# Protein quality evaluation of animal protein ingredients applied in extruded dry dog food using mink (*Neovison vison*) as a model species

Vurdering av proteinkvalitet i animalske proteinråvarer benyttet i ekstrudert tørrfôr til hund med mink (*Neovison vison*) som modell

Philosophiae Doctor (PhD) Thesis

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*In memory of*

*my dear grandmother and grandfather,*

*Mosse and Odd,*

*who learned me all about goodness.*

*Thank you for everything,*

*I will for always keep you in my heart.*

*Love*

~ ~ ~ ~ ~

*From the movie Lady and the Tramp, Walt Disney (1955):*

*"In the whole history of the world*

*there is but one thing that money can not buy...*

*to wit - the wag of a dog's tail."*

*Josh Billings*

*To my special, four-legged companions, this work is especially dedicated to you:*

*Panter, Zorro, Tara, Ludde and Chanel*

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Råde, August 2016

Maria Therese Tjernsbekk

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# List of abbreviations

| | |
|---|---|
| AA | Amino acids |
| AAFCO | Association of American Feed Control Officials |
| AID | Apparent ileal digestibility |
| Asp | Aspartic acid |
| ATTD | Apparent total tract digestibility |
| BW | Body weight |
| CP | Crude protein |
| Cys | Cysteine |
| DM | Dry matter |
| FEDIAF | European Pet Food Industry Federation |
| FM | Fish meal |
| Glu | Glutamic acid |
| IAAO | Indicator amino acid oxidation |
| LM | Lamb meal |
| Lys | Lysine |
| ME | Metabolizable energy |
| Met | Methionine |
| MJ | Megajoule |
| MSC | Mechanically separated chicken meat |
| N | Nitrogen |
| $NH_3$ | Ammonia |
| NRC | National Research Council |
| PER | Protein efficiency ratio |
| PM | Poultry meal |
| Ser | Serine |
| SID | Standardized ileal digestibility |
| SPH | Salmon protein hydrolysate |
| Thr | Threonine |

# List of papers

I. Tjernsbekk, M. T., A.-H. Tauson and Ø. Ahlstrøm. 2014. Ileal, colonic and total tract nutrient digestibility in dogs (*Canis familiaris*) compared with total tract digestibility in mink (*Neovison vison*). *Archives of Animal Nutrition* 68: 245-261.

II. Tjernsbekk, M. T., A.-H. Tauson, C. F. Matthiesen and Ø. Ahlstrøm. 2016. Protein and amino acid bioavailability of extruded dog food with protein meals of different quality using growing mink (*Neovison vison*) as a model. *Journal of Animal Science* doi: 10.2527/jas.2016-0526.

III. Tjernsbekk, M. T., A.-H. Tauson, O. F. Kraugerud and Ø. Ahlstrøm. Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility. Accepted, revised manuscript submitted to *Journal of Animal Physiology and Animal Nutrition*.

# Abstract

Determination of protein and amino acid (AA) digestibility is essential for protein quality evaluation of dog foods, and should preferably be measured as standardized ileal digestibility (SID). Assessment of protein utilization in the body, through measures like nitrogen (N) balance and protein efficiency ratio (PER), will provide valuable additional information on the bioavailability of protein in extruded dog foods. However, use of dogs in experimental studies is considered ethically questionable. In particular, ileal digestibility determination in dogs is debatable, as invasive methods like ileal cannulation or euthanization are required. Finding and testing of alternative, non-invasive methods is, therefore, important. Use of animal models could be such an alternative. The need for reliable methods for protein quality evaluation of dog food is emphasized by the great variation in AA composition and bioavailability known to occur between animal protein ingredients applied in such foods. The main objective of this thesis was to provide more knowledge about the protein quality of animal protein ingredients used in extruded dog food, by use of mink (*Neovison vison*) as a model species for the dog (*Canis familiaris*).

In a comparative study (Paper I), nutrient digestibility was determined as apparent total tract digestibility (ATTD) in adult mink and as apparent ileal digestibility (AID), SID, apparent colonic digestibility and ATTD in adult dogs. Three experimental diets were produced by extrusion and were formulated to have similar contents of crude protein (CP) (ranged from 24.9 to 25.5%, as-fed basis) and crude fat (ranged from 18.6 to 20.3%, as-fed basis), respectively, but different AA composition and digestibility. Lamb meal (LM), poultry meal (PM) and fish meal (FM), with an ATTD of CP in adult mink of 67.7, 80.9 and 87.5%, respectively, were used as protein ingredients in the respective diets. In dogs, AID of CP (74.4%) was, as expected, lower ($P < 0.001$) than ATTD (83.5%), and similar results were found for the individual AA. The AID of CP in dogs did not differ ($P > 0.05$) from ATTD of CP in mink (77.8%). For several AA, AID in dogs and ATTD in mink were also similar ($P > 0.05$), but the AID values in dogs were in general numerically lower than the corresponding ATTD values in mink. The SID of CP (79.6%) and AA in dogs was very close ($P > 0.05$) to ATTD in mink, except for threonine and serine. The different digestibility measurements were significantly correlated ($P \leq 0.01$) for digestibility of CP and most AA and for the ranking of AA with respect to digestibility levels.

The LM, PM and FM diets applied in the comparative study with dogs and mink were further utilized in a growth-study with mink kits (Paper II), and the known differences in supply of bioavailable AA between the diets were reflected in the N balance and growth rate data obtained. For the LM, PM and FM diets, retention of N was 0.66, 1.04 and 1.18 g/kg$^{0.75}$/day, body weight gain was 8.2, 26.8 and 35.3 g/day, PER was 0.38, 1.39 and 1.71 and ATTD of CP was 66.8, 73.8 and 82.1%, respectively. The SID data previously obtained for the dogs (Paper I) were presented in more detail in Paper II, to provide bioavailability estimates of protein and the individual AA in the LM, PM and FM diets. The diets differed ($P \leq 0.017$) with respect to SID of CP and AA, which was lowest for the LM diet and highest for the FM diet. The SID of CP in the LM, PM and FM diets was 71.5, 80.2 and 87.0%, respectively. The bioavailability estimates were utilized to demonstrate how extruded dog foods with similar protein content can supply widely different levels of bioavailable AA and, thereby, the limitations of basing nutritional adequacy of dog foods on chemical content only.

In a third study (Paper III), adult mink were used for protein digestibility determination of relevant animal protein ingredients available for use in extruded dog food. The protein ingredients evaluated were mechanically separated chicken meat (MSC), salmon protein hydrolysate (SPH) and PM. Mechanically separated chicken meat and SPH were chosen because of the increasing interest in using such high-quality ingredients in extruded dog foods, at the expense of rendered ingredients like PM. Composition of AA and ATTD of CP and AA in mink were determined both for protein quality evaluation of the respective ingredients (used as the only protein source in a wet diet), and in extruded dog foods where MSC or SPH provided 25% of the dietary CP by partial replacement of the PM applied in the previous studies (Paper I and II). The PM diet applied in the two first studies was used as a control diet. For the PM, MSC and SPH ingredients, content of dry matter (DM) was 944.0, 358.0 and 597.4 g/kg, content of CP was 670.7, 421.2 and 868.9 g/kg DM and content of crude fat was 141.4, 547.8 and 18.5 g/kg DM, respectively. The SPH deviated from the MSC and PM with a lower content of total essential AA (g/100 g CP) of more than 10.0 percentage units. The ATTD of CP differed ($P < 0.001$) between ingredients, and was 80.9, 88.2 and 91.3% for the PM, MSC and SPH, respectively. Similarly, ATTD of AA was generally lowest ($P < 0.05$) for the PM. In the extruded diets, the ATTD of CP was 80.3, 81.3 and 79.0% for the PM, MSC and SPH diets, respectively, and for several AA, ATTD was numerically highest for the PM diet. The difference

in ATTD of CP and AA between ingredients was, therefore, not reflected in the extruded diets. Extrusion possibly affected ATTD of CP and AA in the MSC and SPH diets differently than for the PM diet, due to differences in ingredient properties or previous processing.

In conclusion, reliable estimates of AID and SID of CP and AA in dogs can be obtained by determination of ATTD in adult mink, and growth assays with mink kits can provide valuable additional information on possible limitations in the supply of bioavailable AA from extruded dog foods. Rendered animal protein ingredients vary widely with respect to protein quality, whereas a high protein quality can be expected for MSC, SPH and similar ingredients. The protein quality of extruded dog foods depends mainly on the protein quality of the ingredients used, but may possibly also be negatively affected by the extrusion process. Protein quality of animal protein ingredients and extruded dog foods is primarily affected by AA composition and digestibility, which should be determined to ensure nutritional adequacy of dog foods.

# Sammendrag

Bestemmelse av protein- og aminosyrefordøyelighet er vesentlig ved evaluering av proteinkvalitet i hundefôr og bør fortrinnsvis måles som standardisert ileal fordøyelighet (SID). Vurdering av proteinutnyttelse i kroppen, ved hjelp av mål som nitrogenbalanse og "protein efficiency ratio" (PER), vil gi verdifull tilleggsinformasjon om biotilgjengelighet av protein i ekstruderte hundefôr. Bruken av hunder i eksperimentelle forsøk er imidlertid regnet som etisk betenkelig. Bestemmelse av ileal fordøyelighet hos hunder er spesielt omstridt, siden dette krever invasive metoder som kannulering av tynntarm eller avlivning. Det å finne og teste alternative, ikke-invasive metoder er derfor viktig. Bruk av modelldyr kan være et slikt alternativ. Behovet for pålitelige metoder for å evaluere proteinkvalitet i hundefôr understrekes av den store variasjonen i sammensetning og biotilgjengelighet av aminosyrer (AA) som man vet forekommer mellom animalske proteinråvarer brukt i slike fôr. Hovedmålet med denne avhandlingen var å skaffe til veie mer kunnskap om proteinkvalitet i animalske proteinråvarer benyttet i ekstruderte hundefôr ved å bruke mink (*Neovison vison*) som modell for hund (*Canis familiaris*).

I et komparativt studie (Artikkel I) ble fordøyelighet av næringsstoffer bestemt som apparent totalfordøyelighet (ATTD) hos voksne mink og som apparent ileal fordøyelighet (AID), SID, apparent colon fordøyelighet og ATTD hos voksne hunder. Tre forsøksfôr ble produsert ved ekstrudering og formulert til å ha likt innhold av henholdsvis råprotein (CP) (varierte fra 24.9 til 25.5%) og råfett (varierte fra 18.6 to 20.3%), men ulik sammensetning og fordøyelighet av AA. Lammemel (LM), fjørfemel (PM) og fiskemel (FM), med en ATTD av CP på henholdsvis 67.7, 80.9 og 87.5% hos voksne mink, ble brukt som proteinråvarer i de respektive fôrene. Hos hunder var AID av CP (74.4%) som forventet lavere ($P < 0.001$) enn ATTD (83.5%), og lignende resultater ble funnet for de individuelle AA. Apparent ileal fordøyelighet av CP hos hunder var ikke forskjellig ($P > 0.05$) fra ATTD av CP hos mink (77.8%). Apparent ileal fordøyelighet hos hunder og ATTD hos mink var også like ($P > 0.05$) for mange av AA, men AID verdiene hos hundene var generelt numerisk lavere enn de tilvarende ATTD verdiene hos mink. Standardisert ileal fordøyelighet av CP (79.6%) og AA hos hunder lå veldig tett opptil ($P > 0.05$) ATTD hos mink, bortsett fra for treonin og serin. De ulike fordøyelighetsmålene var

signifikant korrelerte ($P \leq 0.01$) for fordøyelighet av CP og de fleste AA, og for rangering av AA med hensyn til fordøyelighetsnivåene.

Lammemel, PM og FM fôrene brukt i den komparative studien med hunder og mink ble videre utnyttet i et vekst-forsøk med minkvalper (Artikkel II), og de kjente forskjellene i tilførsel av biotilgjengelige AA mellom fôrene ble gjenspeilet i resultatene for nitrogenbalanse og tilvekst. Nitrogenavleiringen var 0.66, 1.04 og 1.18 g/kg$^{0.75}$/dag, tilveksten var 8.2, 26.8 og 35.3 g/dag, PER var 0.38, 1.39 og 1.71 og ATTD av CP var 66.8, 73.8 og 82.1% for henholdsvis LM, PM og FM fôret. Verdiene for SID, som tidligere ble bestemt for hundene (Artikkel I), ble presentert i mer detalj i Artikkel II for å gi estimater for biotilgjengelighet av protein og de individuelle AA i LM, PM og FM fôrene. Det var forskjell ($P \leq 0.017$) mellom fôrene med tanke på SID av CP og AA, som var lavest for LM fôret og høyest for FM fôret. Standardisert ileal fordøyelighet av CP i LM, PM og FM fôret var på henholdsvis 71.5, 80.2 og 87.0%. Estimatene for biotilgjengelighet ble utnyttet til å demonstrere hvordan ekstruderte hundefôr med likt proteininnhold kan tilføre svært forskjellige mengder med biotilgjengelige AA, og dermed, begrensningene i det å basere ernæringsmessig tilstrekkelighet av hundefôr kun på kjemisk innhold.

I en tredje studie (Artikkel III), ble voksne mink brukt for å bestemme proteinfordøyelighet av relevante animalske proteinråvarer tilgjengelige for bruk i ekstruderte hundefôr. Proteinråvarene som ble evaluert var mekanisk utbeinet kyllingkjøtt (MSC), lakseproteinhydrolysat (SPH) og PM. Mekanisk utbeinet kyllingkjøtt og SPH ble valgt ut på grunn av den økende interessen for å bruke slike kvalitetsråvarer i ekstruderte hundefôr på bekostning av tørkede mel slik som PM. Aminosyresammensetning og ATTD av CP og AA hos mink ble bestemt for å evaluere proteinkvalitet både i de respektive proteinråvarene (brukt som eneste proteinkilde i våtfôr) og i ekstruderte hundefôr der MSC eller SPH tilførte 25% av fôrets CP innhold ved å delvis erstatte det PM som ble benyttet i de foregående studiene (Artikkel I og II). Fjørfemelfôret benyttet i de to første studiene ble brukt som kontrollfôr. Innholdet av tørrstoff (DM) var 944.0, 358.0 og 597.4 g/kg, innhold av CP var 670.7, 421.2 og 868.9 g/kg DM og innhold av råfett var 141.4, 547.8 og 18.5 g/kg DM for henholdsvis PM, MSC og SPH. Lakseproteinhydrolysatet skilte seg ut fra MSC og PM med et lavere innhold av totalt essensielle AA (g/100g CP) på mer enn 10.0 prosentenheter. Apparent totalfordøyelighet av CP var ulik ($P < 0.001$) mellom råvarene og var på henholdsvis 80.9, 88.2 og 91.3% for PM, MSC og SPH. På

lignende vis var ATTD av AA generelt lavest ($P < 0.05$) for PM. I de ekstruderte fôrene var ATTD av CP på henholdsvis 80.3, 81.3 og 79.0% for PM, MSC og SPH fôrene, og for flere av AA var ATTD numerisk høyest for PM fôret. Forskjellen i ATTD av CP og AA mellom råvarene ble derfor ikke gjenspeilet i de ekstruderte fôrene. Ekstruderingen påvirket muligens ATTD av CP og AA i MSC og SPH fôrene annerledes enn for PM fôret på grunn av forskjeller i råvarenes egenskaper eller tidligere prosessering.

Det kan konkluderes med at pålitelige estimater for AID og SID av CP og AA hos hunder kan skaffes til veie ved å bestemme ATTD hos voksne mink, og vekst-studier med minkvalper kan gi verdifull tilleggsinformasjon om mulige begrensinger i tilførselen av biotilgjengelige AA i ekstruderte hundefôr. Tørkede animalske proteinråvarer varierer mye med hensyn til proteinkvalitet, mens MSC, SPH og lignende ingredienser derimot kan forventes å ha en høy proteinkvalitet. Proteinkvaliteten i ekstruderte hundefôr avhenger hovedsakelig av proteinkvaliteten til de benyttede råvarene, men kan muligens også bli negativt påvirket av ekstruderingsprosessen. Proteinkvaliteten til animalske proteinråvarer og ekstruderte hundefôr blir hovedsakelig påvirket av aminosyresammensetning og fordøyelighet, som bør bestemmes for å sikre at næringsbehovet dekkes.

# 1. General introduction

The global pet food retail sale amounted to around USD 70 billion, or around NOK 565 billion, in 2015 (Phillips-Donaldson, 2016). According to Phillips-Donaldson (2016), dog food accounts for most of the pet food retail sale globally, and extruded dry food has the greatest market share when considering the different pet food categories. The dog food retail sale in Norway amounts to around NOK 1 billion annually. In 2010, 50 269 metric tons of pet food was produced or imported in Norway, of which 61% was dog food. Extruded dry food constituted around 73% of the dog food sale, and the majority (78%) of the extruded food was imported (Norwegian Food Safety Authority, 2010). The global value of the pet food ingredients market was set to USD 28.6 billion, or around NOK 230 billion, in 2014, and animal by-products had the greatest market share with around 48.7% (Markets and Markets, 2015). Globally, chicken is the most widely used protein ingredient in pet food, also in extruded dog food (Phillips-Donaldson, 2016).

From the data above, it is clear that the market for extruded dog foods is extensive, and animal protein ingredients have a vital role in the formulation of such foods. The options in selection of relevant animal protein ingredients are great many, and include the range of different species available, what parts of the animal to use, and if, and then how, the animal protein ingredients should be treated prior to inclusion in the food (Swanson et al., 2013). Naturally, such a wide range of options form the basis for a great variation in protein quality between animal protein ingredients and between extruded dog foods containing these ingredients.

The main objective of the present thesis was to provide more knowledge about the protein quality of animal protein ingredients used in extruded dog foods, and for this purpose, the relevance of using mink (*Neovison vison*) as an animal model for protein quality evaluation of extruded dog foods.

# 2. Background

## 2.1 Protein

Protein is an essential nutrient for growth, maintenance and health in human and animal nutrition, as it has a wide range of vital functions in the body. Enzymes, antibodies and some hormones, like insulin, are proteins. Furthermore, actin and myosin in muscles, collagen in the connective tissue, hemoglobin in blood and keratin in skin, hair and nails are all examples of proteins in the body. In addition, protein can also be utilized as a source of energy. The building blocks of protein are the amino acids (AA), and body protein is built up by 20 different AA. For dogs, ten of the AA are considered as essential, and must be provided by the food. These include arginine, histidine, isoleucine, leucine, lysine (Lys), methionine (Met), phenylalanine, threonine (Thr), tryptophan and valine (National Research Council (NRC), 2006).

## 2.2 Protein quality evaluation

### 2.2.1 Definition of protein quality

Protein quality has been defined by Boye et al. (2012) as "*the ability of a food protein to meet the body's metabolic demand for AA and nitrogen (N) and is determined by the AA composition and digestibility of the protein as well as the bioavailability of the individual AA*". The term bioavailability could be defined as: "*the proportion of the total AA that is digested and absorbed in a form suitable for protein synthesis*" (Batterham, 1992). According to Fuller and Tomé (2005), the term bioavailability can be divided in three parts, including *"digestibility, chemical integrity and freedom from interference in metabolism"*. Of these, digestibility is usually considered as the most important part (Fuller and Tomé, 2005).

### 2.2.2 Determination of protein and amino acid digestibility in dogs

#### *2.2.2.1 Apparent total tract digestibility*

In dogs, protein digestibility has traditionally been measured as apparent total tract digestibility (ATTD). The ATTD is a simple measure based on the difference between the amount of nutrient consumed and the amount of nutrient excreted in feces. Recording of accurate feed intake combined with total collection of feces is commonly used to determine ATTD. An alternative method is the marker method, where an inert indigestible marker is used to estimate digestibility by determination of the marker concentration in food and feces (McDonald et al., 2002). For dogs, chromic oxide has regularly been used as an inert marker in the diet, but yttrium oxide has been shown to be a viable alternative yielding similar digestibility values as total collection of feces, both for dogs and for relevant model species (Vhile et al., 2007; Sundling et al., 2012). Although determination of ATTD is a very gentle and non-invasive procedure, it is not considered accurate, as ATTD may be largely confounded by the microbial fermentation occurring in the large intestine of dogs.

The residence time of digesta in the large intestine of dogs has been found to increase with body size, and varied from 9.1 hours for Miniature Poodles to 39.4 hours for Giant Schnauzers (Hernot et al., 2006). With basis in a medium sized dog of around 13-14 kg, an estimated large intestinal passage rate of 4.3 cm/hour has been calculated by Hendriks et al. (2012). According to Hernot et al. (2006), the length of the large intestine should increase with increased body size, and the rate of the passage of digesta through the large intestine will, therefore, probably be similar between dogs of varying body size. During the time digesta resides in the large intestine of dogs, a significant amount of unabsorbed AA of dietary or endogenous origin are deaminated by the large intestinal microbiota, and the resulting ammonia ($NH_3$) is absorbed from the large intestine (Hendriks et al., 2012). An apparent dietary N disappearance in the large intestine of dogs as high as 46% has been estimated (Hendriks et al., 2012). Although the dog colon mucosa probably is able to transport AA (Robinson et al., 1973), the absorption of AA from the large intestine of dogs is considered negligible (Hendriks et al., 2012). The N absorbed from the large intestine is, therefore, mainly $NH_3$, which is subsequently secreted in the urine (Hendriks et al., 2012). Thus, N absorbed from the large intestine is of no value for the dog, but increases the ATTD values of crude protein (CP). Of the N excreted in feces of dogs, around 50% has been estimated to be of microbial origin (Karr-Lilienthal et al., 2004). Microbial

degradation and synthesis of AA will, therefore, significantly influence the AA composition of the fecal N and affect the ATTD values of the individual AA.

#### *2.2.2.2 Ileal digestibility*

Digestibility measured at the end of the small intestine (ileum) is unaffected by the large intestinal microbiota, and ileal digestibility values are, therefore, preferred for more accurate estimation of protein and AA bioavailability in dogs. Lower apparent ileal digestibility (AID) values than ATTD values of N, with an average difference of 9.4 percentage units, has been reported for dogs (Hendriks et al., 2012). For the individual AA, inconsistent results have been reported, and both lower and higher levels of AID than ATTD have been observed (Hendriks and Sritharan, 2002; Hendriks et al., 2013). The latter could be explained by the microbial degradation and synthesis of AA occurring in the large intestine. In general, the difference between AID and ATTD of CP and AA will decrease with higher levels of AID (Hendriks et al., 2012).

The AID values of CP and AA are affected by the endogenous AA present in the ileal digesta, referred to as ileal endogenous AA losses (Stein et al., 2007a; Stein et al., 2007b). As reviewed in the latter studies, the ileal endogenous AA losses could be divided into basal and specific, respectively. The basal ileal endogenous losses are principally affected by dry matter (DM) intake, whereas the specific ileal endogenous losses are affected by the ingredient composition of the food. The endogenous losses of AA are not corrected for when AID is determined, and the AID values are, therefore, lower than true ileal digestibility values that are corrected for both basal and specific ileal endogenous losses. When a correction is made for the basal endogenous losses only, standardized ileal digestibility (SID) values are obtained. In pig nutrition, knowledge of specific endogenous losses induced by different feed ingredients, and thus, true ileal digestibility values, are limited, and SID values are preferred for determination of ileal digestibility (Stein et al., 2007a; Stein et al., 2007b). As compared with the AID values of feed ingredients, SID values are more accurate due to the advantage of being more additive when feed ingredients are used in mixed diets (Stein et al., 2005). Considering dogs, recent studies have focused on SID values for estimation of AA bioavailability in dog foods (Hendriks et al., 2013; Hendriks et al., 2015). As only basal, and not specific, endogenous losses of AA are included in the minimal requirement estimates of AA in dogs set by the NRC (2006), SID values

are the most accurate to use when AA bioavailability in dog foods is estimated (Hendriks et al., 2013; Hendriks et al., 2015).

Although determination of ileal digestibility is preferred rather than ATTD to estimate protein and AA bioavailability for dogs, this is a practice belonging more or less to the past, mainly due to ethical reasons. Ileal digestibility in dogs reported in scientific studies has generally been determined by use of the T-cannulation method (e.g. Murray et al., 1997; Johnson et al., 1998; Bednar et al., 2000; Faber et al., 2010; Hendriks et al., 2013) or by dissection of the end of ileum in euthanized animals (Hendriks and Sritharan, 2002). Both methods require the use of an indigestible marker and have their advantages and limitations, as discussed by Nyachoti et al. (1997). No significant differences in AID of CP and AA digestibility have been found when the two methods have been compared in pig studies (Moughan and Smith, 1987; Donkoh et al., 1994; Pedersen et al., 2010). As reported by Hill et al. (1996), the cannulation method in dogs is highly associated with different complications, including severe excoriation and development of ulcers in the skin. It could, therefore, be argued that dissection of the intestine after euthanization is a less invasive and troublesome technique for the animals. Still, however, neither of the two methods can be used for routine measurements, as they are both economically costly and ethically questionable.

#### *2.2.2.3 Estimation of ileal digestibility in dogs*

From existing literature data, Hendriks et al. (2015) have developed a regression equation for estimation of standardized ileal outflow of N from apparent fecal outflow of N in dogs. Based on N intake and the estimated standardized ileal outflow of N, SID of N can, then, be calculated (Hendriks et al., 2015). Regression equations for estimation of SID of individual AA based on the SID of N were also developed in the latter study. According to Hendriks et al. (2015), a significant linear relationship between apparent fecal and standardized ileal outflow of N was found, but the variability in the data increased with increased N outflow. With respect to the linear relationship between SID of N and SID of individual AA, only a limited dataset was available to determine the relationship, and variable coefficients of determination ($R^2$), ranging from 0.61 to 0.93, were found (Hendriks et al., 2015). Based on the results of Hendriks et al. (2015) it is possible to estimate SID of CP and AA from fecal N content, although inaccuracies are likely to occur.

Use of animal models is another alternative for estimation of ileal digestibility in dogs. True AA digestibility in cecectomized roosters has been found to be highly correlated with AID of AA in dogs (Johnson et al., 1998), and Folador et al. (2006) and Faber et al. (2010) also used cecectomized roosters to determine AA digestibility of potential protein ingredients available for use in dog foods. Mink has also been verified as a possible model for CP and AA digestibility in dogs (Ahlstrøm and Skrede, 1998; Vhile et al., 2005), but only ATTD and not ileal digestibility was determined in the latter studies. Mink has a short digestive tract, the caecum is lacking and the large intestine with a length of approximately 10 cm has minimal microbial activity (Skrede, 1979; Szymeczko and Skrede, 1990). The total digestive tract of the mink is, therefore, not so different from the small intestine of dogs, and as hypothesized by Vhile (2007), it is possible that total tract digestibility determined in mink could be relevant for estimation of ileal digestibility of AA in dogs.

By use of *in vitro* methods for estimation of ileal digestibility in dogs, experiments with laboratory animals could be avoided. A dynamic *in vitro* model simulating the stomach and small intestine of dogs has been described (Smeets-Peeters et al., 1999), but according to Butts et al. (2012), such dynamic models are expensive to operate, and may not be appropriate for routine digestibility measurements. Less complex *in vitro* enzymatic methods for CP digestibility determination of dog foods have also been described (Tonglet et al., 2001; Hervera et al., 2009), but the results of the latter methods were only compared with *in vivo* measures of ATTD, and not AID, in dogs. Such an insufficient validation of *in vitro* methods developed for ileal digestibility determination is common, and reports of the repeatability and optimization of the *in vitro* assays are usually also inadequate in scientific studies (Butts et al., 2012). As discussed by Butts et al. (2012), rapid and inexpensive *in vitro* digestibility assays could be a useful tool when evaluating protein and AA digestibility of different food ingredients, and could at least be used to rank the ingredients with respect to digestibility level. However, as for humans (Butts et al., 2012), a standardized and validated *in vitro* model is at present needed to increase the relevance of using *in vitro* measures as an alternative to *in vivo* measures in estimation of ileal digestibility in dogs.

### 2.2.3 Bioavailability of amino acids

Besides digestibility, chemical integrity and freedom from interference in metabolism are influential aspects of AA bioavailability (Fuller and Tomé, 2005). The term "chemical integrity" is related to the structural changes of AA that may occur during processing of food proteins, whereas "freedom from interference in metabolism" is related to the potential influence of substances, other than protein, in the evaluated food protein source on AA bioavailability (Fuller and Tomé, 2005). For processed dog food, the aspect of chemical integrity is important, as heat processing, like rendering of the protein ingredients and extrusion of the food, may affect AA bioavailability considerably (Björck and Asp, 1983; Papadopoulos, 1989; Moughan, 2003). As reviewed by Papadopoulos (1989) and Moughan (2003), cross-linkages formed between AA side chains during food processing can reduce the ileal digestibility. Furthermore, food proteins can react with a number of other nutritional compounds during processing, of which reducing sugars could be considered as the most important (Moughan, 2003). The resultant changes in the chemical structure of the AA may not affect the digestibility and absorption, but could render the AA unavailable for metabolism in the body. Therefore, ileal digestibility values may overestimate the availability of AA, and arginine, Lys, Thr, Met, cysteine (Cys) and tryptophan seem to be especially vulnerable for such detrimental chemical changes during processing (Batterham, 1992; Moughan, 2003). Of the latter AA, bioavailability of Lys has been most extensively studied. Lysine has a reactive epsilon amino group, which readily reacts with reducing sugars during heat treatment, resulting in the formation of early Maillard reaction products (Batterham, 1992; Moughan, 2003; van Rooijen, 2015). As described by the latter authors, such Maillard reaction products may be partly absorbed in the small intestine, but are nutritionally unavailable and excreted in the urine. However, during conventional AA analyses with strong acid hydrolysis, the Maillard reaction products are converted back to Lys. When content of Lys in diet and ileal digesta is analyzed by the conventional method, ileal Lys digestibility, therefore, overestimates Lys bioavailability (Moughan, 2003). As reviewed by Moughan (2003), more accurate estimation of Lys bioavailability can be performed by analysis of reactive Lys content in food and ileal digesta, and then, ileal digestibility determination of the reactive Lys, whereas as for AA other than Lys, more research is needed considering the bioavailability in processed food protein.

