based eluents and post-column derivatization with ninhydrin (Physiological Fluid Chemical Kit, Biochrom Ltd). Data were analysed against external standards (Sigma amino acid standard solutions: acidics, neutrals and basics, supplemented with glutamine, tryptophan and S-2-aminoethyl-1-cysteine; all purchased from Sigma Chemical, St. Louis, MO, USA) using the Chromeleon® Chromatography Management Software (Dionex Ltd, Surrey, UK).

2.4 Calculations

ATTD (%) of nutrients was calculated as:

((nutrient ingested (g) – nutrient in faeces (g))/nutrient ingested (g)) · 100.

Dietary metabolizable energy (ME) content was calculated based on ATTD data, using the following equation:

ME (kJ) = g CP · 18.42 kJ + g digestible EE · 39.76 kJ + g digestible CHO · 17.58 kJ (Lassén et al. 2012).

Data on N balance were calculated in relation to the current metabolic size of the mink kits, so that comparisons could be made across periods. Digested N (DN, g/kg BW $^{0.75}$/d) was calculated as:

N intake (g/d) – faecal N (g/d).

Retained N (RN, g/kg BW$^{0.75}$/d) was calculated as:

N intake (g/d) – (faecal N (g/d) + urinary N (g/d)).

PER was calculated as:

g growth/g protein ingested

BV was calculated as:

((N-intake (g/d) - (faecal N (g/d) - endogenous faecal N (g/d)) - (urinary N (g/d) - endogenous urinary N (g/d)) / (N-intake (g/d) - (faecal N (g/d) - endogenous faecal N (g/d)).

Factors applied for endogenous faecal N in mink was 278 mg/100g dry matter consumed (Skrede 1979) and 280 mg N /kg BW $^{0.75}$ for endogenous urinary N (Tauson et al. 2001).

2.5 Statistical analyses

Statistical analyses of data were performed with the SAS 9.3 computer software (SAS Institute Inc., Cary, NC, USA) using different models. In the N balance study with growing mink the MIXED procedure was applied with the following model: $Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(l)} + \tau_l + \varepsilon_{ijkl}$, where μ = general mean, α_i = fixed effect of diet, β_j = fixed effect of period, $(\alpha\beta)_{ij}$ = effect of interaction between α_i and β_j, $\gamma_{k(l)}$ = random effect of animal nested within replicate (litter), τ_l = random effect of the lth replicate and ε_{ijkl} = random error component. The model was reduced in cases of non-significance for the random effects and the interaction effect.

The GLM procedure was applied for testing differences in nutrient ATTD (%) and ME content between diets and between adults and young mink kits with the model $Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijkl}$ where μ = general mean, α_i = fixed effect of diet,, β_j = fixed effect of age, $(\alpha\beta)_{ij}$ = interaction effect diet x age, ε_{ijkl} = random error component. Interaction effect was not applied in the model for testing differences in dietary ME content. The results are presented as least-square means, and significant differences between means ($p < 0.05$) were found with the PDIFF option using the Tukey adjustment. Measure of variance is presented as the standard error of the mean (SEM).

3.0 Results

3.1 Apparent total tract digestibility of diets in kits and adults

The general trend in main nutrient and AA ATTD values was that the LM diet showed the lowest values, PM intermediate and FM the significantly highest values, and that adult mink revealed significantly higher values than kits (Table 3). (TABLE 3 HERE)

The difference in ATTD of AA between kits and adults was most pronounced for Met with an average of 7.7 percentage units lower in the kits. The difference in Met ATTD was, however, dependent on diet, since it decreased from 11.6 percentage units with the LM diet, to 7.3 and 4.1 percentage units with the PM and the FM diets, respectively.

