Protein Digestibility and Quality of Selected Proteins for Humans, Dogs, and Pigs

by

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Dissertation

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Abstract

Four experiments were conducted to determine the protein quality, digestibility, and/or health effects of selected proteins for humans, dogs, and pigs, including salmon protein hydrolysates (SPH), seaweeds and seaweed co-products, and egg proteins. Experiment 1 used the digestible indispensable amino acid score (DIAAS) method to test the hypothesis that the protein in salmon protein hydrolysate concentrate (SPHC) and two salmon protein hydrolysate isolates (SPHI1 and SPHI2) can supplement lower-quality proteins. Twelve growing gilts with a T-cannula installed in the distal ileum were used. There were eight observations per treatment. The standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA), and DIAAS were calculated. For children and older individuals, SPHC had greater ($P < 0.05$) DIAAS than SPHI1 and SPHI2, and SPHI2 had greater ($P < 0.05$) DIAAS than SPHI1. For children, leucine was the first limiting AA in SPHC, and tryptophan was the first limiting AA in SPHI1 and SPHI2. For older individuals, there was no limiting AA (DIAAS ≥ 100) for SPHC, but for both SPHI, leucine was the first limiting AA. Results indicated that SPHC can be used to compensate for lower protein quality in other ingredients to produce a balanced diet that meets requirements for all indispensable AA for older individuals. Experiment 2 was conducted to determine apparent total tract digestibility (ATTD), oxidative stress and inflammatory biomarkers, skin barrier function, and fecal metabolites and microbiota of dogs fed SPHC and SPHI, testing the hypothesis that SPH improves digestibility and health outcomes compared with chicken meal. Thirty adult dogs were used. There were 10 observations per treatment. Feces, blood, and skin parameters were collected on days 0, 45, and 90, with total fecal collection conducted during the last five days. Dogs fed SPHI had greater ($P < 0.05$) ATTD of dry matter and organic matter, while both SPH had greater ($P < 0.05$) ATTD of CP and gross energy than the control diet.

Malondialdehyde (MDA) was greater in SPHC over time (interaction, $P < 0.05$). Glutathione was greater ($P < 0.05$) in the control diet compared with the SPHC. Skin hydration in the inguinal location was greater ($P < 0.05$) in SPHC than in control and SPHI diets. The transepidermal water loss (TEWL) was reduced in the pinnae location over time in SPHC and control diets (interaction, $P < 0.05$). Phenols and indoles were lower ($P < 0.05$) in SPHI than in the control and SPHC diets. There was an increase in BCFA in the control diet on day 90 compared with days 0 and 45, but a decrease on day 90 compared with day 0 in the SPHC (interaction, $P < 0.05$). There was a partial separation ($P < 0.05$) in beta diversity between the control and SPHC diets and between both SPH diets. Results indicated that SPH in diets for dogs improved digestibility and health outcomes compared with chicken meal and demonstrated the importance of defining functional targets when selecting protein hydrolysates for pet foods. Experiment 3 was conducted to test the hypothesis that protein quality of seaweed species from three different color groups is less than that of casein and whey protein isolate (WPI) as indicated by the DIAAS method. The second hypothesis tested was that seaweed polysaccharides (i.e., carrageenan, agar, alginate, and cellulose) decrease AA digestibility of high-quality proteins, including casein and WPI. Twelve growing gilts with a T-cannula installed in the distal ileum were used. There were six observations per treatment. The SID of AA and DIAAS was calculated. Digestibility of most AA was less than 80%, and DIAAS was less than 75 for the three seaweeds. The SID of AA in casein or WPI was not reduced when carrageenan, agar, or cellulose was added to the diet, but the SID of most indispensable AA was reduced ($P < 0.05$) when alginate was added to the casein diet, indicating a reduction in intestinal absorption. Results indicated that, due to the low DIAAS, no claims regarding protein quality can be made for seaweeds, demonstrating that native cell wall structure reduces AA digestibility, whereas

soluble fibers in seaweed polysaccharides did not reduce AA digestibility in high-quality proteins, except for alginate, which reduced AA digestibility in casein. Experiment 4 was conducted to test the hypothesis that the SID of AA in pasteurized egg products is sufficient for these ingredients to serve as a high-quality protein source in weanling pig diets; and that the SID of AA in trypsin-inhibitor-free egg protein (TFE) does not differ from that of casein, and is greater than the SID of AA in egg products containing residual trypsin inhibitor activity (TIA). Egg proteins included raw egg powder (REP), dry-pasteurized egg powder (DPE), liquid pasteurized egg powder (LPE), and TFE. Twelve weanling pigs with a T-cannula in the distal ileum were used. There were twelve observations per treatment. The SID of CP and AA was calculated. The SID of most AA was greater ($P < 0.05$) in TFE and casein compared with REP, DPE, and LPE, indicating that the residual TIA reduced AA digestibility. However, the SID of Lys, Cys, and Ser was not different between TFE and LPE. The SID of His, Lys, Asp, and Ser was less ($P < 0.05$) in TFE than in casein, whereas the SID of other AA and CP in TFE was not different from that in casein. Results indicated that all egg products had excellent SID of CP (> 82.0 %) and AA (> 80.0 %), but only when TIA were reduced, as in TFE, were the SID of AA in egg protein not different from the SID of AA in casein. Overall, protein quality differs among protein sources such as salmon and seaweeds for human nutrition, whereas salmon and egg proteins can serve as effective protein sources in diets for dogs and young pigs, respectively.
Keywords: dog; digestibility; egg proteins; protein quality; pig; salmon hydrolysate; seaweed

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To my husband and my family.

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Chapter 1: Introduction

Global protein availability is critical as dietary protein plays an important role in combating hunger, malnutrition, and food insecurity (Food and Agriculture Organization – FAO, 2010). Global per capita dietary protein supply has increased by approximately 7% since 2010, with variations across regions, with Europe and North America leading with over 100 grams per capita daily, whereas consumers in Africa consume less than 70 grams (Burlingame et al., 2024). Low-income countries derive only 18% of their protein from animal sources, compared with 63% in high-income nations, highlighting disparities in nutritional equity and access (Burlingame et al., 2024).

The demand for protein is rapidly increasing due to population growth, rising incomes, and the livestock/pet food industry, driving the need for sustainable and equitable protein production systems (Burlingame et al., 2024). In the pet food industry, in particular, this demand follows the continuing growth of pet ownership around the globe (Hobbs et al., 2024). The need for protein will continue to increase for humans, companion animals, and livestock, and it is projected that the demand for animal-derived protein will double by 2050; therefore, innovative solutions will be essential to ensure that this growing demand is met without compromising the environment (Henchion et al., 2017).

Novel protein sources for humans and animals are important to contribute to the increased demand (Salter and Lopez-Viso, 2021), but these proteins must address affordability, cultural acceptance, and nutritional adequacy to make a significant impact (Burlingame et al., 2024). Examples of novel or alternative proteins include proteins coming from aquaculture, such as algae, as well as protein hydrolysates and other alternatives to traditional livestock meat (Hou et al., 2017; Williamson et al., 2024). Aquaculture production is estimated to increase to

contribute to the growing demand of future generations, and compared with meat from terrestrial animals, these proteins represented approximately 15% of all crude protein produced in 2018 (Boyd et al., 2022). In addition, protein hydrolysates produced from animal or plant sources have the potential to improve the nutrition and health of domestic and companion animals (Hou et al., 2017).

Protein is an essential nutrient for all living organisms, including humans and animals. It is present in skeletal muscle, mammary glands, liver, and the small intestine, as well as animal products such as milk, meat, and eggs (Hou et al., 2017). The composition of the protein affects its nutritional value, biological activity, and functional role as a food component (Sikorski and Sinkiewicz, 2023). In addition, the nutritional value of a food protein is determined by the protein content (i.e., percent protein per gram of food source) and protein quality (Williamson et al., 2024).

Protein quality is determined by factors including amino acid (AA) composition and digestibility (Schönfeldt and Hall, 2012; Williamson et al., 2024). In animal nutrition, diets are usually formulated based on the digestibility of each indispensable amino acid (IAA), which makes it possible to match diet composition to requirements for the IAA that cannot be synthesized by the body (Stein et al., 2007). In contrast, the protein value of foods for humans relies on a single value for digestible nitrogen, which is not accurate in predicting the digestibility of each IAA (FAO, 2013; Stein, 2024). Therefore, it is important to determine protein quality in food for humans because the methodology used to determine AA digestibility is identical to that used to determine the digestibility of feed ingredients for animals (Stein, 2024), and the pig has been recognized as the best model for humans (FAO, 2013; Hodgkinson et al., 2022).

Therefore, the objectives of this dissertation are 1) to test the hypothesis that the protein value of selected proteins for both humans and pigs can be obtained from the ileal digestibility of amino acids determined in pigs; 2) to test the hypothesis that the use of selected proteins fed to dogs has similar or improved effects on protein digestibility and health status compared with a traditional protein source.

Literature Cited

Boyd, C. E., McNevin, A. A., and Davis, R. P. 2022. The contribution of fisheries and aquaculture to the global protein supply. Food Secur. 14:805-827. doi:10.1007/s12571-021-01246-9

Burlingame, B., Moltedo, A., and Cafiero, C. 2024. Global protein sustainability and the United Nations, through to the 2030 agenda. Front. Nutr. 11:1383898. doi:10.3389/fnut.2024.1383898

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation #92. Rome

FAO. FAOSTAT Food Balances. 2010. New food balance sheet methodology. https://www.fao.org/faostat/en/#data/FBS (accessed Jan 2025)

Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., and Tiwari, B. 2017. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods. 6:53. doi:10.3390/foods6070053

Hobbs, L., Shanoyanb, A., and Aldrich, G. 2024. Assessing research needs for informing pet food industry decisions. Food Agribus. Manag. Rev. 27:903-936. doi:10.22434/ifamr2023.0004

Hodgkinson, S. M., Stroebinger, N., Van Der Wielen, N., Mensink, M., Montoya, C., Hendriks, W. H., De Vries, S., Stein, H. H., and Moughan, P. J. 2022. Comparison of true ileal amino acid digestibility between adult humans and growing pigs. J. Nutr. 152:1635-1646. doi:10.1093/jn/nxac077

Hou, Y., Wu, Z., Dai, Z., Wang, G., and Wu, G. 2017. Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. J. Anim. Sci. Biotechnol. 8:209-232. doi:10.1186/s40104-017-0153-9

Salter, A.M., and Lopez-Viso, C., 2021. Role of novel protein sources in sustainably meeting future global requirements. Proc. Nutr. Soc. 80:186-194. doi:10.1017/S0029665121000513

Schönfeldt, H. C., and Hall, N. G. 2012. Dietary protein quality and malnutrition in Africa. Br. J. Nutr. 108:S69-S76. doi:10.1017/S0007114512002553

Sikorski, Z., and Sinkiewicz, I. 2023. Role of proteins in food. Chemical and functional properties of food components. 4th edition. CRC Press 155-201. ISBN: 978100326595

Stein, H. H. 2024. The pig is an excellent model to determine amino acid digestibility of human foods and to generate data needed to meet human amino acid requirements. Front. Nutr. 11:1434430. doi: 10.3389/fnut.2024.1434430

Van der Spiegel, M., Noordam, M. Y., and Van der Fels-Klerx, H. J. 2013. Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr. Rev. Food Sci. Food Saf. 12:662-678. doi:10.1111/1541-4337.12032

Williamson, E., Ross, I. L., Wall, B. T., and Hankamer, B. 2024. Microalgae: Potential novel protein for sustainable human nutrition. Trends in Plant Sci. 29:370-382. doi:10.1016/j.tplants.2023.08.006

Chapter 2: The Importance of Amino Acid Composition and Digestibility of Proteins for Humans and Animals: Literature Review

Introduction

Protein is a macronutrient that is essential for human and animal tissue growth, repair, and maintenance. Amino acids (AA), the building blocks of protein, are indispensable for metabolic processes, and some of them must be adequately supplied through the diet (Lopez and Mohiuddin, 2024). Protein-energy malnutrition remains a significant global health concern, particularly in developing nations. It results from insufficient protein and energy intake and can severely impact physical and cognitive development (Batool et al., 2015). Therefore, ensuring sufficient protein intake is critical for maintaining healthy muscle mass and preventing illness among susceptible populations (Millward et al., 2008).

Adequate protein consumption is a function of many factors, with disposable income being one of the most important factors (Aggarwal and Drewnowski, 2019). People living in Europe consistently consume the most animal-based protein meals, whereas people living in Southeast Asia or Africa consume the least (Henchion et al., 2021). Furthermore, the search for alternative protein sources and sustainability concerns has influenced protein consumption in livestock and companion animals. Processing methods, including enzymatic hydrolysis, have also been employed to extract proteins and bioactive peptides, offering an alternative to conventional proteins (Hou et al., 2017; Hefferon et al., 2023).

Characteristics of a protein regarding its AA composition as well as digestibility dictate protein quality. Protein quality is crucial for both humans and animals, as it directly influences growth, maintenance, and overall health (FAO, 2013). In humans, high-quality proteins provide indispensable amino acids (IAA) in adequate proportions to support muscle function, immune

response, and metabolic processes (Stein, 2024). For animals, particularly in livestock and companion animals, protein quality affects not only growth performance and feed efficiency but also gut health and immune function (Oba et al., 2020). Therefore, selecting protein sources with an optimal AA profile and high digestibility is essential to ensuring efficient nutrient absorption, promoting well-being, and supporting sustainable food production systems.