### 2.2.4 Methods for protein quality evaluation

In addition to determination of AA composition and digestibility, a wide range of methods can be used in protein quality evaluation (Boye et al., 2012; Elango et al., 2012). Although protein quality evaluation of dog foods mainly is restricted to digestibility determination, information on protein utilization for bodily needs may provide valuable additional information with respect to protein quality of dietary protein, especially since ileal digestibility may overestimate the bioavailability of AA (chapter 2.2.3). In studies reporting protein quality of protein sources or diets for dogs, a wide range of different measures, including both *in vitro* methods and *in vivo* growth assays, have been used for evaluation of protein quality (Burns et al., 1982; Hegedűs et al., 1998; Dust et al., 2005; Folador et al., 2006; Cramer et al., 2007). The *in vivo* growth assays have included measures like N balance, protein efficiency ratio (PER), net protein ratio, biological value and net protein utilization, with PER values reported in all of the latter studies. Burns et al. (1982) performed a comparative study with growing dogs and rats, whereas others have used growing rats (Hegedűs et al., 1998) or growing chickens (Dust et al., 2005; Folador et al., 2006; Cramer et al., 2007) as animal models for protein quality evaluation of protein ingredients or diets for dogs.

The growth assays mentioned above measure protein utilization in the body and are used for estimation of protein bioavailability (Elango et al., 2012). Measures like the PER provide valuable information with respect to total protein utilization, but bioavailability of the individual AA is not measured. With slope-ratio assays, however, determination of individual AA bioavailability is possible, as described by Batterham (1992). In such assays, the response (like growth or feed conversion efficiency) to an increased intake of a test protein source is compared with the response to an increased intake of a reference protein. In each assay performed, bioavailability is determined for the first limiting AA in the diets. Based on the principle of slope-ratio assays, an alternative method, called the indicator AA oxidation (IAAO) method, has been developed for determination of individual AA bioavailability (Elango et al., 2012). As reviewed by Elango et al. (2012), the IAAO method is based on the concept that when one AA is limiting for protein synthesis, the other AA, including the indicator AA, are in excess and will be oxidized. The oxidation of the indicator AA is inversely proportional to the rate of protein synthesis, and the ratio between the IAAO response of the test protein source and the reference protein is calculated for determination of AA bioavailability. The IAAO method is less time-

consuming than the slope-ratio assays based on responses like growth or feed conversion efficiency, and bioavailability of several AA can, therefore, be determined over a relatively short time period. However, certain criteria have to be met for the diets used in slope-ratio assays, including that the AA in question has to be first limiting and supplied in deficient amounts as compared with the requirement of the animals. Furthermore, the dietary contents of nutrients other than the tested AA have to be similar between the test diet and the reference diet, to be sure that the observed response is caused by the intake of the AA tested (Elango et al., 2012).

## 2.3 Protein quality of extruded dog foods

### 2.3.1 Protein and amino acid requirements of dogs

Following the definition given in chapter 2.2.1, protein quality of dog foods concerns the ability of the food proteins to cover the protein and AA requirements of dogs. Research on the protein and AA requirements of dogs has been reviewed by the NRC (2006), and summarized and presented in tabular form as the "Minimal Requirement" of CP and AA in dogs (NRC, 2006). The CP and AA requirements are generally higher for puppies and pregnant or lactating bitches than for adult dogs at maintenance. The minimal requirement estimates are based on the bioavailable amounts of the nutrients, and these estimates have been added a safety margin to obtain the standards of recommended nutrient intake, called the "Recommended Allowance" values (NRC, 2006). In addition to the NRC, the Association of American Feed Control Officials (AAFCO) and the European Pet Food Industry Federation (FEDIAF) are the two other authoritative organizations that provide recommended values for nutrient content in dog foods. As for the recommended allowance values of the NRC (2006), the "Dog Food Nutrient Profiles" of the AAFCO (2016), and the "Minimum Recommended" values of the FEDIAF (2014), are based on the minimal requirement estimates of the NRC (2006) and a safety margin accounting for the nutrient bioavailability. The authoritative organizations use different estimates for bioavailability and differences in the recommendations for CP and AA content are, therefore, apparent between the NRC, AAFCO and FEDIAF. For CP, the recommended allowance set by the NRC (2006) for adult dogs at maintenance is 10% of DM (6.0 g/megajoule (MJ) metabolizable energy (ME)), whereas the AAFCO (2016) and the FEDIAF (2014) recommend 18% of DM (10.8 g/MJ ME).

### 2.3.2 Animal protein ingredients used in extruded dog foods

When dog owners discuss dog food and pet food producers present their products, dietary protein quality and protein ingredients are one of the most engaging subjects. In commercial, extruded dog foods, protein is usually provided as a blend of animal and vegetable protein sources. Considering the animal protein ingredients, large amounts of animal by-products, which are materials of animal origin not consumed by humans (European Commission, 2016), are available for utilization in pet food. In the European Union, more than 20 million tons of animal by-products are generated annually (European Commission, 2016), with similar amounts produced in the United States (Meeker and Hamilton, 2006). In the future, the amounts of animal by-products will probably increase further, as meat consumption worldwide is expected to increase in concert with the worlds increasing human population (Food and Agriculture Organization of the United Nations, 2009).

By-products from livestock and poultry industry include products like skin, feet, feathers, bone, blood, contents from the abdomen or intestines, viscera and meat, whereas the fish industry mainly generates muscle-trimmings, viscera, bones and heads, as reviewed by Martínez-Alvarez et al. (2015). Most commonly, the animal by-products used in extruded dog foods have been rendered to animal by-product meals. The rendering process involves cooking and separation of fat, followed by dehydration of the animal by-products (Meeker and Hamilton, 2006). The nutrient content of the final product varies, but the DM content is usually above 90%, CP content is 50% or higher, fat content is around 10% and the ash content may constitute up to around 25% on an as-fed basis (NRC, 2006). A range of different rendered animal meals are commonly used in extruded dog food, including products like meat and bone meal, meat meal, lamb meal (LM), poultry by-product meal, poultry meal (PM) and fish meal (FM) (Aldrich, 2006). Definitions of the different types of meals are given by the AAFCO. The rendered animal meals are a heterogeneous group of protein ingredients, and may consist of different parts of the animals. For example, PM "*is the dry rendered product from a combination of clean flesh and skin with or without accompanying bone, derived from the parts of whole carcasses of poultry or a combination thereof, exclusive of feathers, heads, feet, and entrails*" (AAFCO, 2016).

The protein quality of heat-treated ingredients may be reduced during heat processing, as described in chapter 2.2.3. Another factor that may affect the protein quality of rendered animal meals is the ash content, as an increased ash content is associated with generally lower levels of

essential AA, and higher levels of several non-essential AA on a CP basis (Shirley and Parsons, 2001). Differences in raw material composition and the processing conditions used in production of animal meal ingredients are, therefore, the main reasons for the great variation observed with respect to the protein quality of such products (Johnson and Parsons, 1997; Johnson et al., 1998; Wang and Parsons, 1998; Shirley and Parsons, 2000; Hendriks et al., 2002a; Cramer et al., 2007). In general, however, rendered animal meals are excellent sources of nutrients, including essential AA, essential fatty acids, vitamins and minerals, and considerable improvements in the AA digestibility have been observed since the 1980s (Meeker and Hamilton, 2006; Meeker and Meisinger, 2015). Furthermore, the use of rendered animal protein meals in companion animal diets is recognized as a highly sustainable utilization of the great amounts of animal by-products produced annually (Meeker and Meisinger, 2015). If not rendered, valuable protein ingredients would have been lost and the large amounts of animal by-products would have to be disposed off by alternative methods. As discussed by Meeker and Meisinger (2015), such methods are associated with environmental pollution and health risks for the public.

Consumers' (dog owners) demands affect the dog food market, and as a result of the increased humanization of dogs, the use of natural pet foods made of human-grade ingredients is a growing trend. Despite the high value of using rendered, animal by-products in extruded dog food, such rendered meals are, therefore, increasingly being replaced by human-grade meat products (Buff et al., 2014; Carter et al., 2014). According to the definition of "meat" ingredients given by the AAFCO (2016), meat is mainly the raw muscle tissue of animals without accompanying bones, whereas meat by-products mainly is the remains of the animals when muscle tissue is removed. Compared with rendered animal by-products, the use of human-grade meat ingredients in dog food is not a sustainable alternative, as it puts dog food and human food up against each other and requires more meat to be produced (Carter et al., 2014; Deng and Swanson, 2015; Meeker and Meisinger, 2015). According to Carter et al. (2014), however, consumers prefer raw (fresh) meat ingredients, which are considered as more natural ingredients with a higher quality than animal by-products.

A high protein quality of meat ingredients was reported by Faber et al. (2010), who found AID values of CP and AA close to or above 90% for dogs fed extruded foods in which good-quality cuts of animal meats or skinless fish fillets were used as the single protein source in addition to the protein provided from grain ingredients. The high inclusion rate of the animal

protein sources used by Faber et al. (2010) was promoted by an additional processing step where the raw protein ingredients were dried at low temperature and ground prior to extrusion. According to the AAFCO (2016), dried ingredients are not considered as fresh, and such ingredients would not be in accordance with the consumers' preference for raw meat products. As opposed to dried or rendered ingredients, the use of raw meat ingredients is challenging for the extrusion process, as the high contents of fat and water in the raw meat reduce the friction in the extruder (Beaton, 2016). The inclusion rate of the meat ingredients may, therefore, be restricted to promote an optimal extrusion process, and the contribution of AA from the meat in the final, extruded food will then be limited. Reports on the protein quality of extruded food containing raw meat ingredients, as compared with rendered animal meals, are scarce, and a clear difference in AID of CP and AA between diets containing such ingredients have not been found when fed to dogs (Murray et al., 1997).

Animal protein hydrolysates are alternative animal protein ingredients commonly applied in diets for dogs with food allergies. In addition, animal protein hydrolysates are used as palatants and possibly also function as nutraceuticals in diets for pets (Martínez-Alvarez et al., 2015). Besides the potential positive health effects of applying animal protein hydrolysates in dog diets, they generally also provide highly digestible AA (Gilbert et al., 2008; Martínez-Alvarez et al., 2015). As reviewed by Martínez-Alvarez et al. (2015), hydrolysates may be produced from by-products of the livestock, poultry or fish industry. Animal protein hydrolysates used in dog food are, therefore, alternative, high-quality protein ingredients, which promote the sustainability of dog food production. Reports regarding the use of animal protein hydrolysates in extruded dog foods are scarce, but Folador et al. (2006) reported a high palatability of an extruded food containing salmon protein hydrolysate (SPH) when fed to dogs. With respect to protein quality, Verlinden et al. (2006) and Zinn et al. (2009) reported protein digestibility in dogs fed extruded foods containing animal protein hydrolysates, but only ATTD of CP was determined. Recently, van Rooijen (2015) found that *in vitro* digestibility of CP, Lys and reactive Lys decreased after extrusion of a diet containing a fish protein hydrolysate, and it was suggested that protein hydrolysates are more easily negatively affected with respect to protein quality than intact protein ingredients during extrusion of dog foods.

### 2.3.3 Labelling and declaration of protein content in commercial dog foods

For commercial dog foods sold in the United States, it is required that all ingredients are listed on the pet food label in descending order, as determined by their weight on an "as-formulated" basis. Considering the nutrient content, only the minimum percent of CP and crude fat, and the maximum percent of crude fiber and moisture are required. A statement of nutritional adequacy is also required, and the food can be labelled as "complete and balanced" if the nutrient contents meet the "Dog Food Nutrient Profiles" published by the AAFCO or if the food has passed a feeding trial as defined by the AAFCO (AAFCO, 2016). In Europe, labelling of ingredients resembles the practice in the United States, whereas as for nutrients, analytical values of CP, crude fiber, crude fat and crude ash are required. Labelling of moisture content is not required as long as the moisture content is 14% or lower. In contrast to the labelling requirements in the United States, a statement of nutritional adequacy is not required in Europe (FEDIAF, 2011), but pet food manufacturers should follow the nutritional guidelines set by the FEDIAF and validate the nutritional adequacy of dog foods by at least chemical analyses (FEDIAF, 2014).

As discussed by Morris and Rogers (1994), the practice of validating nutritional adequacy of dog foods based on nutrient content only is inaccurate, as nutrient bioavailability is not accounted for. The limitations of using chemical content as the basis for nutritional adequacy was demonstrated by Huber et al. (1986), showing how puppies fed diets labelled with similar nutrient contents experienced different growth rates. Similar results were also reported by Huber et al. (1991). The safety margin incorporated in the nutrient recommendations of the NRC, AAFCO and FEDIAF should ensure that the minimal requirements of nutrients in dogs are met, although diets differ in nutrient bioavailability. As demonstrated by Hendriks et al. (2015), however, the protein and AA bioavailability accounted for as safety margins by the NRC is too high. The AAFCO and the FEDIAF have also accounted for a too high bioavailability of most of the AA (Hendriks et al., 2015). The findings of Hendriks et al. (2015) strengthens the uncertainty inherent with the practice of validating nutritional adequacy of dogs food solely based on the chemical content of nutrients assessed against the nutrient recommendations set by the NRC, AAFCO or FEDIAF.

Since labelling of AA content and bioavailability of protein and AA is not required, consumers are only informed about the protein sources used in the food and the CP content. The opportunity of the consumers to assess the protein quality of a dog food based on the labelling is,

therefore, severely restricted. This is not satisfactory, especially when considering the great variation in protein quality found between different animal protein ingredients, which is reflected in the protein quality of commercial dog foods. Although a CP digestibility ≥ 80.0% is considered as normal in extruded dog foods (FEDIAF, 2014), a range in AID of CP from 66.2 to 83.3% has been reported for five commercial dry foods fed to dogs (Hendriks et al., 2013). Similarly, Krogdahl et al. (2004) found ATTD in 12 commercial dry dog foods to vary from 72.7 to 83.8% when fed to mink.

A high protein content will in most cases probably compensate for the variable protein quality of commercial dog foods. According to the NRC (2006), the CP content of extruded dog foods commonly range between 18-32% of DM, and CP content of the five commercial extruded diets evaluated by Hendriks et al. (2013) varied from 24.3 to 32.7% of DM. In the diets evaluated by Krogdahl et al. (2004), a CP content of 23.7% (DM-basis) or higher was found. These levels exceed the recommended levels of 10% of DM (NRC, 2006) or 18% of DM (FEDIAF, 2014; AAFCO, 2016). The high CP levels usually found in extruded diets are part of the consumer trends, where a high CP content is associated with quality (Carter et al., 2014). As discussed by several authors, a high CP supply exceeding the minimal requirements may have beneficial effects on dogs' health, and this should be explored in future studies (Swanson et al., 2013; Buff et al., 2014). However, oversupply of CP increases the amount of N voided in urine and feces, and a lowered CP content in dog foods would be beneficial from a sustainability point of view (Swanson et al., 2013; Deng and Swanson, 2015).

# 3. Objectives of the thesis and main hypotheses

The main objective of this thesis was to contribute with increased knowledge regarding the protein quality of animal protein ingredients used in extruded dog food, by use of mink as an animal model for protein quality evaluation.

The thesis included three studies with these objectives:

1. To evaluate if ATTD determination of CP and AA in adult mink can be used for estimation of AID and SID of CP and AA in dogs.
2. To investigate if differences in the supply of bioavailable AA between extruded dog foods are reflected in the growth rates and N balance data obtained with mink kits and, thereby, to evaluate if growing mink is a relevant model for protein quality evaluation of extruded dog foods.
3. To evaluate the protein quality of mechanically separated chicken meat (MSC) and SPH, and of extruded dog foods containing MSC or SPH.

The main hypotheses:

1. ATTD of CP and AA in mink is highly correlated with AID and SID of CP and AA in dogs.
2. Growing mink kits will show growth response in accordance with the protein quality of an extruded dog food.
3. Raw animal protein and animal protein hydrolysate ingredients have a superior protein quality, and can partially replace rendered animal ingredients and improve the protein quality of extruded dog foods.

# 4. Summary of papers I-III

## 4.1 Paper I

Ileal, colonic and total tract nutrient digestibility in dogs (*Canis familiaris)* compared with total tract digestibility in mink (*Neovison vison*).

The main objective of this study was to compare ATTD of CP and AA in mink with AID in dogs, to test the hypothesis that the mink is a suitable model for estimation of AID of CP and AA in dogs. In addition, SID of CP and AA in dogs was calculated and compared with ATTD in mink. Furthermore, apparent colonic digestibility and ATTD in dogs were determined in order to study the level of CP and AA degradation taking place in the hindgut. The study included 12 dogs and 12 mink, respectively divided in three groups of four animals fed one out of three experimental diets differing in CP digestibility (LM, PM and FM diets).

*Main results*

- AID of CP (74.4%) was lower ($P < 0.001$) than ATTD of CP (83.5%) in dogs, and similar results were found for all AA.
- For CP, AID in dogs did not differ ($P > 0.05$) from ATTD in mink (77.8%). Non-significant differences between AID in dogs and ATTD in mink were also found for several AA, although AID of most AA was numerically lower than ATTD in mink.
- SID in dogs and ATTD in mink were numerically very close ($P > 0.05$) for CP and all AA, except for Thr and serine (Ser).
- The different digestibility measurements were highly correlated with respect to the digestibility of CP and most AA ($P < 0.01$) and for ranking of AA based on the digestibility levels ($P < 0.001$).

*Conclusion*

Apparent ileal digestibility of CP and most AA in dogs was significantly correlated to ATTD in mink. Furthermore, ATTD in mink was numerically very close to SID in dogs for CP and AA, except for Thr and Ser. The results suggest that ATTD in mink can be a highly relevant and efficient tool for determination of AID and SID of CP and AA in diets for dogs. This would enable reliable estimates of CP and AA digestibility levels in dogs to be obtained in a gentle manner, without the use of surgery.

## 4.2 Paper II

### Protein and amino acid bioavailability of extruded dog food with protein meals of different quality using growing mink (*Neovison vison*) as a model

The main objective of the study was to investigate if the growth response in mink kits is sensitive to variations in the supply of bioavailable AA between extruded dog foods and, therefore, the suitability of using a growing mink assay in protein quality evaluation of extruded dog foods. The mink study included 12 kits aged eight weeks when the study started and was organized as a 3 × 3 Latin square, which lasted until the kits were 11 weeks old. Three extruded dog foods with similar CP content but of different protein quality were used (same diets as in Paper I). Protein meals with low (LM), intermediate (PM) and high (FM) protein quality were applied as protein sources in the respective diets. Nitrogen balance, body weight (BW) gain, PER and ATTD were used as measures of protein and AA bioavailability in growing mink. Bioavailability of protein and AA in the extruded foods was also evaluated for adult dogs, by a more detailed presentation of the SID determined in Paper I. Dietary contents of CP and AA were compared with nutrient recommendations for adult dogs (NRC, 2006; FEDIAF, 2014; AAFCO, 2016), whereas the digestible CP and AA contents (based on SID) in the diets were compared with the minimal requirement for adult dogs (NRC, 2006).

*Main results*

- The LM diet resulted in lowest ($P < 0.001$) values for N retention, utilization of digested N for retention, BW gain and PER in growing mink, whereas non-significant differences ($P > 0.05$) were found between the PM and FM diets. The values of N retention, BW gain and PER were, however, numerically lower for the PM diet than the FM diet. The observed values for the LM, PM and FM diets, respectively, were as following: retention of N: 0.66, 1.04 and 1.18 g/kg$^{0.75}$/day; BW gain: 8.2, 26.8 and 35.3 g/day; PER: 0.38, 1.39 and 1.71.
- For growing mink, the ATTD of CP and all AA, except for hydroxyproline, differed between diets ($P < 0.001$), and was lowest for the LM diet and highest for the FM diet.

- SID of CP and AA in dogs differed between diets ($P \leq 0.017$) and was lowest for the LM diet and highest for the FM diet. The SID of CP was 71.5, 80.2 and 87.0% for the LM, PM and FM diets, respectively.
- Dietary contents of CP and AA in all diets were above the NRC and the AAFCO recommended levels set for adult dogs, but digestible content of Met + Cys in the LM diet was below the minimal requirement for adult dogs (NRC, 2006).

*Conclusion*

Differences in protein quality between foods of similar protein content clearly affected N retention, BW gain and PER in mink kits. These results imply that growing mink readily respond to limitations in the supply of bioavailable AA from extruded dog foods and suggest that growth studies with mink kits can provide valuable information in protein quality assessment of such foods. Differences in AA composition and digestibility between the protein sources were the main factors affecting protein quality of the experimental diets. Information on these factors is crucial to ensure nutritional adequacy of dog foods and to be able to compare the protein quality between foods.

## 4.3 Paper III

### Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility

The main objective of the study was to evaluate the protein quality of MSC and SPH as ingredients, and as part of extruded dog foods where MSC or SPH partially replaced protein from a rendered PM and provided around 25% of the dietary protein content. Protein quality of the ingredients and the extruded foods was evaluated by analysis of AA composition and determination of ATTD of CP and AA in mink. Six experimental diets were used; three wet diets with PM, MSC or SPH as sole protein sources for determination of ATTD of CP and AA in the protein ingredients, and three extruded dog foods containing the respective protein ingredients. Groups of four mink were fed the experimental diets for determination of ATTD.

*Main results*

- Nutrient composition varied between the protein ingredients. Content of DM was 944.0, 358.0 and 597.4 g/kg, content of CP was 670.7, 421.2 and 868.9 g/kg DM and content of crude fat was 141.4, 547.8 and 18.5 g/kg DM for the PM, MSC and SPH, respectively.
- The total essential AA content in g/100 g CP was more than 10.0 percentage units lower in SPH than in PM and MSC.
- The ingredients differed ($P < 0.001$) with respect to ATTD of CP, which was 80.9, 88.2 and 91.3% for the PM, MSC and SPH, respectively. A non-significant difference ($P > 0.05$) was found for ATTD of total AA between the MSC and SPH, whereas it was lower ($P < 0.001$) for the PM.
- For the extruded diets, a similar ($P > 0.05$) ATTD of CP of 80.3, 81.3 and 79.0% was found for the PM, MSC and SPH diets, respectively. The ATTD of several AA was also similar ($P > 0.05$) between diets. For some AA, ATTD was numerically highest for the PM diet.

*Conclusion*

The MSC and SPH ingredients had a higher ATTD of CP and AA than PM when used in wet, untreated diets. In extruded foods, the expected contribution to a higher ATTD of CP and AA when MSC and SPH partially replaced PM and provided 25% of the dietary CP was not observed. Possibly, extrusion affected ATTD of CP and AA in the diets differently due to differences in properties and previous processing of the protein ingredients. Further studies are warranted to assess the effects of the extrusion process on protein quality of raw animal protein ingredients and animal protein hydrolysates.

# 5. General discussion

## 5.1 Nutrient digestibility

### 5.1.1 Apparent ileal and total tract digestibility of crude protein and amino acids in dogs

The results presented in **Paper I** confirmed that AID is lower than ATTD of CP in dogs, as reviewed by Hendriks et al. (2012). The difference between ATTD and AID of CP is expected to decrease with increasing AID (Hendriks et al., 2012), which was supported by the results in **Paper I**. However, even for the FM diet, with a relatively high AID of CP of 81.8% in average, the difference between ATTD and AID averaged to 6.9 percentage units. This demonstrates that ATTD is an inaccurate measure of protein bioavailability in dogs, also for ingredients or diets of high protein quality.

Equations for estimation of AID of CP and AA from ATTD values were presented in **Paper I**. These regression equations, which were based on the results obtained with the LM, PM and FM diets varying only with respect to protein quality, show that it is possible to predict AID of CP and AA from ATTD. However, the difference between AID and ATTD may be affected by several other factors than protein quality only. For example, the continuous microbial breakdown and synthesis of AA in the large intestine affects the AA composition and the ATTD values (Hendriks and Sritharan, 2002; Hendriks et al., 2013; **Paper I**). Dietary factors, like protein intake (Yamka et al., 2003), fibers (Muir et al., 1996; Silvio et al., 2000; Burkhalter et al., 2001) and starch source (Murray et al., 1999) have been shown to influence AID or ATTD of CP and AA. The practical value of the regression equations in **Paper I** is, therefore, probably limited. As described in chapter 2.2.2.3, however, more extensive equations for the determination of SID of CP and AA, based on fecal excretion of N, have been presented by Hendriks et al. (2015). Although measuring of fecal content of N is a non-invasive and very gentle procedure, it still requires that dogs are applied as experimental units. An alternative option for ileal digestibility determination in dogs could, therefore, be the use of animal models.

### 5.1.2 Mink as a model for estimation of apparent and standardized ileal digestibility of crude protein and amino acids in dogs

Determination of ATTD in adult mink can be used to obtain reliable estimates of AID and SID of CP and AA in dogs (**Paper I**). The experimental diets used in **Paper I** resembled commercial diets, except for containing only one protein ingredient in addition to the protein provided from grains. Usually, commercial diets contain several protein ingredients with complementary AA composition, and vegetable protein sources like soybean meal are commonly included in the diet formulations. Vhile et al. (2005) reported ATTD levels of CP and AA between 85 and 90% for extruded diets containing soybean meal in mink, and ATTD of CP in mink was slightly lower than ATTD in dogs. The latter study, therefore, implies that mink have a high capacity for digestion of vegetable protein sources, and the lower ATTD observed in mink than in dogs corresponds well with the expected lower values of AID than ATTD in dogs. The results of **Paper I** also show that AID in dogs and ATTD in mink of CP and AA are highly correlated for diets of varying protein quality, which strengthens the relevance of the mink as an animal model for estimation of AID and SID of CP and AA in dogs.

As compared with the calculation of AID or SID from ATTD in dogs, the confounding factor of microbial fermentation in the large intestine and its associated effect on AA composition is limited when ATTD is measured in mink. Considering the relevant methods that are available for determination of ileal CP and AA digestibility in dogs, the use of mink is, therefore, a suitable alternative. As reported in **Paper I-III**, protein ingredients and extruded dog foods were palatable and highly accepted by the mink. Furthermore, with mink it is possible to do rapid measurements at a low cost and with few animals, and individual values can be obtained at standardized conditions. Digestibility can in addition be measured by a non-invasive method. For these reasons, it could also be suggested that mink may be a useful *in vivo* model for comparative purposes in the development of relevant *in vitro* digestibility methods applicable for extruded dog foods. Additionally, it is worth mentioning that data on CP and AA digestibility for a number of protein ingredients applied in mink feed can be obtained from Nordic fur animal associations and others, and some of these ingredients are similar to those applied in dog food (Rouvinen-Watt et al., 2005; Kopenhagen Fur, 2016).

### 5.1.3 Digestibility of dry matter, crude fat, starch and carbohydrates in dogs and mink

For dogs, significant differences in AID and ATTD were observed for DM, starch and carbohydrates, and ATTD was higher than AID as a consequence of the microbial fermentation of nutrients in the large intestine (**Paper I**). The difference between AID and ATTD of starch was small and of little practical importance, as a high average AID value of 96.9% was observed. For mink, ATTD of DM was similar to AID in dogs, whereas ATTD of crude fat was lower and ATTD of starch and carbohydrates was higher than the corresponding AID values in dogs (**Paper I**). However, the differences between AID in dogs and ATTD in mink for crude fat and starch, especially, were numerically small, and the results in **Paper I** imply that digestibility measurements with mink could provide reliable information with respect to the AID of main nutrients other than CP in dogs also.

### 5.1.4 Digestibility of individual amino acids as compared with crude protein digestibility

The digestibility of individual AA differs. Some AA have a higher digestibility than CP, whereas others have lower (**Paper I - III**). Digestibility of aspartic acid (Asp) and Cys was especially low in **Paper I - III**. As discussed in **Paper I**, endogenous secretions of AA influence apparent AA digestibility levels. Endogenous secretions in ileum of dogs and feces of mink contain high levels of Thr, glutamic acid (Glu), Asp and Ser (Skrede, 1979; Hendriks et al., 2002b). In line with this, digestibility of Thr and Ser increased the most when SID values in dogs were calculated from AID (**Paper I**). For Glu, however, only a small increase in digestibility was observed when SID was calculated. This was probably caused by the high dietary intake of Glu, leaving the endogenous Glu content to be less influential. As discussed in **Paper I**, an opposite effect would possibly be apparent for Cys, for which endogenous secretions most likely would reduce the AID significantly, due to low dietary contents of Cys. This was supported by the SID values of Cys presented in **Paper II**, which were 8.4, 6.8 and 7.1 percentage units higher than the AID found for the LM, PM and FM diets, respectively (results for AID of Cys in the individual diets not shown in the papers). Despite the corrections for basal endogenous losses, digestibility of Asp and Cys was still low (**Paper I** and **II**), implying that the bioavailability of these AA really was poor as compared with the other AA.

Heat treatment has been shown to markedly reduce Asp and Cys digestibility in FM. (Ljøkjel et al., 2000). It is, therefore, possible that the rendering of most of the protein ingredients used in **Paper I - III** can explain the low digestibility values observed for Asp and Cys. The results of **Paper III** support this, as ATTD of Asp and Cys was 27.0 and 26.5 percentage units higher in MSC than in PM, respectively. Similarly, Cramer et al. (2007) measured true AA digestibility in intact roosters, and found Asp and Cys to be the AA with lowest digestibility in rendered meals. In raw animal by-products, Cys was generally also the AA with lowest digestibility, but the Asp and Cys digestibility values of the raw ingredients were in average 16.2 and 22.5 percentage units higher, respectively, than the average Asp and Cys digestibility values in the rendered meals (Cramer et al., 2007). With respect to Cys, hairs in feces could potentially also contribute to a lowered Cys digestibility since hair protein contain high levels of Cys (Hendriks et al., 1998), but the fecal samples obtained in **Paper I-III** were sifted to remove hairs prior to chemical analyses.