Differences in ATTD of main nutrients between diets were highest for EE, with ATTD ranging from 73.2 % (LM) to 90.5 % (FM) (p<0.01), but the individual variation in ATTD of EE was huge for the PM (6.3 – 91.4 %) and LM (34.4 – 80.5 %) diets and large for the FM diet (77.4 – 94.4 %). Also, between adults and kits, the ATTD of EE differed with almost 15 percentage units, adults having superior digestive capacity (Table 3). This affected the dietary ME contents, which were significantly higher (p<0.05) for the adults than for the kits with all diets except for the PM diet (p<0.06) (data not shown). The mean difference between adults and kits was greatest for the LM diet and smallest for the FM diet. (Figure 1). (FIGURE 1 HERE)

3.2 N balance and growth response in kits

In the N balance study, the DM intake was similar ($p > 0.05$) among diets (Table 4). Intake of ME differed ($p < 0.05$) between diets and was lowest with the LM diet and highest with the FM diet. As expected, excretion of faecal N differed between diets, and was highest for the LM diet and lowest for the FM diet. Excretion of urinary N was greater ($p < 0.05$) for the FM diet than for the PM diet, with an intermediate excretion for the LM diet. Retained N was lower ($p< 0.05$) for the LM diet compared with the PM and FM diets, and the utilization of digested N for retention (RNDN) was approximately 10 percentage units lower ($p< 0.05$) with the LM diet than with the PM or FM diets. (TABLE 4 HERE).

The FM diet resulted in a higher ($p < 0.05$) average BW of the mink kits than with the PM and LM diets (Table 4). However, daily BW gain did not differ significantly between the PM and FM diets (p=0.11). Since PER values are influenced by the weight gain of the animals also the PER values were higher ($p< 0.05$) for the PM and FM diets than for the LM diet. The BV was lowest for the LM diet (p<0.05), while the values were higher and similar for the PM and FM diets.

The mean dietary ATTD contents of EAA for kits were generally lowest with the LM diet (Table 5), while the PM and FM diets were quite similar. The most pronounced difference between the LM diet and the two other diets was for the ATTD content of Met, which was approximately 35 % and 50 % lower than with the PM and FM diets, respectively (Table 5). (TABLE 5 HERE)

3.3 Amino acids in blood plasma

The total concentration of plasma AA did not differ significantly between diets (Table 6). The concentration of EAA was significantly lower for the LM diet than the for PM diet, while the FM diet did not differ from the other diets. For the plasma concentration of NEAA, the FM diet showed lower values than the LM diet. (TABLE 6 HERE)

3.4 Amino acid requirement for young growing mink kits

The dietary supply of EAA plus Cys and Tyr compared with the current standards for young growing mink kits (Sandbøl, 2012) indicated that the provision of Arg, Lys and Thr met the requirement with all three diets, while that of Ile met the requirement with the PM and FM, but not with the LM diet. The intake with the PM and FM diets covered about 80 % of the recommended provision of His, Leu, Phe and Val while the Met provided from below 60 % (LM) to about 105 % (FM) of the recommendation. For Cys the LM diet provided less than 10 % and the PM and FM diets only about 50 % of the recommended intake while the intake of Tyr was between 80 % and 100 % of recommendation (Figure 2). (FIGURE 2 HERE)

4.0 Discussion

4.1 Apparent total tract digestibility of amino acids and major nutrients – effect of diet and age

The expected differences in dietary protein quality measured as lower contents of EAA and higher contents of NEAA combined with lower ATTD of N and AA in LM than in PM and FM were confirmed. The ATTD of the other main nutrients and energy was poorest for the LM diet. This difference was especially prominent for ATTD of EE, probably due to more saturated fat in the LM. However, individual EE ATTD showed a remarkable variation. Young mink kits have a low, immature ability to digest EE, and others have reported low EE ATTD in mink kits of the same age as in the present experiment (Hellwing et al. 2008; Hellwing et al. 2009).

Reports regarding differences in AA digestibility between mink kits and adults are scarce. A study examining differences in CP ATTD of six fish by-products in 7, 16- and 38-weeks old mink kits showed that the 38 weeks old animals that had reached adult age had 2-3 percentage units higher ATTD values than the younger kits (Skrede 1978). In the present experiment, ATTD of CP was on average 3.5 percentage units lower in kits than in adults. ATTD values of CP and AA will be influenced by DM intake because of effect on endogenous secretion. In the present study, DM intakes in adult mink were very similar to that of the kits, about 65 g/d for all diets (data not shown), and it is therefore not likely that different DM intakes have been an important factor for the lower ATTD values in kits compared with adults. The lower ATTD of CP in kits than in adults is in accordance with previous studies showing about 30 % lower proteolytic activity in kits (Elnif et al. 1988).