This literature review aims to explore the critical role of AA and protein ingredients in human and animal nutrition, emphasizing digestibility, protein quality, and the potential of incorporating selected protein sources into diets to address nutritional needs.

Importance of Amino Acids vs. Protein

The English term "protein" comes from the Greek word "proteios", which means "primary", which is particularly suitable in nutrition because protein is an essential component of tissues in both humans and animals (Wu, 2016). A protein contains varying quantities of 20 distinct AA connected by peptide bonds, and the uniqueness of each protein is determined by the AA it possesses (WHO, 2007; Wu, 2016). Humans and animals have nutritional requirements for AA, not protein per se (WHO, 2007; Stein, 2024). Therefore, understanding AA biochemistry is important for optimizing protein intake and assessing its health effects.

Each AA has a carboxyl group, a primary α-amino group, and a side chain that is distinct for each AA (Lopez and Mohiuddin, 2024). The AA sequence determines the three-dimensional structure of the protein (Lopez and Mohiuddin, 2024). The IAA are AA that are not produced by animal cells and must be obtained from diets. Semi-indispensable AA are designed as indispensable during periods of growth or physiological stress, where the utilization happens at a

greater rate than synthesis (Wu, 2009). Dispensable AA, on the other hand, are those that humans and animals are able to synthesize (WHO, 2007; Lopez and Mohiuddin, 2024).

Over the past 30 years, research has used AA tracers and nitrogen balance techniques to establish the dietary requirements of protein and AA for humans and animals (Wu, 2009; Lopez and Mohiuddin, 2024). When all AA in the diet are provided in quantities needed for protein synthesis, oxidation of AA is minimal. However, excess AA is not stored in the body but is oxidized and converted into carbon dioxide, water, ammonia, and urea (Wu, 2016; Stein, 2024). Likewise, if one AA is insufficient, the oxidation of all other AA is increased (Wu, 2016). As a result, AA must be supplied in each meal to fulfill requirements and prevent limitation of protein synthesis (Schoenfeld and Aragon, 2018).

Because the side chains of AA are different, each AA has specific functions in nutrition and health (Wu, 2009). In addition to their role as protein building blocks, AA are important regulators of key metabolic pathways and for the synthesis of signaling hormones and neurotransmitters (Suenaga et al., 2008; Wu, 2016). General symptoms of inadequate intake of IAA may include low appetite, vomiting, weakness, stunted growth, diseases, and depression (Lopez and Mohiuddin, 2024). Dietary AA intake depends on the dietary AA content and food consumption. Therefore, when formulating diets for humans or animals, many factors must be considered, such as endogenous AA synthesis, presence of antinutritional factors, amount of dietary fiber, interactions among food ingredients, digestibility, and bioavailability of nutrients (Li and Wu, 2023). In some cases, supplementation of crystalline AA to meet nutritional requirements is also needed.

Amino Acid Digestibility and Protein Quality

Dietary protein has no nutritional value unless it is digested by proteases and peptidases into AA, dipeptides, or tripeptides; thus, digestibility and relative proportions of AA in dietary protein determine its nutritional value (Tomé, 2013). Protein quality also relates to the nutritional value of foods because it links the digestible IAA to the AA's body requirements (FAO, 2013). Therefore, digestibility and protein quality are important factors in meeting physiological needs and minimizing AA deficits (FAO, 2013). In animal nutrition, formulating diets with sufficient quantities of digestible IAA is also critical for maintaining health, growth, and productivity. There are several factors that affect AA digestibility and protein quality, including the ingredient source and its chemical composition, processing methods applied, and the age or physiological status of the individual (Stein et al., 2007; Oba et al., 2020).

The small intestine is the major site for protein digestibility and absorption in humans and animals (Stoll et al., 1998; Wu, 2016). Dietary AA bioavailability is defined as the proportion of ingested AA that are absorbed in a chemical form that makes them appropriate for metabolism or protein synthesis (Batterham, 1992). However, determination of bioavailability of AA is tedious and expensive and not practical for routine protein evaluation (Stein et al., 2007). Therefore, *in vivo* methods to determine AA digestibility have been developed and are used to estimate AA bioavailability (Sauer and Ozimek, 1986; Stein et al., 2007). Because AA are only absorbed in the small intestine and because hindgut fermentation affects AA concentration, ileal digestibility is a more accurate measure of AA bioavailability than total tract (fecal) digestibility (Sauer and Ozimek, 1986; Hendriks et al., 2013).

Determining ileal digestibility involves collecting digesta from the terminal ileum of humans or animals to measure the proportion of dietary AA absorbed in the small intestine by

subtracting the amount of AA in the ileal digesta from the total AA intake, then dividing this difference by the total AA intake, which generates values known as apparent ileal digestibility (AID) of AA (Stein et al., 2007). However, when a protein is fed to humans or animals, the AA that reach the distal ileum include dietary undigested AA plus endogenous AA that were included in proteins secreted into the intestinal lumen without being digested and re-absorbed such as mucoproteins, serum albumin, sloughed cells, digestive enzymes, bacterial protein, and ingested hair (Stein et al., 2007). Therefore, the endogenous losses affect the calculated AID of AA and result in values not being additive in mixed meals (Stein et al., 2005).

Ileal endogenous protein losses are divided into basal losses, which are losses of proteins secreted into the small intestine in response to dry matter, and specific losses, which are losses of proteins secreted into the small intestine in response to specific dietary factors (Nyachoti et al., 1997). However, because the basal endogenous losses are independent of diet composition and only secreted in response to dry matter intake, these losses can be disregarded in the calculation of AA digestibility, which generates values known as standardized ileal digestibility (SID) of AA that are additive in mixed diets (Stein et al., 2005; Stein et al., 2007). Basal endogenous losses can be measured using a nitrogen-free diet or a diet that contains highly digestible ingredients (Jansman et al., 2002). Values for basal endogenous losses of AA determined in humans and animals when using a nitrogen-free diet are presented in Table 2.1. Examples of differences in AID and SID values for protein and AA in common diets fed to animals are also demonstrated in Table 2.2.

Humans

Over the past decades, a few methods have been developed to assess AA digestibility in humans, but the application of *in vivo* assays has been relatively laborious (FAO, 2013). Direct

methods via sampling ileal digesta involve the use of humans with nasoileal intubation or ileostomates. Although the use of humans with nasoileal intubation provides valuable information, it is invasive because it involves the use of a tube from the nose throughout the terminal ileum and requires that the particle size of the digesta is fine due to the size of the tube required to perform this assay (Gaudichon and Calvez, 2021; Hodgkinson et al., 2022). The use of ileostomates involves using people who had their large intestine removed and their terminal ileum exteriorized for medical reasons such as colorectal cancer, obstruction, ulcerative colitis, and other conditions. The advantage of this procedure is that the protein source is not limited by particle size, but the biggest disadvantage is finding suitable individuals who do not have other medical conditions or are using medications and are willing to participate in a research study (Hodgkinson et al., 2022).

Indirect methods to determine AA digestibility in humans that are less invasive also exist. The indicator AA oxidation method is used to measure the relative change in net protein synthesis based on the fact that when a single AA is limiting for protein synthesis, all other AA are oxidized (FAO, 2013). From this method, digestibility is calculated by comparing the slope of the oxidation curve when increasing the intake of a limiting AA from a food source with the slope of oxidation when the same AA is provided in a fully digestible crystalline form. This is done by measuring CO_2 in breath samples, as the oxidation of the indicator AA decreases when more of the limiting AA is absorbed and used for protein synthesis (FAO, 2013). Although this assay is considered relatively non-invasive, it only determines one single AA at a time; thus, multiple studies are required to evaluate all IAA of a food (Hodgkinson et al., 2022).

Another less invasive method to determine AA digestibility is the dual-isotope procedure. This assay consists of measuring plasma AA and uses intrinsic labeling of AA to allow studying

the digestion of a protein in a meal and another intrinsic labeling of a highly digestible protein to account for and correct for the metabolism of AA in the gut and the liver (FAO, 2013). Protein oxidation can also be estimated from breath samples. However, the main limitation of this approach is the cost of obtaining stable isotope-labeled proteins, plus the cost of analysis, and the need for several validation processes (FAO, 2013; Hodgkinson et al., 2022).

Although approaches for determining AA digestibility in humans are available, they cannot be considered standard procedures and must be extensively evaluated. In addition, the use of *in vitro* assays has been proposed, but a standardized, accurate, and validated method is not available (FAO, 2013). As a result, an animal model was proposed to determine AA digestibility in proteins for humans (FAO, 2013; Hogkinson et al., 2020). The growing pig has been recognized as the best model to determine the ileal digestibility of AA because digestive functions and consumption patterns are similar in pigs and humans (Hogkinson et al., 2020). Indeed, a very close agreement between the ileal digestibility of AA in the growing pig and humans has been demonstrated, and it is concluded that values for digestibility obtained in pigs can be directly used for humans (Deglaire et al., 2009; Hodgkinson et al., 2022; Stein, 2024).

Since 1991, the protein digestibility corrected amino acid score (PDCAAS) method has been used to determine protein quality in human foods for regulatory purposes in the United States. However, several limitations to the PDCAAS procedure have been documented (Schaafsma, 2000; Mathai et al., 2017). These limitations include the fact that one single value obtained from the total tract digestibility of protein in rats is assumed to represent the ileal digestibility of each AA, which results in an overestimation of protein quality of lower-quality foods (FAO, 2013; Mathai et al., 2017). To address concerns about PDCAAS, the digestible indispensable amino acid score (DIAAS) method was proposed as a more accurate assessment of

protein quality because it considers the ileal digestibility of each IAA as an individual nutrient (FAO, 2013).

Dogs

The most frequent consideration of dog food manufacturers when using a protein source is regarding its digestibility (Oberbauer and Larsen, 2021). The total tract digestibility assay is usually used for measuring protein digestibility in feed ingredients for dogs because measuring ileal AA digestibility requires the use of ileal cannulated dogs, and ethical and animal welfare concerns have prevented their use (Li and Wu, 2023). Although the canine large intestine is relatively short, there is no significant AA absorption in the large intestine, but because some AA are fermented in the hindgut, the use of the total tract digestibility assay may overestimate the digestibility of AA (Hendriks et al., 2013).

Alternative approaches for assessing AA digestibility in proteins fed to dogs without the influence of hindgut microbiota are by using cecectomized roosters or minks as indirect models (Tjernsbekk et al., 2017; Oba et al., 2020). Indeed, a high correlation between rooster and dog data exists, and the precision-fed cecectomized rooster assay is less expensive and less time-consuming than using ileal cannulated dogs (Johnson et al., 1998; Li and Wu, 2023).

The DIAAS method, originally developed to assess protein quality in human foods, has also been used to determine protein quality in feed ingredients for companion animals (Oba et al., 2019; Reilly et al., 2020). However, regulatory agencies have not yet established a methodology specifically for companion animals, and its use is often intended to align with human nutritional trends and pet humanization practices. Therefore, AA composition continues to be used as the primary basis for establishing nutritional guidelines in diets for companion animals (Hendrik et al., 2013).

Pigs

Measures of AA digestibility in pigs have traditionally been made *in vivo*, and assays that determine SID of AA are used for pigs of all ages (Stein et al., 2007). Ileal digesta from pigs can be collected either by the slaughter method or by inserting a T-cannula in their distal ileum, with the latter being the preferred method because this is considered a less invasive approach, and cannulated pigs can be used repeatedly in a number of studies (Stein et al., 1998; King and Adeola, 2014). In addition, when calculating SID of AA, the use of indigestible markers is preferred over the total collection procedure because it reduces labor (Kong and Adeola, 2014).

Protein quality is not typically measured in feed ingredients used in livestock because diet formulation relies on SID values that are additive in mixed diets (Stein et al., 2005). Unlike protein scores, which attempt to evaluate overall protein quality, the formulation of diets for monogastric livestock is based on meeting the specific requirements of each IAA. This is achieved by calculating the SID values of AA from each ingredient and ensuring that the total diet meets the IAA requirements (Stein et al., 2007).

Protein Ingredients for Humans and Animals

Protein ingredients play a critical role in human and animal nutrition, serving as a primary source of IAA that are necessary for growth, maintenance, and overall health (Wu, 2016). A list of the most common traditional protein ingredients used for humans and animals is provided in Table 2.3 and Table 2.4, respectively.

The consumption of plant proteins is common among humans and animals (Hayes, 2018), but plant foods, unlike animal foods, may contain mycotoxins or anti-nutritional factors (Sapkota et al., 2007). Proteins from plants may also have low concentrations of some IAA (Hoffman and

Falvo, 2004). As an example, legumes are limiting in sulfur AA, and cereal grains have low concentrations of lysine, and corn is also low in tryptophan (Oberbauer and Larsen, 2021). Indeed, low DIAAS values for plant proteins were reported when compared with animal-based proteins, indicating that protein quality in these ingredients is low if used as the only source of digestible AA in diets for humans (Cervantes-Pahm et al., 2014; Mathai et al., 2017; Han et al., 2019). However, plant proteins are less expensive and more abundant than animal proteins (Hayes, 2018), and they have not been associated with negative connotations of animal diseases (Hill and Pas, 2004).