## 5.2 Bioavailability of amino acids

From the results of **Paper I**, it was already known that the AA composition and digestibility varied considerably between the experimental diets applied in **Paper II**. As expected, this difference in protein quality between the extruded diets was reflected in the measures of N balance, BW growth and PER obtained in growing mink (**Paper II**). Methionine was probably the first limiting AA in the diets and responsible for the different growth responses observed. The content of digestible Met was 0.17, 0.26 and 0.33 g/MJ ME in the LM, PM and FM diets, respectively, and for the LM and PM diets, this was lower than the 0.31 g digestible Met/MJ ME recommended in the early growth period of mink (Lassén et al., 2012). The results of **Paper II** imply that the growth response in mink kits is sensitive to limitations in the supply of bioavailable AA from extruded dog foods. However, to obtain a clear growth response, the protein content in the food must be considerably below the recommended level for growing mink of 45% of ME (Lassén et al., 2012). Assays involving growing mink are not suited for routine measurements, as mink has a one-year cycle and 8-11 weeks old kits, like them we applied in our study, are only available during July in the Northern hemisphere. Furthermore, the growth results in mink kits are not directly transferable to dogs, as they have a lower protein and AA requirement than mink kits (NRC, 2006; Lassén et al., 2012). Still, a growing mink assay is an

efficient tool that can be used in comparison of protein quality between extruded dog foods, and it could, for instance, be useful in assessment of novel protein ingredients.

## 5.3 Animal protein ingredients in extruded dog foods

Animal protein ingredients usually provide a considerable amount of the protein in extruded dog foods, but the protein quality of these ingredients is known to vary. The LM, PM, FM, MSC and SPH ingredients applied in **Paper I-III** had an ATTD of CP in adult mink of 67.7, 80.9, 87.5, 88.2 and 91.3%, respectively. A lower protein quality for rendered animal meals than for raw animal by-products has been reported by Cramer et al. (2007). As demonstrated in **Paper I** and **II**, however, the protein quality between rendered meals can vary considerably, mainly because of differences in the raw materials used and processing conditions (Johnson and Parsons, 1997; Johnson et al., 1998; Wang and Parsons, 1998; Shirley and Parsons, 2000; Hendriks et al., 2002a; Cramer et al., 2007). Raw material composition of the LM, PM and FM applied in **Paper I** and **II** was unknown, and the same was also true for processing details of the PM and FM. The protein quality of the LM was especially low. The total AA content of the LM consisted to 37.8% of essential AA and to 62.2% of non-essential AA. Similar numbers were 43.2 and 56.8% for the PM and 48.9 and 51.1% for the FM. The high ash content of 26.7% in the LM was probably a contributing factor to the lower levels of essential AA and higher levels of non-essential AA, and thus, the lower PER values observed for the LM than the PM and FM (Shirley and Parsons, 2001). On the contrary, both Johnson et al. (1998) and Shirley and Parsons (2001) reported that AA digestibility of rendered animal meals was unaffected by ash content. The reason for the low CP and AA digestibility of the LM is uncertain, but processing of the meal, at 133°C at 3.0 bar for 20 minutes, possibly had a negative effect on the digestibility (Johnson et al., 1998; Wang and Parsons, 1998; Shirley and Parsons, 2000).

The FM applied in **Paper I** and **II** was a high-quality FM exposed to a gentle drying process, and the ATTD of CP was similar to the ATTD of the MSC product evaluated in **Paper III**. Although it is possible for rendered animal meals to have a high protein quality (**Paper II**), the use of such products is often negatively conceived by consumers and associated with low nutritional quality (Carter et al., 2014). Rendered animal meals are, therefore, increasingly being replaced with raw meat products in extruded pet food (Buff et al., 2014; Carter et al., 2014). For the MSC diet presented in **Paper III**, MSC constituted 33.1% of the dietary formulation and

would appear first on the ingredient list if the diet was commercially available. The contribution to a higher protein quality in the diet was, however, marginal, as the MSC provided only 25% of the dietary CP content. This implies that the contribution of MSC and similar raw meat products to an increased protein quality in extruded dog foods might be limited, as a high inclusion rate of raw meat ingredients is challenging for the extrusion process (Beaton, 2016). Protein quality in extruded dog foods incorporated with meat ingredients will, therefore, to some extent depend on the protein quality of the other protein ingredients also applied in the food.

As presented in **Paper III**, the ATTD of CP and AA in the SPH ingredient was, as expected, high. However, for both the MSC and SPH ingredients evaluated in **Paper III**, the high ATTD of CP and AA in the ingredients was not reflected in the ATTD of the respective extruded dog foods in which they were incorporated. Especially the SPH diet had lower digestibility levels than expected based on the ATTD of CP and AA in the PM and SPH ingredients. As discussed in **Paper III**, it is possible that the extrusion process had a greater negative effect on ATTD of CP and AA in the untreated MSC and the SPH with high levels of short peptides than the already rendered PM. As the present study did not specifically aim at studying the effects of extrusion on protein digestibility in animal protein ingredients, however, more controlled studies are warranted to examine this further. At least, determination of CP and AA digestibility in the feed mash prior to extrusion would have provided more information. Others have reported that the protein quality of animal protein ingredients can be differently affected during extrusion (Opstvedt et al., 2003; Tran, 2008; van Rooijen, 2015), which points to the importance of controlling protein quality of the final extruded dog food.

## 5.4 Nutritional adequacy of extruded dog foods

As demonstrated in **Paper II,** extruded dog foods with a similar CP content varied significantly with respect to protein quality. In compliance with the "Dog Food Nutrient Profiles" established by the AAFCO (2016), all three diets evaluated in **Paper II** could, based on chemical analyses, be considered as complete and balanced for adult dogs, with respect to the CP and AA contents. The contents of CP and AA in all the experimental diets also met the recommended allowance values for adult dogs set by the NRC (2006), but the digestible content of Met + Cys in the LM diet was below the minimal requirement for adult dogs (NRC, 2006). The results presented in

**Paper II**, therefore, highlighted the inaccuracy of basing nutritional adequacy of dog foods on nutrient content only, which is an option in the todays practice.

From the results of **Paper II,** it was implied that the estimate used in the nutrient recommendations of the NRC (2006) and the AAFCO (2016) for Met + Cys bioavailability is too high, in accordance with the results of Hendriks et al. (2015). The CP and AA contents of the experimental diets were also compared with the minimum recommended levels for adult dogs set by the FEDIAF (2014) (**Paper II**). In contrast to the comparison with recommended levels set by the NRC (2006) and the AAFCO (2016), where Met and Met + Cys content in the LM diet was just sufficient (117 and 104% of the recommended levels, respectively), it was only 96 and 88% of the recommended levels set by the FEDIAF (2014). The variation between the recommended levels used by the different authoritative associations bear evidence of the lack of scientific veracity embedded in these data, as pointed out by Morris and Rogers (1994) and Hendriks et al. (2015). Considering Met, it should be noted that the recommended level set by the FEDIAF (2014) was based on a bioavailability of 66.7%, which was lower than the earlier bioavailability estimate of 84.0% reported as too high by Hendriks et al. (2015). The updated Met bioavailability of 66.7 % seems to be rational (Hendriks et al. 2015; **Paper II**).

The too high bioavailability generally applied by the different authoritative associations (Hendriks et al., 2015) imply that the current recommended levels of most AA should be elevated to secure a reliable safety margin. This would give a better assurance for an adequate AA supply from diets with a low protein quality, like the LM diet applied in **Paper I** and **II**. The results of **Paper II**, however, demonstrated how extruded dog foods with a CP content typical that of commercial diets may provide a surplus of most AA. The digestible contents of individual AA in all three experimental diets, with exceptions for Met and Met + Cys, were twice or more the minimal requirement for adult dogs. One should, however, have in mind that SID and bioavailability of several AA could differ. With respect to Lys, determination of ileal digestibility of reactive Lys is preferred (Moughan, 2003). In **Paper I-III**, only a conventional AA analysis was performed, and the bioavailable Lys content in the experimental diets was, therefore, most likely overestimated (Moughan, 2003). A reactive to total Lys ratio as low as 0.44 has been found in a commercial extruded dog food (Williams et al., 2006). Assuming such a low ratio for the LM diet applied in **Paper II**, and similar SID of reactive as for total Lys, content of digestible reactive Lys in the LM diet would supply 150% of the minimum

bioavailable Lys content required. Content of Lys, and possibly other AA, in the LM, PM and FM diets would, therefore, probably be closer to the minimal requirement level if the real bioavailability was accounted for, but still, it seems that the AA supply would be more than adequate.

Since nutritional adequacy of extruded dog foods can be based solely on chemical content, and since proclamation of nutrient availability is not required on dog food labels, extruded dog foods with high protein contents of low quality can be commercially available. A high supply of protein and AA with a low bioavailability will be of little value for the dogs and will result in excretion of high levels of N from indigestible and metabolically unavailable AA in feces and urine, respectively. However, if declaration of bioavailability of protein and AA in extruded dog foods was required, in addition to the chemical content, a much closer control with the protein quality of the food and a better assurance of nutritional adequacy would have been achieved. Such a practice would probably also promote the use of high-quality protein ingredients in extruded dog foods. In turn, the CP content of the foods could be reduced without jeopardizing a sufficient supply of bioavailable AA, which would be favorable from a sustainability point of view. For example, the CP content of the FM diet applied in **Paper I** and **II** could theoretically be reduced to 16% (as-fed basis) and still supply sufficient amounts of bioavailable Met + Cys (based on SID) to cover the minimal requirement for adult dogs (NRC, 2006).

Several methods can be used for bioavailability estimation. The IAAO method is a promising method, where both digestibility and metabolic utilization of the limiting AA in a diet is taken into account (Elango et al., 2012). However, as discussed by Elango et al. (2012), bioavailability of only one AA can be measured at a time, and several dietary adjustments are necessary to meet the criteria required for the method. For commercial dog foods, determination of SID is, therefore, probably more efficient and relevant, especially as the bioavailability of all individual AA is measured simultaneously. As presented in this thesis, SID determination in dogs is a questionable procedure, but other options are available. In practical terms, determination and labelling of protein and AA bioavailability in extruded dog foods would be advantageous, but also a challenge and a task for the future, as a unison agreement of standardized and validated methods applicable for protein and AA bioavailability determination would be required.

# 6. Concluding remarks and future perspectives

- Apparent total tract digestibility of CP and AA measured with adult mink is a reliable model for estimation of AID and SID of CP and AA in dogs. The use of adult mink for digestibility determination of CP and AA is highly relevant for evaluation and comparison of protein quality between different protein ingredients and extruded dog foods. Furthermore, a growing mink assay is an efficient tool for a more in-depth evaluation of possible limitations in the supply of bioavailable AA from extruded dog foods. The latter assay could be useful for instance in evaluation of novel protein ingredients relevant for use in dog food.
- As *in vivo* digestibility trials with mink yield reliable results in a rapid, gentle and effective manner, they can be a useful basis for comparison in the development of *in vitro* methods applicable for CP and AA digestibility determination of extruded dog foods.
- The protein quality of different rendered animal meals can vary considerably, whereas raw meat products and animal protein hydrolysates, in this thesis represented by MSC and SPH, respectively, generally have a high protein quality.
- Inclusion rate of raw meat products in extruded dog foods might be restricted due to high contents of fat and water in the meat. As a limited amount of CP can be supplied from raw meat, the protein quality of the extruded food will to some extent be dependent on the protein quality of the remaining protein ingredients used in the food.
- Raw meat ingredients and animal protein hydrolysates might be vulnerable for a reduction in protein quality during extrusion of dog foods, but further studies are needed to confirm this hypothesis.
- The variation in protein quality between animal protein ingredients and possible negative effects of processing on protein quality in extruded dog foods emphasizes the importance of evaluating protein quality, at least by means of AA composition and CP and AA digestibility determination, of both ingredients and extruded foods for dogs.
- Assessment of nutritional adequacy of CP and AA for dogs based on nutrient content only is inaccurate. A more accurate assurance of nutritional adequacy in dog foods would be that documentation of protein and AA bioavailability was required in addition to the chemical

content, although approved, standard methods for bioavailability determination then would have to be specified. If data on bioavailability of protein and AA, in addition to AA composition, were included on the pet food label, consumers would be able to compare different foods with respect to protein quality. Increased knowledge of protein quality could contribute to a closer adjustment of dietary protein content in relation to AA requirements, and an oversupply of N could be avoided.

# 7. References

Ahlstrøm, Ø. and A. Skrede. 1998. Comparative nutrient digestibility in dogs, blue foxes, mink and rats. *The Journal of Nutrition* 128: 2676S-2677S.

Aldrich, G. 2006. Rendered products in pet food. In: D. L. Meeker (ed.) Essential Rendering. All About the Animal By-Products Industry. p 159-177. The National Renderers Association, Arlington, Virginia.

Association of American Feed Control Officials (AAFCO). 2016. 2016 Official Publication. AAFCO, Atlanta, GA. p. 689.

Batterham, E. S. 1992. Availability and utilization of amino acids for growing pigs. *Nutrition Research Reviews* 5: 1-18.

Beaton, L. 2016. The challenges of extruding high-meat pet food. http://www.petfoodindustry.com/articles/5690-the-challenges-of-extruding-high-meat-pet-food (Accessed 9 August 2016).

Bednar, G. E., S. M. Murray, A. R. Patil, E. A. Flickinger, N. R. Merchen and G. C. Fahey Jr. 2000. Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of ileally cannulated dogs. *Archives of Animal Nutrition* 53: 127-140.

Björck, I. and N.-G. Asp. 1983. The effects of extrusion cooking on nutritional value - A literature review. *Journal of Food Engineering* 2: 281-308.

Boye, J., R. Wijesinha-Bettoni and B. Burlingame. 2012. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition* 108: S183-S211.

Buff, P. R., R. A. Carter, J. E. Bauer and J. H. Kersey. 2014. Natural pet food: A review of natural diets and their impact on canine and feline physiology. *Journal of Animal Science* 92: 3781-3791.

Burkhalter, T. M., N. R. Merchen, L. L. Bauer, S. M. Murray, A. R. Patil, J. L. Brent Jr and G. C. Fahey Jr. 2001. The ratio of insoluble to soluble fiber components in soybean hulls affects ileal and total-tract nutrient digestibilities and fecal characteristics of dogs. *The Journal of Nutrition* 131: 1978-1985.

Burns, R. A., M. H. LeFaivre and J. A. Milner. 1982. Effects of dietary protein quantity and quality on the growth of dogs and rats. *The Journal of Nutrition* 112: 1843-1853.

Butts, C. A., J. A. Monro and P. J. Moughan. 2012. *In vitro* determination of dietary protein and amino acid digestibility for humans. *British Journal of Nutrition* 108: S282-S287.

Carter, R. A., J. E. Bauer, J. H. Kersey and P. R. Buff. 2014. Awareness and evaluation of natural pet food products in the United States. *Journal of the American Veterinary Medical Association* 245: 1241-1248.

Cramer, K. R., M. W. Greenwood, J. S. Moritz, R. S. Beyer and C. M. Parsons. 2007. Protein quality of various raw and rendered by-product meals commonly incorporated into companion animal diets. *Journal of Animal Science* 85: 3285-3293.

Deng, P. and K. S. Swanson. 2015. Companion animals symposium: Future aspects and perceptions of companion animal nutrition and sustainability. *Journal of Animal Science* 93: 823-834.

Donkoh, A., P. J. Moughan and W. C. Smith. 1994. Comparison of the slaughter method and simple T-piece cannulation of the terminal ileum for determining ileal amino acid digestibility in meat and bone meal for the growing pig. *Animal Feed Science and Technology* 49: 43-56.

Dust, J. M., C. M. Grieshop, C. M. Parsons, L. K. Karr-Lilienthal, C. S. Schasteen, J. D. Quigley III, N. R. Merchen and G. C. Fahey Jr. 2005. Chemical composition, protein quality, palatability, and digestibility of alternative protein sources for dogs. *Journal of Animal Science* 83: 2414-2422.

Elango, R., C. Levesque, R. O. Ball and P. B. Pencharz. 2012. Available versus digestible amino acids - new stable isotope methods. *British Journal of Nutrition* 108: S306-S314.

European Commission. 2016. Animal by-products. http://ec.europa.eu/food/safety/animal-by-products/index_en.htm (Accessed 8 August 2016).

European Pet Food Industry Federation (FEDIAF). 2011. Code of good labelling practice for pet food. http://www.fediaf.org/self-regulation/labelling/ (Accessed 9 August 2016).

European Pet Food Industry Federation (FEDIAF). 2014. Nutritional guidelines for complete and complementary pet food for cats and dogs. FEDIAF, Brussels, Belgium. p. 99.

Faber, T. A., P. J. Bechtel, D. C. Hernot, C. M. Parsons, K. S. Swanson, S. Smiley and G. C. Fahey Jr. 2010. Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays. *Journal of Animal Science* 88: 1421-1432.

Folador, J. F., L. K. Karr-Lilienthal, C. M. Parsons, L. L. Bauer, P. L. Utterback, C. S. Schasteen, P. J. Bechtel and G. C. Fahey Jr. 2006. Fish meals, fish components, and fish protein hydrolysates as potential ingredients in pet foods. *Journal of Animal Science* 84: 2752-2765.

Food and Agriculture Organization of the United Nations. 2009. How to feed the world in 2050. http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf (Accessed 8 August 2016).

Fuller, M. F. and D. Tomé. 2005. In vivo determination of amino acid bioavailability in humans and model animals. *Journal of AOAC International* 88: 923-934.

Gilbert, E. R., E. A. Wong and K. E. Webb Jr. 2008. Board-invited review: Peptide absorption and utilization: Implications for animal nutrition and health. *Journal of Animal Science* 86: 2135-2155.

Hegedűs, M., S. Fekete, L. Solti, E. Andrásofszky and L. Pallós. 1998. Assessment of nutritional adequacy of the protein in dog foods by trials on growing rats. *Acta Veterinaria Hungarica* 46: 61-70.

Hendriks, W. H., E. J. Bakker and G. Bosch. 2015. Protein and amino acid bioavailability estimates for canine foods. *Journal of Animal Science* 93: 4788-4795.

Hendriks, W. H., C. A. Butts, D. V. Thomas, K. A. C. James, P. C. A. Morel and M. W. A. Verstegen. 2002a. Nutritional quality and variation of meat and bone meal. *Asian-Australasian Journal of Animal Sciences* 15: 1507-1516.

Hendriks, W. H. and K. Sritharan. 2002. Apparent ileal and fecal digestibility of dietary protein is different in dogs. *The Journal of Nutrition* 132: 1692S-1694S.

Hendriks, W. H., K. Sritharan and S. M. Hodgkinson. 2002b. Comparison of the endogenous ileal and faecal amino acid excretion in the dog (*Canis familiaris*) and the rat (*Rattus rattus*) determined under protein-free feeding and peptide alimentation. *Journal of Animal Physiology and Animal Nutrition* 86: 333-341.

Hendriks, W. H., M. F. Tarttelin and P. J. Moughan. 1998. The amino acid composition of cat (*Felis catus*) hair. *Animal Science* 67: 165-170.

Hendriks, W. H., D. G. Thomas, G. Bosch and G. C. Fahey Jr. 2013. Comparison of ileal and total tract nutrient digestibility of dry dog foods. *Journal of Animal Science* 91: 3807-3814.

Hendriks, W. H., J. van Baal and G. Bosch. 2012. Ileal and faecal protein digestibility measurement in humans and other non-ruminants - a comparative species view. *British Journal of Nutrition* 108: S247-S257.

Hernot, D. C., H. J. Dumon, V. C. Biourge, L. J. Martin and P. G. Nguyen. 2006. Evaluation of association between body size and large intestinal transit time in healthy dogs. *American Journal of Veterinary Research* 67: 342-347.

Hervera, M., M. D. Baucells, G. González, E. Pérez and C. Castrillo. 2009. Prediction of digestible protein content of dry extruded dog foods: comparison of methods. *Journal of Animal Physiology and Animal Nutrition* 93: 366-372.

Hill, R. C., G. W. Ellison, C. F. Burrows, J. E. Bauer and B. Carbia. 1996. Ileal cannulation and associated complications in dogs. *Laboratory Animal Science* 46: 77-80.

Huber, T. L., D. P. Laflamme, L. Medleau, K. M. Comer and P. M. Rakich. 1991. Comparison of procedures for assessing adequacy of dog foods. *Journal of the American Veterinary Medical Association* 199: 731-734.

Huber, T. L., R. C. Wilson and S. A. McGarity. 1986. Variations in digestibility of dry dog foods with identical label guaranteed analysis. *Journal of the American Animal Hospital Association* 22: 571-575.

Johnson, M. L. and C. M. Parsons. 1997. Effects of raw material source, ash content, and assay length on protein efficiency ratio and net protein ratio values for animal protein meals. *Poultry Science* 76: 1722-1727.

Johnson, M. L., C. M. Parsons, G. C. Fahey Jr., N. R. Merchen and C. G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. *Journal of Animal Science* 76: 1112-1122.

Karr-Lilienthal, L. K., C. M. Grieshop, J. K. Spears, A. R. Patil, G. L. Czarnecki-Maulden, N. R. Merchen and G. C. Fahey Jr. 2004. Estimation of the proportion of bacterial nitrogen in canine feces using diaminopimelic acid as an internal bacterial marker. *Journal of Animal Science* 82: 1707-1712.

Kopenhagen Fur. 2016. Fordøjelighedsforsøg. http://www.kopenhagenfur.com/da/minkavl/fagligt/fordoejelighedsforsoeg (Accessed 22 August 2016).

Krogdahl, Å., Ø. Ahlstrøm and A. Skrede. 2004. Nutrient digestibility of commercial dog foods using mink as a model. *The Journal of Nutrition* 134: 2141S-2144S.

Lassén, T. M., A.-H. Tauson, Ø. Ahlstrøm, K. Rouvinen-Watt, P. Sandbøl, N. Koskinen and M. L. E. Christiansen. 2012. Energy and main nutrients in feed for mink and foxes. 2nd ed. In: A. Skrede (ed.) Nordic Association of Agricultural Scientists, Subsection for Fur Animals, Report No. 63, SL Grafik, Copenhagen, Denmark. p. 98.

Ljøkjel, K., O. M. Harstad and A. Skrede. 2000. Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows. *Animal Feed Science and Technology* 84: 83-95.

Markets and Markets. 2015. Pet food ingredients market by source (animal derivatives, plant derivatives & additives), form (wet & dry), animal (dog, cat, bird, fish & others), & by region - global trends and forecasts to 2020. http://www.researchandmarkets.com/research/8rp5cz/pet_food (Accessed 19 August 2016).

Martínez-Alvarez, O., S. Chamorro and A. Brenes. 2015. Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review. *Food Research International* 73: 204-212.

McDonald, P., R. A. Edwards, J. F. D. Greenhalgh and C. A. Morgan. 2002. Animal Nutrition. 6th ed. Prentice Hall, Harlow, England. p. 693.

Meeker, D. L. and C. R. Hamilton. 2006. An overview of the rendering industry. In: D. L. Meeker (ed.) Essential Rendering. All About the Animal By-Products Industry. p 1-16. The National Renderers Association, Arlington, Virginia.

Meeker, D. L. and J. L. Meisinger. 2015. Companion animals symposium: Rendered ingredients significantly influence sustainability, quality, and safety of pet food. *Journal of Animal Science* 93: 835-847.

Morris, J. G. and Q. R. Rogers. 1994. Assessment of the nutritional adequacy of pet foods through the life cycle. *The Journal of Nutrition* 124: 2520S-2534S.

Moughan, P. J. 2003. Amino acid availability: aspects of chemical analysis and bioassay methodology. *Nutrition Research Reviews* 16: 127-141.

Moughan, P. J. and W. C. Smith. 1987. A note on the effect of cannulation of the terminal ileum of the growing pig on the apparent ileal digestibility of amino acids in ground barley. *Animal Production* 44: 319-321.

Muir, H. E., S. M. Murray, G. C. Fahey Jr., N. R. Merchen and G. A. Reinhart. 1996. Nutrient digestion by ileal cannulated dogs as affected by dietary fibers with various fermentation characteristics. *Journal of Animal Science* 74: 1641-1648.

Murray, S. M., G. C. Fahey Jr., N. R. Merchen, G. D. Sunvold and G. A. Reinhart. 1999. Evaluation of selected high-starch flours as ingredients in canine diets. *Journal of Animal Science* 77: 2180-2186.

Murray, S. M., A. R. Patil, G. C. Fahey Jr., N. R. Merchen and D. M. Hughes. 1997. Raw and rendered animal by-products as ingredients in dog diets. *Journal of Animal Science* 75: 2497-2505.

National Research Council (NRC). 2006. Nutrient Requirements of Dogs and Cats. The National Academies Press, Washington, DC. p. 398.

Norwegian Food Safety Authority. 2010. Feed statistics 2010. http://www.mattilsynet.no/dyr_og_dyrehold/for/forstatistikk_2010.7329/binary/F%C3%B4rstatistikk%202010 (Accessed 19 August 2016).

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. *Canadian Journal of Animal Science* 77: 149-163.

Opstvedt, J., E. Nygård, T. A. Samuelsen, G. Venturini, U. Luzzana and H. Mundheim. 2003. Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed. *Journal of the Science of Food and Agriculture* 83: 775-782.

Papadopoulos, M. C. 1989. Effect of processing on high-protein feedstuffs: A review. *Biological Wastes* 29: 123-138.

Pedersen, C., E. Vinyeta and R. Gerritsen. 2010. Comparison of the slaughtering and the T-cannulation methods on ileal digestibility of nutrients in pigs. In: G. Matteo Crovetto (ed.). 3rd EAAP International Symposium on Energy and Protein Metabolism and Nutrition, EAAP publication No. 127, Parma, Italy. p. 411-412.

Phillips-Donaldson, D. 2016. Global pet food trends: sales and volume rose 4 % in 2015. http://www.petfoodindustry.com/blogs/7-adventures-in-pet-food/post/5609-global-pet-food-trends-sales-and-volume-rose-4-in-2015 (Accessed 19 August 2016).

Robinson, J. W. L., A.-L. Luisier and V. Mirkovitch. 1973. Transport of amino-acids and sugars by the dog colonic mucosa. *Pflügers Archiv-European Journal of Physiology* 345: 317-326.

Rouvinen-Watt, K., M. B. White and R. Campbell. 2005. Mink Feeds and Feeding. Ontario Ministry of Agriculture and Food, through the Agricultural Research Institute of Ontario, and the Nova Scotia Agricultural College, Truro, Canada. p. 182.

Shirley, R. B. and C. M. Parsons. 2000. Effect of pressure processing on amino acid digestibility of meat and bone meal for poultry. *Poultry Science* 79: 1775-1781.

Shirley, R. B. and C. M. Parsons. 2001. Effect of ash content on protein quality of meat and bone meal. *Poultry Science* 80: 626-632.

Silvio, J., D. L. Harmon, K. L. Gross and K. R. McLeod. 2000. Influence of fiber fermentability on nutrient digestion in the dog. *Nutrition* 16: 289-295.

Skrede, A. 1979. Utilization of fish and animal byproducts in mink nutrition. IV. Fecal excretion and digestibility of nitrogen and amino acids by mink fed cod (*Gadus morrhua*) fillet or meat-and-bone meal. *Acta Agriculturae Scandinavica* 29: 241-257.

Smeets-Peeters, M. J. E., M. Minekus, R. Havenaar, G. Schaafsma and M. W. A. Verstegen. 1999. Description of a dynamic *in vitro* model of the dog gastrointestinal tract and an evaluation of various transit times for protein and calcium. *Atla-Alternatives to Laboratory Animals* 27: 935-949.

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández and C. F. M. de Lange. 2007a. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. *Livestock Science* 109: 282-285.

Stein, H. H., C. Pedersen, A. R. Wirt and R. A. Bohlke. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. *Journal of Animal Science* 83: 2387-2395.

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan and C. F. M. de Lange. 2007b. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. *Journal of Animal Science* 85: 172-180.

Sundling, L., Ø. Ahlstrøm and A.-H. Tauson. 2012. Yttrium oxide as an inert marker for nutrient digestibility in ferrets (*Mustela putoris furo*), mink (*Neovison vison*) and cats (*Felis catus*). In: P. F. Larsen et al. (eds.). Proceedings of the Xth International Scientific Congress in fur animal production, Copenhagen, Denmark. p. 91-96.

Swanson, K. S., R. A. Carter, T. P. Yount, J. Aretz and P. R. Buff. 2013. Nutritional sustainability of pet foods. *Advances in Nutrition: An International Review Journal* 4: 141-150.

Szymeczko, R. and A. Skrede. 1990. Protein digestion in mink. *Acta Agriculturae Scandinavica* 40: 189-200.

Tonglet, C., I. Jeusette, L. Istasse and M. Diez. 2001. Prediction of protein digestibility in dog food by a multi-enzymatic method: a useful technique to develop. *Journal of Animal Physiology and Animal Nutrition* 85: 189-194.

Tran, Q. D. 2008. Extrusion processing: effects on dry canine diets. Ph.D. Thesis, Wageningen University, Wageningen, Netherlands.

van Rooijen, C. 2015. Maillard reaction products in pet foods. Ph.D. Thesis, Wageningen University, Wageningen, Netherlands.

Verlinden, A., M. Hesta, J. M. Hermans and G. P. J. Janssens. 2006. The effects of inulin supplementation of diets with or without hydrolysed protein sources on digestibility, faecal characteristics, haematology and immunoglobulins in dogs. *British Journal of Nutrition* 96: 936-944.