Hedemann et al. (2011) found that pancreatic lipase activity in kits at the same age as in the present study, was 20-35 times lower than in adults. The difference in ATTD in the present experiment was most pronounced for EE, which averaged 14.8 percentage units. The difference in EE ATTD between adult mink and mink kits was in line with the results reported by others (Skrede 1978; Tauson 1988). Both the latter studies found a numerically higher EE ATTD in adults than kits, but not to the same extent as in the present experiment. This divergence may partly be explained by differences regarding other dietary factors like fat sources and ash content.

The difference in ME content between adults and kits, was greatest with the LM diet ($p<0.05$), intermediate with the PM diet ($p<0.06$) and smallest with the FM diet ($p<0.05$) (Figure 1). This shows that ingredients and diets with poor ATTD in adults will have even poorer ATTD in kits. Table values on digestibility of feed ingredients are normally based on values found in adult animals, which ought to be taken into consideration when composing feed for young growing animals. Such age difference should also be a concern for the pet food industry when making diet formulations for puppies and kittens.

4.2 N balance, growth response, protein efficiency ratio and biological value

Different ATTD of CP and AA between the diets was, as expected, reflected in the N balance study. The amount of N retained was found to be similar when kits were fed the PM and FM diets, with an average of 1.04 and 1.18 g//BW$^{0.75}$/d, respectively. Previous studies of N retention in mink kits at the same age as in the present study have examined the effect of different dietary CP levels using the same high-quality protein sources such as a combination of raw chicken meat and fishmeal (Matthiesen et al. 2012; Larsson et al. 2012; Vesterdorf et al. 2014). With this experimental design, N retention was lower for the diets with the low CP content when diets contributed to 18 versus 32 % of total ME (Matthiesen et al. 2012) and 24 versus 42 % of total ME (Larsson et al. 2012; Vesterdorf et al. 2014). The N retentions for the PM and FM diets were close to the 1.09 g/kg BW$^{0.75}$/d provided by a diet with 0.33 g Met /MJ ME and CP contributing to 24 % of total ME (Matthiesen et al. 2012). The efficiency of utilization of digested N for retention (RNDN) was on average 48.6 % for kits fed the PM and FM diets, in good agreement with 49.3 when CP contributing to 18 % of ME (Matthiesen et al. 2012) and 49.4 % when CP contributing to 24 % of total ME (Vesterdorf et al. 2014). Higher N retention (close to 2.5 g/kg BW$^{0.75}$/d) and RNDN (55.7 %) has, however, been reported for mink kits fed a diet with CP contributing to 32 % of total ME (Matthiesen et al. 2012).

Based on the N balance data and growth rates, it was apparent that the LM diet did not support the potential for N retention and growth in young mink kits. Still, the level of RNDN and high growth rates observed for PM and FM diets indicate a high availability of EAA, and that the growth response reflected the dietary ATTD AA supply.

PER and biological values of the diets corresponded mostly with the N retention values, but the BV of the FM diet was not different from the PM diet. This can be due to that the FM diet supplied AA above the optimal level for N retention while the supply with the PM diet may have been slightly below.

4.3 Amino acids in blood plasma

Concentrations of AA in blood plasma of cats and mink have been found to increase with increasing dietary protein intake (Green et al. 2008; Hellwing et al. 2008). In the present experiment, digestible AA intake differed significantly between diets. Still, AA intake was only modestly reflected in the concentration of AA in blood plasma. The lack of more clear-cut differences in blood plasma AA concentrations between diets could be due to the low and relatively similar CP content in the diets used, as other studies that have shown effects have applied widely different protein concentrations (Green et al. 2008; Hellwing et al. 2008). However, there are inconsistent reports of whether there exists a relationship between dietary intake of AA and the concentration of AA in plasma (Fernández-Fígares et al. 1993).