Animal-based foods provide the majority of AA in foods consumed by the United States population, and a significant amount of rendered animal products or byproducts from the human industry is incorporated into animal feed (Sapkota et al., 2007). Proteins from animal foods usually contain more AA and have a greater protein quality compared with plant proteins (Hoffman and Falvo, 2004; Mathai et al., 2017), but some animal proteins are sometimes linked to cardiovascular diseases or food-borne illnesses (Hoffman and Falvo, 2004). Environmental concerns are also sometimes linked to animal-based proteins, especially regarding the use of land, water, and energy (Hayes, 2018).

Alternative Proteins

Feed for livestock occupies approximately 40% of the world's total cropland, and an increasing population has brought attention to protein substitutes (Hefferon et al., 2023). The search for alternative solutions has led to the emergence of new technologies, including fermentation and cell-based cultivation (Hayes, 2018). An interest in powders with high protein content has also emerged (Hefferon et al., 2023). Alternative proteins may have high

concentrations of AA, but digestibility of protein and AA needs to be determined before nutritional recommendations can be made. Potential hazards of using these proteins also need to be considered. Although some safety aspects are intrinsic to the ingredient, many hazards may occur due to production and processing methods (Van der Spiegel et al., 2013).

Salmon protein hydrolysate

Characteristics and applications

Fish protein hydrolysates are produced by enzymatic or microbial hydrolysis followed by partial or total removal of fat and dehydration (Kristinsson and Rasco, 2000; Hou et al., 2017). Autolytic hydrolysis in fish products may also be performed, which involves the use of endogenous proteases (Kristinsson and Rasco, 2000). Offal from the fish industry, including viscera, skin, heads, and skeleton are underutilized, but they may be used in animal diets (Folador et al., 2006). Variations in chemical composition and protein quality of salmon protein hydrolysates may occur due to the environmental conditions, farming type, specific part of fish used in preparation, degree and type of hydrolysis, and quality of water (Folador et al., 2006; Hou et al., 2017). In human nutrition, fish hydrolyzed proteins have been used in food applications as flavor enhancers, protein supplements, and as functional ingredients (Kristinsson and Rasco, 2000). In pet nutrition, they are being increasingly used as food palatants, to manage food sensitivities, and to enhance nutrient digestibility (Folador et al., 2006; Freiche et al., 2020).

Advantages

The hydrolysis process allows the protein to be hydrolyzed into smaller peptides, which may be digested in the small intestine with a subsequent absorption of AA (Folador et al., 2006; Hou et al., 2017). Indeed, when evaluating digestibility and protein quality in salmon protein hydrolysate, it was demonstrated that this protein has greater IAA concentration, with greater

concentrations of arginine, leucine, and lysine, as well as greater AA digestibility compared with other fish substrates and traditional rendered foods (Folador et al., 2006; Tjernsbekk et al., 2017). Salmon protein hydrolysate also contains a considerable amount of omega-3 fatty acids and bioactive peptides that may confer health benefits (Folador et al., 2006; Hou et al., 2017). The use of enzymatic and microbial hydrolysis also aids in removing hyperallergenic and antinutritional factors, and the use of autolytic hydrolysis is simple, and there are no costs associated with exogenous enzymes (Kristinsson and Rasco, 2000; Hou et al., 2017).

Disadvantages

Salmon protein hydrolysate contains non-protein nitrogen (NPN), which results in a lower ratio between IAA and dispensable AA due to the increased presence of free AA and small peptides generated during enzymatic hydrolysis, as well as a high concentration of biogenic amines due to AA decarboxylation, where AA lose their carboxyl groups through the action of decarboxylase enzymes (Folador et al., 2006; Tjernsbekk et al., 2017). There is also potential for enzyme inhibitors to be present in raw protein materials if enzymatic hydrolysis is used (Hou et al., 2017). If microbial hydrolysis is performed, changes in microbial activity may result in inconsistency in the production of peptides and free AA (Hou et al., 2017). If an autolytic hydrolysis is utilized, different requirements for endogenous proteases are needed and can vary within species and in the same reaction, which makes the process challenging to control (Kristinsson and Rasco, 2000). Another limiting factor is that some fish protein hydrolysates may have a strong odor and bitter taste due to processing compromising palatability and acceptance as a food or feed ingredient (Kristinsson and Rasco, 2000).

Seaweed

Characteristics and applications

Seaweed is a multicellular organism form of algae that uses photosynthesis and grows in a marine environment (from capture, wild-harvested), but can also be cultivated in salt water (from aquaculture), with approximately 97% of its harvest weight derived from aquaculture (Van der Spiegel et al., 2013; Boyd et al., 2022). Currently, approximately 10,000 different species of seaweed have been described, and they produce proteins with different AA compositions than in terrestrial plants and AA composition may change depending on season, light intensity, temperature, place of collection, and nutrient concentration in seawater (Hayes, 2018). To quantify protein in seaweeds, conversion factors of 5.38, 4.92, and 5.13 are proposed for brown, red, and green species, respectively, due to the amount of NPN in these types of algae (Biancarosa et al., 2017).

Seaweeds are used in fresh or dried forms for direct human consumption, processed to provide hydrocolloids that do not contain protein such as agar, carrageenan, and alginate to be incorporated in animal diets as feed additives or used as functional ingredients, and in the treatment of industrial effluents, and fertilizers (Murphy et al., 2008; de Oliveira et al., 2009; Boyd et al., 2022). However, the use of a new processing method or application to an existing seaweed species may characterize them as novel proteins (Van der Spiegel et al., 2013).

Advantages

Seaweed does not compete with arable land needed for human food or animal feed, nor does it require fertilizers or pesticides. It is also believed that seaweed plays a role in the sequestration of carbon dioxide, nitrogen, and phosphorus in the ocean (Boyd et al., 2022; Hefferon et al., 2023). Moreover, seaweeds are capable of adapting to harsh conditions of the

marine environment and compared with terrestrial crops, they are easier to cultivate and have greater photosynthetic efficiency and productivity with faster growth and reproduction rates (Hayes, 2018). Biancarosa et al. (2017) demonstrated that several species of seaweeds are excellent sources of IAA, and that most species have concentrations of leucine, phenylalanine, threonine, tyrosine, and valine that are greater than protein-rich ingredients such as soybean meal and fishmeal.

Disadvantages

There are some safety concerns when including seaweed in food applications if environmental conditions are not well controlled such as the presence of anti-nutritional factors (i.e., tannins, lectins, phytic acid, trypsin, and alpha-amylase inhibitors), iodine, ammonium (from the seawater), dioxins (from industrial origin), and radioactive isotopes (Rees, 2003; de Oliveira et al., 2009; Van der Spiegel et al., 2013). In addition, if wild-harvested, seaweed can be exposed to pollution and heavy metals such as arsenic, cadmium, nickel, mercury, and others, depending on the species, growing phase, collection time, and the levels of heavy metals present in the water (Smith et al., 2010).

Egg-derived products

Characteristics and applications

Eggs are consumed throughout the world, and egg-derived products are widely used in human and animal nutrition. These products vary in composition, with egg whites being rich in albumen proteins like ovalbumin and ovotransferrin, whereas egg yolk contains lipids, phospholipids, and immunoglobulins (Miranda et al., 2015). In general, the use of egg powders and pasteurized liquid eggs is more common than the use of fresh eggs. In the food industry, eggs are commonly used for thickening, emulsifying, gelling, and coloring properties (Miranda

et al., 2015). Heat-treated eggs are used to improve foaming stability and reduce microbial load, and pasteurized liquid eggs are used to ensure safety in food and feed applications (Miranda et al., 2015). The use of hyperimmunized-hen eggs, egg byproducts, and egg hydrolysates for livestock has also been reported (Schmidt et al., 2003; Heo et al., 2015; Matsuoka et al., 2019). Egg powders are usually used in the manufacturing of companion animal foods, but there is an increase in the use of egg powder in diets for swine (Ma et al., 2017).

Advantages

Eggs have no use restrictions by religions compared with other animal proteins (Miranda et al., 2015). Egg-derived products usually have an excellent AA profile (Schmidt et al., 2003). Digestibility of AA in either raw or cooked eggs is high, and protein quality is considered excellent (Miranda et al., 2015; Fanelli et al., 2024). Bioactive compounds with antioxidant and immunomodulator properties have also been identified in egg-derived products (Abeyrathne et al., 2013). In addition, eggs are easy to handle and store and are considered highly nutritious foods that are low in ash and carbohydrates and high in vitamins and minerals (Miranda et al., 2015).

Disadvantages

Eggs contain saturated fat and cholesterol, and consumption of eggs and egg-derived products may result in food-borne illnesses, primarily caused by S*almonella* (Miranda et al., 2015). Raw eggs may also contain anti-nutritional factors such as ovomucoid proteins that can inhibit trypsin or avidin that can bind to biotin (Miranda et al., 2015). In addition, fat-soluble environmental contaminants such as persistent organic pollutants (i.e., pesticides and heavy metals) or dioxins (industrial byproducts) can accumulate in eggs through laying hen chickens by

feed contamination and soil exposure, which may affect growth, reproduction, and immune function of livestock (Piskorska-Pliszczynska et al., 2014).

Conclusions

To address the increasing global demand for protein, the use of alternative protein sources is necessary in both human and animal nutrition. Therefore, robust data for the nutritional value of alternative proteins needs to be generated to make sure these proteins can be included in diets for humans and animals without compromising the protein quality of the diet. More specifically, protein and AA digestibility must be assessed to determine the nutritional value of alternative protein sources to provide data for dietary formulation that meets nutritional requirements.

Tables

Table 2.1. Basal ileal endogenous losses of amino acids (AA) determined in humans and animals using a nitrogen-free diet[1]

Nutrient, µg/g dry matter intake	Human	Dog	Pig
Indispensable AA			
Arginine	949	370	375
Histidine	289	268	173
Isoleucine	478	362	327
Leucine	809	560	535
Lysine	601	441	416
Methionine	232	119	133
Phenylalanine	561	465	348
Threonine	872	1168	505
Tryptophan	263	-	134
Valine	731	536	471
Dispensable AA			
Alanine	659	515	532
Aspartic acid + asparagine	1290	960	800
Glutamic acid + glutamine	1310	1089	885
Glycine	-	600	1053
Proline	-	643	3104
Serine	741	925	460
Tyrosine	-	444	213

[1]Values adapted from Hendriks et al. (2002) and Hodgkinson et al. (2022).

Table 2.2. Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of amino acids (AA) fed to dogs or pigs[1]

	Adult dogs		Finishing-pigs	
Nutrient, %	AID	SID	AID	SID
Crude protein	76.2	81.4	80.7	87.8
Indispensable AA				
Arginine	87.3	89.6	91.0	94.9
Histidine	61.4	66.1	87.5	91.3
Isoleucine	77.7	81.6	81.9	87.5
Leucine	79.4	82.3	85.0	89.1
Lysine	76.8	80.0	83.8	88.9
Methionine	82.6	85.1	86.4	90.1
Phenylalanine	80.3	84.7	85.2	89.4
Threonine	62.0	75.1	78.5	86.2
Tryptophan	-	-	85.7	82.4
Valine	76.7	80.2	83.1	90.0
Dispensable AA				
Alanine	77.7	80.8	78.9	85.9
Aspartic acid + asparagine	67.0	71.1	82.8	88.0
Cysteine	56.5	-	79.9	87.6
Glutamic acid + glutamine	81.6	84.3	87.1	90.8
Glycine	73.1	76.7	74.3	89.1
Proline	78.8	82.6	80.5	104.2

Table 2.2 (cont.)

	Adult dogs		Finishing-pigs	
Nutrient, %	AID	SID	AID	SID
Serine	69.0	78.1	84.7	90.6
Tyrosine	77.2	82.5	85.1	90.5

[1]Values are based on commercial diets adapted from Stein et al. (2005) and Hendriks et al. (2013).

Table 2.3. Most common protein food ingredients used for humans[1]

Category	Raw material	Examples
Plant-derived	Whole grains	Quinoa, buckwheat, brown rice, oats
	Legumes and tubers	Lentils, chickpeas, black beans, soybeans
	Nuts and seeds	Almonds, peanuts, chia seeds, peanuts
	Vegetables	Broccoli, spinach, brussels sprouts
Animal-derived	Meat	Beef, chicken, pork, lamb
	Fish and seafood	Salmon, tuna, shrimp, cod
	Dairy products	Milk, cheese, yogurt
	Eggs	Chicken eggs

[1]Adapted from Hoffman and Falvo, 2004.

Table 2.4. Most common protein feed ingredients used for animals[1]

Category	Raw material	Examples
Plant-derived	Grains and grain byproducts	Corn, corn gluten meal, wheat gluten, distiller grains with solubles
	Legumes and tubers	Soybeans, soybean meal, peas, pea protein concentrate, potato protein
Animal-derived	Meat and meat byproducts	Meat, meat byproducts (lungs, spleen, liver, blood, plasma, bone, etc.),
	Poultry and poultry byproducts	Poultry, poultry byproducts (heads, feet, viscera)
	Rendered products	Meat meal, meat and bone meal, poultry byproduct meal, lamb meal, fish meal

[1]Adapted from National Research Council – NRC, 2006; 2012.