Vhile, S. G. 2007. Nutrient digestibility in dogs (*Canis familiaris)* and model carnivore species. Comparative studies on protein and amino acid digestibility in dogs, blue foxes (*Alopex lagopus*) and mink (*Mustela vison*) fed various protein sources using different determination methods. Ph.D. Thesis, Norwegian University of Life Sciences, Ås, Norway.

Vhile, S. G., A. Skrede, Ø. Ahlstrøm and K. Hove. 2005. Comparative apparent total tract digestibility of major nutrients and amino acids in dogs (*Canis familiaris*), blue foxes (*Alopex lagopus*) and mink (*Mustela vison*). *Animal Science* 81: 141-148.

Vhile, S. G., A. Skrede, Ø. Ahlstrøm and K. Hove. 2007. Yttrium oxide ($Y_2O_3$) as an inert marker in digestibility studies with dogs, blue foxes and mink fed diets containing different protein sources. *Journal of Animal Physiology and Animal Nutrition* 91: 381-389.

Wang, X. and C. M. Parsons. 1998. Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. *Poultry Science* 77: 834-841.

Williams, P. A., S. M. Hodgkinson, S. M. Rutherfurd and W. H. Hendriks. 2006. Lysine content in canine diets can be severely heat damaged. *The Journal of Nutrition* 136: 1998S-2000S.

Yamka, R. M., U. Jamikorn, A. D. True and D. L. Harmon. 2003. Evaluation of low-ash poultry meal as a protein source in canine foods. *Journal of Animal Science* 81: 2279-2284.

Zinn, K. E., D. C. Hernot, N. D. Fastinger, L. K. Karr-Lilienthal, P. J. Bechtel, K. S. Swanson and G. C. Fahey Jr. 2009. Fish protein substrates can substitute effectively for poultry by-product meal when incorporated in high-quality senior dog diets. *Journal of Animal Physiology and Animal Nutrition* 93: 447-455.

# Paper I

**Ileal, colonic and total tract nutrient digestibility in dogs (*Canis familiaris*) compared with total tract digestibility in mink (*Neovison vison*)**

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# Ileal, colonic and total tract nutrient digestibility in dogs (*Canis familiaris*) compared with total tract digestibility in mink (*Neovison vison*)

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Mink (*Neovison vison*) was studied as a model for the determination of ileal crude protein (CP) and amino acid (AA) digestibility in dogs (*Canis familiaris*). Apparent ileal digestibility (AID) and apparent colonic digestibility (ACD) in dogs and apparent total tract digestibility (ATTD) in dogs and mink were measured for dry matter (DM), main nutrients and AA. Standardised ileal digestibility (SID) of CP and AA in dogs was calculated. Twelve dogs and 12 mink divided into three groups were fed one out of three diets differing in CP digestibility. In dogs, AID of CP was lower (74.4%) than ATTD (83.5%) ($p < 0.001$). The ATTD of CP in mink (77.8%) did not differ from AID, ACD (78.5%) and SID (79.6%) in dogs. Digestibility of AA followed the same pattern, and, except for Thr and Ser, ATTD in mink was very close to SID in dogs. Also, AID was close to ATTD in mink for several AA. High correlations were found between methods for digestibility of CP and most AA ($p < 0.01$) and for AA ranking with respect to digestibility level ($p < 0.001$). In dogs, ether extract digestibility was approximately 96% at all sites, while DM, starch and total carbohydrate digestibility increased from ileal to faecal level ($p < 0.01$). Mink ATTD of DM and main nutrients was closest to ACD in dogs. It was concluded that mink is a suitable model for the determination of AID and SID of CP and AA in dogs.

**Keywords:** amino acids; comparisons; digestibility; dogs; methodology; mink

## 1. Introduction

Amino acid (AA) digestibility is an important criterion in dietary protein evaluation. Since the digestion and absorption of AA mainly take place in the small intestine, ileal digestibility values are considered more reliable than total tract values that are based on faecal AA content. Faecal contents of AA are influenced by microbial breakdown and transformation in the large intestine and will therefore not give a true picture of absorption. Nevertheless, apparent total tract digestibility (ATTD) has been the usual measure of nutrient digestibility in dogs. ATTD measurements in dogs overestimate apparent ileal digestibility (AID) of crude protein (CP) (Hendriks et al. 2012), while both overestimation and underestimation have been detected for different AA (Hendriks and Sritharan 2002; Hendriks et al. 2013). Furthermore, the difference between AID and ATTD is not constant, and it appears to decrease with increasing CP and AA digestibility of the diet (Hendriks et al. 2012). Estimation of AID from ATTD values is therefore an uncertain procedure, and the most accurate and preferred method to use when estimating the availability of CP and AA in dog foods is to measure AID. However, AID measurements are complicated, expensive and invasive. The cannulation method has been

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used in several experiments studying CP and AA digestibility of different protein sources and dry dog foods (Murray et al. 1997; Johnson et al. 1998; Bednar et al. 2000; Hendriks et al. 2013). Another approach is to dissect the end of ileum in euthanised animals (Hendriks and Sritharan 2002). Both of these methods are ethically questionable and not suitable for routine application. Clearly, no appropriate method for routine AID measurements in dogs exists.

Several species have been studied as potential model animals for dog digestibility. However, the number of studies concerning model animals for the determination of AID of CP and AA in dogs is scarce. One exception is a study by Johnson et al. (1998), where a high correlation was found between true AA digestibility in caecectomised roosters and AID of AA in dogs. It would, however, be preferable if the model animals could be used without methods involving surgery. In that respect, the mink (*Neovison vison*) is an interesting candidate and a relevant model animal. Mink has a short digestive tract, lacks caecum and has minimal microbial activity in the large intestine (Skrede 1979; Szymeczko and Skrede 1990). Thus, the total digestive tract of mink resembles the small intestine of dogs. The potential of mink as a suitable model animal for AID of CP and AA in dogs has been hypothesised (Vhile 2007). However, no published data exist to confirm this hypothesis, as comparative digestibility studies with dogs and mink have included measurements of ATTD only (Ahlstrøm and Skrede 1998; Vhile et al. 2005). A high correlation between ATTD of CP in mink and dogs has been found, although mink ATTD was lower than that of dogs (Ahlstrøm and Skrede 1998). The lower ATTD of CP in mink than in dogs was confirmed in the study by Vhile et al. (2005), but in contrast to the findings by Ahlstrøm and Skrede (1998), it did not reveal any significant correlation between the two species regarding CP digestibility. ATTD of several essential and some non-essential AA was, however, significantly correlated in dogs and mink, and for some AA, ATTD in dogs was lower than in mink (Vhile et al. 2005). Since ATTD values in dogs overestimate AID of CP, while AA have been both over- and underestimated, these results suggest that mink may be a suitable model animal for AID of CP and AA in dogs. This can be further supported by results demonstrating ATTD in mink to be very similar to AID in pigs for CP and AA (Skrede et al. 1998).

Digestibility values for CP and AA can be given as apparent, standardised or true values, according to the correction made for the endogenous part of the digesta or faeces collected (Stein, Fuller et al. 2007; Stein, Sève et al. 2007). Standardised digestibility values are apparent digestibility (AD) values corrected for the basal endogenous losses of protein or AA, and standardised ileal digestibility (SID) values are preferred to apparent or true ileal digestibility values in diet formulation for pigs (Stein, Fuller et al. 2007; Stein, Sève et al. 2007). In dogs, SID of CP and AA has been reported (Hendriks et al. 2013), and because SID is a more precise measure in protein evaluation, it is likely that it will be applied to higher degree in dog diet formulation in the future.

The main objective of the present study was to test the hypothesis that the mink is a suitable model for AID in dogs. In addition to AID measured in dogs, SID was calculated and compared with ATTD in mink. Also, the apparent colonic digestibility (ACD) and ATTD in dogs were determined in order to study the level of CP and AA degradation taking place in the hindgut.

## 2. Material and methods

### *2.1. Diets*

Three experimental dry diets with known and different levels of ATTD of CP (low, medium and high) were produced by extrusion at Centre for Feed Technology,

Norwegian University of Life Sciences, Ås, Norway (Table 1). The diets were formulated to contain equal amounts of CP and crude fat (ether extract, EE). Lamb meal was used as the main protein source in the food with low CP digestibility (LM diet), whereas poultry meal (PM diet) and fish meal (FM diet) were the main protein sources in the foods with medium and high CP digestibility, respectively. Selection of the protein meals used in the diets was based on earlier digestibility studies with mink, where ATTD of CP was found to be 67.7%, 80.9% and 87.5% for lamb meal, poultry meal and fish meal, respectively. Chemical composition of the protein meals is presented in Table 2. Yttrium oxide was added to the diets as a marker for digestibility determination (Vhile et al. 2007).

### 2.2. *Production of experimental diets*

Dry ingredients were mixed in a Tatham Forberg twin-shaft mixer (1992 OB-1078, 400 l, Rochdale, UK). Prior to mixing, yttrium oxide was hand-mixed into a small sample of the batch, ensuring a homogeneous distribution of yttrium oxide in the foods. The mixed ingredients were conditioned in a Bühler two-stage preconditioner (BCTC-10, Uzwil, Switzerland) and extruded in a Bühler twin-screw extruder (BCTG 62/20 D, Uzwil, Switzerland) with an 8-mm die. The extrudates were pre-dried in a Bühler fluid-bed dryer (OTW 50 05TSR2, Uzwil, Switzerland). Drying was completed in rectangular batch drying cabinets (of about 0.3 $m^2$, holding up to 40 kg), mounted with 10-kW heated fans. Poultry fat was added to the extrudates in a Dinnissen (Sevenum, Holland) vacuum coater. After fat addition, the food was packed in airtight bags and frozen-stored until use.

Table 1. Diet formulation [g/kg].

| | Diet | | |
|---|---|---|---|
| | Lamb meal | Poultry meal | Fish meal |
| Lamb meal* | 344.9 | | |
| Poultry meal† | | 291.1 | |
| Fish meal‡ | | | 268.8 |
| Poultry fat | 165.3 | 164.9 | 178.6 |
| Wheat | 315.1 | 349.9 | 355.5 |
| Corn | 90.0 | 100.0 | 101.6 |
| Rice flour | 27.0 | 30.0 | 30.5 |
| Beet pulp | 9.0 | 10.0 | 10.2 |
| Salmon oil | 13.5 | 15.0 | 15.2 |
| Limestone meal | 6.9 | 7.7 | 7.8 |
| Monocalcium phosphate | 9.5 | 10.5 | 10.7 |
| Sodium chloride | 6.3 | 7.0 | 7.1 |
| Betaine | 1.3 | 1.4 | 1.5 |
| Vitamin E# | 1.9 | 2.1 | 2.1 |
| Mineral premix‖ | 2.1 | 2.3 | 2.3 |
| Vitamin premix§ | 7.1 | 7.9 | 8.0 |
| Yttrium oxide | 0.1 | 0.1 | 0.1 |

Notes: *Lamb meal, Norsk Protein AS, Ingeberg, Norway; †Poultry meal, Low Ash, GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG, Diepholz, Germany; ‡Fish meal, Norse-LT 94, Norsildmel AS, Bergen, Norway; #Normin AS, Hønefoss, Norway, 100,000 mg vitamin E per kg; ‖Normin AS, Hønefoss, Norway. Containing per kg: 11 g Cu, 115 g Zn, 35 g Mn, 1.5 g I, 100 g Fe; §Normin AS, Hønefoss, Norway. Containing per kg: 1376 mg vitamin A, 10 mg vitamin $D_3$, 100,000 mg vitamin E, 12,000 mg thiamine, 24,000 mg riboflavin, 150,000 mg niacin, 60,000 mg pantothenic acid, 30,000 mg vitamin $B_6$, 64 mg vitamin $B_{12}$, 4000 mg folic acid, 1500 mg biotin.

Table 2. Analysed chemical composition of protein meals used in the experimental diets [g/kg].

| | Protein meals | | |
|---|---|---|---|
| | Lamb meal | Poultry meal | Fish meal |
| 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 |
| Hyp | 24.4 | 20.0 | 3.6 |
| Pro | 41.4 | 40.2 | 28.6 |
| Ser | 26.0 | 30.2 | 30.3 |
| Tyr | 11.9 | 19.0 | 20.8 |

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

### *2.3. Animals*

The experimental procedures were approved by the Norwegian Animal Research Authority and were performed in accordance with institutional and national guidelines for the care and use of animals (the Norwegian Animal Welfare Act and the Norwegian Regulation on Animal Experimentation).

The digestibility experiments in dogs were carried out at a sled dog kennel at Harestua, Oppland, Norway. Twelve privately owned dogs (*Canis familiaris*) of the mixed breed Alaskan Husky, including seven males and five females, were used in the experiment. The age of the dogs varied from 1.5 to 13 years, with an average of 7 years. Body weight (BW) was on average 23.6 ± 1.8 kg for the males and 20.3 ± 3.0 kg for the females. The experimental dogs were all healthy, and most of them were former performance sled dogs, but for different reasons, their owners had decided to euthanise them. The dogs were divided into three groups of four animals balanced as good as possible for age and sex. Each group received one of the experimental diets. During the experimental period, dogs were housed outdoors in separate dog houses placed in rows. The dogs were tied to their houses with a leash of 4 m, and they were out of reach of food other than their own. Food was offered once a day, in amounts adjusted to cover the maintenance energy requirement (525 kJ ME/kg $BW^{0.75}$ per day, Burger 1994). The dogs had free access to

drinking water. The experimental period lasted for 10 days. Faeces from each dog were sampled on day 7 and frozen-stored (−20°C). On day 10, the dogs were fed at different times that corresponded with the time of euthanisation which was accomplished 4 h after the last meal. The dogs were euthanised one by one within a 30-min interval by a veterinarian inside a building at the kennel. The dogs were sedated with xylazine (Narcoxyl Vet, Merck/MSD Animal Health, Summit, NJ, USA, 1 mg/kg BW) prior to euthanisation with pentobarbital (Mebumal, 100 mg/kg BW). The intestine of the dogs was dissected out shortly after the dogs were put to sleep, and intestinal content was sampled from the last part of ileum and from colon, respectively. Intestinal content was immediately put in plastic containers and frozen in liquid nitrogen, before being frozen-stored (−20°C). Faeces and intestinal content were freeze-dried and ground prior to chemical analyses. To avoid contamination with hair, the samples were sifted after grinding.

The digestibility experiment in mink was carried out in a laboratory at the research farm at Norwegian University of Life Sciences, Ås, Norway. Twelve adult male mink (*Neovison vison*), 2 years of age and with a BW of 2.1 ± 0.2 kg, were used in the experiment. As for the dogs, the mink were divided into three groups of four animals, one group for each of the experimental diets. During the experiment, the animals were kept in metabolic cages equipped for total collection of faeces, feed residuals and separation of urine. The experimental period lasted for 7 d, including a 3-d adaption period followed by a 4-d period with feed intake registrations and collection of faeces that was frozen-stored (−20°C). To make the dog food more convenient to eat and more palatable, the food pellets were added water to obtain a food:water ratio of 1:3 and mixed to a porridge-like consistency. The mink were fed once a day in order to meet their daily maintenance energy requirement, approximately 530 kJ ME/kg $BW^{0.75}$ (Chwalibog et al. 1980), and had free access to drinking water. At the end of the experimental period, faeces from each animal was freeze-dried, ground and sifted, before chemical analyses.

### *2.4. Chemical analyses*

Diets and freeze-dried intestinal content and faeces were analysed for dry matter (DM) (ISO 6496 1999) and ash (ISO 5984 2002). CP was determined as Kjeldahl-N · 6.25 (AOAC International 2002, method 2001.11), and EE was determined after extraction with petroleum ether and acetone in an Accelerated Solvent Extractor (ASE 200) from Dionex (Sunnyvale, CA, USA). Starch was analysed according to the method described by McCleary et al. (1994). Content of total carbohydrates (CHO) was calculated by difference:

$$\mathrm{CHO} = \mathrm{DM} - (\mathrm{CP} + \mathrm{EE} + \mathrm{ash}).$$

AA were analysed according to ISO 13903 (2005) (not Trp). For the determination of yttrium, samples were digested with concentrated ultrapure $HNO_3$ at 250°C using a Milestone microwave UltraClave III (Milestone Srl, Sorisole, Italy). Samples were then diluted (to 10% $HNO_3$ concentration), and yttrium was analysed by inductively coupled plasma optical emission spectrometry (ICP-OES analysis) with a PerkinElmer Optima 5300 DV (PerkinElmer Inc., Shelton, CT, USA).

### *2.5. Calculations*

AD values were calculated based on the concentration of nutrients and yttrium in food and faeces or intestinal content using the following the equation:

$$\text{AD } [\%] = \frac{\left\{\left(\frac{\text{Con}_{\text{nutr}} \text{ in food}}{\text{Con}_{\text{Y}} \text{ in food}}\right) - \left(\frac{\text{Con}_{\text{nutr}} \text{ in faeces or intestinal content}}{\text{Con}_{\text{Y}} \text{ in faeces or intestinal content}}\right)\right\}}{\left(\frac{\text{Con}_{\text{nutr}} \text{ in food}}{\text{Con}_{\text{Y}} \text{ in food}}\right)} \cdot 100\%$$

where $\text{Con}_{\text{nutr}}$ is the concentration of nutrient and $\text{Con}_{\text{Y}}$ is the concentration of yttrium.

SID of CP and AA in dogs was calculated as follows (Stein, Sève et al. 2007):

$$\text{SID } [\%] = \text{AID } [\%] + \left(\frac{\text{BL of nutrient [g/kg DM intake]}}{\text{Con}_{\text{nutr}} \text{ in food [g/kg DM]}}\right) \cdot 100\%$$

where BL is the basal ileal endogenous loss and $\text{Con}_{\text{nutr}}$ is the concentration of nutrient.

The applied estimates of ileal-basal endogenous losses of protein and AA in dogs were from Hendriks et al. (2002), as determined in dogs fed a protein-free diet.

### *2.6. Statistical analyses*

Data were analysed by the use of the SAS 9.3 computer software (SAS Institute Inc., Cary, NC, USA). The general linear model procedure was used for the conduction of analysis of variance (ANOVA). The effect of diets (LM, PM and FM) and the method for the determination of digestibility (AID, ACD, ATTD and SID in dogs and ATTD in mink) on nutrient digestibilities were tested by two-way ANOVA using the following equation:

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk},$$

where $\mu$ is the general mean, $\tau_i$ is the fixed effect of diet, $\beta_j$ is the fixed effect of method, $(\tau\beta)_{ij}$ is the effect of interaction between $\tau_i$ and $\beta_j$ and $\varepsilon_{ijk}$ is the random error component. Within diet, effect of method on CP and AA digestibility was tested by one-way ANOVA using the equation:

$$Y_{jk} = \mu + \beta_j + \varepsilon_{jk},$$

where $\mu$ is the general mean, $\beta_j$ is the fixed effect of method and $\varepsilon_{jk}$ is the random error component. The results were expressed as least-square means, with the variance presented as pooled standard error of the means (SEM) or as means with standard deviation. Significant ($p < 0.05$) differences between means were ranked by Tukey's multiple comparison tests. Pearson correlation coefficients were used to express the covariance in CP and AA digestibilities between methods. In addition, covariance for the ranking of AA with respect to digestibility level was analysed and expressed as Pearson correlation coefficients between methods. The linear relationships between AID of CP and AA in dogs and ATTD in mink and between AID and ATTD in dogs were found by using the regression procedure and were presented by regression equations.

## 3. Results

### *3.1. Diets*

The diets were well accepted and readily consumed by the dogs. Feed consumption in mink averaged to 99%, except for one animal eating about 80% of the feed given.

Table 3. Analysed chemical composition of experimental diets [g/kg].

| | Diet | | |
|---|---|---|---|
| | Lamb meal | Poultry meal | Fish meal |
| 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 |
| 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 essential amino acids | 94.3 | 105.1 | 109.9 |
| Total non-essential amino acids | 161.5 | 145.1 | 134.5 |
| Total amino acids | 255.8 | 250.2 | 244.4 |

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

Chemical analyses showed that the contents of main nutrients were similar for the three diets, except for an expected higher ash content in the LM diet (Table 3). The total content of essential AA was lowest in the LM diet and highest in the FM diet. Especially the contents of Ile, Leu, Lys and Met were low in the LM diet. For the non-essential AA, differences were most pronounced for Gly, Hyp and Pro, the contents of which were highest in the LM diet and lowest in the FM diet.

### *3.2. Nutrient digestibility*

Digestibility of DM, main nutrients and AA is presented in Table 4. One animal receiving the FM diet was excluded from the calculation of ileal DM, EE and CHO digestibility because the sample of ileal effluent was too small to allow for the DM and fat analyses.

As expected, the different protein sources used in the diets had a significant effect on CP and AA digestibility, which were found to be lowest in the LM diet and highest in the FM diet (Table 4, Figure 1). Digestibility of EE and starch was high in all diets, and only

Table 4. Least-square means of digestibility of main nutrients and amino acids in diets with different protein sources, measured in dogs and mink [%].

| | Dog | | | | Mink | Diet | | | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AID* | ACD[†] | ATTD[‡] | SID[#] | ATTD | LM[‖] | PM[§] | FM[$] | SEM[♦] | Diet | Method[◇] | Diet × Method |
| Dry matter | 73.2$^{c}$ | 76.7$^{b}$ | 79.8$^{a}$ | | 75.0$^{bc}$ | 69.5$^{C}$ | 77.7$^{B}$ | 81.3$^{A}$ | 0.54 | <0.001 | <0.001 | 0.494 |
| Crude protein | 74.4$^{c}$ | 78.5$^{b}$ | 83.5$^{a}$ | 79.6$^{b}$ | 77.8$^{bc}$ | 71.1$^{C}$ | 79.3$^{B}$ | 85.9$^{A}$ | 0.84 | <0.001 | <0.001 | 0.730 |
| Ether extract | 96.4$^{a}$ | 95.2$^{ab}$ | 96.6$^{a}$ | | 92.7$^{b}$ | 93.7$^{B}$ | 95.2$^{AB}$ | 96.7$^{A}$ | 0.88 | 0.037 | 0.017 | 0.178 |
| Starch | 96.9$^{c}$ | 98.2$^{b}$ | 99.1$^{a}$ | | 97.8$^{b}$ | 97.7$^{B}$ | 98.3$^{A}$ | 98.0$^{AB}$ | 0.18 | 0.050 | <0.001 | 0.453 |
| Carbohydrates | 73.9$^{d}$ | 81.0$^{b}$ | 84.3$^{a}$ | | 78.5$^{c}$ | 76.3$^{B}$ | 80.4$^{A}$ | 81.7$^{A}$ | 0.61 | <0.001 | <0.001 | 0.494 |
| Essential amino acids | | | | | | | | | | | | |
| Arg | 85.1$^{c}$ | 88.3$^{ab}$ | 90.2$^{a}$ | 87.2$^{b}$ | 87.3$^{b}$ | 82.6$^{C}$ | 88.3$^{B}$ | 92.0$^{A}$ | 0.43 | <0.001 | <0.001 | 0.183 |
| His | 76.0$^{c}$ | 80.8$^{b}$ | 86.9$^{a}$ | 80.4$^{b}$ | 78.8$^{bc}$ | 71.1$^{C}$ | 81.9$^{B}$ | 88.8$^{A}$ | 0.79 | <0.001 | <0.001 | 0.065 |
| Ile | 78.7$^{b}$ | 82.9$^{a}$ | 85.1$^{a}$ | 82.0$^{a}$ | 83.7$^{a}$ | 74.7$^{C}$ | 83.0$^{B}$ | 89.7$^{A}$ | 0.71 | <0.001 | <0.001 | 0.948 |
| Leu | 80.2$^{c}$ | 84.6$^{ab}$ | 86.8$^{a}$ | 83.0$^{b}$ | 84.6$^{ab}$ | 76.6$^{C}$ | 84.6$^{B}$ | 90.3$^{A}$ | 0.61 | <0.001 | <0.001 | 0.704 |
| Lys | 78.9$^{b}$ | 81.3$^{ab}$ | 83.8$^{a}$ | 81.7$^{ab}$ | 82.6$^{a}$ | 71.6$^{C}$ | 83.0$^{B}$ | 90.4$^{A}$ | 0.73 | <0.001 | <0.010 | 0.718 |
| Met | 82.0$^{b}$ | 84.5$^{ab}$ | 86.1$^{a}$ | 84.3$^{ab}$ | 85.3$^{a}$ | 76.5$^{C}$ | 85.5$^{B}$ | 91.3$^{A}$ | 0.68 | <0.001 | <0.010 | 0.753 |
| Phe | 81.9$^{b}$ | 84.8$^{a}$ | 86.4$^{a}$ | 86.1$^{a}$ | 85.2$^{a}$ | 79.6$^{C}$ | 85.2$^{B}$ | 89.9$^{A}$ | 0.57 | <0.001 | <0.001 | 0.934 |
| Thr | 69.7$^{c}$ | 78.4$^{b}$ | 82.6$^{a}$ | 81.1$^{ab}$ | 70.9$^{c}$ | 66.4$^{C}$ | 77.3$^{B}$ | 85.9$^{A}$ | 0.88 | <0.001 | <0.001 | 0.578 |
| Val | 77.8$^{b}$ | 81.7$^{a}$ | 84.5$^{a}$ | 81.9$^{a}$ | 81.7$^{a}$ | 74.2$^{C}$ | 81.6$^{B}$ | 88.8$^{A}$ | 0.65 | <0.001 | <0.001 | 0.834 |
| Non-essential amino acids | | | | | | | | | | | | |
| Ala | 80.5$^{b}$ | 83.9$^{ab}$ | 87.3$^{a}$ | 83.5$^{b}$ | 83.8$^{b}$ | 78.2$^{C}$ | 83.9$^{B}$ | 89.3$^{A}$ | 0.77 | <0.001 | <0.001 | 0.678 |
| Asp | 52.0$^{c}$ | 68.9$^{b}$ | 78.3$^{a}$ | 56.3$^{c}$ | 57.0$^{c}$ | 42.9$^{C}$ | 63.7$^{B}$ | 80.8$^{A}$ | 1.56 | <0.001 | <0.001 | <0.010 |
| Cys | 54.5$^{c}$ | 66.5$^{b}$ | 71.0$^{a}$ | | 53.8$^{c}$ | 43.6$^{C}$ | 64.6$^{B}$ | 76.1$^{A}$ | 0.99 | <0.001 | <0.001 | 0.051 |
| Glu | 82.2$^{c}$ | 86.2$^{b}$ | 88.9$^{a}$ | 84.5$^{bc}$ | 85.3$^{b}$ | 78.2$^{C}$ | 86.2$^{B}$ | 91.8$^{A}$ | 0.59 | <0.001 | <0.001 | 0.393 |
| Gly | 76.2$^{c}$ | 82.2$^{b}$ | 88.3$^{a}$ | 78.9$^{bc}$ | 79.9$^{bc}$ | 75.2$^{C}$ | 81.1$^{B}$ | 87.0$^{A}$ | 1.05 | <0.001 | <0.001 | 0.598 |
| Hyp | 79.5$^{c}$ | 87.0$^{b}$ | 94.4$^{a}$ | | 84.2$^{bc}$ | 82.0$^{B}$ | 86.4$^{AB}$ | 90.4$^{A}$ | 1.39 | <0.001 | <0.001 | 0.909 |
| Pro | 81.5$^{c}$ | 86.6$^{b}$ | 89.9$^{a}$ | 85.2$^{b}$ | 84.2$^{bc}$ | 81.1$^{C}$ | 85.8$^{B}$ | 89.6$^{A}$ | 0.64 | <0.001 | <0.001 | 0.576 |
| Ser | 70.5$^{c}$ | 78.3$^{b}$ | 82.3$^{a}$ | 78.1$^{b}$ | 74.8$^{b}$ | 67.1$^{C}$ | 77.8$^{B}$ | 85.5$^{A}$ | 0.82 | <0.001 | <0.001 | 0.551 |
| Tyr | 77.4$^{b}$ | 82.1$^{a}$ | 84.5$^{a}$ | 82.7$^{a}$ | 83.0$^{a}$ | 74.6$^{C}$ | 82.8$^{B}$ | 88.4$^{A}$ | 0.64 | <0.001 | <0.001 | 0.947 |

Notes: *AID, Apparent ileal digestibility; [†]ACD, Apparent colonic digestibility; [‡]ATTD, Apparent total tract digestibility; [#]SID, Standardised ileal digestibility; [‖]LM, Lamb meal; [§]PM, Poultry meal; [$]FM, Fish meal; [♦]SEM, Pooled standard error of the mean; [◇]Digestibility measurement (AID, ACD, ATTD and SID in dogs and ATTD in mink); [a,b,c,d]Least-square means in the same row within digestibility measurement not sharing the same superscript differ at $p < 0.05$; [A,B,C]Least-square means in the same row within diet not sharing the same superscript differ at $p < 0.05$.