4.4 Dietary protein and amino acids in relation to requirements in mink kits

The nutrient composition of the diets used in the present experiment was not optimal for young growing mink kits. The mink is a strict carnivore with high obligatory N losses, and a dietary supply of CP of minimum 45 % of total ME is recommended for mink kits from 8 weeks to 10 weeks of age. The corresponding recommendation for fat supply is 35-50 % of total ME, while carbohydrates can contribute with a maximum of 20 % of total ME (Lassén et al. 2012). Thus, the dietary CP content (contributing 23 % of total ME), was considerably lower than recommended, whereas the carbohydrate content (contributing 37-38 % of tptal ME) was far higher than the maximum recommended level.

Studies concerning AA requirements for growth in mink kits are scarce and have mainly concerned the entire growing-furring period and are primarily focused on the requirement of Met (Glem-Hansen 1982) in combination with other essential AA and Cys (Børsting and Clausen 1996, Sandbøl et al. 2009). The recommended provision of ATTD Met of 0.31 g/MJ ME (Sandbøl 2012) is based on a review by Børsting and Clausen (1996), and it concurs with the growth response and N retention in the present study with 8-11 weeks old kits. Kits given the LM diet (0.17g ATTD Met/MJ ME) had low N retention and growth rate and excreted the same amount of urinary N as with the PM and FM diets despite digested N was significantly lower (Table 4). It is therefore very likely that the poor growth rate was primarily related to the low dietary Met content combined with the low Cys content. Significantly higher and similar (p=0.11) N retention and growth rate was obtained for the PM and FM diets containing 0.26 and 0.33 g ATTD Met/MJ ME, respectively, and with higher Cys levels. This suggests that the recommended level of 0.31 g ATTD Met/MJ ME covers the minimum requirement with a safety margin (Sandbøl 2009).

In relation to the current recommendation for EAA provision to mink kits all diets used here were deficient in His, Phe, Tyr, Leu and Val. However, the N retention and growth response of the kits fed the PM and FM diets with average concentrations (g ATTD/MJ ME) of 0.33 His, 0.58 Phe, 0.43 Tyr, 1.07 Leu and 0.66 Val, imply that the minimum requirement of these AA in the early growth period is lower than the previously suggested levels (Børsting and Clausen, 1995; 1996).

5.0 Conclusion

ATTD of main nutrients and AA was lower in 8-11 weeks old mink kits than in adults, and the difference increased when ATTD values were low. This age difference must be considered when composing optimal diets for young mink kits. The results of this study show that the currently recommended level of Met, 0.31g/MJ ME in 8-11 weeks old mink kits, covers the requirement with a safety margin. The study showed that a supply of His, Phe, Tyr, Leu and Val 0.33, 0.58, 0.43, 1.07, 0.66 g ATTD/MJ ME, respectively, was sufficient to kits of this age. These values were lower than previously suggested requirement figures.

6.0 Declarations

6.1 Ethical approval

The Danish part of the experiment followed the guidelines of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe, 1986). National permission number: 2012-15-2934-00394. The digestibility study performed in Norway was in accordance with the institutional and national guidelines for the care and use of animals (Norwegian Ministry of Agriculture and Food, 1996, 2009). The laboratory has a general permission to carry out digestibility determinations in mink as the size of cages are identical to those approved for production animals.

6.2 Consent for publication

Not applicable.

6.3 Availability of data and material

The datasets used during this study are available from the corresponding author on reasonable request.

6.4 Declaration of interests

None.

6.5 Sources of funding

Sources of funding have been the Norwegian University of Life Sciences, Ås, Norway and Felleskjøpet Fôrutvikling, Trondheim, Norway.

6.6 Study design

Study design and interpretation of the results have been performed by the researchers. Planning, design and performance of the Norwegian study was done by MTT, AHT and ØA. The Danish part of the study was planned by AHT and ØA and carried out by AHT and CFM. Writing of the manuscript was mainly done by MTT with contributions from AHT, CFM and ØA.