Literature Cited

Abeyrathne, E. D. N. S., Lee, H. Y., and Ahn, D. U. 2013. Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents: A review. Poult. Sci. 92:3292-3299. doi:10.3382/ps.2013-03391

Aggarwal, A., and Drewnowski, A. 2019. Plant-and animal-protein diets in relation to sociodemographic drivers, quality, and cost: Findings from the Seattle Obesity Study. Am. J. Clin. Nutr. 110:451-460. doi:10.1093/ajcn/nqz064

Batterham, E. S. 1992. Availability and utilization of AA for growing pigs. Nutr. Res. Rev. 5:1-18. doi:10.1079/NRR19920004

Batool, R., Butt, M. S., Sultan, M. T., Saeed, F., and Naz, R. 2015. Protein–energy malnutrition: A risk factor for various ailments. Crit. Rev. Food Sci. Nutr. 55:242-253. doi:10.1080/10408398.2011.651543

Biancarosa, I., Espe, M., Bruckner, C. G., Heesch, S., Liland, N., Waagbø, R., Torstensen, B., and Lock, E. J. 2017. Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. J. Appl. Phycol. 29:1001-1009. doi:10.1007/s10811-016-0992-1

Cervantes-Pahm, S. K., Liu, Y., and Stein, H. H. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/S0007114513004273

Deglaire, A., Bos, C., Tomé, D., and Moughan, P. J. 2009. Ileal digestibility of dietary protein in the growing pig and adult human. Br. J. Nutr. 102:1752-1759. doi:10.1017/S0007114509991279

de Oliveira, M. N., Freitas, A. L. P., Carvalho, A. F. U., Sampaio, T. M. T., Farias, D. F., Teixeira, D. I. A., Gouveia, S. T., and Pereira, J. G. 2009. Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. Food Chem. 115:254-259. doi:10.1016/j.foodchem.2008.11.081

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation #92. Rome

Fanelli, N. S., Martins, J. C., and Stein, H. H. 2024. The digestible indispensable amino acid score (DIAAS) in eggs and egg-containing breakfast meals is greater than in toast breads or hash browns served without eggs. J. Nutr. Sci. 13:e68. doi:10.1017/jns.2024.52

Folador, J. F., Karr-Lilienthal, L. K., Parsons, C. M., Bauer, L. L., Utterback, P. L., Schasteen, C. S., Bechtel, P. J., and Fahey, G. C. 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.2005-636

Freiche, V., Fontaine, J., and Blanchard, G. 2020. Dietary management of food allergies in dogs and cats: Hydrolyzed proteins. Vet. Clin. North Am. Small Anim. Pract. 50:1109-1125. doi:10.1016/j.cvsm.2020.06.007

Han, F., Han, F., Wang, Y., Fan, L., Song, G., Chen, X., Jiang, P., Miao, H., and Han, Y. 2019. Digestible indispensable amino acid scores of nine cooked cereal grains. Br. J. Nutr. 121:30-41. doi:10.1017/S0007114518003033

Hayes, M. 2018. Current and future trends in protein use and consumption. In: Novel proteins for food, pharmaceuticals and agriculture. 1st ed. Hoboken, NJ. doi:10.1002/9781119385332

Hefferon, K. L., De Steur, H., Perez-Cueto, F. J., and Herring, R. 2023. Alternative protein innovations and challenges for industry and consumer: An initial overview. Front. Sustain. Food Syst. 7:1038286. doi: 10.3389/fsufs.2023.1038286

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

Hendriks, W. H., Sritharan, K., and Hodgkinson, S. M. 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. doi:10.1046/j.1439-0396.2002.00385.x

Henchion, M., Moloney, A. P., Hyland, J., Zimmermann, J., and McCarthy, S. 2021. Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. Anim. 15:1751-7311. doi:100287.10.1016/j.animal.2021.100287

Heo, J. M., Woyengo, T. A., Kahindi, R. K., Kiarie, E., Maiti, P. K., and Nyachoti, C. M. 2015. Ileal amino acid digestibility in egg from hyperimmunized hens fed to weaned pigs and piglet response to diets containing egg products. Anim. Feed Sci. Technol. 204:52-61. doi:10.1016/j.anifeedsci.2015.03.011

Hill, D., and Pas, D. A. C. A. N. 2004. Alternative proteins in companion animal nutrition. In: Pet Food Association of Canada Fall Conference. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=7264b18da36a825ce8607fdac8e17025bad0d858 (accessed Jan. 2025)

Hodgkinson, S. M., Stroebinger, N., Van Der Wielen, N., Mensink, M., Montoya, C., Hendriks, W. H., De Vries, S., Stein, H. H., and Moughan, P. J. 2022. Comparison of true ileal

amino acid digestibility between adult humans and growing pigs. J. Nutr. 152:1635-1646. doi:10.1093/jn/nxac077

Hoffman, J. R., and Falvo, M. J. 2004. Protein–which is best? J. Sports Sci. Med. 3:118-130. PMCID: PMC3905294

Hou, Y., Wu, Z., Dai, Z., Wang, G., and Wu, G. 2017. Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. J. Anim. Sci. Biotechnol. 8:209-232. doi:10.1186/s40104-017-0153-9

Hodgkinson, S. M., Stein, H. H., de Vries, S., Hendriks, W. H., and Moughan, P. J. 2020. Determination of true ileal amino acid digestibility in the growing pig for calculation of digestible indispensable amino acid score (DIAAS). J. Nutr. 150:2621-2623. doi:10.1093/jn/nxaa210

Jansman, A. J. M., Smink, W., van Leeuwen, P., and Rademacher, M. 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Anim. Feed Sci. Technol. 98:49-60. doi:10.1016/S0377-8401(02)00015-9

Johnson, M. L., Parsons, C. M., Fahey, G. C. Jr., Merchen, N. R., and 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. J. Anim. Sci. 76:1112-1122. doi:10.2527/1998.7641112x

Kong, C., and Adeola, O. 2014. Evaluation of amino acid and energy utilization in feedstuffs for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

Kristinsson, H. G., and Rasco, B. A. 2000. Fish protein hydrolysates: production, biochemical, and functional properties. Crit. Rev. Food Sci. Nutr. 40:43-81. doi:10.1080/10408690091189266

Li, P., and Wu, G. 2023. Amino acid nutrition and metabolism in domestic cats and dogs. J. Anim. Sci. Biotechnol. 14:19-39. doi:10.1186/s40104-022-00827-8

Lopez, M. J., and Mohiuddin, S. S. 2024. Biochemistry, essential amino acids. In: StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557845/?report=classic (accessed Jan. 2025)

Ma, X. K., Zhang, S., Pan, L., and Piao, X. S. 2017. Effects of lysozyme on the growth performance, nutrient digestibility, intestinal barrier, and microbiota of weaned pigs fed diets containing spray-dried whole egg or albumen powder. Can. J. Anim. Sci. 97:466-475. doi:10.1139/cjas-2016-0143

Mathai, J. K., Liu, Y., and Stein, H. H. 2017. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br. J. Nutr. 117:490-499. doi:10.1017/S0007114517000125

Matsuoka, R., Kurihara, H., Nishijima, N., Oda, Y., and Handa, A. 2019. Egg white hydrolysate retains the nutritional value of proteins and is quickly absorbed in rats. Sci. World J. 2019. doi: 10.1155/2019/5475302

Millward, D. J., Layman, D. K., Tomé, D., and Schaafsma, G. 2008. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am. J. Clin. Nutr. 87:1576S-1581S. doi:10.1093/ajcn/87.5.1576S

Miranda, J. M., Anton, X., Redondo-Valbuena, C., Roca-Saavedra, P., Rodriguez, J. A., Lamas, A., Franco, C. M., and Cepeda, A. 2015. Egg and egg-derived foods: effects on human health and use as functional foods. Nutr. 7:706-729. doi:10.3390/nu7010706

Murphy, V., Hughes, H., and McLoughlin, P. 2008. Comparative study of chromium biosorption by red, green, and brown seaweed biomass. Chemosphere 70:1128-1134. doi:10.1016/j.chemosphere.2007.07.044

NRC. 2006. Nutrient requirements for dogs and cats. 6th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/10668

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

Nyachoti, C. M., de Lange, C. F. M., McBride, B. W., and Schulze, H. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Can. J. Anim. Sci. 77:149-163. doi:10.4141/A96-044

Oba, P. M., Utterback, P. L., Parsons, C. M., de Godoy, M. R. C., and Swanson, K. S. 2019. Chemical composition, true nutrient digestibility, and true metabolizable energy of chicken-based ingredients differing by processing method using the precision-fed cecectomized rooster assay. J. Anim. Sci. 97:998-1009. doi:10.1093/jas/sky461

Oba, P. M., Utterback, P. L., Parsons, C. M., and Swanson, K. S. 2020. True nutrient and amino acid digestibility of dog foods made with human-grade ingredients using the precision-fed cecectomized rooster assay. Transl. Anim. Sci. 4:442-451. doi:10.1093/tas/txaa167

Oberbauer, A. M., and Larsen, J. A. 2021. Amino acids in dog nutrition and health. In: Amino acids in nutrition and health: Amino acids in the nutrition of companion, zoo and farm animals. 1st ed. Cham, Switzerland. doi:10.1007/978-3-030-54743-0_12

Piskorska-Pliszczynska, J., Mikolajczyk, S., Warenik-Bany, M., Maszewski, S., and Strucinski, P. 2014. Soil as a source of dioxin contamination in eggs from free-range hens on a Polish farm. Sci. Total Environ. 466-467:447-454. doi:10.1016/j.scitotenv.2013.07.022

Rees, T. A. V. 2003. Safety factors and nutrient uptake by seaweeds. Mar. Ecol. Prog. Ser. 263:29-42. doi:10.3354/meps263029

Reilly, L. M., von Schaumburg, P. C., Hoke, J. M., Davenport, G. M., Utterback, P. L., Parsons, C. M., and de Godoy, M. R. 2020. Use of precision-fed cecectomized rooster assay and digestible indispensable amino acid scores to characterize plant- and yeast-concentrated proteins for inclusion in canine and feline diets. Transl. Anim. Sci. 4:txaa133. doi:10.1093/tas/txaa133

Sauer, W. C., and Ozimek, L. 1986. Digestibility of amino acids in swine: Results and their practical applications. A review. Livest. Prod. Sci. 15:367-388. doi:10.1016/0301-6226(86)90076-X

Schoenfeld, B. J., and Aragon, A. A. 2018. How much protein can the body use in a single meal for muscle-building? Implications for daily protein distribution. J. Int. Soc. Sports Nutr. 15:1-6. doi:10.1186/s12970-018-0215-1

Schaafsma, G. 2000. The protein digestibility–corrected amino acid score. J. Nutr. 130:1865S-1867S. doi:10.1093/jn/130.7.1865S

Schmidt, L. S., Nyachoti, C. M., and Slominski, B. A. 2003. Nutritional evaluation of egg byproducts in diets for early-weaned pigs. J. Anim. Sci. 81:2270-2278. doi:10.2527/2003.8192270x

Smith, J. L., Summers, G., and Wong, R. 2010. Nutrient and heavy metal content of edible seaweeds in New Zealand. N. Z. J. Crop Hortic. Sci. 38:19-28. doi:10.1080/01140671003619290

Stein, H. H., Pedersen, C., Wirt, A. R., and Bohlke, R. A. 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. doi:10.2527/2005.83102387x

Stein, H. H., Seve, B., Fuller, M. F., Moughan, P. J., and de Lange, C. F. M. 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

Stein, H. H., Shipley, C. F., and Easter, R. A. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436. doi:10.2527/1998.7651433x

Stein, H. H. 2024. The pig is an excellent model to determine amino acid digestibility of human foods and to generate data needed to meet human amino acid requirements. Front. Nutr. 11:1434430. doi: 10.3389/fnut.2024.1434430

Stoll, B., Burrin, D. G., Henry, J., Yu, H., Jahoor, F., and Reeds, P. J. 1998. Dietary amino acids are the preferential source of hepatic protein synthesis in piglets. J. Nutr. 128:1517-1524. doi:10.1093/jn/128.9.1517

Suenaga, R., Tomonaga, S., Yamane, H., Kurauchi, I., Tsuneyoshi, Y., Sato, H., Denbow, D. M., and Furuse, M. 2008. Intracerebroventricular injection of L-arginine induces sedative and hypnotic effects under an acute stress in neonatal chicks. Amino Acids. 35:139-146. doi:10.1007/s00726-007-0614-4

Tjernsbekk, M. T., Tauson, A. H., Kraugerud, O. F., and Ahlstrøm, Ø. 2017. Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility. J. Anim. Physiol. Anim. Nutr. 101:323-331. doi:10.1111/jpn.12608

Tomé, D. 2013. Digestibility issues of vegetable versus animal proteins: protein and amino acid requirements-functional aspects. Food Nutr. Bull. 34:272-274. doi:10.1177/156482651303400225

Van der Spiegel, M., Noordam, M. Y., and Van der Fels-Klerx, H. J. 2013. Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr. Rev. Food Sci. Food Saf. 12:662-678. doi:10.1111/1541-4337.12032

WHO. 2007. Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. World Health Organization

Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino acids. 37:1-17. doi:10.1007/s00726-009-0269-0

Wu, G. 2016. Dietary protein intake and human health. Food Funct. 7:1251-1265. doi:10.1039/c5fo01530h

Chapter 3: Determination of Digestible Indispensable Amino Acid Score for Salmon Hydrolysate Proteins[1]

Abstract

Salmon products are excellent foods that contain indispensable nutrients including fatty acids and amino acids (AA), but during processing, salmon co-products that cannot be used for the primary purpose are also generated. Examples of such co-products include salmon protein hydrolysate concentrate (SPHC) and salmon protein hydrolysate isolate (SPHI), but there is no information about the protein quality of these co-products. Therefore, the objective of this experiment was to use the digestible indispensable amino acid score (DIAAS) method to test the hypothesis that the protein in SPHC and two sources of SPHI (SPHI1 and SPHI2) can supplement lower-quality proteins. For children from 6 months to 3 years old and individuals older than 3 years, SPHC had greater ($P < 0.05$) DIAAS than SPHI1 and SPHI2, and SPHI2 had greater ($P < 0.05$) DIAAS than SPHI1. For children from 6 months to 3 years, leucine was the first limiting AA in SPHC, and tryptophan was the first limiting AA in SPHI1 and SPHI2. For individuals older than 3 years, there was no limiting AA (DIAAS ≥ 100) for SPHC, but for SPHI1 and SPHI2 leucine was the first limiting AA. All sources of salmon protein hydrolysates had excellent AA digestibility, and SPHC can be used to compensate for lower protein quality in other ingredients to produce a balanced diet that meets requirements for all indispensable AA for individuals older than 3 years.