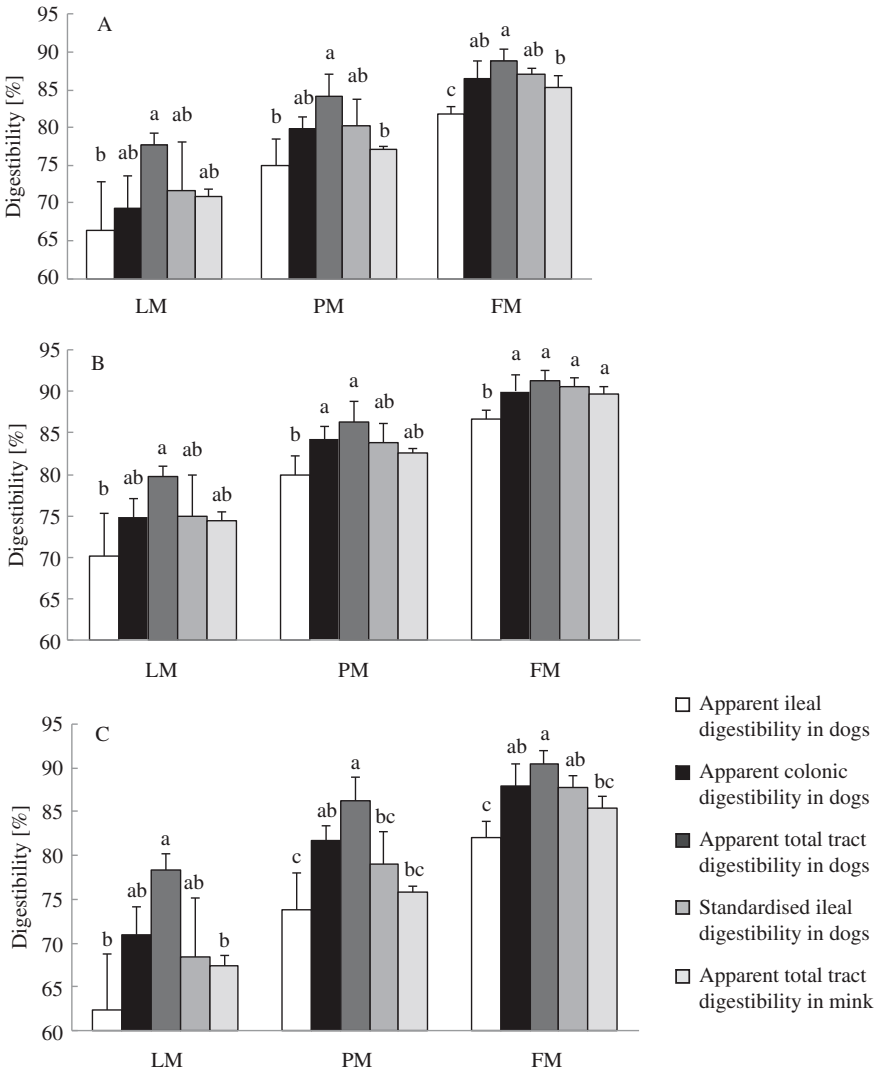


Figure 1. Digestibility in dogs and mink receiving diets with lamb meal (LM), poultry meal (PM) or fish meal (FM) as protein sources. (A) Crude protein; (B) essential amino acids and (C) non-essential amino acids.
Notes: Values are means, with standard deviations represented by vertical bars ($n$ = 4). [a,b,c]Means within diet not sharing the same superscript differ at $p < 0.05$.

small but significant differences were found. DM and CHO digestibility differed significantly among the diets.

AID of CP and AA in dogs was significantly lower than ATTD (Table 4, Figure 1). Values of ACD and SID were intermediate, although not different from AID or ATTD values for some AA. When SID values were calculated from AID, the increase in digestibility was especially apparent for Thr (11.4 percentage units) and to a certain extent for Ser (7.6 percentage units). The digestibility level for the remaining AA and CP increased with an average of 3.5 percentage units. Generally, ATTD in mink showed

the closest similarity to ACD and SID values in dogs. The numerical difference between SID in dogs and ATTD in mink ranged from −1.7 to +1.8 percentage units for CP and all AA, exceptions being Thr and Ser. ATTD of CP, His, Thr, Ala, Asp, Cys, Gly, Hyp and Pro in mink was, however, not found to be different from AID in dogs, while ATTD of Ile, Leu, Lys, Met, Phe, Val and Tyr was similar in mink and dogs. Irrespective of the measuring site in dogs, the lowest AD level of AA was observed for Asp, Cys, Thr and Ser, while the remaining AA had a higher digestibility level than for CP. The same pattern was observed in mink.

Digestibility of EE was high, approximately 96%, and did not differ with the measuring site in dogs (Table 4). In mink, the digestibility of EE (92.7%) was significantly lower than ileal and total tract digestibility in dogs, but similar to colonic digestibility. High values for starch digestibility were also found (96.9–99.1%), with small, but significant differences between ileum, colon and total tract in dogs. Starch digestibility in mink was similar to starch digestibility measured in colon in dogs. Digestibility of DM and CHO was significantly lowest when measured in ileum and highest when measured over the total tract in dogs, with mink digestibility being the closest to dog ileal and colonic digestibility values. The interaction effect between diet and method was non-significant for all main nutrients and AA, except for Asp (Table 4).

### *3.3. Correlations and regression equations*

All methods were highly correlated with respect to CP digestibility, with Pearson correlation coefficients ranging from 0.81 between both AID and SID, and ATTD in mink ($p < 0.01$) to 0.94 between ACD in dogs and mink ATTD ($p < 0.001$). For most of the AA, all methods were highly correlated ($p < 0.001$), with Pearson correlation coefficients generally higher than 0.9. Gly, Hyp and Pro deviated from this, with lower or non-significant correlation between the methods. Regression equations for linear relationships between AID in dogs and ATTD in mink and between AID and ATTD in dogs are shown in Tables 5 and 6, respectively.

High coefficients of correlation were found between the methods for the ranking of the AA with respect to digestibility level, when all individual observations were included in the calculations (Table 7). As shown in Figure 2, the AA digestibilities varied almost in the same manner when comparing mean values for AID in dogs with ATTD in mink ($r = 0.989$, $p < 0.001$).

## 4. Discussion

### *4.1. Apparent ileal and total tract digestibility of crude protein and amino acids in dogs*

As expected, AID of CP was lower than ATTD in dogs in the present study. The difference was on average 9.1 percentage units. This was close to the average value of 9.4 percentage units reported in a review of the difference between AID and ATTD of CP from 30 studies with dogs (Hendriks et al. 2012). However, this difference varied to a large extent, ranging from −4.1 to 31.3 percentage units (Hendriks et al. 2012). The digestibility of the protein sources influences the difference between AID and ATTD of CP, and as pointed out by Hendriks et al. (2012), the difference generally decreases with increased digestibility of the CP in the diet. In accordance with the latter study, the present study showed that the average difference between AID and ATTD of CP was 11.4, 9.1

Table 5. Linear relationship between apparent ileal digestibility of crude protein and amino acids in dogs and apparent total tract digestibility in mink.

| | Model* | *p*-value | $R^{2\dagger}$ | SE‡ intercept | SE slope |
|---|---|---|---|---|---|
| Crude protein | $y = 0.99x - 2.36$ | <0.010 | 0.66 | 17.63 | 0.23 |
| Essential amino acids | | | | | |
| Arg | $y = 1.06x - 7.10$ | <0.001 | 0.79 | 15.20 | 0.17 |
| His | $y = 0.90x + 5.44$ | <0.001 | 0.82 | 10.64 | 0.13 |
| Ile | $y = 1.08x - 11.24$ | <0.001 | 0.80 | 14.34 | 0.17 |
| Leu | $y = 1.02x - 5.73$ | <0.001 | 0.79 | 13.86 | 0.16 |
| Lys | $y = 1.09x - 11.41$ | <0.001 | 0.84 | 12.50 | 0.15 |
| Met | $y = 1.25x - 24.97$ | <0.001 | 0.85 | 14.26 | 0.17 |
| Phe | $y = 1.02x - 4.66$ | <0.001 | 0.72 | 16.89 | 0.20 |
| Thr | $y = 1.06x - 5.16$ | <0.001 | 0.81 | 11.47 | 0.16 |
| Val | $y = 1.06x - 8.99$ | <0.001 | 0.83 | 12.55 | 0.15 |
| Non-essential amino acids | | | | | |
| Ala | $y = 1.09x - 10.78$ | <0.010 | 0.61 | 23.19 | 0.28 |
| Asp | $y = 1.12x - 11.97$ | <0.001 | 0.84 | 9.05 | 0.15 |
| Cys | $y = 0.86x + 8.21$ | <0.001 | 0.92 | 4.36 | 0.08 |
| Glu | $y = 1.10x - 12.07$ | <0.001 | 0.81 | 14.25 | 0.17 |
| Gly | $y = 1.11x - 12.49$ | <0.050 | 0.38 | 36.02 | 0.45 |
| Hyp | $y = 0.25x + 58.35$ | >0.050 | 0.01 | 55.50 | 0.66 |
| Pro | $y = 1.04x - 6.08$ | <0.050 | 0.48 | 28.90 | 0.34 |
| Ser | $y = 1.07x - 9.62$ | <0.001 | 0.80 | 12.91 | 0.17 |
| Tyr | $y = 1.02x - 7.36$ | <0.001 | 0.82 | 12.68 | 0.15 |

Notes: *$y$ is apparent ileal digestibility in dogs when apparent total tract digestibility in mink is $x$; †$R^2$, Coefficient of determination; ‡SE, Standard error.

and 6.9 percentage units for LM, PM and FM diets, respectively. A significant reduction in the difference between these two measuring sites was, however, not revealed, mainly due to large variation in AID observed for the animals receiving the LM diet. As for CP, the difference in digestibility of the AA between the measuring sites generally decreased with increased digestibility, but this effect was only significant for Asp.

The significantly lower AID than ATTD observed for all the AA in dogs in the present study was in contrast to the results of Hendriks and Sritharan (2002), where a significantly lower AID than ATTD was found only for Thr, Asp, Gly, Pro and Ser. Furthermore, AID was found to be higher than ATTD for Arg, His, Ile, Lys, Met, Phe and Tyr, although this difference was only significant for Met (Hendriks and Sritharan 2002). Lower AID than ATTD for all AA was observed in another study, but the difference was not significant for Ile, Lys, Met, Phe and Ala (Hendriks et al. 2013). Thus, the results of Hendriks et al. (2013) were more in line with the results presented herein.

Despite the partly conflicting results between the present study and the results presented by others (Hendriks and Sritharan 2002; Hendriks et al. 2013), there seems to be a certain pattern in the difference between AID and ATTD for most of the AA. In all three studies, AA found to have the largest difference between AID and ATTD included Thr, Asp, Gly and Ser, although their ranking order varied. Also, Cys and His were among the AA found to differ greatly between AID and ATTD in the present study and in the study by Hendriks et al. (2013). This was, however, contradictory to the results found by Hendriks and Sritharan (2002), where AID and ATTD of His were close to equal, whereas Cys digestibility was not reported. The results presented herein showed that the AA found to have the smallest differences between AID and ATTD were Met, Phe, Lys, Arg and Ile.

Table 6. Linear relationship between apparent ileal and total tract digestibility of crude protein and amino acids in dogs.

| | Model* | *p*-value | $R^{2\dagger}$ | SE‡ intercept | SE slope |
|---|---|---|---|---|---|
| Crude protein | $y = 1.37x - 39.95$ | <0.001 | 0.82 | 16.82 | 0.20 |
| Essential amino acids | | | | | |
| Arg | $y = 1.54x - 53.38$ | <0.001 | 0.90 | 14.96 | 0.17 |
| His | $y = 1.62x - 64.80$ | <0.001 | 0.86 | 18.11 | 0.21 |
| Ile | $y = 1.30x - 32.17$ | <0.001 | 0.85 | 14.95 | 0.18 |
| Leu | $y = 1.38x - 39.85$ | <0.001 | 0.88 | 14.36 | 0.17 |
| Lys | $y = 1.33x - 32.67$ | <0.001 | 0.90 | 11.90 | 0.14 |
| Met | $y = 1.36x - 35.52$ | <0.001 | 0.82 | 17.45 | 0.20 |
| Phe | $y = 1.22x - 23.32$ | <0.001 | 0.79 | 17.41 | 0.20 |
| Thr | $y = 1.43x - 48.52$ | <0.001 | 0.90 | 12.81 | 0.15 |
| Val | $y = 1.32x - 34.10$ | <0.001 | 0.88 | 12.98 | 0.15 |
| Non-essential amino acids | | | | | |
| Ala | $y = 1.72x - 69.51$ | <0.001 | 0.80 | 23.79 | 0.27 |
| Asp | $y = 2.24x - 123.39$ | <0.001 | 0.93 | 15.70 | 0.20 |
| Cys | $y = 1.15x - 27.39$ | <0.001 | 0.89 | 9.01 | 0.13 |
| Glu | $y = 1.55x - 55.71$ | <0.001 | 0.90 | 14.86 | 0.17 |
| Gly | $y = 1.92x - 93.42$ | <0.001 | 0.75 | 30.80 | 0.35 |
| Hyp | $y = 1.94x - 104.17$ | <0.050 | 0.42 | 68.51 | 0.73 |
| Pro | $y = 1.47x - 50.71$ | <0.001 | 0.74 | 24.74 | 0.27 |
| Ser | $y = 1.35x - 40.53$ | <0.001 | 0.92 | 10.48 | 0.13 |
| Tyr | $y = 1.22x - 25.76$ | <0.001 | 0.85 | 13.68 | 0.16 |

Notes: *$y$ is apparent ileal digestibility in dogs when apparent total tract digestibility in dogs is $x$; †$R^2$, Coefficient of determination; ‡SE, Standard error.

Table 7. Pearson correlation coefficients ($r$) between methods for the ranking pattern of amino acid digestibilities.

| | AID*, dog | | ACD†, dog | | ATTD‡, dog | | SID#, dog | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *p*-value | *r* | *p*-value | *r* | *p*-value | *r* | *p*-value |
| ACD, dog | 0.936 | <0.001 | | | | | | |
| ATTD, dog | 0.871 | <0.001 | 0.931 | <0.001 | | | | |
| SID, dog | 0.980 | <0.001 | 0.921 | <0.001 | 0.854 | <0.001 | | |
| ATTD, mink | 0.926 | <0.001 | 0.927 | <0.001 | 0.872 | <0.001 | 0.894 | <0.001 |

Notes: *AID, Apparent ileal digestibility; †ACD, Apparent colonic digestibility; ‡ATTD, Apparent total tract digestibility; #SID, Standardised ileal digestibility.

Although in another order, these were the AA with the highest positive difference between AID and ATTD in the study by Hendriks and Sritharan (2002). Also Hendriks et al. (2013) found the smallest difference in AID and ATTD to include mainly the same AA. The comparison of the results in the present experiment with the results of others (Hendriks and Sritharan 2002; Hendriks et al. 2013) thus shows a possible pattern of those AA mainly used or produced by the microflora in the large intestine of dogs. However, the difference between AID and ATTD of AA in the three studies discussed varied in magnitude and direction. The cause of this variation is uncertain, but contributing factors may be diet composition, experimental procedures and composition of the microflora in the hindgut. The variation emphasises the importance of using AID values when assessing CP and AA availability of protein sources and diets for dogs.

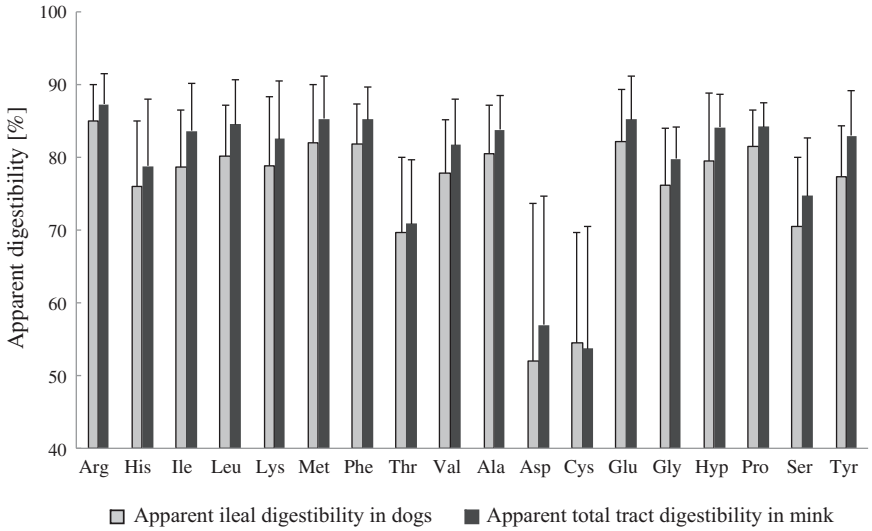


Figure 2. Ranking pattern of apparent digestibility of amino acids in dogs and mink given three different dry dog foods.
Notes: Values are means, with standard deviations represented by vertical bars ($n$ = 12). Pearson correlation coefficient ($r$) = 0.989, $p < 0.001$.

The high correlation found between AID and ATTD in dogs indicates that it might be possible to predict AID of CP and AA from ATTD. However, although quality of protein sources might be one of the main factors affecting the difference in AID and ATTD, several other dietary factors have also been shown to influence apparent CP and AA digestibility at the ileal or faecal level. Protein intake (Yamka et al. 2003), dietary fibre (Muir et al. 1996; Silvio et al. 2000; Burkhalter et al. 2001) and source of dietary starch flours (Murray et al. 1999) are examples of such factors, which influence CP and AA digestibility and can complicate the prediction of AID from ATTD values.

### *4.2. Apparent colonic digestibility of crude protein and amino acids in dogs*

To our knowledge, ACD values in dogs have not been reported earlier. However, since apparent CP and AA digestibility increased from ileal to faecal level, the ACD values found to be in between these were as we expected. The same pattern in CP digestibility has also been shown in weanling pigs (Asche et al. 1989). The disappearance of nitrogen through the colon intestinal wall was probably a result of absorption of ammonia produced from deamination of AA entering the colon (Hendriks et al. 2012). There is also a possibility that a small amount of AA are absorbed from the colon (Blachier et al. 2007; Hendriks et al. 2012). An *in vitro* study by Robinson et al. (1973) demonstrated the ability of the dog colon mucosa to transport AA. However, the contribution of AA absorption in colon is considered very limited and of no importance compared with ileal absorption (Hendriks et al. 2012).

### *4.3. Apparent digestibility of crude protein and amino acids in dogs compared with mink*

The higher ATTD of CP observed in dogs compared with mink is in agreement with earlier comparative studies (Ahlstrøm and Skrede 1998; Vhile et al. 2005). As opposed to

dogs, post-ileal fermentation in the mink is limited (Skrede 1979; Szymeczko and Skrede 1990), and this is most probably the main reason for a lower ATTD of CP in mink than in dogs. The numerically lower ATTD of all AA observed in mink than in dogs in the present experiment was in contrast to the previous results where a significantly higher ATTD of several AA was found in mink than in dogs (Vhile et al. 2005). The AA having significantly higher ATTD in mink than in dogs exceeded ATTD in dogs with 0.9 to 1.8 percentage units and included Ile, Lys, Met and Phe (Vhile et al. 2005). ATTD of these AA was not significantly different between mink and dog in the present experiment. In addition, it is interesting to note that the same AA were among those showing the lowest disappearance between ileal and faecal level in dogs in the present experiment and in the study by Hendriks et al. (2013) and among the ones with higher AID than ATTD in the study by Hendriks and Sritharan (2002). Several studies are therefore pointing in the direction of a net synthesis of these AA in the large intestine of dogs.

A significant correlation between ATTD of CP in dogs and mink was in accordance with the results of Ahlstrøm and Skrede (1998), but in contrast to Vhile et al. (2005). The results obtained by Vhile et al. (2005) also showed a generally lower and non-significant correlation between dog and mink ATTD for several of the AA, compared with the results in the present study. These differences can probably be attributed to different diet properties. One reason could be that the selected difference in digestibility levels used in the present experiment promoted a more marked relationship between dog and mink ATTD than the diets with generally higher and more similar digestibility used by Vhile et al. (2005). The regression equations for the comparison of AID of CP and AA in dogs with ATTD in mink generally had a lower $R^2$-value than the corresponding regression equations for the comparison between AID and ATTD in dogs. The higher values observed for the comparison of AID and ATTD in dogs can probably be explained by the fact that AID values and the corresponding ATTD values were determined in the same animal. This comparison was thereby less influenced by individual variation within diet groups than the comparison of dog AID with mink ATTD. In addition, AID of CP and AA for the dogs receiving the LM diet varied to a large extent, with standard deviations averaging to 6.0, thereby influencing the $R^2$-values negatively.

Based on the present results, AID of CP and most AA in dogs seems to be numerically lower than ATTD in mink, although non-significant differences were detected for CP and several AA. Furthermore, the linear relationship between AID in dogs and ATTD in mink was significant for CP and all AA, except for Hyp. The AID and ATTD measurements in dogs and mink, respectively, were also highly correlated for the ranking of AA according to digestibility level. A similarly high correlation for the ranking order of AA has been found previously, when comparing AID in pigs with ATTD in mink (Skrede et al. 1998).

### *4.4. Apparent digestibility values and endogenous amino acids*

The AA composition of endogenous secretions affects AD values to some extent, and this is probably especially important for AID in dogs and ATTD in mink where the additional effect of microbial fermentation is considered limited. Studies have revealed high endogenous concentrations of Thr, Glu, Asp and Ser both in ileum of dogs and in faeces of mink (Skrede 1979; Hendriks et al. 2002). In line with this, AID in dogs and ATTD in mink of these AA, except for Glu, were especially low in the present study. The high digestibility levels found for Glu can be explained by the high dietary content and thus intake of Glu, leaving the endogenous secretions of this AA less influential on the AD level. The opposite effect was probably present for Cys, for which the content in

endogenous secretions assumable was less than the content of the other AA mentioned here (Skrede 1979). Still, the endogenous secretions most likely considerably affected the AD level of Cys, which was very low, due to the low dietary content of this AA.

### *4.5. Standardised ileal digestibility of crude protein and amino acids in dogs*

AD values can be corrected for the content of CP and AA in endogenous secretions to obtain the more precise values of standardised or true digestibility. SID in dogs has been reported in one study (Hendriks et al. 2013) and is the preferred choice of digestibility measurement in diet formulation for pigs (Stein, Fuller et al. 2007; Stein, Sève et al. 2007). SID values are, in contrast to AID values, more additive in mixed diets (Stein et al. 2005). Compared with true digestibility values that are corrected for the total amount of endogenous losses, SID values include specific endogenous losses, while a correction is made for the basal endogenous losses (Stein, Fuller et al. 2007; Stein, Sève et al. 2007). Since knowledge of specific endogenous losses related to different ingredients currently is limited, SID values are for the time being considered the most proper approach for assessing CP and AA availability in pigs (Stein, Fuller et al. 2007; Stein, Sève et al. 2007). In dogs, it was proposed to update the present assumptions for CP and AA availability used in nutritional guidelines for commercial dog foods, since the values of SID found in dry dog foods were contradictory to the availability estimates currently used (Hendriks et al. 2013). SID values can therefore be considered to give a more precise picture of AA availability also in dogs. The close similarity of SID of CP and AA in dogs and ATTD in mink shown in the present study strengthens the relevance of the mink as a suitable model animal for the evaluation of AID and SID of CP and AA in dogs.

### *4.6. Ether extract, starch, carbohydrate and dry matter digestibility in dogs and mink*

The high and similar EE digestibility between measuring sites in dogs was in accordance with Faber et al. (2010), who reported ileal and total tract digestibility values for diets containing different animal ingredients. A tendency for a higher ileal than total tract digestibility of EE in dry dog foods has previously been observed (Hendriks et al. 2013). As discussed by Hendriks et al. (2013), this can be explained by the production of short-chain fatty acids by microorganisms in colon, in addition to the contribution of the fat content in the microorganisms themselves. Digestion and absorption of dietary fat is therefore more or less completed by the time digesta enters the colon. The high, but still lower, total tract digestibility of EE observed in mink compared with dogs was in accordance with previous results (Ahlstrøm and Skrede 1998; Vhile et al. 2005), although only Ahlstrøm and Skrede (1998) found the difference to be significant as in the present study.

In accordance with others, ileal digestibility of starch was almost complete and only slightly lower than total tract digestibility in dogs (Zuo et al. 1996; Murray et al. 1999; Silvio et al. 2000). Fermentation in the hindgut can explain the small increase in starch digestibility when measured over the total digestive tract. The same explanation is applicable to the difference observed between ileal and total tract digestibility of CHO and DM, which was in accordance with the results reported by Hendriks et al. (2013). The lower total tract digestibility of starch, CHO and DM measured in mink than in dogs in the present study has also been shown by others (Ahlstrøm and Skrede 1998; Vhile et al. 2005). This difference can be explained by the higher microbial activity in the large intestine of dogs than in mink.

## 5. Conclusion

The present results showed that AID of CP and most AA in dogs was significantly correlated to ATTD in mink. Furthermore, ATTD in mink was numerically very close to SID in dogs for CP and AA, except for Thr and Ser. The present results thus suggest that ATTD in mink can be a highly relevant and efficient tool for the determination of AID and SID of CP and AA in diets for dogs. This would enable reliable estimates of CP and AA digestibility levels in dogs to be obtained in a gentle manner, without the use of surgery.

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## Disclosure statement

The authors declare no financial interest or benefit from the direct applications of the research.

## References

Ahlstrøm Ø, Skrede A. 1998. Comparative nutrient digestibility in dogs, blue foxes, mink and rats. J Nutr. 128:2676S–2677S.

AOAC International. 2002. Official methods of analysis of AOAC International. 17th ed. Gaithersburg (MD): AOAC International.

Asche GL, Lewis AJ, Peo ER Jr. 1989. Protein digestion in weanling pigs: effect of feeding regimen and endogenous protein secretion. J Nutr. 119:1083–1092.

Bednar GE, Murray SM, Patil AR, Flickinger EA, Merchen NR, Fahey GC Jr. 2000. Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of ileally cannulated dogs. Arch Anim Nutr. 53:127–140.

Blachier F, Mariotti F, Huneau JF, Tomé D. 2007. Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids. 33:547–562.

Burger IH. 1994. Energy needs of companion animals: matching food intakes to requirements throughout the life cycle. J Nutr. 124:2584S–2593S.

Burkhalter TM, Merchen NR, Bauer LL, Murray SM, Patil AR, Brent JL Jr, Fahey GC Jr. 2001. The ratio of insoluble to soluble fiber components in soybean hulls affects ileal and total-tract nutrient digestibilities and fecal characteristics of dogs. J Nutr. 131:1978–1985.

Chwalibog A, Glem-Hansen N, Henckel S, Thorbek G. 1980. Energy metabolism in adult mink in relation to protein-energy levels and environmental temperature. In: Mount LE, editor. Proceedings of the 8th Symposium on Energy Metabolism; EAAP Publ. no. 26. London: Butterworths; p. 283–286.

Faber TA, Bechtel PJ, Hernot DC, Parsons CM, Swanson KS, Smiley S, Fahey GC Jr. 2010. Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays. J Anim Sci. 88:1421–1432.

Hendriks WH, Sritharan K. 2002. Apparent ileal and fecal digestibility of dietary protein is different in dogs. J Nutr. 132:1692S–1694S.

Hendriks WH, Sritharan K, Hodgkinson SM. 2002. Comparison of the endogenous ileal and faecal amino acid excretion in the dog (*Canis familiaris*) and the rat (*Rattus rattus*) determined under protein-free feeding and peptide alimentation. J Anim Physiol Anim Nutr. 86:333–341.

Hendriks WH, Thomas DG, Bosch G, Fahey GC Jr. 2013. Comparison of ileal and total tract nutrient digestibility of dry dog foods. J Anim Sci. 91:3807–3814.

Hendriks WH, van Baal J, Bosch G. 2012. Ileal and faecal protein digestibility measurement in humans and other non-ruminants – a comparative species view. Br J Nutr. 108:S247–S257.
[ISO] International Organization for Standardization. 1999. Animal feeding stuffs – determination of moisture and other volatile matter content, ISO 6496. Geneva: ISO.
[ISO] International Organization for Standardization. 2002. Animal feeding stuffs – determination of crude ash, ISO 5984. Geneva: ISO.
[ISO] International Organization for Standardization. 2005. Animal feeding stuffs – determination of amino acids content, ISO 13903. Geneva: ISO.
Johnson ML, Parsons CM, Fahey GC Jr, Merchen NR, Aldrich CG. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. J Anim Sci. 76:1112–1122.
McCleary BV, Solah V, Gibson TS. 1994. Quantitative measurement of total starch in cereal flours and products. J Cereal Sci. 20:51–58.
Muir HE, Murray SM, Fahey GC Jr, Merchen NR, Reinhart GA. 1996. Nutrient digestion by ileal cannulated dogs as affected by dietary fibers with various fermentation characteristics. J Anim Sci. 74:1641–1648.
Murray SM, Fahey GC Jr, Merchen NR, Sunvold GD, Reinhart GA. 1999. Evaluation of selected high-starch flours as ingredients in canine diets. J Anim Sci. 77:2180–2186.
Murray SM, Patil AR, Fahey GC Jr, Merchen NR, Hughes DM. 1997. Raw and rendered animal by-products as ingredients in dog diets. J Anim Sci. 75:2497–2505.
Robinson JWL, Luisier AL, Mirkovitch V. 1973. Transport of amino-acids and sugars by the dog colonic mucosa. Pflügers Archiv Eur J Physiol. 345:317–326.
Silvio J, Harmon DL, Gross KL, McLeod KR. 2000. Influence of fiber fermentability on nutrient digestion in the dog. Nutrition. 16:289–295.
Skrede A. 1979. Utilization of fish and animal byproducts in mink nutrition. IV. Fecal excretion and digestibility of nitrogen and amino acids by mink fed cod (*Gadus morrhua*) fillet or meat-and-bone meal. Acta Agric Scand. 29:241–257.
Skrede A, Berge GM, Storebakken T, Herstad O, Aarstad KG, Sundstøl F. 1998. Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. Anim Feed Sci Technol. 76:103–116.
Stein HH, Fuller MF, Moughan PJ, Sève B, Mosenthin R, Jansman AJM, Fernández JA, de Lange CFM. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest Sci. 109:282–285.
Stein HH, Pedersen C, Wirt AR, Bohlke RA. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J Anim Sci. 83:2387–2395.
Stein HH, Sève B, Fuller MF, Moughan PJ, de Lange CFM. 2007. Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J Anim Sci. 85:172–180.
Szymeczko R, Skrede A. 1990. Protein digestion in mink. Acta Agric Scand. 40:189–200.
Vhile SG. 2007. Nutrient digestibility in dogs (*Canis familiaris)* and model carnivore species. Comparative studies on protein and amino acid digestibility in dogs, blue foxes (*Alopex lagopus*) and mink (*Mustela vison*) fed various protein sources using different determination methods [dissertation]. Ås (Norway): Norwegian University of Life Sciences.
Vhile SG, Skrede A, Ahlstrøm Ø, Hove K. 2005. Comparative apparent total tract digestibility of major nutrients and amino acids in dogs (*Canis familiaris)*, blue foxes (*Alopex lagopus*) and mink (*Mustela vison*). J Anim Sci. 81:141–148.
Vhile SG, Skrede A, Ahlstrøm Ø, Hove K. 2007. Yttrium oxide ($Y_2O_3$) as an inert marker in digestibility studies with dogs, blue foxes and mink fed diets containing different protein sources. J Anim Physiol Anim Nutr. 91:381–389.
Yamka RM, Jamikorn U, True AD, Harmon DL. 2003. Evaluation of low-ash poultry meal as a protein source in canine foods. J Anim Sci. 81:2279–2284.
Zuo Y, Fahey GC Jr, Merchen NR, Bajjalieh NL. 1996. Digestion responses to low oligosaccharide soybean meal by ileally-cannulated dogs. J Anim Sci. 74:2441–2449.