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Table 1. Analysed chemical composition of protein meals used in the experimental diets [g/kg].

	Lamb meal	Poultry meal	Fishmeal
Composition			
Dry matter	952.7	944.0	911.3
Crude protein	496.7	633.1	662.3
Ether extract	120.3	133.5	78.6
Ash	266.7	119.3	148.6
Carbohydrates*	69.0	58.1	21.8
Essential amino acids			
Arg	38.2	44.6	43.3
His	10.2	15.7	15.1
Ile	16.1	26.1	31.9
Leu	34.6	47.4	54.6
Lys	28.2	43.8	51.4
Met	7.5	14.1	20.0
Phe	17.8	25.8	29.1
Thr	21.0	28.5	31.1
Val	23.0	30.0	38.8
Non-essential amino acids			
Ala	38.0	42.0	38.8
Asp	40.6	56.9	68.0
Cys	5.3	6.9	5.9
Glu	70.0	88.5	90.4
Gly	66.4	59.5	42.5
Pro	41.4	40.2	28.6
Ser	26.0	30.2	30.3
Tyr	11.9	19.0	20.8

Notes: *Calculated by difference: Carbohydrates = dry matter – (crude protein + ether extract + ash); diet composition is given in Tjernsbekk et al. (2014).

Table 2. Analysed chemical composition of experimental diets [g/kg].

	Diet		
	Lamb meal	Poultry meal	Fishmeal
Dry matter	943.3	914.1	922.0
Crude protein	255.1	248.7	251.3
Ether extract	202.8	186.1	187.7
Starch	257.9	268.8	269.1
Ash	119.6	72.3	70.3
Carbohydrates*	365.8	407.0	412.7
Essential amino acids			
Arg	17.2	16.3	14.9
His	5.0	6.1	6.1
Ile	8.5	10.8	11.6
Leu	17.3	19.0	19.8
Lys	12.8	15.1	17.6
Met	3.6	5.0	6.2
Phe	9.5	10.6	10.5
Thr	8.8	9.7	10.2
Val	11.6	12.5	13.0
Total essential amino acids	94.3	105.1	109.9
Non-essential amino acids			
Ala	17.8	15.6	15.2
Asp	18.8	20.5	22.4
Cys	2.7	3.2	3.1
Glu	40.9	42.8	44.1
Gly	30.1	21.1	15.7
Hyp	11.1	5.5	1.9
Pro	21.6	17.0	12.6
Ser	11.5	11.2	11.3
Tyr	7.0	8.2	8.2
Total non-essential amino acids	161.5	145.1	134.5
Total amino acids	255.8	250.2	244.4

*Calculated by difference: carbohydrates = dry matter – (crude protein + ether extract + ash).

Table 3. Least square means of apparent total tract digestibility of main nutrients and amino acids in mink kits and adults for the lamb meal (LM), poultry meal (PM) and fishmeal (FM) diets [%].