Keywords: amino acids; digestibility; digestible indispensable amino acid score; fish; protein quality

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Introduction

Protein is an essential nutrient required by the human body due to requirements for indispensable amino acids (AA) that are needed for protein synthesis and other functions (Millward et al., 2008). To ensure the correct balance among AA, protein quality in human foods needs to be determined. The digestible indispensable amino acid score (DIAAS) is the preferred method to evaluate protein quality in foods because it is based on determination of AA absorption before the end of the small intestine (the ileum), which is more accurate than determining total tract digestibility of AA due to microbial fermentation in the hindgut (FAO, 2013). Because of the difficulty of determining ileal digestibility of AA in humans, the pig is often used as the preferred model (FAO, 2013), and values for AA digestibility in pigs are in close agreement with values obtained in humans (Hodgkinson et al., 2022).

Salmon provides essential nutrients to diets, including indispensable AA, omega-3fatty acids, vitamins, and minerals, which make salmon an excellent dietary choice, especially for young children and people with high AA requirements (Khalili et al., 2018). Processing of salmon to generate salmon fillets or other products generates co-products, which can be used to produce salmon hydrolysate proteins. Hydrolysate proteins are considered superior to intact proteins because they are pre-digested by enzymes, resulting in smaller peptides that presumably have improved digestibility of AA (Koopman et al., 2009; Khalili et al., 2018). Indeed, greater digestibility of AA has been demonstrated for salmon, squid, and shrimp protein hydrolysates compared with intact fish meals when fed to pigs, dogs, or roosters (Gottlob et al., 2006; Faber et al., 2010; NRC 2012; Sulabo et al., 2013; Guilherme-Fernandes et al., 2024).

Protein quality of porcine and bovine protein hydrolysates has been reported (Bindari et al., 2018), but no data demonstrating protein quality in salmon protein hydrolysates for humans

are available, which restricts their use in meals that are prepared with the objective of balancing indispensable AA. Therefore, the objective of this experiment was to determine DIAAS in three novel sources of salmon protein hydrolysates and to test the hypothesis that these proteins can be used to supplement lower-quality proteins and create meals that are balanced in AA.

Material and Methods

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois prior to initiation of the experiment. Female pigs that were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

Ingredients and experimental diets

Three novel salmon protein hydrolysates, including one source of salmon protein hydrolysate concentrate (SPHC) and two sources of salmon protein hydrolysate isolates (SPHI1 and SPHI2), were produced by Biomega Group AS, Skageneset, Norway (Table 3.1). The SPHC was produced from raw food-grade salmon materials, and included bone, offcuts, and viscera. The viscera went through autolysis (hydrolysis by endogenous proteases) before being heated to at least 85 °C and separated into three fractions: oil, water-soluble, and water-insoluble fractions. The oil was removed, whereas the other two fractions were mixed with the bones and non-soluble fraction from the offcut line (leftover trimmings and scraps) before being dried in a disc-drier and milled.

The SPHI1 and SPHI2 were produced from raw food-grade salmon materials (offcut without viscera) using a commercial food-grade protease in a patented process (Biomega Group AS). After enzymatic hydrolysis, all fractions were heated to at least 85 °C before the water-

soluble content was separated from the oil and non-soluble fractions by centrifugal force. In the production of SPHI1, the water-soluble fraction was spray-dried without prior filtration, but in the production of SPHI2 the water-soluble fraction was ultrafiltered, and the permeate from the ultrafiltration was passed through a nano-filter. The retentate from the nanofiltration was concentrated in an evaporator before spray-drying.

Three diets were formulated by including each of the three salmon protein hydrolysates in one diet as the only source of crude protein (CP) and AA. A nitrogen-free diet was also formulated and used to determine basal endogenous losses of CP and AA. Therefore, a total of four diets were used (Tables 3.2 and 3.3). Diet formulation was adjusted to obtain approximately 10% CP in all diets [dry matter (DM) basis] as recommended (FAO, 2014). All diets included vitamins and minerals to meet or exceed nutrient requirement estimates for swine (NRC, 2012), and 0.40% titanium dioxide was used as an indigestible marker. Samples of each ingredient and diet were collected at the time of diet mixing and used for chemical analysis.

Experimental design and digestibility experiment

Twelve growing gilts (average initial body weight: 85.8 ± 9.1 kg) with a T-cannula in the distal ileum were randomly allotted to quadruplicated 3 × 2 Youden squares, which included three salmon protein diets and two 7-day periods, for a total of eight observations per treatment. All pigs received a nitrogen-free diet during the third period to determine basal endogenous AA losses, allowing each pig to serve as its own control for calculating standardized ileal digestibility (SID; FAO, 2014). The T-cannulas were surgically installed in the pigs when they had an average initial body weight of 30 kg (Stein et al., 1998); the pigs had been used in two previous experiments before the current experiment. However, before being used in the present experiment, they were fed a common grower phase diet for 2 weeks.

Pigs were housed in individual pens (1.5 × 2.5 m) in an environmentally controlled room. Pens had smooth sides and partially slatted floors, and a feeder and a nipple drinker were installed in each pen. All pigs were fed their assigned diets in a daily amount equivalent to 8% of body weight$^{0.75}$ calculated on a DM basis. The daily feed provisions were divided into two equal meals that were provided every day at 0700 and 1600 h (FAO, 2014). Water was available at all times and the amount of feed supplied each day was recorded. Pig weights were recorded at the end of each experimental period to calculate feed allowance for the following period.

The initial 5 days of each period were considered an adaptation period to the diet, and ileal digesta were collected for 9 h on days 6 and 7 according to standard procedures (Stein et al., 1998; Hodgkinson et al., 2020). A plastic bag was attached to the cannula barrel, and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta, or at least once every 30 min, and immediately frozen at −20 °C to prevent bacterial degradation of the AA in the digesta. At the conclusion of the experiment, pigs had an average final body weight of 92.4 ± 10.0 kg.

Chemical analysis

At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a subsample was collected for chemical analysis. Ileal digesta samples were lyophilized (freeze-dryer Gamma 1-16 LSCplus, IMA Life, Christ, Osterode amHarz, Germany) and finely ground prior to chemical analysis. Samples of all ingredients, diets, and ileal digesta were analyzed for DM (AOAC Official Method 930.15; AOAC, 2019), and AA were analyzed according to AOAC Official Method, 2019 982.30E (a, b, c) on an AA analyzer (model L8800, Hitachi High Technologies America, Inc., Pleasanton, CA, USA). Nitrogen was analyzed by combustion (Method 990.03; AOAC, 2019) using a LECO FP628 nitrogen analyzer (LECO

Corp., Saint Joseph, MI, USA). Crude protein in ingredients, diets, and ileal digesta was calculated as nitrogen × 6.25, except for SPHI2, for which a nitrogen conversion factor of 5.60 was used according to the manufacturer's specifications. Diets and ileal digesta samples were also analyzed for titanium (Myers et al., 2004).

Calculations

The apparent ileal digestibility (AID), basal endogenous losses, and SID of CP and all AA in each diet were calculated (Stein et al., 2007). Subsequently, the quantity (mg) of SID AA per gram of protein in each diet was calculated by multiplying the digestibility for each AA by the AA concentration in each protein. This quantity was then divided by the reference value for each indispensable AA to calculate the digestible indispensable AA reference ratio for each AA using the following equation (FAO, 2013):

Digestible indispensable AA reference ratio = Digestible indispensable AA content in 1 g protein of food (mg) / mg of the same dietary indispensable AA in 1 g of reference protein.

The DIAAS values were calculated for children (from 6 months to 3 years old) and for older children, adolescents, and adults (individuals older than 3 years) using the following equation (FAO, 2013):

DIAAS (%) = 100 × Lowest value of digestible indispensable AA reference ratio.

The DIAAS values for the salmon protein hydrolysates were calculated based on the CP content using the 6.25 nitrogen conversion factor as recommended (FAO, 2013). However, for SPHI2, the DIAAS values were also calculated using the 5.60 nitrogen factor to provide a more accurate value for this ingredient.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (9.4 version, SAS Institute, Cary, NC, USA) using the pig as the experimental unit. Normality of residuals and homogeneity of variances were confirmed using the UNIVARIATE procedure of SAS. Brown and Forsythe's test was used to confirm variance homogeneity and, when this assumption was not met, data were transformed using the BOXCOX procedure, and assumptions were verified. Outliers were detected as observations that deviated from 1st or 3rd quartiles by ±3 times the interquartile range and removed from the treatments. The statistical model included diet as the fixed effect, whereas pig, square, and period were considered random effects. Treatment means were calculated using LSMeans and, if significant, means were separated using the PDIFF option in the MIXED procedure. Results were considered significant at $P < 0.05$.

Results

The AID of most AA was greater ($P < 0.05$) in SPHC compared with SPHI1 and SPHI2, with the exception that for the AID of methionine no difference was observed between SPHC and SPHI1, and for the AID of serine no difference was observed between SPHC and SPHI2 (Table 3.4). The AID of CP was greater ($P < 0.05$) for SPHC and SPHI1 than for SPHI2. The AID of arginine and glycine were greater ($P < 0.05$) for SPHI1 and SPHI2 compared with SPHC. No differences were observed for AID of lysine, alanine, and glutamic acid among the three salmon hydrolysate proteins.

The SID of CP, histidine, methionine, and tryptophan were greater ($P < 0.05$) in SPHC and SPHI1 compared with SPHI2 (Table 3.5). The SID of threonine, cysteine, and serine were greater ($P < 0.05$) for SPHC than for SPHI1 and SPHI2, but for the SID of aspartic acid no

difference was observed between SPHC and SPHI1. In addition, no differences among the three salmon hydrolysate proteins were observed for the SID of the remaining AA.

For children from 6 months to 3 years old and individuals older than 3 years, SPHC had greater ($P < 0.05$) DIAAS compared with SPHI1 and SPHI2, and SPHI2 had greater ($P < 0.05$) DIAAS than SPHI1 (Table 3.6). For children, leucine was the first limiting AA for SPHC and tryptophan was the first limiting AA for SPHI1 and SPHI2. For individuals older than 3 years, there was no limiting AA (DIAAS ≥ 100) for SPHC, but for SPHI1 and SPHI2 leucine was the first limiting AA.

Discussion

Salmon is a widely consumed food and includes several species that may be caught in the wild or produced in fisheries or aquaculture systems. Since the mid-1990s, production of farmed salmon has surpassed that of wild-caught salmon and now represents 80% of the global salmon supply (Ziegler and Hilborn, 2023). Salmon is primarily used to produce salmon fillets or steaks, but co-products are also generated, although considered low-value products. However, hydrolyzation of some of the co-products including heads, bones, and skin may increase their values (Folador et al., 2006). The process of making salmon protein hydrolysates involves hydrolyzing the proteins into smaller peptides and AA, which enhances their nutritional and functional properties (Chalamaiah et al., 2012; Malcorps et al., 2021). This process uses specific enzymes to hydrolyze the proteins at specific sites (Vázquez et al., 2019). The choice of temperature, enzymes, pH, and process duration are critical factors that influence the yield, molecular weight distribution, and bioactive properties of the resulting hydrolysates. After enzymatic hydrolysis, the mixture undergoes centrifugation or filtration to remove insoluble

materials (Vázquez et al., 2019). The liquid hydrolysates can then be dried to obtain a powdered form that can be incorporated into food preparations. Therefore, protein hydrolysates may be used in food and beverage formulations, as well as dietary supplements. There is, however, a lack of knowledge about the protein quality of salmon hydrolysis co-products for human consumption, and the current research was conducted to help fill this gap.