# Paper II

**Protein and amino acid bioavailability of extruded dog food with protein meals of different quality using growing mink (*Neovison vison*) as a model**

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# Protein and amino acid bioavailability of extruded dog food with protein meals of different quality using growing mink (*Neovison vison*) as a model[1,2]

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**ABSTRACT:** The present study evaluated growing mink (*Neovison vison*) as a model for dietary protein quality assessment of protein meals used in extruded dog foods. Three foods with similar CP content but of different protein quality were produced using different protein meals. The protein meals varied with respect to CP digestibility and AA composition and included lamb meal (LBM), poultry meal (PM), and fish meal (FM) with low, intermediate, and high protein quality, respectively. Nitrogen balance, BW gain, protein efficiency ratio (PER), and apparent total tract digestibility (ATTD) were used as measures of protein and AA bioavailability in growing mink. Standardized ileal digestibility (SID) was used to measure protein and AA bioavailability in adult dogs (*Canis familiaris*). The mink study (3 × 3 Latin square design) included 12 kits aged 8 to 11 wk. The dog study included 12 dogs divided in 3 groups allocated to 1 of the experimental diets. The growing mink responded in accordance with the different AA supply between diets, as determined by the first limiting AA. The LBM diet deviated from the other diets with lower ($P < 0.001$) values for N retention, BW gain, and PER, and the diets differed ($P < 0.001$) in ATTD of CP and all AA, except for hydroxyproline. Retention of N was 0.66, 1.04, and 1.18 $g \cdot kg^{-0.75} \cdot d^{-1}$; BW gain was 8.2, 26.8, and 35.3 g/d; PER was 0.38, 1.39, and 1.71; and ATTD of CP was 66.8, 73.8, and 82.1% for the LBM, PM, and FM diets, respectively. In dogs, SID of CP and AA differed ($P \leq 0.017$) between diets and was generally lowest for the LBM diet, intermediate for the PM diet, and greatest for the FM diet. For CP, SID was 71.5, 80.2, and 87.0% for the LBM, PM, and FM diets, respectively. The contents of digestible CP and AA (based on SID) covered the minimal requirement for adult dogs set by the NRC for all diets, except for the content of digestible Met + Cys in the LBM diet. Despite this, dietary content of Met + Cys in the LBM diet agreed with the recommended level set by the NRC and the Association of American Feed Control Officials for adult dogs but was below the level recommended by the European Pet Food Industry Federation. It was concluded that growth studies with mink kits can provide valuable information in protein quality assessment of extruded dog foods. Furthermore, the study showed that to ensure nutritional adequacy of dog food and to be able to compare protein quality of dog foods, information on AA composition and digestibility is crucial.



**Key words:** amino acids, bioavailability, dogs, growing mink, protein efficiency ratio, standardized ileal digestibility



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## INTRODUCTION

Digestibility, N balance, BW gain, and protein efficiency ratio (**PER**) provide valuable information on protein and AA bioavailability of dietary protein. Due to ethical, economic, or commercial reasons, such measures are obtained in dogs only to a certain extent, and both in vitro methods and animal models have been used to assess protein quality of individual feedstuffs or diets for dogs (Burns et al., 1982; Hegedűs et al., 1998; Dust et al., 2005; Folador et al., 2006; Cramer et al., 2007). Apparent total tract digestibility (**ATTD**) in the mink has been shown to be a reliable model for estimation of apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of CP and AA in dogs (Tjernsbekk et al., 2014). Growing mink have a greater dietary protein and AA requirement than adult dogs (NRC 2006; Lassén et al., 2012), and they are, therefore, likely to show a pronounced growth response to differences in dietary protein quality. The main objective of the present study was to investigate if the growth response in mink kits is sensitive to variations in the supply of bioavailable AA between extruded dog foods and, therefore, the suitability of using a growing mink assay in protein quality evaluation of extruded dog foods. To strengthen the value of the protein quality measurements obtained in growing mink, SID of CP and AA was determined in adult dogs to obtain valid estimates of protein and AA bioavailability in the extruded foods. Protein quality for dogs was assessed by comparison of digestible CP and AA in the foods with the minimal requirement (NRC, 2006). The differences in protein quality between the extruded dog foods used in the present study were used to demonstrate the importance of protein quality assessment and the limitations of using dietary content as a measure of nutritional adequacy.

## MATERIALS AND METHODS

The mink experiment was performed in Denmark and followed the guidelines of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe, 1986) and Danish national legislation. The dog experiment was performed in Norway and was approved by the Norwegian Animal Research Authority (Brumunddal, Norway). The experimental procedures were performed in accordance with institutional and national guidelines for the care and use of animals (Norwegian Ministry of Agriculture and Food, 1996, 2009).

### *Diets and Protein Meals*

Three extruded dog foods with different protein sources were used. The selected protein sources were intended for the pet food market and included lamb meal (**LBM**), poultry meal (**PM**), and fish meal (**FM**). The diets were produced at the Centre for Feed Technology, Norwegian University of Life Sciences, Ås, Norway. Details of the food production, ingredient composition of the experimental diets, and chemical composition of the protein meals and their respective diets are described elsewhere (Tjernsbekk et al., 2014). The diets were formulated to have similar CP content but different AA composition and digestibility. Content of ME in the LBM, PM, and FM diets was calculated to be 13.7, 15.0, and 16.5 MJ/kg, respectively, for growing mink and 15.6, 16.0, and 16.6 MJ/kg, respectively, for adult dogs. The ATTD of CP in the protein meals was determined in a pre-experimental screening using adult mink. Based on AA composition and the ATTD of CP of 67.7, 80.9, and 87.5% in the LMB, PM, and FM, respectively, protein quality was considered to be low, intermediate, and high for the LMB, PM, and FM, respectively.

### *Bioavailability of Protein and AA in Growing Mink*

The experiment was performed at University of Copenhagen, Fur Animal Laboratory, Rørrendegård, Taastrup, Denmark. Three male mink kits (*Neovison vison*) from 4 litters ($n$ = 12) were used in the experiment. The kits were of the standard brown color type and were 8 wk of age at the start of the experiment, which lasted until the age of 11 wk. During the experiment, the kits were housed in metabolic cages equipped for total collection of feces, urine, and feed residuals. The experiment was designed as a 3 × 3 Latin square, where the 3 kits from each litter received different diets during 3 balance periods. Each of the 3 balance periods lasted for 7 d. The balance periods were initiated when the kits were 8, 9, and 10 wk of age and are denoted as period 1, 2, and 3, respectively. The Latin square was replicated 4 times, with the 3 kits from 1 of the 4 litters in each replicate.

Bioavailability of protein and AA for mink kits was measured as N balance, BW gain, PER, and ATTD during each of the 3 balance periods. The balance periods included a 3-d adaptation period and a 4-d collection period. Body weight was registered on the first and last day of the 4-d collection period. Before mink kits were fed, the experimental diets were mixed with water in a ratio of 1:2 to achieve a suitable consistency and to make the feed more palatable. The kits were fed a specific amount of food that was slightly greater than the amount expected to be consumed. Feed was offered once a day, and the kits had free access to drinking water. Daily feed intake was accurately recorded by weighing the feed rations offered and the feed residues. Feces and urine were quantitatively collected once daily. Urine was collected in vials containing 10 mL of 5% sulfuric acid. When the daily collection of feces

and urine was completed, collection screens and funnels were rinsed in a 1% citric acid solution to minimize losses of N and the citric acid rinse was quantitatively collected. Each day, collected feces, urine, and citric acid rinse were weighed and recorded and then stored at −18°C. Feeding, collection, and recording procedures were performed between 0830 and 1100 h. When the experimental period ended, collected feces, urine, and citric acid rinse from each animal were thawed and mixed separately for withdrawal for samples used for analyses. Before analyses, a representative fecal sample from each animal was freeze-dried, ground, and sifted to remove hairs.

### *Bioavailability of Protein and AA in Adult Dogs*

Apparent ileal digestibility, SID, and ATTD of CP and AA in the experimental diets were determined in dogs. The diets differed with respect to CP and AA digestibility, as published by Tjernsbekk et al. (2014). In the present paper, a more detailed presentation of the SID of CP and AA in each diet is given. The dog experiment was performed at a sled dog kennel at Harestua, Oppland, Norway, using 12 Alaskan huskies (*Canis familiaris*). The dogs were all healthy and mainly former performance sled dogs, but for different reasons their owners had decided to euthanize them. The group of 12 dogs consisted of 7 males and 5 females aged 1.5 to 13 yr, with an average BW of 23.6 ± 1.8 kg for the males and 20.3 ± 3.0 kg for the females. During the experiment, the dogs were divided into 3 groups of 4 animals, and each group was allocated to 1 of the experimental diets. The dogs were housed separately in outdoor houses placed in rows, and each dog had a free range area of 4 m. They had access only to their own food, which was offered once daily in the amount necessary to cover the maintenance energy requirement of 525 kJ ME·kg$^{-0.75}$·d$^{-1}$ (Burger, 1994). Drinking water was available at all times. The experimental period lasted for 10 d. For determination of ileal digestibility, contents of the ileum were collected after euthanization. The dogs were brought inside a building at the kennel one by one for euthanization by a veterinarian on the last day of the experimental period. Each dog was offered food 4 h before euthanization. Xylazine (Narcoxyl Vet; Merck/MSD Animal Health, Summit, NJ; 1 mg/kg BW) was used for sedation of the dogs followed by euthanization with injection of pentobarbital (Mebumal; Oak Pharmaceuticals Inc., Lake Forest, IL;100 mg/kg BW). Intestinal content from approximately the last 15 cm of the distal ileum was sampled after dissection of the intestine. This content was immediately put in plastic containers and frozen in liquid N and then stored (−20°C). Before chemical analyses, the samples of intestinal contents were freeze-dried, ground, and sifted to remove hairs.

### *Chemical Analyses*

The extruded dog foods were analyzed for DM, ash, N, crude fat, starch, and AA. The feed and water mix given to the mink kits was analyzed for DM. Fresh samples of feces from mink were analyzed for DM and N, whereas the freeze-dried samples of feces were analyzed for DM, ash, crude fat, GE, and AA. Urine and citric acid rinse from the mink study were analyzed for N content. For determination of SID, the intestinal content from the dogs was analyzed for N and AA. In addition, yttrium concentration in diets and intestinal content was analyzed as described by Tjernsbekk et al. (2014). Dry matter was determined by drying of the samples to constant weight at 103°C. For determination of ash content, samples were combusted at 550°C for 10 h. Nitrogen in the diets and intestinal contents was determined by the micro-Kjeldahl procedure, using a Kjeltec 1015 Digester at 420°C and a Kjeltec Auto 2400/2600 (Foss Tecator AB, Höganäs, Sweden). Nitrogen content of feces, urine, and citric acid rinse was also determined as Kjeldahl-N, by use of a 2020 Digestor at 420°C and a 2200 Kjeltec Auto Distillation Unit (Foss Tecator AB). Crude protein was determined as Kjeldahl-N × 6.25. Crude fat content in diets was determined by extraction with petroleum ether and acetone, using an Accelerated Solvent Extractor (ASE 200) from Dionex Corp. (Sunnyvale, CA). Crude fat in feces was analyzed by hydrolysis of the samples with 3 *M* hydrochloric acid in a Soxtec 1047 Hydrolyzing Unit (Foss Tecator AB) followed by extraction with petroleum ether in a Soxtec HT 1043 Extraction Unit (Foss Tecator AB). Starch was analyzed according to the description given by McCleary et al. (1994), whereas content of total carbohydrates in the samples was calculated by the following formula: carbohydrates = DM − (CP + crude fat + ash). Gross energy was determined with an adiabatic bomb calorimeter (IKA Calorimeter System C 5000; IKA KG, Staufen, Germany). The analysis of AA followed the European Commission Directive 98/64/EC (European Commission, 1998), with norvaline used as an internal standard.

### *Calculations*

In mink, ATTD of nutrients was calculated as ATTD (%) = {[nutrient ingested (g) − nutrient in feces (g)]/nutrient ingested (g)} × 100. The content of ME in the diets was calculated based on ATTD data, using the following equation: ME (kJ) = digestible protein (g) × 18.42 kJ + digestible fat (g) × 39.76 kJ + digestible carbohydrate (g) × 17.58 kJ (Lassén et al., 2012). Data of N balance were calculated in relation to metabolic body size of the mink kits, so that comparisons could be made across periods. Digested N (g·kg$^{-0.75}$·d$^{-1}$) was

calculated as N intake − fecal N, whereas retained N ($g \cdot kg^{-0.75} \cdot d^{-1}$) was calculated as N intake − (fecal N + urinary N). The utilization of digested N for retention (**RNDN**) was calculated as the fraction of retained N in percent of the digested N. Protein efficiency ratio was calculated as BW gain (g/d)/CP intake (g/d).

In dogs, SID of CP and AA was calculated as follows (Stein et al., 2007): SID (%) = AID (%) + {[basal ileal endogenous loss of nutrient (g/kg DMI)/concentration of nutrient in food (g/kg DM)] × 100}. The applied estimates of ileal basal endogenous losses of protein and AA in dogs were from Hendriks et al. (2002b), as determined in dogs fed a protein-free diet. For Cys, an estimate of endogenous excretion in the ileum of 239.4 µg/g DMI of a protein-free diet was used (Hendriks et al., 2015). Dietary contents of CP and AA (g/MJ ME) were determined by use of the following equation for calculation of ME in the diets: ME (kJ) = digestible protein (g) × 18.6 kJ + digestible fat (g) × 39.3 kJ + digestible carbohydrate (g) × 17.2 kJ (NRC, 2006). The digestibility values used in the equation were the SID obtained for CP and the AID (not presented) obtained for crude fat and carbohydrates in the respective diets.

**Table 1.** Least squares means of feed intake, N balance, BW, BW gain, and protein efficiency ratio (PER) in growing mink fed extruded dog foods with different protein meals ($g \cdot kg^{-0.75} \cdot d^{-1}$, unless otherwise denoted)

| | Diet[1] | | | | *P*-values | |
|---|---|---|---|---|---|---|
| Item | LBM | PM | FM | SEM | Diet | Period |
| DMI | 63.8 | 66.9 | 68.0 | 2.21 | 0.128 | 0.028 |
| ME intake,[2] $MJ \cdot kg^{-0.75} \cdot d^{-1}$ | 0.93$^{c}$ | 1.10$^{b}$ | 1.22$^{a}$ | 0.04 | <0.001 | 0.039 |
| N balance | | | | | | |
| N intake | 2.76 | 2.91 | 2.97 | 0.10 | 0.079 | 0.029 |
| Fecal N | 0.91$^{a}$ | 0.76$^{b}$ | 0.53$^{c}$ | 0.03 | <0.001 | 0.405 |
| Digested N | 1.85$^{c}$ | 2.15$^{b}$ | 2.43$^{a}$ | 0.08 | <0.001 | 0.011 |
| Urinary N | 1.19$^{ab}$ | 1.11$^{b}$ | 1.25$^{a}$ | 0.05 | 0.006 | 0.714 |
| Retained N | 0.66$^{b}$ | 1.04$^{a}$ | 1.18$^{a}$ | 0.05 | <0.001 | 0.005 |
| RNDN,[3] % | 35.2$^{b}$ | 48.6$^{a}$ | 48.5$^{a}$ | 1.56 | <0.001 | 0.006 |
| BW,[4] g | 1,127$^{c}$ | 1,174$^{b}$ | 1,242$^{a}$ | 57.49 | <0.001 | <0.001 |
| BW gain, g/d | 8.2$^{b}$ | 26.8$^{a}$ | 35.3$^{a}$ | 2.90 | <0.001 | 0.051 |
| PER[5] | 0.38$^{b}$ | 1.39$^{a}$ | 1.71$^{a}$ | 0.14 | <0.001 | 0.001 |

$^{a–c}$Least squares means in the same row with different superscripts differ ($P < 0.05$).

[1]LBM = lamb meal; PM = poultry meal; FM = fish meal.

[2]Calculated based on feed intake and ME content of the diets.

[3]RNDN = utilization of digested N for retention.

[4]Significant ($P < 0.05$) interaction effect of diet × period.

[5]PER = BW gain (g/d)/CP intake (g/d).

### *Statistical Analyses*

Statistical analyses of data were performed with the SAS 9.3 computer software (SAS Inst. Inc., Cary, NC). Data from the mink experiment were analyzed by use of the MIXED procedure according to the following model: $y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(l)} + \delta_l + \varepsilon_{ijkl}$, in which $\mu$ = the general mean, $\alpha_i$ = the fixed effect of diet, $\beta_j$ = the fixed effect of period, $(\alpha\beta)_{ij}$ = the effect of the interaction between $\alpha_i$ and $\beta_j$, $\gamma_{k(l)}$ = the random effect of animal nested within replicate (litter), $\delta_l$ = the random effect of the $l$th replicate, and $\varepsilon_{ijkl}$ = the random error component. The model was reduced in cases of nonsignificant random and interaction effects. The effect of diet on BW gain and PER values within periods was tested by use of the following model: $y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$, in which $\mu$ = the general mean, $\alpha_i$ = the fixed effect of diet, $\beta_j$ = the random effect of the $j$th replicate, and $\varepsilon_{ijk}$ = the random error component. The results are presented as least squares means, and significant differences between means ($P \leq 0.05$) were found with the PDIFF option using the Tukey adjustment. Variance is given as SEM. The results showing the effect of diet on BW gain and PER values within periods are presented as means with SD.

Data of SID in dogs were analyzed by use of the GLM procedure. Effect of diets on SID of CP and AA was tested by 1-way ANOVA, according to the following model: $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, in which $\mu$ = the general mean, $\alpha_i$ = the fixed effect of diet, and $\varepsilon_{ij}$ = the random error component. The results are expressed as least squares means, with the variance presented as SEM. Significant differences between means ($P \leq 0.05$) were found with the PDIFF option and ranked by use of Tukey adjustment. A 2-way ANOVA was performed to compare ATTD of CP and AA in mink kits with SID in dogs, using the following equation: $y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, in which $\mu$ = the general mean, $\alpha_i$ = the fixed effect of diet, $\beta_j$ = the fixed effect of species, $(\alpha\beta)_{ij}$ = the effect of interaction between $\alpha_i$ and $\beta_j$, and $\varepsilon_{ijk}$ = the random error component. A significance level of $P \leq 0.05$ was used to denote differences between the obtained least squares means.

## RESULTS

### *Bioavailability of Protein and AA in Growing Mink*

The diets were well accepted, and DMI was similar ($P = 0.128$) between diets (Table 1). Because of different ME content, the intake of ME differed ($P < 0.001$) between diets and was lowest with the LBM diet and greatest with the FM diet. A similar tendency was observed for the N intake ($P = 0.079$). As expected, excretion of fecal N differed ($P < 0.001$) between diets and was greatest for the LBM diet, intermediate for the PM diet, and lowest for the FM diet. As a result of these differences, the amount of digested N differed

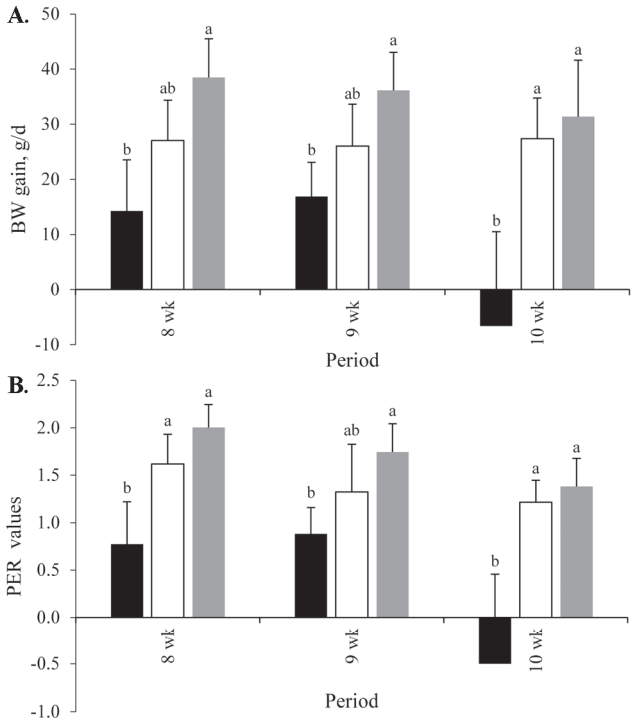


**Figure 1.** (A) Body weight gain (g/d) and (B) protein efficiency ratio (PER) in growing mink of 8, 9, and 10 wk of age fed extruded dog foods with lamb meal (■), poultry meal (□), and fish meal (▪) as protein sources. Values are means, with SD represented by vertical bars. [a,b]Means within period with different superscripts differ ($P < 0.05$).

**Table 2.** Least squares means of apparent total tract digestibility of main nutrients and AA in growing mink fed extruded dog foods with different protein meals (%)

| | Diet[1] | | | | *P*-values | |
|---|---|---|---|---|---|---|
| Item | LBM | PM | FM | SEM | Diet | Period |
| DM | 66.7[c] | 75.5[b] | 81.5[a] | 0.96 | <0.001 | 0.314 |
| CP | 66.8[c] | 73.8[b] | 82.1[a] | 0.54 | <0.001 | 0.004 |
| Crude fat | 68.1[b] | 77.0[ab] | 88.5[a] | 5.97 | 0.001 | 0.396 |
| Carbohydrates | 79.5[b] | 82.8[a] | 83.9[a] | 0.44 | <0.001 | 0.703 |
| GE | 71.0[c] | 77.8[b] | 84.4[a] | 1.89 | <0.001 | 0.522 |
| Essential AA | | | | | | |
| Arg | 82.6[c] | 87.2[b] | 91.1[a] | 0.64 | <0.001 | 0.838 |
| His | 72.8[c] | 80.5[b] | 86.2[a] | 1.20 | <0.001 | 0.221 |
| Ile | 72.7[c] | 81.3[b] | 88.4[a] | 0.84 | <0.001 | 0.023 |
| Leu | 76.7[c] | 83.3[b] | 89.6[a] | 0.71 | <0.001 | 0.258 |
| Lys | 74.1[c] | 82.1[b] | 89.9[a] | 1.11 | <0.001 | 0.212 |
| Met | 66.5[c] | 78.6[b] | 87.8[a] | 1.03 | <0.001 | 0.360 |
| Phe | 79.4[c] | 83.6[b] | 87.8[a] | 0.70 | <0.001 | 0.450 |
| Thr | 64.4[c] | 75.6[b] | 81.2[a] | 1.04 | <0.001 | 0.353 |
| Val | 69.2[c] | 77.2[b] | 85.4[a] | 1.03 | <0.001 | 0.022 |
| Nonessential AA | | | | | | |
| Ala | 77.2[c] | 81.6[b] | 87.4[a] | 0.81 | <0.001 | 0.006 |
| Asp | 42.7[c] | 58.5[b] | 75.6[a] | 2.04 | <0.001 | 0.062 |
| Cys | 3.3[b] | 32.7[a] | 36.7[a] | 3.44 | <0.001 | 0.124 |
| Glu | 77.3[c] | 84.1[b] | 90.1[a] | 0.81 | <0.001 | 0.039 |
| Gly | 74.8[c] | 77.5[b] | 82.0[a] | 1.04 | <0.001 | 0.030 |
| Hyp[2] | 54.2 | 55.7 | 49.9 | 3.37 | 0.270 | 0.095 |
| Pro | 78.5[c] | 82.1[b] | 85.2[a] | 0.72 | <0.001 | 0.028 |
| Ser | 71.1[c] | 80.3[b] | 84.5[a] | 1.12 | <0.001 | 0.010 |
| Tyr[3] | 72.1[c] | 79.2[b] | 84.9[a] | 0.99 | <0.001 | 0.375 |

[a–c]Least squares means in the same row with different superscripts differ ($P < 0.05$).

[1]LBM = lamb meal; PM = poultry meal; FM = fish meal.

[2]Hyp = hydroxyproline.

[3]Interaction effect of diet × period significant for Tyr ($P = 0.01$).

($P < 0.001$) between diets. Excretion of urinary N was greater ($P < 0.01$) for the FM diet than for the PM diet, with an intermediate amount of excretion for the LBM diet. Retention of N was less ($P < 0.001$) for the LBM diet compared with the PM and FM diets. The RNDN was also less ($P < 0.001$) for the LBM diet than for the PM or FM diet. Period affected ($P \leq 0.039$) dietary intakes of DM, ME, and N, in addition to the amount of digested N, retained N, and RNDN, all of which declined from period 1 to 3.

Average BW of the mink kits was affected by diet ($P < 0.001$) and was greatest with the FM diet and lowest with the LBM diet (Table 1). Body weight gain was less ($P < 0.001$) with the LBM diet compared with the PM and FM diets. The low average BW gain observed when animals were fed the LBM diet was caused by the weight loss in 3 out of the 4 animals fed the LBM diet in period 3 (Fig. 1a). However, the pattern with lower ($P < 0.01$) BW gain for animals fed the LBM diet compared with animals fed the FM diet was significant during period 1 and 2, with an intermediate BW gain observed for animals fed the PM diet (Fig. 1a). In period 3, BW gain was greater ($P < 0.01$) for animals fed the PM and FM diets than for animals fed the LBM diet. Because PER values are influenced by the BW gain of the animals, it was shown that the PER values were also greater ($P < 0.001$) for animals fed the PM and FM diets than for animals fed the LBM diet (Table 1). In addition, PER values were greater ($P \leq 0.011$) during periods 1 and 2 than period 3. Differences between diets in PER values during the 3 separate experimental periods showed the same pattern as for the BW gain values (Fig. 1b).

Apparent total tract digestibility of main nutrients and all AA was different ($P \leq 0.001$) between diets, except for hydroxyproline (Table 2). Generally, digestibility was lowest for the LBM diet, intermediate for the PM diet, and greatest for the FM diet. Period affected ($P \leq 0.039$) only the digestibility of CP and some of the AA, with an average decrease in digestibility values of 2.3 percentage units from period 1 to 3. An exception to this was Ser, for which digestibility in period 3 was greater ($P < 0.01$) than in period 2, with an intermediate level during period 1. For the digestibility of Tyr, there was a significant ($P = 0.01$) interaction effect between diet and period.

**Table 3.** Least squares means of standardized ileal digestibility of CP and AA in dogs fed extruded dog foods with different protein meals (%)

| | Diet[1] | | | | *P*-values |
|---|---|---|---|---|---|
| Item | LBM | PM | FM | SEM | Diet |
| CP | 71.5[b] | 80.2[a] | 87.0[a] | 2.14 | 0.002 |
| Essential AA | | | | | |
| Arg | 81.2[c] | 88.2[b] | 92.2[a] | 0.96 | <0.001 |
| His | 71.0[c] | 80.7[b] | 89.5[a] | 1.98 | <0.001 |
| Ile | 74.1[b] | 82.6[a] | 89.4[a] | 1.86 | <0.001 |
| Leu | 75.2[b] | 84.0[a] | 89.8[a] | 1.58 | <0.001 |
| Lys | 71.2[b] | 83.2[a] | 90.7[a] | 1.93 | <0.001 |
| Met | 75.8[b] | 85.6[a] | 91.5[a] | 1.82 | <0.001 |
| Phe | 80.7[b] | 86.7[a] | 91.1[a] | 1.46 | 0.002 |
| Thr | 70.6[c] | 81.7[b] | 91.0[a] | 2.22 | <0.001 |
| Val | 74.1[c] | 82.0[b] | 89.5[a] | 1.63 | <0.001 |
| Nonessential AA | | | | | |
| Ala | 76.6[b] | 83.9[ab] | 89.9[a] | 2.06 | 0.005 |
| Asp | 32.5[c] | 57.4[b] | 79.1[a] | 4.27 | <0.001 |
| Cys | 44.3[c] | 64.2[b] | 77.5[a] | 1.50 | <0.001 |
| Glu | 76.4[c] | 85.4[b] | 91.8[a] | 1.56 | <0.001 |
| Gly | 71.4[b] | 78.8[ab] | 86.5[a] | 2.91 | 0.017 |
| Pro | 79.5[b] | 85.4[ab] | 90.6[a] | 1.70 | 0.004 |
| Ser | 67.2[c] | 79.3[b] | 87.7[a] | 1.97 | <0.001 |
| Tyr | 75.4[b] | 83.3[a] | 89.4[a] | 1.58 | <0.001 |

[a–c]Least squares means in the same row with different superscripts differ ($P < 0.05$).

[1]LBM = lamb meal; PM = poultry meal; FM = fish meal.