	Diet			Age		Pooled	*p*-values		
	LM	PM	FM	Kits	Adults	SEM	Diet	Age	Diet x Age
Dry matter	66.9[c]	75.8[b]	81.4[a]	74.6	75.0	3.0	<0.001	NS	NS
CP	67.8[c]	74.8[b]	82.9[a]	74.3	77.8	1.9	<0.001	<0.001	NS
EE	73.2[b]	81.0[ab]	90.5[a]	77.9	92.7	9.0	<0.01	<0.01	NS
Total CHO	78.3[c]	82.0[b]	83.3[a]	82.1	78.5	1.5	<0.001	<0.001	<0.05
Essential amino acids									
Arg	82.5[c]	87.3[b]	91.3[a]	87.0	87.3	1.5	<0.001	NS	NS
His	71.5[c]	80.5[b]	86.8[a]	79.9	78.8	2.8	<0.001	NS	<0.01
Ile	73.5[c]	82.0[b]	89.1[a]	80.8	83.7	2.5	<0.001	<0.01	NS
Leu	76.8[c]	83.8[b]	90.1[a]	83.2	84.6	1.8	<0.001	<0.05	NS
Lys	73.8[c]	82.5[b]	90.3[a]	82.0	82.6	2.5	<0.001	NS	NS
Met	69.4[c]	80.5[b]	88.8[a]	77.6	85.3	3.2	<0.001	<0.001	NS
Phe	79.6[c]	84.1[b]	88.5[a]	83.7	85.2	1.8	<0.001	<0.05	NS
Thr	63.5[c]	74.4[b]	81.2[a]	73.8	70.9	3.2	<0.001	<0.01	NS
Val	70.5[c]	78.4[b]	86.4[a]	77.3	81.7	2.8	<0.001	<0.001	NS
Non-essential amino acids									
Ala	77.6[c]	82.1[b]	88.0[a]	82.1	83.8	2.0	<0.001	<0.05	NS
Asp	41.2[c]	58.0[b]	76.3[a]	59.0	57.0	4.7	<0.001	NS	NS
Cys	18.4[b]	44.3[a]	54.4[a]	24.3	53.8	3.2	<0.001	<0.001	NS
Glu	77.5[c]	84.6[b]	90.6[a]	83.9	85.3	2.0	<0.001	<0.05	NS
Gly	75.0[c]	77.8[b]	82.8[a]	78.1	79.9	2.4	<0.001	<0.05	NS
Hyp	60.9	62.5	59.6	53.3	84.1	9.0	NS	<0.001	NS
Pro	79.1[c]	82.5[b]	86.0[a]	82.0	84.2	2.0	<0.001	<0.01	NS
Ser	69.8[c]	78.9[b]	84.4[a]	78.7	74.8	3.7	<0.001	<0.01	NS
Tyr	72.9[c]	80.5[b]	86.1[a]	78.8	83.0	3.3	<0.001	<0.001	NS

[a, b, c] Values that share no common superscript differ significantly ($p<0.05$)

Table 4. Least square means of dry matter (DM) intake, metabolizable energy (ME) intake, nitrogen (N) balance, body weight, body weight gain, protein efficiency ratio (PER) and biological value in growing mink kits with the lamb meal (LM), poultry meal (PM) and fishmeal (FM) diets [g ·BW-0.75 ·d-1, unless otherwise denoted].

	Diet				*p*-values	
	LM	PM	FM	SEM†	Diet	Period×
DM intake	63.8	66.9	68.0	2.21	NS	<0.05
ME intake	0.93^{c}	1.10^{b}	1.22^{a}	0.04	<0.001	<0.05
Nitrogen balance						
N intake	2.76	2.91	2.97	0.10	NS	<0.05
Faecal N	0.91^{a}	0.76^{b}	0.53^{c}	0.03	<0.001	NS
Digested N (DN)	1.85^{c}	2.15^{b}	2.43^{a}	0.08	<0.001	<0.05
Urinary N	1.19^{ab}	1.11^{b}	1.25^{a}	0.05	<0.01	NS
Retained N (RN)	0.66^{b}	1.04^{a}	1.18^{a}	0.05	<0.001	<0.01
RN, % of DN	35.2^{b}	48.6^{a}	48.5^{a}	0.016	<0.001	<0.01
Body weight (g)	1127^{b}	1174^{b}	1242^{a}	57.49	<0.001	<0.001
Body growth (g/d)	8.2^{b}	26.8^{a}	35.3^{a}	2.90	<0.001	NS
*PER	0.38^{b}	1.39^{a}	1.71^{a}	0.14	<0.001	<0.001
Biological value	0.55^{b}	0.63^{a}	0.62^{a}	0.02	<0.01	NS

Notes: † Pooled standard error of the mean; ×The animals received each of the diets in three periods in a Latin square design; ^{a,b,c} Least square means in the same row not sharing the same superscript differ at $p<0.05$. *PER [g growth / g protein ingested].

Table 5. Mean apparent total tract digestible amino acid content in the lamb meal, poultry meal and fishmeal diets for mink kits [g/MJ].