The AA composition of the salmon protein hydrolysates indicated that tryptophan and cysteine were the AA with lowest concentrations, which is in agreement with published data for these proteins (Sandbakken et al., 2023). Nutrient composition analysis indicated that the intended concentrations of CP and AA were present in all diets. Pigs that received the diet with SPHC readily consumed their assigned meal, but pigs receiving diets containing SPHI1 or SPHI2 did not consume all of their daily allotted feed (average refusal SPHI1: 40%; SPHI2: 38%), which may be attributed to palatability issues because fish protein hydrolysates may have a bitter taste due to the presence of hydrophobic AA that are exposed during the hydrolysis process (Kristinsson and Rasco, 2000). However, the recovery of the indigestible marker was as expected and digestibility of AA was not believed to have been affected by the reduced feed intake of these proteins.

The SID of AA demonstrated that threonine was the indispensable AA with the lowest digestibility, which is because the greatest source of endogenous protein is mucin, which is high in threonine (Adeola et al., 2016; Park et al., 2024). Values for digestibility of AA in the salmon proteins were also generally in agreement with published data for digestibility of AA in salmon protein hydrolysate, with cysteine having the lowest SID among all AA (Gottlob et al., 2006; Tjernsbekk et al., 2017; Sandbakken et al., 2023). The lysine:CP for all salmon proteins was between 5.7% and 7.7%, compared with 5.5% in a regular salmon protein hydrolysate, indicating

that the drying methods used in the production of these proteins did not destroy lysine. The SID for most indispensable AA was not different among the three hydrolysates, but the observation that SID of histidine, methionine, and tryptophan were less in SPHI2 compared with SPHC and SPHI1 may be due to the addition of the evaporation step before spray-drying, which may have resulted in protein aggregation reducing enzyme digestibility, but more research is needed to confirm this hypothesis.

Based on the calculated DIAAS values, SPHC can be considered a 'good' quality protein for children from 6 months to 3 years old and an 'excellent' quality protein for individuals older than 3 years, and may be used to complement lower quality proteins (FAO, 2013). Viscera were used in the manufacture of SPHC and, despite the fact that viscera are sometimes considered low-value co-products, they contain more AA than proteins in heads and bones, indicating that inclusion of viscera in a mixed protein may contribute to improved protein quality (Siddik et al., 2021; Nikoo et al., 2023). It is therefore likely that it was the presence of viscera in SPHC that resulted in this protein having the greatest DIAAS among the three hydrolysates.

No claims regarding protein quality can be made for SPHI1 and SPHI2 for both age groups, because DIAAS was less than 75 for these two proteins (FAO, 2013). However, if a nitrogen factor of 5.60 is used for calculating DIAAS, SPHI2 can be considered as having a 'good' protein quality for individuals older than 3 years. Although FAO recommends the use of 6.25 as the nitrogen factor for standardization purposes, ingredients that have a lower nitrogen conversion factor may have their protein quality underestimated if a greater conversion factor is used, because the ingredient AA composition is an important factor used in DIAAS calculations (FAO, 2013). A greater DIAAS was also demonstrated for pistachio nuts if a nitrogen conversion factor of 5.30 was used rather than 6.25 (Bailey and Stein, 2020).

According to published data, the DIAAS in salmon fillet powder used in food preparations is between 86 and 93, having leucine or valine as first limiting AA (Desai et al., 2018). In the present work, leucine or tryptophan was the first limiting AA. Leucine is the AA with the greatest requirements by humans due to its role in muscle protein synthesis (Mero, 1999); although the concentration of digestible leucine was relatively high in the hydrolysates, the high requirement for leucine caused this AA to be limiting for both age groups. Tryptophan was also a limiting AA in SPHI1 and SPHI2 for children from 6 months to 3 years old because of its low concentration in salmon hydrolysates and its low SID compared with other indispensable AA, which is common in animal coproducts because of the high concentration of collagen in connective tissue, cartilage, and skin (Rojas et al., 2013). Therefore, although both salmon hydrolysate isolates had a high protein concentration, the digestible concentration of leucine and tryptophan was not sufficient to meet requirements for the age groups used in DIAAS calculations. It is likely that the absence of viscera in both protein isolates also contributed to the reduced quality compared with the SPHC.

If the sum of analyzed AA in an ingredient is not close to the CP, it is likely that the protein contains non-protein nitrogen (NPN). The NPN in salmon hydrolysates may include free nitrates, biogenic amines, and nucleotides due to the presence of bones and cartilage in fish co-products (Hendriks et al., 2002; Nguyen et al., 2022). The presence of bones may also reduce protein quality because AA in collagen have low digestibility (Hendriks et al., 2002; Rojas et al., 2013). The SPHC and SPHI1 products contained approximately 7% and 20% NPN, respectively, likely due to the presence of biogenic amines. Indeed, salmon protein hydrolysates had the highest concentration of biogenic amines when compared with other fish co-products (Folador et al., 2006). Differences in NPN were previously observed among different types of fish co-

products, and were affected by the fish species being processed as well as the raw materials and processing used, with NPN content ranging from 4% to 19% (Folador et al., 2006; Nguyen et al., 2022). In porcine and bovine hydrolysates, the NPN content ranged from 1% to 17% and also depended on the processing and raw materials used (Bindari et al., 2018).

The lower DIAAS values in the SPHI1 product compared with SPHI2 may be due to the high content of NPN and the lower concentration of indispensable AA relative to CP, which is one of the factors that determine DIAAS. The fact that SPHI1 was not ultrafiltrated after separation may have contributed to the increased NPN compared with SPHI2, because NPN compounds are released into the liquid processing streams with most of the water, including potential impurities and larger peptide fragments (Nguyen et al., 2022), indicating that the ultrafiltration step in the production of SPHI2 enhanced protein quality. This clearly demonstrates that differences in salmon co-products exist, which may affect their protein quality. It was therefore concluded that the hypothesis that salmon protein hydrolysates can improve protein quality of a meal containing low-quality proteins can only be partially accepted, because the hypothesis was true for SPHC consumed by individuals older than 3 years, but not if consumed by younger children. For SPHI1 and SPHI2, the hypothesis was rejected because the DIAAS values were too low to allow these proteins to complement other low-quality proteins.

Conclusions

The three salmon hydrolysate proteins used in this experiment have SID values for most AA greater than 85%, indicating excellent digestibility of AA. When compared with FAO requirements for AA, no claims regarding protein quality can be made for SPHI1 because DIAAS was less than 75 for both children and individuals older than 3 years. However, SPHI2

was considered a ‘good’ quality protein for individuals older than 3 years if a lower Jones factor than 6.25 was used, and SPHC was considered a ‘good’ quality protein for children from 6 months to 3 years old and an ‘excellent’ quality protein for individuals older than 3 years. Therefore, SPHC can be used to compensate for ingredients with lower protein quality to produce a diet for individuals older than 3 years that is balanced in all AA.

Tables

Table 3.1. Analyzed nutrient composition of ingredients (as-fed basis)[1]

Item, %	SPHC	SPHI1	SPHI2
Dry matter	96.69	96.16	96.37
Crude protein[2]	68.63	92.75	91.95
Indispensable amino acids			
Arginine	4.10	5.28	6.63
Histidine	1.59	1.63	2.07
Isoleucine	2.83	2.21	2.92
Leucine	4.40	4.15	4.83
Lysine	4.76	5.33	7.08
Methionine	1.83	1.75	2.34
Phenylalanine	2.54	2.14	2.66
Threonine	2.82	2.81	3.66
Tryptophan	0.92	0.47	0.66
Valine	3.27	2.84	3.69
Dispensable amino acids			
Alanine	4.24	6.28	7.18
Aspartic acid	5.91	6.70	9.16
Cysteine	0.65	0.33	0.53
Glutamic acid	7.87	10.54	13.61
Glycine	6.15	11.32	13.10
Proline	3.46	5.48	6.79

Table 3.1 (cont.)

Item, %	SPHC	SPHI1	SPHI2
Serine	2.43	2.83	3.53
Tyrosine	1.49	0.99	1.50
Total amino acids	61.26	73.08	91.94
Lysine:crude protein ratio	6.94	5.75	7.70
Non-protein nitrogen[3]	7.37	19.67	0.01

[1]SPHC, salmon protein hydrolysate concentrate; SPHI1, salmon protein hydrolysate isolate 1; SPHI2, salmon protein hydrolysate isolate 2.

[2]Crude protein for SPHC and SPHI1 was calculated as nitrogen × 6.25, but for SPHI2, crude protein was calculated as nitrogen × 5.60.

[3]Calculated as: crude protein – total amino acids.

Table 3.2. Ingredient composition of experimental diets (as-fed basis)[1]

Ingredient, %	SPHC	SPHI1	SPHI2	Nitrogen-free
Experimental protein	14.00	10.80	10.80	-
Corn starch	66.25	68.00	67.50	78.10
Sucrose	10.00	10.00	10.00	10.00
Canola oil	5.00	5.00	5.00	5.00
Solka floc	3.00	3.00	3.00	3.00
Dicalcium phosphate	-	1.00	1.50	1.70
Ground limestone	0.25	0.80	0.50	0.40
Sodium chloride	0.40	0.40	0.40	0.40
Magnesium oxide	0.10	0.10	0.10	0.10
Potassium carbonate	0.10	-	0.30	0.40
Titanium dioxide	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.50	0.50	0.50	0.50

[1]SPHC, salmon protein hydrolysate concentrate; SPHI1, salmon protein hydrolysate isolate 1; SPHI2, salmon protein hydrolysate isolate 2. All diets, except the nitrogen-free diet, were formulated to contain approximately 10% crude protein (dry matter basis).

[2]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg;

Table 3.2 (cont.)

biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 3.3. Analyzed nutrient composition of experimental diets (as-fed basis)[1]

Item, %	SPHC	SPHI1	SPHI2	Nitrogen-free
Dry matter	92.63	92.26	91.98	91.40
Crude protein[2]	9.76	10.00	9.88	0.27
Indispensable amino acids				
Arginine	0.51	0.55	0.69	0.01
Histidine	0.23	0.19	0.24	0.01
Isoleucine	0.40	0.26	0.35	0.01
Leucine	0.63	0.49	0.58	0.03
Lysine	0.67	0.62	0.81	0.02
Methionine	0.25	0.21	0.25	0.01
Phenylalanine	0.36	0.24	0.31	0.02
Threonine	0.38	0.32	0.42	0.01
Tryptophan	0.11	0.05	0.07	0.02
Valine	0.46	0.33	0.43	0.02
Dispensable amino acids				
Alanine	0.60	0.73	0.84	0.02
Aspartic acid	0.84	0.78	1.07	0.02
Cysteine	0.08	0.04	0.06	0.01
Glutamic acid	1.17	1.29	1.63	0.04
Glycine	0.85	1.30	1.52	0.01
Proline	0.49	0.63	0.78	0.02
Serine	0.35	0.36	0.45	0.01

Table 3.3 (cont.)

Item, %	SPHC	SPHI1	SPHI2	Nitrogen-free
Tyrosine	0.18	0.10	0.14	0.01
Total amino acids	8.56	8.49	10.64	0.30

[1]SPHC, salmon protein hydrolysate concentrate; SPHI1, salmon protein hydrolysate isolate 1; SPHI2, salmon protein hydrolysate isolate 2.

[2]Crude protein for the SPHC and SPHI1 diets was calculated as nitrogen × 6.25, but for SPHI2 diet, crude protein was calculated as nitrogen × 5.60

Table 3.4. Apparent ileal digestibility of crude protein and amino acids in experimental ingredients[1]

Item, %	SPHC	SPHI1	SPHI2	SEM	*P*-value
Crude protein	73.3[a]	74.6[a]	68.5[b]	1.97	0.048
Indispensable amino acids					
Arginine	81.9[b]	87.2[a]	87.1[a]	1.65	0.009
Histidine	83.3[a]	79.7[b]	79.9[b]	0.87	0.005
Isoleucine	84.0[a]	77.1[c]	80.3[b]	0.93	0.001
Leucine	85.5[a]	81.1[b]	82.0[b]	0.71	0.001
Lysine	83.3	82.9	83.5	0.95	0.884
Methionine	88.3[a]	86.2[ab]	84.5[b]	0.87	0.013
Phenylalanine	84.2[a]	77.8[c]	80.5[b]	0.91	0.001
Threonine	76.1[a]	67.8[c]	72.0[b]	1.33	0.001
Tryptophan	83.6[a]	69.3[b]	72.4[b]	2.33	<0.0001
Valine	83.0[a]	76.9[b]	79.3[b]	0.90	0.001
Dispensable amino acids					
Alanine	80.6	83.8	82.3	1.60	0.131

Table 3.4 (cont.)

Item, %	SPHC	SPHI1	SPHI2	SEM[2]	*P*-value
Aspartic acid	70.9[a]	60.6[b]	55.5[b]	3.00	0.005
Cysteine	56.1[a]	-7.4[c]	18.2[b]	3.86	<0.0001
Glutamic acid	83.7	83.2	83.4	0.94	0.912
Glycine	70.5[b]	78.8[a]	76.2[a]	2.96	0.032
Serine	75.6[a]	72.0[b]	73.7[ab]	1.46	0.032
Tyrosine	79.9[a]	64.5[c]	72.0[b]	1.46	<0.0001

[a-c]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI1, salmon protein hydrolysate isolate 1; SPHI2, salmon protein hydrolysate isolate 2. Data are means of 8 observations per treatment, except for the SPHI1 that had 6 observations per treatment due to statistical outliers.