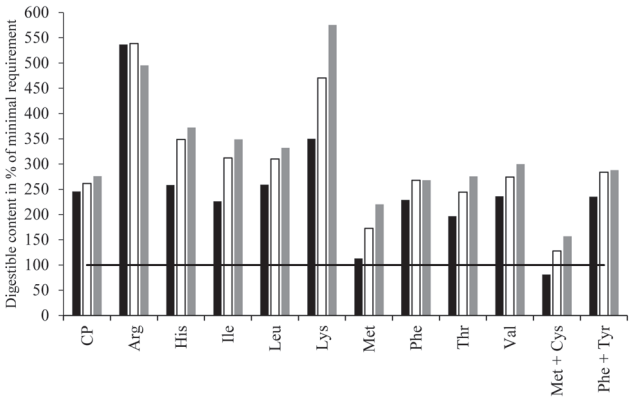


**Figure 2.** Digestible (based on standardized ileal digestibility in dogs) content of CP and AA in extruded dog foods with lamb meal (■), poultry meal (□), and fish meal (■) as protein sources (g/MJ ME), in percent of the minimal requirement for adult dogs at maintenance (set to 100%; NRC, 2006).

### *Bioavailability of Protein and AA in Adult Dogs*

Standardized ileal digestibility of CP and all AA differed between diets ($P \leq 0.017$; Table 3). The SID values were generally lowest for the LBM diet and greatest for the FM diet, with intermediate values found for the PM diet. Standardized ileal digestibility of CP and AA in dogs was compared with ATTD values found in the mink kits, and lower ($P \leq 0.003$) digestibility values were found for CP, Met, Phe, Thr, Val, Cys, Pro, and Tyr in the mink kits than in dogs. A significant ($P < 0.05$) interaction effect between diet and species was found for Asp and Gly.

The dietary contents of CP and AA (g/MJ ME) in the PM and FM diets were above the current recommended levels proposed by the NRC (2006), the Association of American Feed Control Officials (**AAFCO**; 2016), and the European Pet Food Industry Federation (**FEDIAF**; 2014) for adult dogs. For the LBM diet, however, levels of Met (0.23 g/MJ ME) and Met + Cys (0.40 g/MJ ME) were just at the levels recommended by the NRC (2006) and the AAFCO (2016) and below the FEDIAF (2014) recommended levels. When accounting for CP and AA bioavailability, based on the SID values in dogs, the digestible contents of CP and AA in the experimental diets were generally twice or more the minimal requirement for adult dogs set by the NRC (2006; Fig. 2). An exception to this was the contents of digestible Met and Met + Cys. For the LBM diet, the content of digestible Met of 0.18 g/MJ ME was slightly above the minimal requirement of 0.16 g/MJ ME, whereas the content of digestible Met + Cys of 0.25 g/MJ ME was below the minimal requirement of 0.31 g/MJ ME.

## DISCUSSION

### *Bioavailability of Protein and AA in Growing Mink*

The choice of using growing mink as an animal model for protein and AA bioavailability in dogs was based on the finding that adult male mink are suitable models for AID and SID determination of CP and AA in dogs (Tjernsbekk et al., 2014). The BW gain in mink kits is high in the period shortly after weaning, and a dietary supply of a minimum of 45% of ME from protein is recommended in the period from 8 to 10 wk of age to ensure optimal growth (Lassén et al., 2012). The protein content in the experimental diets was considerably lower than this, as protein supplied only 23% of ME. In addition, the diets markedly differed with respect to AA composition and digestibility, as protein meals of widely different quality were used as protein sources in the 3 experimental diets. The restricted CP level and the variable AA supply between diets were, therefore, expected to be reflected in the BW gain of the mink kits.

The supply of AA from the LBM diet was clearly too low to support the potential for N retention and growth in young mink kits, as reflected in lower N retention, RNDN, BW gain, and PER for this diet compared with those for the PM and FM diets. A low DMI with an average of 55.7 $g \cdot kg^{-0.75} \cdot d^{-1}$ in period 3 reduced the average values of feed intake, N balance, and growth data reported for the LBM diet and was the main cause for the significant effects between periods. However, the LBM diet showed lowest values of BW gain and PER also in period 1 and

2. Values of N retention followed the same pattern, and RNDN was around 10.0 percentage units lower for the LBM diet than for the PM and FM diets in both period 1 and period 2. For these periods, dietary intake between diets differed only with respect to ME, although a similar ME intake was observed for the LBM and PM diets in period 1 (results not shown). The results confirmed the intermediate position of the PM diet with respect to protein quality. Although the retention of N, BW gain, and PER were not significantly different between the PM and FM diets, they were numerically lower for the PM diet. Furthermore, mink kits fed the FM diet had the greatest BW. Therefore, the results for the FM diet were in accordance with the greater availability of essential AA from this diet. Studies of essential AA requirements in mink have focused mainly on Met, and a supply of 0.31 g digestible Met/MJ ME is recommended in the early growth period (Lassén et al., 2012). The digestible Met content in the LBM, PM, and FM diets was 0.17, 0.26, and 0.33 g/MJ ME, respectively, and Met was probably the first limiting AA responsible for the observed differences in N retention, BW gain, and PER between diets.

The use of mink kits as models in growth studies for assessment of protein quality in foods for dogs is a new approach, as others have used rats (Burns et al., 1982; Hegedűs et al., 1998) and chickens (Dust et al., 2005; Folador et al., 2006; Cramer et al., 2007). Only Burns et al. (1982) performed a comparative experiment with rats and dogs. Based on the N balance data, BW gain, and PER obtained in the present study, it was apparent that the mink kits responded according to the 3 diets in accordance with the different AA supply, as determined by the first limiting AA of the respective diets. Therefore, the protein content constituting 23% of ME in the experimental diets was optimal to reveal the difference in protein quality between the diets. This implies that growth studies involving mink kits can provide valuable information with respect to possible limitations in the supply of bioavailable AA of extruded dog foods, as the contents of CP and ME in the experimental diets were within the range normally found in commercial dry dog foods (NRC, 2006).

### *Bioavailability of Protein and AA in Adult Dogs*

As expected, the difference in protein quality between the experimental diets was also reflected in the SID values obtained from the dogs. Standardized ileal digestibility is preferred for determination of AA bioavailability in dog foods (Hendriks et al., 2013, 2015), and adult mink can be used to obtain reliable estimates for SID of CP and AA in dogs (Tjernsbekk et al., 2014). However, the present study revealed that mink kits seem less suitable than adult mink as models for SID determination in dogs, based on the lower ATTD of CP and several AA observed in mink kits compared with SID in dogs. The lower digestive capacity in kits can be explained by the lower proteolytic activity compared with that of adult mink (Elnif et al., 1988).

Adult dogs have a lower protein and AA requirement than mink kits (NRC, 2006; Lassén et al., 2012), and contents of most AA in all 3 experimental diets were well above the requirements in adult dogs when SID was accounted for. However, the inadequate content of digestible Met + Cys in the LBM diet implies that the safety margin for sufficient AA supply was smaller with the LBM diet than with the PM and FM diets, in line with the results obtained in growing mink.

### *Importance of Protein Quality Assessment in Commercial Dog Foods*

The rendered animal meals selected as protein sources in the present study substantially varied with respect to AA composition and protein digestibility level. Such a variation in quality of protein sources is common and generally can be related to differences in the raw materials used and the processing conditions (Johnson and Parsons, 1997; Johnson et al., 1998; Wang and Parsons, 1998; Hendriks et al., 2002a; Cramer et al., 2007). The variability of AA composition and availability in protein sources are reflected in the protein quality of commercial dog foods, and Hendriks et al. (2013) found that AID of CP in 5 commercial dog foods varied from 66.2 to 83.3% in dogs. Despite the variation in CP and AA digestibility between different diets, declaration of CP and AA availability is not required to claim nutritional adequacy of a dog food. Therefore, it is sufficient that the nutrient content meets the Dog Food Nutrient Profiles established by the AAFCO for a commercial dog food to be labeled as "complete and balanced" when sold in the United States (AAFCO, 2016). This practice has been criticized for its inaccuracy (Morris and Rogers, 1994). The criticism by Morris and Rogers (1994) was supported by the results of the present study, which demonstrated how the protein quality of diets with similar CP content can vary. All diets in the present study would have passed the Dog Food Nutrient Profiles of the AAFCO (2016) when considering dietary contents of CP and AA. Still, the content of digestible Met + Cys in the LBM diet was below the minimal requirement set for adult dogs (NRC, 2006). This is of concern and emphasizes that knowledge of CP and AA content gives only limited information with respect to AA availability. Similarly, Huber et al. (1986) found that variation in protein digestibility was an important factor for the differences in growth response in puppies fed foods with similar label guarantees. Also, Huber et al. (1991) demonstrated that the practice of nutritional

evaluation only by chemical content is inadequate for assessment of the feeding value of dog food.

The nutrient profiles or recommendations set by the NRC, AAFCO, and FEDIAF include the minimal requirement estimates (NRC, 2006) in addition to a safety margin accounting for the bioavailability of nutrients. As presented herein, dietary content of Met + Cys in the LBM diet was in agreement with the recommendation set by the NRC (2006) and the AAFCO (2016), whereas the digestible content of Met + Cys was below the minimal requirement for adult dogs (NRC, 2006). This suggests that the estimate used by the NRC (2006) and the AAFCO (2016) for bioavailability of Met + Cys is too high. This agrees with the results of Hendriks et al. (2015), who showed that the estimated bioavailability used by the NRC, AAFCO, and FEDIAF was too high for most of the AA.

### *Conclusion*

As shown by the results of the present study, differences in protein quality between foods of similar protein content clearly affected N retention, BW gain, and PER in mink kits. These results imply that growing mink readily respond to limitations in the supply of bioavailable AA from extruded dog foods and suggest that growth studies with mink kits can provide valuable information in protein quality assessment of such foods. Differences in AA composition and digestibility between the protein sources were the main factors affecting protein quality of the experimental diets. Information on these factors is crucial to ensure nutritional adequacy of dog foods and to be able to compare the protein quality between foods.



## LITERATURE CITED


Association of American Feed Control Officials (AAFCO). 2016. 2016 Official publication. AAFCO, Atlanta, GA.

Burger, I. H. 1994. Energy needs of companion animals: Matching food intakes to requirements throughout the life cycle. J. Nutr. 124:2584S–2593S.

Burns, R. A., M. H. LeFaivre, and J. A. Milner. 1982. Effects of dietary protein quantity and quality on the growth of dogs and rats. J. Nutr. 112:1843–1853.

Council of Europe. 1986. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. European Treaty Series, no. 123. Council of Europe, Strasbourg, France.

Cramer, K. R., M. W. Greenwood, J. S. Moritz, R. S. Beyer, and C. M. Parsons. 2007. Protein quality of various raw and rendered by-product meals commonly incorporated into companion animal diets. J. Anim. Sci. 85:3285–3293. doi:10.2527/jas.2006-225

Dust, J. M., C. M. Grieshop, C. M. Parsons, L. K. Karr-Lilienthal, C. S. Schasteen, J. D. Quigley III, N. R. Merchen, and G. C. Fahey Jr. 2005. Chemical composition, protein quality, palatability, and digestibility of alternative protein sources for dogs. J. Anim. Sci. 83:2414–2422.

Elnif, J., N. E. Hansen, K. Mortensen, and H. Sørensen. 1988. Production of digestive enzymes in mink kits. In: B. D. Murphy and D. B. Hunter, editors, Biology, pathology and genetics of fur bearing animals. Proc. IV Int. Congr. Fur Anim. Prod., Ontario, Canada. p. 320–326.

European Commission. 1998. Commission Directive 98/64/EC of 3 September 1998 establishing community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feedingstuffs and amending, Directive 71/393/EEC. Off. J. Eur. Communities 257:14–28.

European Pet Food Industry Federation (FEDIAF). 2014. Nutritional guidelines for complete and complementary pet food for cats and dogs. FEDIAF, Brussels, Belgium.

Folador, J. F., L. K. Karr-Lilienthal, C. M. Parsons, L. L. Bauer, P. L. Utterback, C. S. Schasteen, P. J. Bechtel, and G. C. Fahey Jr. 2006. Fish meals, fish components, and fish protein hydrolysates as potential ingredients in pet foods. J. Anim. Sci. 84:2752–2765. doi:10.2527/jas.2005-560

Hegedűs, M., S. Fekete, L. Solti, E. Andrásofszky, and L. Pallós. 1998. Assessment of nutritional adequacy of the protein in dog foods by trials on growing rats. Acta Vet. Hung. 46:61–70.

Hendriks, W. H., E. J. Bakker, and G. Bosch. 2015. Protein and amino acid bioavailability estimates for canine foods. J. Anim. Sci. 93:4788–4795. doi:10.2527/jas.2015-9231

Hendriks, W. H., C. A. Butts, D. V. Thomas, K. A. C. James, P. C. A. Morel, and M. W. A. Verstegen. 2002a. Nutritional quality and variation of meat and bone meal. Asian-Australas. J. Anim. Sci. 15:1507–1516. doi:10.5713/ajas.2002.1507

Hendriks, W. H., K. Sritharan, and S. M. Hodgkinson. 2002b. Comparison of the endogenous ileal and faecal amino acid excretion in the dog (*Canis familiaris*) and the rat (*Rattus rattus*) determined under protein-free feeding and peptide alimentation. J. Anim. Physiol. Anim. Nutr. 86:333–341. doi:10.1046/j.1439-0396.2002.00391.x

Hendriks, W. H., D. G. Thomas, G. Bosch, and G. C. Fahey Jr. 2013. Comparison of ileal and total tract nutrient digestibility of dry dog foods. J. Anim. Sci. 91:3807–3814. doi:10.2527/jas.2012-5864

Huber, T. L., D. P. Laflamme, L. Medleau, K. M. Comer, and P. M. Rakich. 1991. Comparison of procedures for assessing adequacy of dog foods. J. Am. Vet. Med. Assoc. 199:731–734.

Huber, T. L., R. C. Wilson, and S. A. McGarity. 1986. Variations in digestibility of dry dog foods with identical label guaranteed analysis. J. Am. Anim. Hosp. Assoc. 22:571–575.

Johnson, M. L., and C. M. Parsons. 1997. Effects of raw material source, ash content, and assay length on protein efficiency ratio and net protein ratio values for animal protein meals. Poult. Sci. 76:1722–1727. doi:10.1093/ps/76.12.1722

Johnson, M. L., C. M. Parsons, G. C. Fahey Jr., N. R. Merchen, and C. G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. J. Anim. Sci. 76:1112–1122.

Lassén, T. M., A.-H. Tauson, Ø. Ahlstrøm, K. Rouvinen-Watt, P. Sandbøl, N. Koskinen, and M. L. E. Christiansen. 2012. Energy and main nutrients in feed for mink and foxes. In: A. Skrede, editor, Nordic Association of Agricultural Scientists, subsection for fur animals, report no. 63. 2nd ed. SL Grafik, Copenhagen, Denmark.

McCleary, B. V., V. Solah, and T. S. Gibson. 1994. Quantitative measurement of total starch in cereal flours and products. J. Cereal Sci. 20:51–58. doi:10.1006/jcrs.1994.1044

Morris, J. G., and Q. R. Rogers. 1994. Assessment of the nutritional adequacy of pet foods through the life cycle. J. Nutr. 124:2520S–2534S.

NRC. 2006. Nutrient requirements of dogs and cats. Natl. Acad. Press, Washington, DC.

Norwegian Ministry of Agriculture and Food. 1996. Regulation 1996-01-15-23: Regulation on Animal Experimentation. https://norecopa.no/files/statute.html (Accessed 26 February 2016.)

Norwegian Ministry of Agriculture and Food. 2009. Law-2009-06-19-97: Animal Welfare Act. https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/ (Accessed 26 February 2016.)

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172–180. doi:10.2527/jas.2005-742

Tjernsbekk, M. T., A.-H. Tauson, and Ø. Ahlstrøm. 2014. Ileal, colonic and total tract nutrient digestibility in dogs (*Canis familiaris*) compared with total tract digestibility in mink (*Neovison vison*). Arch. Anim. Nutr. 68:245–261. doi:10.1080/1745039X.2014.915137

Wang, X., and C. M. Parsons. 1998. Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. Poult. Sci. 77:834–841. doi:10.1093/ps/77.6.834

# Paper III

**Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility**

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# Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility

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## Summary

Protein quality was evaluated for mechanically separated chicken meat (MSC) and salmon protein hydrolysate (SPH), and for extruded dog foods where MSC or SPH partially replaced poultry meal (PM). Apparent total tract digestibility (ATTD) of crude protein (CP) and amino acids (AA) in the protein ingredients and extruded foods was determined with mink (*Neovison vison*). The extruded dog foods included a control diet with protein from PM and grain, and two diets where MSC or SPH provided 25% of the dietary CP. Nutrient composition of the protein ingredients varied, dry matter (DM) was 944.0, 358.0 and 597.4 g/kg, CP; 670.7, 421.2 and 868.9 g/kg DM, crude fat; 141.4, 547.8 and 18.5 g/kg DM and ash; 126.4, 32.1 and 107.0 g/kg DM for PM, MSC and SPH, respectively. The content of essential AA (g/100 g CP) was more than 10.0 percentage units lower in SPH than in PM and MSC. The ATTD of CP differed ($p < 0.001$) between protein ingredients, and was 80.9, 88.2 and 91.3% for PM, MSC and SPH, respectively. The ATTD of total AA was lowest ($p < 0.001$) for PM, and similar ($p > 0.05$) for MSC and SPH. In the extruded diets, the expected higher ATTD of CP and AA from replacement of PM with MSC or SPH was not observed. The ATTD of CP was determined to 80.3, 81.3 and 79.0% for the PM, MSC and SPH extruded foods, respectively. Furthermore, the ATTD of several AA was numerically highest for the PM diet. Possibly, extrusion affected ATTD of the diets differently due to different properties and previous processing of the three protein ingredients.



**Keywords:** Animal protein sources, extrusion, mink, pet food, protein quality

## Introduction

Animal-based protein sources are widely used in dog food, and are most often included as rendered animal by-product meals in extruded dry foods. However, a growing trend is inclusion of ingredients with human-grade quality and replacement of rendered animal meals with raw animal protein sources such as mechanically separated meat (Buff et al., 2014; Carter et al., 2014). Another type of protein sources of current interest for use in pet food is animal protein hydrolysates, which are commonly made by enzymatic hydrolysis of proteins into smaller peptides, followed by fat removal and partial dehydration (Kristinsson and Rasco, 2000). As reviewed by Martínez-Alvarez et al. (2015), animal protein hydrolysates are added to pet diets as palatants, hypoallergenic ingredients and possibly also as nutraceuticals. Furthermore, protein hydrolysates provide AA that are easily absorbed in the small intestine (Gilbert et al., 2008; Martínez-Alvarez et al., 2015).

Raw animal protein sources and animal protein hydrolysates are generally considered as high-quality protein ingredients. Despite the high interest, however, studies evaluating the protein quality of such ingredients when used in extruded dog foods are scarce. Cramer et al. (2007) reported a higher protein quality of raw, freeze-dried animal protein ingredients than of rendered animal meals evaluated in various chick assays, but did not incorporate the ingredients into extruded dog foods. By use of caecectomised roosters, Folador et al. (2006) reported a high, true digestibility of amino acids (AA) in a salmon protein hydrolysate (SPH) product. A high palatability of an extruded dog food containing the SPH was found when offered to dogs, but AA digestibility in the food was not determined (Folador et al., 2006). Murray et al. (1997), however, compared apparent ileal digestibility of crude protein (CP) and AA in dogs fed extruded diets where protein was partially provided from either fresh beef or rendered beef meat and bone meal,

or from fresh or rendered poultry by-products. Digestibility values were similar for the diets containing the beef products, whereas the raw poultry by-products increased the CP and AA digestibility of extruded dog food as compared with the rendered poultry by-products (Murray et al., 1997).

Raw mechanically separated chicken meat (MSC) and SPH are two typical raw animal protein and animal protein hydrolysate ingredients, respectively, that are available for use in pet food. The main objective of the present study was to evaluate the protein quality of MSC and SPH as ingredients, and as part of extruded dog foods where MSC or SPH partially replaced protein from a rendered poultry meal (PM). It was hypothesised that the replacement would improve CP and AA digestibility as compared with a standard PM control food. Analysis of AA composition and measures of AA digestibility are essential for evaluation of protein quality in both ingredients and complete foods for dogs. Considering digestibility in dogs, apparent and standardised ileal digestibility of CP and AA can be estimated by determination of apparent total tract digestibility (ATTD) in adult mink (*Neovison vison*) (Tjernsbekk et al., 2014). Thus, compositional analyses and ATTD of CP and AA obtained with mink were used to determine the protein quality of the individual ingredients and the extruded dog foods.

## Materials and Methods

The experimental procedures were approved by the Norwegian Animal Research Authority and followed institutional and national guidelines for the care and use of animals (the Norwegian Animal Welfare Act and the Norwegian Regulation on Animal Experimentation).

### Protein ingredients

Nutrient composition of PM, MSC and SPH is shown in Table 1. The MSC consisted of chicken meat separated from broiler carcasses. The SPH was produced from a blend of viscera, heads and frames of salmon, which was hydrolysed by use of a commercial enzyme (not disclosed). For determination of ATTD of CP and AA in the PM, MSC and SPH before further processing into extruded foods, they were applied as sole protein sources in wet diets fed to mink (Table 2). The protein ingredients were mixed with standard ingredients including precooked corn starch, cellulose powder, soybean oil and a supplement of vitamins and minerals. Water was added to make a proper consistency.

**Extruded diets**

Three extruded dog foods were produced (Table 3). In one diet, PM was used as the main protein source, contributing with around 73.5% of the protein content. The remaining dietary protein originated from the grain ingredients. In the two other diets, either the MSC or the SPH provided around 25% of the protein content by partial substitution of the PM. The inclusion rates of the MSC and SPH products were limited to 25% because of the high water contents, and also high fat content with the MSC, which would put risk of poor processing conditions and subsequently poor product quality with a higher inclusion level. The diets were formulated to contain similar levels of CP and crude fat. The three diets were produced at Centre for Feed Technology, Norwegian University of Life Sciences, Ås, Norway. The PM diet was produced at an earlier occasion in a study reported by Tjernsbekk et al. (2014), and included as a control in the present study. All the dry ingredients in the PM and SPH diets, respectively, were mixed in a Tatham Forberg twin-shaft mixer (1992 OB-1078, 400 l, Rochdale, UK). The SPH was added by spraying it into the mixer. To mix the ingredients of the MSC diet, only small batches were prepared at a time, to ensure that the MSC was properly mixed with the remaining dry

ingredients. For this purpose, a small twin shaft paddle mixer of 40 l was used (IdeCon, Porsgrunn, Norway). Diets were produced by use of a Bühler two-stage preconditioner (BCTC-10, Uzwil, Switzerland) and a Bühler twin-screw extruder (BCTG 62, L:D 20, Uzwil, Switzerland). Prior to extrusion, the feed mash of the PM, MSC and SPH diets had a crude fat content of 78.9, 116.2 and 63.0 g/kg and a moisture content of 93.3, 293.5 and 140.9 g/kg, respectively. Steam and water was added during processing of the PM and SPH diets to a total moisture content of around 30%. Temperature at the outlet of the conditioner was around 31°C for the MSC diet and above 90°C for the PM and SPH diets. Moisture was not added to the conditioner for production of the MSC diet, as that caused blocking. Temperature in the third section of the extruder varied from 131-132, 102-105 and 108-119°C, and temperature at the die of the extruder varied from 127-128, 107-112 and 94-107°C for the PM, MSC and SPH diet, respectively. Specific mechanical energy (SME) was around 56 Wh/kg for the PM diet and 34 Wh/kg for the MSC and SPH diets. The PM extrudate was pre-dried in a Bühler fluid-bed dryer (OTW 50 05TSR2, Uzwil, Switzerland) prior to drying in rectangular batch drying cabinets (of around 0.3 $m^2$, holding up to 40 kg), mounted with 10-kW heated fans. The MSC and SPH extrudates were dried in a NMBU-FORBERG fluid bed dryer (Forberg, Oslo, Norway). Poultry fat was coated onto the extrudates in a vacuum coater (Dinnissen, Sevenum, Holland). The diets were packed in airtight bags and frozen-stored until use. Prior to feeding of the mink, the extruded diets were added with water in a ratio of 1:2, to obtain a suitable consistency and to avoid spilling.

**Animals**

The digestibility experiment was performed in a laboratory at the research farm at Norwegian University of Life Sciences, Ås, Norway, by use of adult male mink (*Neovison vison*). The

131 animals were two years old, and body weight averaged to 2.2 ± 0.2 kg. Four mink were allocated
132 to each experimental diet (three wet diets for determination of ATTD of CP and AA in protein
133 ingredients, and three extruded dog foods with the respective protein ingredients). During the
134 experiment, the animals were kept in metabolic cages for total collection of faeces and feed
135 residuals, and for separation of urine. The experimental diets were fed for seven days, including
136 a three day adaptation period prior to four days of accurate feed intake registration and total
137 collection of faeces. The daily ratios were approximately 60 g dry matter (DM), which met the
138 daily metabolisable energy requirement of around 530 kJ/kg body weight$^{0.75}$ (Chwalibog et al.,
139 1980). The rations were weighed at the beginning of the experiment and stored at -20°C, and
140 thawed at room temperature one day before use. Collected faeces from each animal was frozen-
141 stored (-20°C). Feed was offered once a day, and drinking water was available at all times. When
142 the experimental period ended, the total amount of the individually collected faeces was freeze-
143 dried, weighed, ground and sifted to remove hairs, followed by chemical analyses.

144 **Chemical analyses**

145 The protein ingredients and the extruded diets were analysed for DM, ash, CP, crude fat and AA.
146 The extruded diets were also analysed for starch. Samples of freeze-dried faeces were analysed
147 for CP and AA. Dry matter was determined by drying of the samples to constant weight at
148 103°C, whereas samples were combusted at 550°C for 10 hours for determination of ash.
149 Nitrogen was analysed by use of a Kjeltec 1015 Digester at 420°C and a Kjeltec Auto 2400/2600
150 (Foss Tecator AB, Höganäs, Sweden), and CP was determined as N × 6.25. Crude fat was
151 determined by extraction with petroleum ether and acetone in an Accelerated Solvent Extractor
152 (ASE 200) from Dionex (Sunnyvale, CA, USA). Analysis of starch followed the description of
153 McCleary et al. (1994), whereas the content of total carbohydrates was calculated by difference:

154 carbohydrates = DM – (CP + crude fat + ash). The content of AA was analysed according to the
155 European Commission Directive 98/64/EC (EC, 1998).

156 **Calculations and statistical analyses**

157 The ATTD was calculated by use of the following equation: ATTD (%) = ((nutrient consumed
158 (g) – nutrient in faeces (g))/nutrient consumed (g)) × 100.

159 The SAS 9.4 computer software (SAS Institute Inc., Cary, NC, USA) was used for statistical
160 analyses. Data were analysed by use of the GLM procedure. The effect of protein ingredient or
161 extruded diet, respectively, on ATTD of CP and AA was tested by one-way ANOVA according
162 to the following model: $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where $\mu$ = general mean, $\alpha_i$ = fixed effect of protein
163 ingredient/extruded diet and $\varepsilon_{ij}$ = random error component. The results are expressed as least-
164 square means, with the variance given as pooled standard error of the means (SEM). Significant
165 differences between means ($p \leq 0.05$) were found and ranked by use of the PDIFF option with
166 Tukey adjustment.

167 **Results**

168 ***Nutrient composition of protein ingredients and extruded diets***

169 The DM content varied between the protein ingredients and was as expected highest for the PM
170 (Table 1). On a DM basis, CP was the major constituent of PM and SPH, whereas the content of
171 crude fat was low, especially for the SPH. For the MSC, the reverse protein:fat relationship was
172 observed, as the crude fat content exceeded that of CP. Ash content was at similar and
173 substantially higher levels in PM and SPH, than in MSC. Calculated content of carbohydrates
174 was negligible in all three ingredients. The AA composition in PM and MSC was very similar,
175 with a difference of only 0.6 percentage units or less for most of the AA. However, total AA

(TAA) in percent of CP was lower in the MSC. Compared with PM and MSC, SPH had a lower level of all essential AA, and the content of total essential AA (TEAA) was more than 10.0 percentage units lower than in PM and MSC. The content of total non-essential AA (TNEAA) was similar in MSC and SPH, whereas PM had a higher TNEAA content.

The contents of DM and main nutrients in the MSC and SPH extruded diets were very similar (Table 4). The PM diet had only a slightly lower DM, CP, crude fat and starch content than the other diets, and a little higher ash content. Differences in AA composition between diets were small, but the SPH diet had a slightly lower concentration of TEAA than the PM and MSC diets.

***Digestibility experiments***

The diets used for determination of ATTD of CP and AA in the protein ingredients were well accepted by the animals, as feed intake on average was more than 99% of the feed ration offered. The protein ingredients differed ($p < 0.001$) with respect to ATTD of CP, which was highest for the SPH and lowest for the PM (Table 5). The ATTD of all, individual AA was affected ($p \leq 0.024$) by protein ingredient. The ATTD of TEAA, TNEAA and TAA was lowest ($p < 0.001$) for the PM. For the individual essential AA, except for Arg and His, the ATTD in the MSC and SPH was on the same level. The ATTD of Cys was markedly lower for the PM and SPH than the MSC, and PM also had a low ATTD of Asp.