	Lamb meal	Poultry meal	Fishmeal
Essential amino acids			
Arg	1.03	0.95	0.82
His	0.26	0.33	0.32
Ile	0.45	0.59	0.62
Leu	0.97	1.06	1.08
Lys	0.69	0.83	0.96
Met	0.17	0.26	0.33
Phe	0.55	0.59	0.56
Thr	0.41	0.49	0.50
Val	0.58	0.64	0.67
Non-essential amino acids			
Ala	1.00	0.85	0.81
Asp	0.58	0.80	1.03
Cys	0.08	1.05	1.13
Glu	2.30	2.40	2.41
Gly	1.64	1.09	0.78
Hyp	0.44	0.20	0.06
Pro	1.23	0.93	0.65
Ser	0.59	0.60	0.58
Tyr	0.37	0.43	0.42

Table 6. Least square means of plasma amino acid (AA) concentration in kits given lamb meal (LM), poultry meal (PM) or fishmeal (FM) diets in three one-week periods in a Latin square design [nmol/l].

	Diet			Period				*p*-value	
	LM	PM	FM	1	2	3	SEM†	Diet	Period
Essential AA									
Arg	99^{b}	176^{a}	126^{b}	116	150	134	16	<0.01	NS
His	69	83	71	80	73	71	5	NS	NS
Ile	61	70	60	67	63	60	4	NS	NS
Leu	117	122	106	123	113	108	8	NS	NS
Lys	84^{b}	143^{a}	161^{a}	127	126	135	12	<0.001	NS
Met	42^{b}	58^{a}	53^{ab}	50	51	52	3	<0.01	NS
Phe	87	93	84	88	93	83	5	NS	NS
Thr$	125^{b}	175^{a}	168^{ab}	120^{B}	173^{A}	176^{A}	13	<0.05	<0.01
Val	153	157	139	153	152	145	8	NS	NS
Total EAA	836^{b}	1072^{a}	969^{ab}	925	981	963	65	<0.05	NS
None-essential AA									
Ala	494	442	397	452	436	446	36	NS	NS
Asp	22	20	19	22^{A}	21^{A}	17^{B}	1	NS	<0.01
Asn	59	64	57	60	60	58	5	NS	NS
Cys	1	1	1	1^{A}	1^{A}	2^{B}	1	NS	<0.001
Glu	116	127	137	141^{A}	27^{AB}	112^{B}	7	NS	<0.01
Gln	602	630	686	617	612	689	35	NS	NS
Gly	766^{a}	614^{b}	442^{c}	640	628	555	33	<0.001	NS
Hyp	232^{a}	205^{a}	120^{b}	208^{A}	198^{A}	150^{B}	13	<0.001	<0.001
Pro	201^{a}	149^{b}	110^{c}	147^{AB}	181^{A}	132^{B}	13	<0.001	<0.01
Ser$	274^{a}	219^{b}	151^{c}	215	222	207	14	<0.001	NS
Tyr	62	73	61	70	66	61	5	NS	NS
Total NEAA	2829^{a}	2544^{ab}	2180^{b}	2574	2551	2428	111	<0.01	NS
Total AA	3665	3620	3149	3499	3543	3391	168	NS	NS

Notes: † Pooled standard error of the mean; $ Interaction effect of D x P significant for Thr and Ser (p <0.05); $^{a, b, c}$ Values that share no common superscript differ significantly (p<0.05), effect of diet; $^{A, B}$ Values that share no common superscript differ significantly (p<0.05), effect of period.