Table 3.5 Standardized ileal digestibility of crude protein and amino acids in experimental ingredients[1,2]

Item, %	SPHC	SPHI1	SPHI2	SEM	*P*-value
Crude protein	94.0[a]	95.0[a]	88.6[b]	2.48	0.032
Indispensable amino acids					
Arginine	99.0	102.8	99.4	2.29	0.100
Histidine	93.5[a]	92.4[a]	89.7[b]	0.86	0.007
Isoleucine	93.9	92.6	91.7	0.83	0.117
Leucine	94.8	93.6	92.5	0.71	0.061
Lysine	94.5	95.4	93.0	0.90	0.141
Methionine	92.8[a]	91.9[a]	89.3[b]	0.87	0.016
Phenylalanine	93.9	93.1	92.3	0.86	0.382
Threonine	92.1[a]	87.0[b]	86.6[b]	1.09	0.022
Tryptophan	93.1[a]	91.5[a]	87.9[b]	1.87	0.011
Valine	93.7	91.9	90.8	0.96	0.077
Dispensable amino acids					
Alanine	94.4	95.6	92.3	1.59	0.091
Aspartic acid	82.2[a]	73.4[ab]	64.6[b]	2.99	0.002

Table 3.5 (cont.)

Item, %	SPHC	SPHI1	SPHI2	SEM[3]	*P*-value
Cysteine	87.2[a]	56.7[b]	62.1[b]	3.43	<0.0001
Glutamic acid	93.7	92.8	90.8	1.00	0.062
Glycine	93.2	93.0	88.7	2.86	0.168
Serine	92.7[a]	89.1[b]	87.1[b]	1.09	0.002
Tyrosine	94.0	91.7	91.2	1.50	0.361

[a-c]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI1, salmon protein hydrolysate isolate 1; SPHI2, salmon protein hydrolysate isolate 2. Data are means of 8 observations per treatment, except for the SPHI1 that had 6 observations per treatment due to statistical outliers.

[2]The standardized ileal digestibility values were calculated by correcting values for basal ileal endogenous losses for each pig as its own control. However, average values of basal ileal endogenous losses (g/kg dry matter intake) were calculated as follows: CP 21.57, Arginine 0.91, Histidine 0.26, Isoleucine 0.43, Leucine 0.65, Lysine 0.82, Methionine 0.12, Phenylalanine 0.38, Threonine 0.66, Tryptophan 0.12, Valine 0.53, Alanine 0.89, Aspartic acid 1.05, Cysteine 0.27, Glutamic acid 1.29, Serine 0.65, and Tyrosine 0.28.

Table 3.6. Reference ratios and digestible indispensable amino acid score (DIAAS) in salmon hydrolysate proteins[1]

Item	SPHC	SPHI1	SPHI2[2]	SEM	*P*-value
		Child reference ratio[3]			
Histidine	1.08	0.81	0.90 [1.01]		
Isoleucine	1.21	0.69	0.82 [0.91]		
Leucine	0.92	0.63	0.66 [0.74]		
Lysine	1.15	0.96	1.13 [1.26]		
SAA	1.23	0.71	0.87 [0.97]		
AAA	1.06	0.60	0.72 [0.80]		
Threonine	1.22	0.85	1.00 [1.11]		
Tryptophan	1.47	0.55	0.66 [0.74]		
Valine	1.04	0.65	0.76 [0.85]		
DIAAS, %	92[a] (Leucine)	55[c] (Tryptophan)	66[b] [74] (Tryptophan)	1.00	<0.0001
		Older child, adolescent, adult reference ratio[4]			
Histidine	1.35	1.01	1.13 [1.26]		
Isoleucine	1.29	0.74	0.87 [0.97]		

Table 3.6 (cont.)

Item	SPHC	SPHI1	SPHI2[2]	SEM	*P*-value
Leucine	1.00	0.69	0.71 [0.80]		
Lysine	1.37	1.14	1.34 [1.49]		
SAA	1.44	0.83	1.02 [1.14]		
AAA	1.35	0.76	0.91 [1.01]		
Threonine	1.51	1.05	1.24 [1.38]		
Tryptophan	1.89	0.70	0.86 [0.96]		
Valine	1.12	0.70	0.82 [0.91]		
DIAAS, %	100[a]	69[c] (Leucine)	71[b] [80] (Leucine)	0.76	<0.0001

[a-c]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]AAA, aromatic amino acids (phenylalanine + tyrosine); SAA, sulfur amino acids (methionine + cysteine); SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI1, salmon protein hydrolysate isolate 1; SPHI2, salmon protein hydrolysate isolate 2. First-limiting AA is in parentheses. DIAAS claims: < 75 = no protein quality claims; between 75 – 99 = "good" protein quality; ≥ 100 = "excellent" protein quality (FAO, 2013).

[2]Values in brackets for SPHI2 represent values for DIAAS calculated using the 5.60 nitrogen conversion factor for CP in this ingredient.

Table 3.6 (cont.)

[3]The DIAAS were calculated using the recommended AA scoring pattern for a child (6 months to 3 years). The indispensable AA reference patterns are expressed as mg AA/g protein: Histidine, 20; Isoleucine, 32; Leucine, 66; Lysine, 57; Sulfur AA, 27; Aromatic AA, 52; Threonine, 31; Tryptophan, 8.5; Valine, 43.2 (FAO, 2013).

[4]The DIAAS were calculated using the recommended AA scoring pattern for an older child, adolescent, and adult (older than 3 years). The indispensable AA reference patterns are expressed as mg AA/g protein: Histidine, 16; Isoleucine, 30; Leucine, 61; Lysine, 48; Sulfur AA, 23; Aromatic AA, 41; Threonine, 25; Tryptophan, 6.6; Valine, 40.2 (FAO, 2013).

Literature Cited

Adeola, O., Xue, P. C., Cowieson, A. J., and Ajuwon, K. M. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221:274-283. doi:10.1016/j.anifeedsci.2016.06.004

AOAC Int. 2019. Official Methods of Analysis of AOAC. 21st ed., Association of Official Analytical Chemists. Rockville, MD

Bailey, H. M., and Stein, H. H. 2020. Raw and roasted pistachio nuts (*Pistacia vera L.*) are 'good' sources of protein based on their digestible indispensable amino acid score as determined in pigs. J. Sci. Food Agric. 100:3878-3885. doi:10.1002/jsfa.10412

Bindari, Y. R., Lærke, H. N., and Nørgaard, J. V. 2018. Standardized ileal digestibility and digestible indispensable amino acid score of porcine and bovine hydrolyzates in pigs. J. Sci. Food Agric. 98:2131-2137. doi:10.1002/jsfa.8697

Chalamaiah, M., Hemalatha, R., and Jyothirmayi, T. 2012. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chem. 135:3020-3038. doi:10.1016/j.foodchem.2012.06.100

Desai, A. S., Brennan, M. A., and Brennan, C. S. 2018. Amino acid and fatty acid profile and digestible indispensable amino acid score of pasta fortified with salmon (*Oncorhynchus tshawytscha*) powder. Eur. Food Res. Technol. 244:1729-1739. doi:10.1007/s00217-018-3083-9

Faber, T. A., Bechtel, P. J., Hernot, D. C., Parsons, C. M., Swanson, K. S., Smiley, S., and Fahey Jr, G. C. 2010. Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays. J. Anim. Sci. 88:1421-1432. doi:doi.org/10.2527/jas.2009-2140

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation #92. Rome

FAO. 2014. Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO expert working group. Rome

Folador, J. F., Karr-Lilienthal, L. K., Parsons, C. M., Bauer, L. L., Utterback, P. L., Schasteen, C. S., Bechtel, P. J., and Fahey, G. C., 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.2005-636

Gottlob, R. O., DeRouchey, J. M., Tokach, M. D., Goodband, R. D., Dritz, S. S., Nelssen, J. L., and Knabe, D. A. 2006. Amino acid and energy digestibility of protein sources for growing pigs. J. Anim. Sci. 84:1396-1402. doi:10.2527/2006.8461396x

Guilherme-Fernandes, J., Aires, T., Fonseca, A. J., Yergaliyev, T., Camarinha-Silva, A., Lima, S. A., Maia, M. R., and Cabrita, A. R. 2024. Squid meal and shrimp hydrolysate as novel protein sources for dog food. Front. Vet. Sci. 11:1360939. doi:10.3389/fvets.2024.1360939

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

Hodgkinson, S. M., Stein, H. H., de Vries, S., Hendriks, W. H., and Moughan, P. J. 2020. Determination of true ileal amino acid digestibility in the growing pig for calculation of digestible indispensable amino acid score (DIAAS). J. Nutr. 150:2621-2623. doi:10.1093/jn/nxaa210

Hodgkinson, S. M., Stroebinger, N., Van Der Wielen, N., Mensink, M., Montoya, C., Hendriks, W. H., De Vries, S., Stein, H. H., and Moughan, P. J. 2022. Comparison of true ileal amino acid digestibility between adult humans and growing pigs. J. Nutr. 152:1635-1646. doi:10.1093/jn/nxac077

Khalili Tilami, S., and Sampels, S. 2018. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Rev. Fish Sci. Aquac. 26:243-253. doi:10.1080/23308249.2017.1399104

Koopman, R., Crombach, N., Gijsen, A. P., Walrand, S., Fauquant, J., Kies, A. K., Lemosquet, S., Saris, W. H., Boirie, Y., and van Loon, L. J. 2009. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. Am. J. Clin. Nutr. 90:106-115. doi:10.3945/ajcn.2009.27474

Kristinsson, H. G., and Rasco, B. A. 2000. Fish protein hydrolysates: production, biochemical, and functional properties. Crit. Rev. Food Sci. Nutr. 40:43-81. doi:10.1080/10408690091189266

Malcorps, W., Newton, R. W., Sprague, M., Glencross, B. D., and Little, D. C. 2021. Nutritional characterization of European aquaculture processing by-products to facilitate strategic utilization. Front. Sustain. Food Syst. 5:720595. doi:10.3389/fsufs.2021.720595

Mero, A. 1999. Leucine supplementation and intensive training. Sports Med. 27:347-358. doi:10.2165/00007256-199927060-00001

Millward, D. J., Layman, D. K., Tomé, D., and Schaafsma, G. 2008. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am. J. Clin. Nutr. 87:1576S-1581S. doi:10.1093/ajcn/87.5.1576S

Myers, W. D., Ludden, P. A., Nayigihugu, V., and Hess, B. W. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179-183. doi:10.2527/2004.821179x

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

Nguyen, H. T., Hilmarsdóttir, G. S., Tómasson, T., Arason, S., and Gudjónsdóttir, M. 2022. Changes in protein and non-protein nitrogen compounds during fishmeal processing - Identification of unoptimized processing steps. Processes 10:621. doi:10.3390/pr10040621

Nikoo, M., Regenstein, J. M., and Yasemi, M. 2023. Protein hydrolysates from fishery processing by-products: production, characteristics, food applications, and challenges. Foods 12:4470. doi:10.3390/foods12244470

Park, N., Kim, H., and Kim, B. G. 2024. Prediction models for basal endogenous losses of crude protein and amino acids in pigs. Anim. Biosci. 37:1962-1969. doi:10.5713/ab.24.0197

Rojas, O. J., and Stein, H. H. 2013. Concentration of digestible and metabolizable energy and digestibility of amino acids in chicken meal, poultry byproduct meal, hydrolyzed porcine intestines, a spent hen–soybean meal mixture, and conventional soybean meal fed to weanling pigs. J. Anim. Sci. 7:3220-3230. doi:10.2527/jas.2013-6269

Sandbakken, I. S., Five, K. K., Bardal, T., Knapp, J. L., and Olsen, R. E. 2023. Salmon hydrolysate as a protein source for Atlantic salmon; prion content and effects on growth, digestibility and gut health. Aquacult. 576:739863. doi:10.1016/j.aquaculture.2023.739863

Siddik, M. A., Howieson, J., Fotedar, R., and Partridge, G. J. 2021. Enzymatic fish protein hydrolysates in finfish aquaculture: A review. Rev. Aquacult. 13:406-430. doi:10.1111/raq.12481

Stein, H. H., Sève, B., Fuller, M. F., Moughan, P. J., and De Lange, C. F. 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

Stein, H. H., Shipley, C. F., and Easter, R. A. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436. doi:10.2527/1998.7651433x

Sulabo, R. C., Mathai, J. K., Usry, J. L., Ratliff, B. W., McKilligan, D. M., Moline, J. D., Xu, G., and Stein, H. H. 2013. Nutritional value of dried fermentation biomass, hydrolyzed porcine intestinal mucosa products, and fish meal fed to weanling pigs. J. Anim. Sci. 91:2802-2811. doi:10.2527/jas2012-5327

Tjernsbekk, M. T., Tauson, A. H., Kraugerud, O. F., and Ahlstrøm, Ø. 2017. Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility. J. Anim. Physiol. Anim. Nutr. 101:323-331. doi:10.1111/jpn.12608