Food consumption was high with the extruded diets also, with an average feed intake of more than 99% of the food offered. One of the animals receiving the SPH diet was excluded from the digestibility calculations, as an unusually high amount of faeces indicated some digestive disorder. The difference in ATTD of CP and AA observed for the protein ingredients was not reflected with the respective extruded diets in which they were included. The ATTD of

CP tended ($p = 0.053$) to be different between the three diets, but ATTD of TEAA, TNEAA and TAA was similar ($p > 0.05$) (Table 6). For the individual AA, ATTD of Arg, His, Met, Thr, Ala, Cys, Pro and Ser was similar ($p > 0.05$) among diets. The ATTD of Ile, Leu and Phe was higher ($p \leq 0.005$) for the PM diet than the SPH diet, but not significantly different from the MSC diet ($p > 0.05$). In addition, ATTD of Tyr was highest for the PM diet ($p \leq 0.002$). For the other AA, ATTD was numerically highest for the MSC diet, but in most cases not significantly different ($p > 0.05$) from either the PM or SPH diet.

## Discussion

As expected, the nutrient composition of the protein ingredients applied in the present study varied. One of the most notable differences between the protein ingredients was the high fat content of the MSC. As reviewed by Trindade et al. (2004), mechanically separated meat from poultry generally has a higher fat content and a lower protein content than meat fillets, and the nutrient composition of the MSC was comparable to the nutrient composition of a similar product reported by Rivera et al. (2000). The low fat content of the PM, and the SPH especially, coincided with the extraction of fat conducted in processing of such products, whereas the deboning process of the MSC was reflected in the low ash content of this ingredient. Considering the AA composition, TAA made up only 80.6% of the CP content in the SPH evaluated herein. Similar, low levels of TAA in SPH ingredients have also been reported by others (Folador et al., 2006; Opheim et al., 2015), and it therefore seems that SPH generally contains a certain amount of non-protein nitrogen. The lower content of essential AA in the SPH than the PM and MSC resulted in a lower ratio between TEAA and TNEAA of only 0.6 for the SPH. A correspondingly low ratio for a SPH ingredient was also found by Folador et al. (2006).

A lower protein quality of rendered animal meals than for raw, freeze-dried animal by-products has been reported (Cramer et al., 2007). In the latter study, true digestibility of TAA in intact roosters varied from 79.2 to 84.8% for rendered meals, and from 90.3 to 95.5% for raw by-products. The findings of Cramer et al. (2007) can be supported by digestibility values reported from several other studies where caecectomised roosters have been used (Johnson et al., 1998; Folador et al., 2006; Faber et al., 2010). Johnson et al. (1998) found true TAA digestibility to vary from 65.5 to 84.0% for different rendered animal meals, whereas corresponding values for dried, good-quality cuts of different meat and fish substrates has been found to vary from 86.9 to 90.4% (Faber et al., 2010). In SPH, a high, true digestibility of TAA of 94.2% has been found by Folador et al. (2006). In the present study, a similar difference with a lower ATTD of CP and AA in the rendered PM than the MSC and SPH ingredients was found.

Based on the ATTD of CP in the PM diet and the MSC and SPH ingredients, the ATTD of CP could be expected to increase with 2.0 and 2.8 percentage units when MSC or SPH, respectively, partially replaced PM and supplied 25% of the CP in the extruded foods. However, the expected increase in ATTD of CP did not occur, and the observed ATTD values were 1.0 and 4.1 percentage units lower than expected for the MSC and SPH diets, respectively. Similar results were found for the AA, and the observed ATTD values were on average 1.6 ± 2.0 percentage units lower than expected for the MSC diet and 2.3 ± 1.2 percentage units lower than expected for the SPH diet. The ATTD of Cys of 57.2 and 55.1% for the MSC and SPH diets, respectively, was especially lower than the expected values of 65.5 and 61.7%. Use of different batches might have affected the results slightly, as the batches of PM, MSC and SPH used to determine digestibility of the protein ingredients were different from the batches used in the extruded foods. In addition, different batches of PM and grain were applied in the extruded PM

244 control diet than the extruded MSC and SPH diets. However, the batch-to-batch variation in
245 nutrient content and digestibility was considered small, as all ingredients used in the diets are
246 subject to a strict quality control prior to incorporation in dog food. Thus, the numerically higher
247 ATTD values observed for the PM diet than the MSC or SPH diets for CP and several AA were
248 unexpected, despite the use of different batches.

249 Processing of the diets might have affected the ATTD levels of CP and AA observed in
250 the present study. Heat treatment, like extrusion cooking, can cause chemical changes to proteins
251 known to reduce AA availability, due to reactions such as cross-linkages between AA and
252 Maillard reactions (Björck and Asp, 1983; Papadopoulos, 1989). Several studies have, however,
253 shown negligible or only small, negative effects of extrusion on CP and AA digestibility in diets
254 based on animal protein sources (Opstvedt et al., 2003; Ljøkjel et al., 2004; Romarheim et al.,
255 2005; Lankhorst et al., 2007; de-Oliveira et al., 2012; van Rooijen, 2015). Of the individual AA,
256 true, total tract digestibility of Cys has been reported to be most negatively affected by extrusion,
257 by up to 6.8 percentage units in diets fed to mink (Ljøkjel et al., 2004). The observed reduction
258 in ATTD of Cys from the expected values in the MSC and SPH diets could therefore be an
259 indicator of a reducing effect of the food processing on AA digestibility of these diets, but
260 dietary Cys levels were low, and analytical inaccuracy may also have contributed to the
261 difference. Although the negative effects of extrusion on CP and AA digestibility generally could
262 be considered as small, the results of others have indicated that animal protein ingredients can be
263 differently affected during extrusion (Opstvedt et al., 2003; van Rooijen, 2015). Opstvedt et al.
264 (2003) reported that the total process of extrusion, drying and fat coating reduced true, total tract
265 digestibility of CP of fish-meal based diets fed to mink by up to 2.4 percentage units, and CP
266 digestibility of the fish meal with the inherently highest quality decreased the most. van Rooijen

(2015) suggested that protein hydrolysates are more exposed to the Maillard reaction during extrusion than intact protein. This was supported by the findings, as extrusion significantly lowered *in vitro* CP digestibility of a diet containing fish protein hydrolysate from 94.1 to 93.1%, and reduced the content and *in vitro* digestibility of both total and reactive Lys. Corresponding values for a diet based on a PM was unaltered by extrusion (van Rooijen, 2015). Tran (2008) also reported a greater reduction in total and reactive Lys for chicken meat than for PM and fish meal after extrusion of the single ingredients only. Based on the results of others (Opstvedt et al., 2003; Tran, 2008; van Rooijen, 2015), it could therefore be speculated that the extrusion process had a greater negative effect on ATTD of CP and AA in the MSC and SPH than the rendered PM evaluated herein, due to the different nature of the protein ingredients. Possibly, untreated ingredients like the MSC, and hydrolysates like SPH with high levels of short peptides, are more easily exposed to chemical changes involving AA during extrusion than already heat-treated, rendered ingredients.

Besides the study reported by van Rooijen (2015), reports on the effects of extrusion on protein digestibility in dog foods containing raw animal protein sources or animal protein hydrolysates are scarce. However, a similar *in vitro* CP digestibility of 48-50% has been reported both prior to and after extrusion at different processing conditions for a diet containing 10.0% of a MSC product (Lankhorst et al., 2007). Differences in diet formulations and methods of digestibility determination might explain the deviating results between the present study and the study reported by Lankhorst et al. (2007). Murray et al. (1997) reported an apparent ileal digestibility of CP of 80.4 and 82.8% in dogs fed extruded diets containing fresh beef or fresh poultry as protein sources, respectively, but digestibility of the protein ingredients or diets prior to extrusion was not reported. Reports focusing on animal protein hydrolysates as protein sources

290 in extruded dog foods have, to our knowledge, reported only ATTD of CP in dogs, and the
291 effects of extrusion were not adressed (Verlinden et al., 2006; Zinn et al., 2009). The results of
292 the present study therefore warrants further, more controlled, studies focusing on the effects of
293 extrusion processing on CP and AA digestibility of raw animal protein sources and animal
294 protein hydrolysates incorporated into extruded dog foods.

295 **Conclusion**

296 The MSC and SPH ingredients had a higher ATTD of CP and AA than PM when used in wet,
297 untreated diets. In extruded foods, the expected contribution to a higher ATTD of CP and AA
298 when MSC and SPH partially replaced PM and provided 25% of the dietary CP was not
299 observed. Possibly, extrusion affected ATTD of CP and AA in the diets differently due to
300 differences in properties and previous processing of the protein ingredients. Further studies are
301 warranted to assess the effects of the extrusion process on protein quality of raw animal protein
302 ingredients and animal protein hydrolysates.

## References


Björck, I.; Asp, N.-G., 1983: The effects of extrusion cooking on nutritional value - A literature review. *Journal of Food Engineering* **2**, 281-308.

Buff, P.R.; Carter, R.A.; Bauer, J.E.; Kersey, J.H., 2014: Natural pet food: A review of natural diets and their impact on canine and feline physiology. *Journal of Animal Science* **92**, 3781-3791.

Carter, R.A.; Bauer, J.E.; Kersey, J.H.; Buff, P.R., 2014: Awareness and evaluation of natural pet food products in the United States. *Journal of the American Veterinary Medical Association* **245**, 1241-1248.

Chwalibog, A.; Glem-Hansen, N.; Henckel, S.; Thorbek, G., 1980: Energy metabolism in adult mink in relation to protein-energy levels and environmental temperature. In: Mount, L.E. (ed.), *Proceedings of the 8th Symposium on Energy Metabolism*. Butterworths, London. European Association for Animal Production Publication No. 26, 283-286.

Cramer, K.R.; Greenwood, M.W.; Moritz, J.S.; Beyer, R.S.; Parsons, C.M., 2007: Protein quality of various raw and rendered by-product meals commonly incorporated into companion animal diets. *Journal of Animal Science* **85**, 3285-3293.

EC, 1998: Commission Directive 98/64/EC of 3 September 1998 establishing community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in

feedingstuffs and amending, Directive 71/393/EEC. Official Journal of the European Communities **257**:14-28.

Faber, T.A.; Bechtel, P.J.; Hernot, D.C.; Parsons, C.M.; Swanson, K.S.; Smiley, S.; Fahey, G.C.Jr, 2010: Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays. *Journal of Animal Science* **88**, 1421-1432.

Folador, J.F.; Karr-Lilienthal, L.K.; Parsons, C.M.; Bauer, L.L.; Utterback, P.L.; Schasteen, C.S.; Bechtel, P.J.; Fahey, G.C.Jr, 2006: Fish meals, fish components, and fish protein hydrolysates as potential ingredients in pet foods. *Journal of Animal Science* **84**, 2752-2765.

Gilbert, E.R.; Wong, E.A.; Webb, K.E.Jr, 2008: Board-invited review: Peptide absorption and utilization: Implications for animal nutrition and health. *Journal of Animal Science* **86**, 2135-2155.

Johnson, M.L.; Parsons, C.M.; Fahey, G.C.Jr; Merchen, N.R.; Aldrich, C.G., 1998: Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. *Journal of Animal Science* **76**, 1112-1122.

Kristinsson, H.G.; Rasco, B.A., 2000: Fish protein hydrolysates: Production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition* **40**, 43-81.

Lankhorst, C.; Tran, Q.D.; Havenaar, R.; Hendriks, W.H.; van der Poel, A.F.B., 2007: The effect of extrusion on the nutritional value of canine diets as assessed by *in vitro* indicators. *Animal Feed Science and Technology* **138**, 285-297.

Ljøkjel, K.; Sørensen, M.; Storebakken, T.; Skrede, A., 2004: Digestibility of protein, amino acids and starch in mink (*Mustela vison*) fed diets processed by different extrusion conditions. *Canadian Journal of Animal Science* **84**, 673-680.

Martínez-Alvarez, O.; Chamorro, S.; Brenes, A., 2015: Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review. *Food Research International* **73**, 204-212.

McCleary, B.V.; Solah, V.; Gibson, T.S., 1994: Quantitative measurement of total starch in cereal flours and products. *Journal of Cereal Science* **20**, 51-58.

Murray, S.M.; Patil, A.R.; Fahey, G.C.Jr; Merchen, N.R.; Hughes, D.M., 1997: Raw and rendered animal by-products as ingredients in dog diets. *Journal of Animal Science* **75**, 2497-2505.

de-Oliveira, L.D.; de Carvalho Picinato, M.A.; Kawauchi, I.M.; Sakomura, N.K.; Carciofi, A.C., 2012: Digestibility for dogs and cats of meat and bone meal processed at two different temperature and pressure levels. *Journal of Animal Physiology and Animal Nutrition* **96**, 1136-1146.

Opheim, M.; Šližytė, R.; Sterten, H.; Provan, F.; Larssen, E.; Kjos, N.P., 2015: Hydrolysis of Atlantic salmon (*Salmo salar*) rest raw materials - Effect of raw material and processing on composition, nutritional value, and potential bioactive peptides in the hydrolysates. *Process Biochemistry* **50**, 1247-1257.

Opstvedt, J.; Nygård, E.; Samuelsen, T.A.; Venturini, G.; Luzzana, U.; Mundheim, H., 2003: Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed. *Journal of the Science of Food and Agriculture* **83**, 775-782.

Papadopoulos, M.C., 1989: Effect of processing on high-protein feedstuffs: A review. *Biological Wastes* **29**, 123-138.

Rivera, J.A.; Sebranek, J.G.; Rust, R.E.; Tabatabai, L.B., 2000: Composition and protein fractions of different meat by-products used for petfood compared with mechanically separated chicken (MSC). *Meat science* **55**, 53-59.

Romarheim, O.H.; Aslaksen, M.A.; Storebakken, T.; Krogdahl, Å.; Skrede, A., 2005: Effect of extrusion on trypsin inhibitor activity and nutrient digestibility of diets based on fish meal, soybean meal and white flakes. *Archives of Animal Nutrition* **59**, 365-375.

van Rooijen, C., 2015: *Maillard reaction products in pet foods*. Ph.D. Thesis, Wageningen University, Wageningen, the Netherlands.

Tjernsbekk, M.T.; Tauson, A.-H.; Ahlstrøm, Ø., 2014: Ileal, colonic and total tract nutrient digestibility in dogs (*Canis familiaris*) compared with total tract digestibility in mink (*Neovison vison*). *Archives of Animal Nutrition* **68**, 245-261.

Tran, Q.D., 2008: *Extrusion processing: effects on dry canine diets*. Ph.D. Thesis, Wageningen University, Wageningen, the Netherlands.

Trindade, M.A.; de Felício, P.E.; Castillo, C.J.C., 2004: Mechanically separated meat of broiler breeder and white layer spent hens. *Scientia Agricola* **61**, 234-239.

Verlinden, A.; Hesta, M.; Hermans, J.M.; Janssens, G.P.J., 2006: The effects of inulin supplementation of diets with or without hydrolysed protein sources on digestibility, faecal characteristics, haematology and immunoglobulins in dogs. *British Journal of Nutrition* **96**, 936-944.

Zinn, K.E.; Hernot, D.C.; Fastinger, N.D.; Karr-Lilienthal, L.K.; Bechtel, P.J.; Swanson, K.S.; Fahey, G.C.Jr, 2009: Fish protein substrates can substitute effectively for poultry by-product meal when incorporated in high-quality senior dog diets. *Journal of Animal Physiology and Animal Nutrition* **93**, 447-455.

417 **Table 1**. Analysed chemical composition of protein ingredients

| | Protein ingredient | | |
|---|---|---|---|
| | PM | MSC | SPH |
| Dry matter (g/kg) | 944.0 | 358.0 | 597.4 |
| In dry matter (g/kg) | | | |
| Crude protein | 670.7 | 421.2 | 868.9 |
| Crude fat | 141.4 | 547.8 | 18.5 |
| Ash | 126.4 | 32.1 | 107.0 |
| Carbohydrates* | 61.5 | 0.0 | 5.6 |
| Essential amino acids (g/100g crude protein) | | | |
| Arg | 6.8 | 6.2 | 5.6 |
| His | 2.4 | 2.6 | 1.8 |
| Ile | 4.0 | 3.8 | 2.3 |
| Leu | 7.2 | 6.8 | 4.5 |
| Lys | 6.7 | 7.3 | 5.8 |
| Met | 2.1 | 2.3 | 1.9 |
| Phe | 3.9 | 3.5 | 2.2 |
| Thr | 4.3 | 4.2 | 3.4 |
| Val | 4.6 | 4.2 | 3.1 |
| Non-essential amino acids (g/100g crude protein) | | | |
| Ala | 6.4 | 6.0 | 6.3 |
| Asp | 8.6 | 8.5 | 7.1 |
| Cys | 1.0 | 1.1 | 0.5 |
| Glu | 13.4 | 13.6 | 11.7 |
| Gly | 9.0 | 7.1 | 11.1 |
| Hyp | 3.0 | 1.9 | 2.4 |
| Pro | 6.1 | 4.7 | 5.5 |
| Ser | 4.6 | 4.0 | 4.1 |
| Tyr | 2.9 | 2.6 | 1.3 |
| Total essential amino acids | 42.0 | 40.9 | 30.6 |
| Total non-essential amino acids | 55.0 | 49.5 | 50.0 |
| Total amino acids | 97.0 | 90.4 | 80.6 |

418 PM, poultry meal; MSC, mechanically separated chicken meat; SPH, salmon protein

419 hydrolysate.

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

421

**Table 2**. Ingredients and calculated chemical composition of diets used for digestibility determination of poultry meal, mechanically separated chicken meat and salmon protein hydrolysate

| | Protein ingredient | | |
|---|---|---|---|
| | PM | MSC | SPH |
| Ingredients (g/kg) | | | |
| PM* | 208.4 | | |
| MSC† | | 604.7 | |
| SPH‡ | | | 391.3 |
| Precooked corn starch | 109.7 | 72.0 | 152.2 |
| Cellulose | 17.6 | 11.5 | 24.4 |
| Soybean oil | 109.7 | | 181.2 |
| Vitamins and minerals§ | 1.1 | 0.7 | 1.5 |
| Water | 553.5 | 311.1 | 249.4 |
| Total | 1000.0 | 1000.0 | 1000.0 |
| Chemical composition | | | |
| Dry matter (g/kg) | 427.0 | 295.6 | 582.4 |
| In dry matter (g/kg) | | | |
| Crude protein | 308.9 | 308.5 | 348.7 |
| Crude fat | 321.1 | 402.2 | 317.5 |
| Ash | 61.9 | 26.8 | 46.6 |
| Carbohydrates | 308.1 | 262.5 | 287.2 |

PM, poultry meal; MSC, mechanically separated chicken meat; SPH, salmon protein hydrolysate.

*Poultry meal, Low Ash, GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG, Diepholz, Germany.

†Mechanically separated chicken meat, Nortura SA, Hærland, Norway.

‡Salmon protein hydrolysate, Marine Bioproducts AS, Skogsvåg, Norway.

§Normin AS, Hønefoss, Norway. Containing per kg: vitamin A, 2 000 000 IE; vitamin $D_3$, 200 000 IE; vitamin E, 50 000 mg; thiamine, 15 000 mg; riboflavin, 3 000 mg; niacin, 5 000 mg; pantothenic acid, 3 330 mg; vitamin $B_6$, 3 000 mg; vitamin $B_{12}$, 20 mg; folic acid, 300 mg; biotin,

30 mg; Iron (II) sulphate, 610 mg; Iron fumarate, 15 280 mg; Iron chelate, 4 110 mg; Copper (II) sulphate, 1 250 mg; Manganese oxide, 7 500 mg; Zinc oxide 10 000 mg; Calcium iodate, 64 mg; Sodium selenite, 100 mg; Cobalt carbonate, 60 mg.

437 **Table 3**. Diet formulation for production of extruded dog foods (g/kg as-fed basis)

| | Diet | | |
|---|---|---|---|
| | PM | MSC | SPH |
| PM* | 291.1 | 152.9 | 183.6 |
| MSC† | | 330.7 | |
| SPH‡ | | | 115.5 |
| Poultry fat | 164.9 | 78.9 | 173.9 |
| Wheat | 349.9 | 281.5 | 339.1 |
| Corn | 100.0 | 80.4 | 96.9 |
| Rice flour | 30.0 | 24.1 | 29.1 |
| Beet pulp | 10.0 | 8.0 | 9.7 |
| Salmon oil | 15.0 | 12.1 | 14.5 |
| Limestone meal | 7.7 | 6.2 | 7.4 |
| Monocalcium phosphate | 10.5 | 8.5 | 10.2 |
| Sodium chloride | 7.0 | 5.6 | 6.8 |
| Betaine | 1.4 | 1.2 | 1.4 |
| Vitamin E§ | 2.1 | 1.7 | 2.0 |
| Mineral premix¶ | 2.3 | 1.9 | 2.2 |
| Vitamin premix** | 7.9 | 6.4 | 7.7 |

438 PM, poultry meal; MSC, mechanically separated chicken meat; SPH, salmon protein

439 hydrolysate.

440 *Poultry meal, Low Ash, GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG,

441 Diepholz, Germany.

442 †Mechanically separated chicken meat, Nortura SA, Hærland, Norway.

443 ‡Salmon protein hydrolysate, Marine Bioproducts AS, Skogsvåg, Norway.

444 §Normin AS, Hønefoss, Norway, 100 000 mg vitamin E per kg.

445 ¶Normin AS, Hønefoss, Norway. Containing per kg: Cu, 11 g; Zn, 115 g; Mn, 35 g; I, 1.5 g; Fe,

446 100 g.

447 **Normin AS, Hønefoss, Norway. Containing per kg: vitamin A, 4 000 000 IE; vitamin $D_3$,

448 400 000 IE; vitamin E, 100 000 mg; thiamine, 12 000 mg; riboflavin, 24 000 mg; niacin, 150 000

449 mg; pantothenic acid, 60 000 mg; vitamin $B_6$, 30 000 mg; vitamin $B_{12}$, 64 mg; folic acid, 4000

450 mg; biotin, 1500 mg.

451 **Table 4**. Analysed chemical composition of extruded diets (g/kg as-fed basis)

| | Diet | | |
|---|---|---|---|
| | PM | MSC | SPH |
| Dry matter | 914.1 | 944.0 | 937.0 |
| Crude protein | 248.7 | 264.0 | 264.0 |
| Crude fat | 186.1 | 226.0 | 223.0 |
| Starch | 268.8 | 281.0 | 278.0 |
| Ash | 72.3 | 64.0 | 59.0 |
| Carbohydrates* | 407.0 | 390.0 | 391.0 |
| Essential amino acids | | | |
| Arg | 16.3 | 16.6 | 15.9 |
| His | 6.1 | 5.6 | 5.4 |
| Ile | 10.8 | 11.4 | 10.3 |
| Leu | 19.0 | 19.6 | 18.3 |
| Lys | 15.1 | 15.2 | 14.3 |
| Met | 5.0 | 5.0 | 5.1 |
| Phe | 10.6 | 11.0 | 10.1 |
| Thr | 9.7 | 10.0 | 9.9 |
| Val | 12.5 | 14.0 | 12.6 |
| Non-essential amino acids | | | |
| Ala | 15.6 | 15.6 | 16.0 |
| Asp | 20.5 | 21.0 | 20.2 |
| Cys | 3.2 | 4.0 | 3.6 |
| Glu | 42.8 | 44.8 | 43.8 |
| Gly | 21.1 | 20.6 | 22.6 |
| Hyp | 5.5 | 4.9 | 5.5 |
| Pro | 17.0 | 18.2 | 19.2 |
| Ser | 11.2 | 12.6 | 12.7 |
| Tyr | 8.2 | 8.5 | 7.9 |
| Total essential amino acids | 105.1 | 108.4 | 101.9 |
| Total non-essential amino acids | 145.1 | 150.2 | 151.5 |
| Total amino acids | 250.2 | 258.6 | 253.4 |

452 PM, poultry meal; MSC, mechanically separated chicken meat; SPH, salmon protein

453 hydrolysate.

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

455

**Table 5**. Least-square means of mink apparent total tract digestibility of crude protein and amino acids in protein ingredients (%)

| | Protein ingredient | | | | p-value |
|---|---|---|---|---|---|
| | PM | MSC | SPH | SEM | Protein ingredient |
| Crude protein | 80.9$^{c}$ | 88.2$^{b}$ | 91.3$^{a}$ | 0.583 | <0.001 |
| Essential amino acids | | | | | |
| Arg | 90.7$^{b}$ | 91.9$^{b}$ | 96.2$^{a}$ | 0.296 | <0.001 |
| His | 82.3$^{c}$ | 93.3$^{a}$ | 89.8$^{b}$ | 0.563 | <0.001 |
| Ile | 87.1$^{b}$ | 93.9$^{a}$ | 92.7$^{a}$ | 0.397 | <0.001 |
| Leu | 88.1$^{b}$ | 93.7$^{a}$ | 94.2$^{a}$ | 0.354 | <0.001 |
| Lys | 88.3$^{b}$ | 94.5$^{a}$ | 95.3$^{a}$ | 0.378 | <0.001 |
| Met | 89.6$^{b}$ | 95.4$^{a}$ | 94.7$^{a}$ | 0.236 | <0.001 |
| Phe | 83.9$^{b}$ | 87.2$^{a}$ | 86.0$^{ab}$ | 0.703 | 0.024 |
| Thr | 78.4$^{b}$ | 86.4$^{a}$ | 85.0$^{a}$ | 0.642 | <0.001 |
| Val | 85.3$^{b}$ | 92.1$^{a}$ | 92.3$^{a}$ | 0.507 | <0.001 |
| Non-essential amino acids | | | | | |
| Ala | 86.8$^{c}$ | 89.5$^{b}$ | 94.6$^{a}$ | 0.481 | <0.001 |
| Asp | 63.9$^{c}$ | 90.9$^{a}$ | 82.5$^{b}$ | 0.824 | <0.001 |
| Cys | 55.7$^{b}$ | 82.2$^{a}$ | 60.7$^{b}$ | 1.617 | <0.001 |
| Glu | 85.7$^{b}$ | 91.9$^{a}$ | 93.2$^{a}$ | 0.458 | <0.001 |
| Gly | 82.9$^{b}$ | 85.0$^{b}$ | 93.3$^{a}$ | 0.672 | <0.001 |
| Pro | 85.0$^{b}$ | 84.5$^{b}$ | 92.5$^{a}$ | 0.715 | <0.001 |
| Ser | 79.2$^{b}$ | 88.5$^{a}$ | 89.8$^{a}$ | 0.572 | <0.001 |
| Tyr | 84.8$^{b}$ | 89.6$^{a}$ | 84.2$^{b}$ | 0.729 | 0.001 |
| Total essential amino acids | 86.0$^{b}$ | 92.0$^{a}$ | 91.8$^{a}$ | 0.424 | <0.001 |
| Total non-essential amino acids | 78.0$^{b}$ | 87.8$^{a}$ | 86.3$^{a}$ | 0.703 | <0.001 |
| Total amino acids | 82.2$^{b}$ | 90.0$^{a}$ | 89.2$^{a}$ | 0.553 | <0.001 |

PM, poultry meal; MSC, mechanically separated chicken meat; SPH, salmon protein hydrolysate; SEM, pooled standard error of the means.

Concentration of Hyp in faecal samples was not determined, and digestibility of Hyp in the protein ingredients is therefore missing.

$^{a,b,c}$Significant ($p \leq 0.05$) differences among least-square means in the same row are indicated by different superscript letters.

**Table 6**. Least-square means of mink apparent total tract digestibility of crude protein and amino acids in extruded diets containing different protein ingredients (%)

| | Diet | | | | p-value |
|---|---|---|---|---|---|
| | PM | MSC | SPH | SEM | Diet |
| Crude protein | 80.3 | 81.3 | 79.0 | 0.539 | 0.053 |
| Essential amino acids | | | | | |
| Arg | 89.2 | 89.5 | 89.2 | 0.311 | 0.676 |
| His | 82.8 | 83.3 | 82.0 | 0.471 | 0.259 |
| Ile | 86.1[a] | 85.5[a] | 83.9[b] | 0.219 | <0.001 |
| Leu | 87.0[a] | 86.3[ab] | 85.5[b] | 0.232 | 0.006 |
| Lys | 85.7[b] | 86.6[a] | 86.3[ab] | 0.163 | 0.007 |
| Met | 88.0 | 88.4 | 87.7 | 0.211 | 0.169 |
| Phe | 87.2[a] | 86.9[a] | 85.6[b] | 0.234 | 0.005 |
| Thr | 74.4 | 74.1 | 74.1 | 0.469 | 0.846 |
| Val | 84.0[ab] | 84.9[a] | 83.1[b] | 0.298 | 0.010 |
| Non-essential amino acids | | | | | |
| Ala | 85.4 | 85.9 | 85.7 | 0.235 | 0.274 |
| Asp | 62.0[b] | 69.2[a] | 63.6[b] | 0.470 | <0.001 |
| Cys | 61.8 | 57.2 | 55.1 | 1.621 | 0.052 |
| Glu | 87.6[b] | 88.4[a] | 87.6[b] | 0.191 | 0.024 |
| Gly | 81.4[b] | 83.8[a] | 83.1[a] | 0.276 | <0.001 |
| Hyp | 84.8[b] | 89.7[a] | 88.0[a] | 0.627 | 0.001 |
| Pro | 85.7 | 85.2 | 85.6 | 0.272 | 0.332 |
| Ser | 77.9 | 78.8 | 78.7 | 0.481 | 0.326 |
| Tyr | 86.2[a] | 84.2[b] | 83.8[b] | 0.275 | <0.001 |
| Total essential amino acids | 84.9 | 85.1 | 84.2 | 0.258 | 0.104 |
| Total non-essential amino acids | 79.2 | 80.3 | 79.0 | 0.371 | 0.084 |
| Total amino acids | 82.1 | 82.7 | 81.6 | 0.298 | 0.101 |

PM, poultry meal; MSC, mechanically separated chicken meat; SPH, salmon protein hydrolysate; SEM, pooled standard error of the means.

[a,b,c]Significant ($p \leq 0.05$) differences among least-square means in the same row are indicated by different superscript letters.