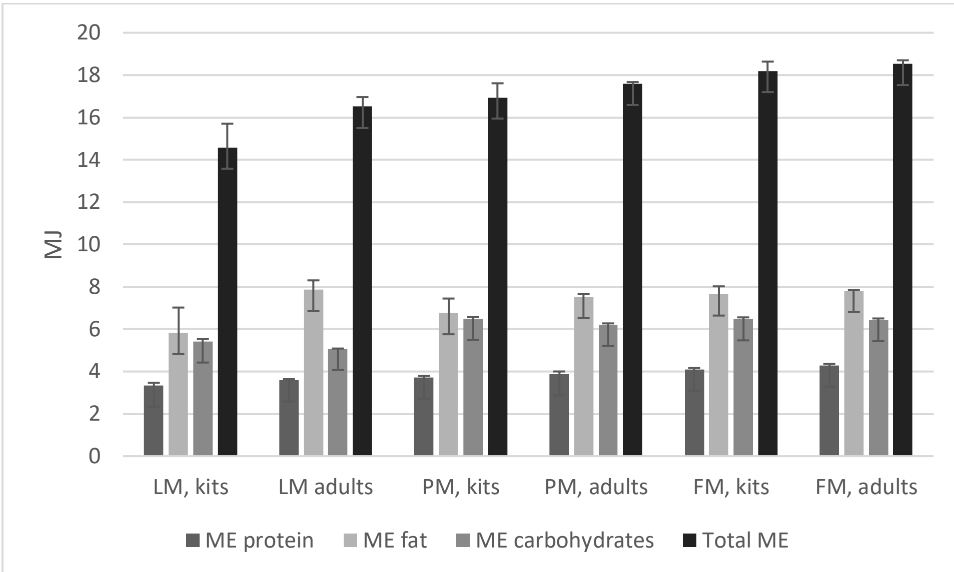

Figure 1. Least square means of metabolizable energy (ME) contribution from protein, fat and carbohydrates (MJ) and total dietary ME content for mink kits and adults with the lamb meal (LM), poultry meal (PM) and fish meal (FM) diets (MJ/kg DM). Notes: Standard deviation represented by lines on top of the bars.

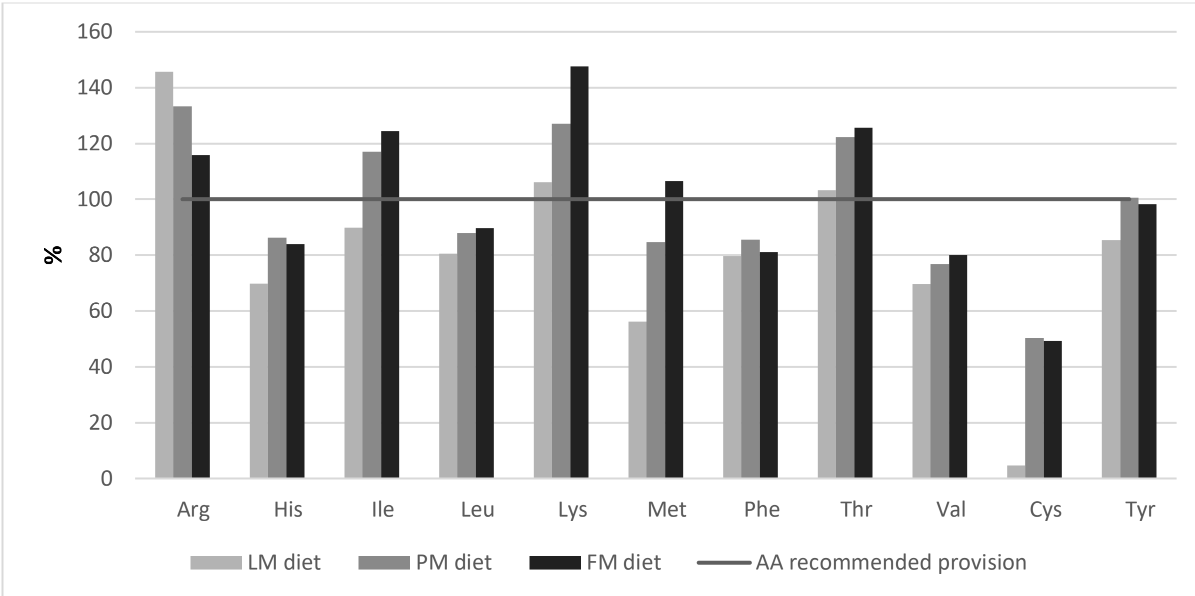

Figure 2. Content of apparent total tract digestible (ATTD) essential amino acids plus cysteine and tyrosine in the lamb meal (LM), poultry meal (PM) and fishmeal (FM) diets compared with recommended provision (Sandbøl 2012) to growing mink kits [%].