Vázquez, J. A., Sotelo, C. G., Sanz, N., Pérez-Martín, R. I., Rodríguez-Amado, I., and Valcarcel, J. 2019. Valorization of aquaculture by-products of salmonids to produce enzymatic hydrolysates: Process optimization, chemical characterization and evaluation of bioactives. Mar. Drugs 17:676. doi:10.3390/md17120676

Ziegler, F., and Hilborn, R. 2023. Fished or farmed: Life cycle impacts of salmon consumer decisions and opportunities for reducing impacts. Sci. Total Environ. 854:158591. doi:10.1016/j.scitotenv.2022.158591

Chapter 4: Effects of Hydrolyzed Salmon Proteins on Macronutrient Digestibility, Fecal Metabolites, Oxidative Stress, Inflammatory Biomarkers, Microbiota, and Skin and Coat Quality in Extruded Diets For Adult Dogs

Abstract

Conventional pet food includes poultry and meat meals as sources of protein, but protein hydrolysates are used due to their improved digestibility and decreased allergenic response. Novel sources of salmon protein hydrolysates (SPH), including salmon protein hydrolysate concentrate (SPHC) and salmon protein hydrolysate isolate (SPHI), have been developed, but data demonstrating their nutritional value remain limited. This study evaluated apparent total tract digestibility (ATTD), oxidative stress and inflammatory biomarkers, skin barrier function, and fecal metabolites and microbiota, testing the hypothesis that SPH improves digestibility and health outcomes relative to chicken meal. Thirty adult dogs were assigned to one of three diets (control, SPHC, or SPHI; n = 10/treatment) for a 90-day experimental period following a 15-day adaptation. Feces, blood, and skin parameters were collected on days 0, 45, and 90, with total fecal collection during the final 5 days. Dogs fed SPHI had greater ($P < 0.05$) ATTD of dry matter and organic matter, while both SPH had greater ($P < 0.05$) ATTD of crude protein (CP) and gross energy than the control diet. Serum malondialdehyde (MDA) was greater in SPHC over time (interaction, $P < 0.05$). Serum reduced glutathione was greater ($P < 0.05$) in the control diet compared with the SPHC, and serum nitric oxide (NO) was greater ($P < 0.05$) in the SPHI than in the control diet. Skin hydration in the inguinal location was greater ($P < 0.05$) in SPHC than in control and SPHI diets. The transepidermal water loss (TEWL) was reduced in the pinnae location over time in SPHC and control diets (interaction, $P < 0.05$). Phenols and indoles were lower ($P < 0.05$) in SPHI than in the control and SPHC diets. There was an increase in BCFA in the control diet on day 90 compared with days 0 and 45, but a decrease on day 90 compared with

day 0 in the SPHC (interaction, $P < 0.05$). There was a partial separation ($P < 0.05$) in beta diversity between the control and SPHC diets and the SPH diets. Fecal microbiota was shifted to a protein-fermenting profile with the presence of *Peptoclostridium* and *Fusobacterium* genus for the control and SPHC diets, respectively, but not for SPHI. In conclusion, these results support the hypothesis that SPH provides improved digestibility and health outcomes relative to chicken meal and demonstrate the importance of defining functional targets when selecting protein hydrolysates for pet foods.

Keywords: digestibility; dog; hydrolysate; salmon; protein

Introduction

The pet food industry has traditionally relied on a wide range of protein sources, including poultry meals, poultry by-product meals, meat and bone meals, soybean meal, and other plant- and animal-derived ingredients (Sieja et al., 2023). In recent decades, however, the use of hydrolyzed proteins has increased due to their favorable effects on palatability and digestibility, as well as their reduced allergenicity, which is attributed to smaller particle sizes and a lower likelihood of eliciting immunoglobulin-mediated allergic responses (Hsu et al., 2024). In this context, fish proteins represent a promising alternative protein source, as they can yield high-quality by-products rich in bioactive peptides, minerals, and fatty acids (Kandyliari et al., 2020). The utilization of fish by-products also contributes to improved waste management within the food industry, as offal from fish processing that remains underutilized may be used for pet food applications (Folador et al., 2006).

Salmon processing generates substantial quantities of by-products, with an estimated 60% of total body weight (BW) comprised of non-fillet components such as heads, skin, viscera,

frames, and bones (Ramakrishnan et al., 2024), that can be converted into high-quality ingredients through hydrolysis to produce salmon protein hydrolysates (SPH). Protein hydrolysates are produced through enzymatic or chemical hydrolysis, followed by partial or complete removal of lipids and subsequent dehydration (Hou et al., 2017; Kristinsson and Rasco, 2000). Autolytic hydrolysis can also occur in fish-derived materials through the action of endogenous proteases naturally present in the tissue (Kristinsson and Rasco, 2000). Variability in the chemical composition of fish protein hydrolysates can arise from environmental conditions, farming practices, the specific fish tissues used, the degree and type of hydrolysis, as well as water quality, which may influence the physiological and health responses in animals (Folador et al., 2006; Hou et al., 2017).

Despite the growing interest in SPH as a functional ingredient in canine nutrition, relatively few studies have evaluated its effects across a broad range of health outcomes. Existing research has focused on specific endpoints or other fish species. For example, SPH has been primarily studied for amino acid digestibility and protein quality in dogs (Folador et al., 2006; Tjernsbekk et al., 2017) or for use in hypoallergenic and elimination diets to mitigate adverse immune responses (Lewis et al., 2025). More recently, mixed fish hydrolysates have been evaluated for their effects on nutrient digestibility, fecal metabolites, palatability, blood profile, and coat quality in dogs (Cabrita et al., 2024); however, integrated assessments of these outcomes using SPH specifically in adult dogs remain limited. Consequently, the physiological responses of dogs to SPH when incorporated into extruded diets, and how these responses compare with those elicited by conventional protein sources, are not well characterized. Therefore, the objectives of this study were to evaluate apparent total tract digestibility (ATTD) of nutrients, oxidative stress, inflammatory biomarkers, skin barrier function, and fecal

metabolites and microbiota in adult dogs fed extruded diets containing SPH, and to test the hypothesis that SPH provides comparable or improved digestibility and health outcomes relative to chicken meal. More specifically, it was hypothesized that SPH would modulate the fecal microbiota toward reduced proteolytic fermentation because, as a hydrolyzed protein, it was expected to have greater protein digestibility. In addition, SPH was expected to promote beneficial effects on skin health and inflammatory markers due to the anti-inflammatory properties of omega-3 fatty acids.

Material and Methods

All animal procedures were revised and approved by the Institutional Animal Care and Use Committee of the University of Illinois Urbana-Champaign under protocol #23113. All methods were performed in accordance with the United States Public Health Service on Humane Care and Use of Laboratory Animals.

Experimental diets

Three experimental dietary treatments were formulated to have similar nutrient and ingredient composition except for the main protein source (Tables 4.1 and 4.2). The control diet contained chicken meal as the primary protein source, and two experimental diets were formulated in which chicken meal was completely substituted with a different SPH. All diets were complete and balanced for adult dogs at maintenance (Association of American Feed Control Officials, 2023) and were extruded at the Feed Technology Center at the University of Illinois Urbana-Champaign. The two sources of SPH (Biomega Group AS, Skageneset, Norway) included one source of salmon protein hydrolysate concentrate (SPHC) that was produced from salmon raw materials, including viscera, subjected to autolytic hydrolysis and dried in a disc-

dryer, and one source of salmon protein hydrolysate isolate (SPHI) that was produced from salmon offcuts without viscera, subjected to controlled enzymatic hydrolysis, and spray-dried (Table 4.3).

Chemical analysis in experimental diets and ingredients

The nutrient composition of diets and ingredients was confirmed prior to the beginning of the study. All diets and ingredient samples were analyzed in duplicates, and a coefficient of variation of less than 5% was considered acceptable. Diets and ingredients were analyzed for dry matter (method 934.01 AOAC Int., 2019) and ash (method 942.05; AOAC Int., 2019). Organic matter was calculated by subtracting analyzed ash values from analyzed dry matter in diets and ingredients. Nitrogen was measured using the combustion procedure (method 990.03; AOAC Int., 2019) in a LECO FP2000 TruMac (LECO Corp., Saint Joseph, MI, USA) and crude protein (CP) was calculated as nitrogen × 6.25. Acid-hydrolyzed fat was analyzed by acid hydrolysis using 3*N* HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY, USA) followed by fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology, Macedon, NY, USA). Insoluble and soluble dietary fibers were analyzed according to the method 991.43 (AOAC Int., 2019) using an Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of insoluble and soluble dietary fibers. The gross energy was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). The nitrogen-free extract was determined by difference, and metabolizable energy was calculated.

Canine study and experimental design

Thirty adult neutered Beagles [average age: 3.2 ± 1.8 years; average BW 9.4 ± 1.3 kg; average body condition score (BCS): 5.3 ± 0.5] were used in a complete randomized design. All dogs were confirmed to be healthy at the beginning of the study based on a comprehensive

veterinary examination. The total study duration was 105 days, consisting of a 15-day adaptation period during which all dogs were fed the control diet, followed by the 90-day randomized experimental phase with 10 dogs assigned to the three experimental diets. Dogs were housed individually in an environmentally controlled animal facility at the University of Illinois Urbana-Champaign with a 12-hour light and 12-hour dark schedule. Pens allowed for nose-nose contact between dogs in adjacent runs and visual contact with all dogs in the room. Dogs were socialized frequently, including human-dog interaction at least twice weekly, and bathed every 15 days. During socialization, dogs were allowed to have physical enrichment (i.e., toys).

The daily feed intake was determined according to the National Research Council's (2006) daily metabolizable energy requirements for adult dogs. Dogs had free access to fresh water and were fed twice a day to maintain BW throughout the study. Feed intake and orts were recorded daily. Dogs were weighed, and BCS was performed once a week. The BCS was assessed on a 9-point scale with '1' being severely malnourished, '5' being an ideal BW, and '9' being severely obese (Laflamme, 1997). Collections of fresh fecal samples (within 15 minutes of defecation), blood samples, and skin parameter data were performed at baseline (day 0), day 45, and day 90. During the final 5 days of the randomized experimental phase, total fecal collections were conducted to determine the ATTD of nutrients for each experimental dietary treatment.

Blood collection and analysis

Blood samples (20 mL) were collected after a 12-h overnight fast by cephalic or jugular venipuncture. During collection, dogs were placed in sternal recumbency on the examination table and manually restrained by holding the neck or muzzle while supporting the back against the handler's body. The venipuncture site (neck or forelimb) was shaved as needed to improve vein visibility and cleansed with alcohol. Blood was collected using a needle (22Gx1 or 21Gx1)

after manual occlusion of the vein. After sample collection, the needle was removed and pressure was applied to the venipuncture site for 30 seconds or until bleeding had stopped. Of the 20 mL collected, 1 mL of whole blood was transferred to a BD Vacutainer® EDTA tube for complete blood count analysis, and the remaining 19 mL was placed into BD Vacutainer® SST tubes and centrifuged to obtain serum for serum chemistry and analysis of protein-related serum metabolites to assess health status. Concentration of oxidative stress biomarkers [e.g., catalase (Cat.No: MBS045476), nitric oxide (NO; Cat.No: MBS015107), cortisol (Cat No. MBS2607560), superoxide dismutase (SOD; Cat No. MBS2104718), malondialdehyde (MDA; Cat No. MBS2605193), reduced glutathione (Cat No. MBS2607626), and thiobarbituric acid reactive species (TBARS; Cat.No MBS1602515)] was analyzed in serum samples using canine-specific commercial ELISA assay kits (MyBioSource, San Diego, CA, USA). Inflammatory cytokines [i.e., interferon-γ (IFN-γ), interleukin- (IL-) 2, 6, 7, 8, 10, 15, 18, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), keratinocyte chemoattractant-like (KC-like), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon ϒ-induced protein-10 (IP-10)] were also analyzed in serum samples using a MILLIPLEX® MAP Kit (EMD Millipore Corporation, Billercia, MA, USA) in a MagPix instrument with ProcartaPlex-multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

Qualitative and quantitative analysis of coat and skin barrier function

Skin and coat were scored by 3 blinded researchers according to Marsh et al. (2000) and Rees et al. (2001) using the following scale: Hair condition score: 1 = dull, coarse, dry, 2 = poorly reflective, non-soft, 3 = medium reflective, medium soft, 4 = highly reflective, very soft, 5 = greasy; Skin condition score: 1 = dry, 2 = slightly dry, 3 = normal, 4 = slightly greasy, 5 =

greasy; Coat quality including glossiness (1 = very dull, 5 = very shiny), softness (1 = very brittle, 5 = very soft), greasiness (1 = very greasy, 5 = not greasy), and scale (1 = excessive scale, 5 = no scale).

Once skin and coat scoring was completed, skin condition was measured for transepidermal water loss (TEWL) with a Tewameter TM 300 MDD (Courage + Khazaka Electronic GmbH, Cologne, Germany), hydration status with a probe Corneometer CM 825 (Courage + Khazaka Electronic GmbH, Cologne, Germany), and sebum concentration using an external Sebumeter SM 815 (Courage + Khazaka Electronic GmbH, Cologne, Germany) for assessment of skin barrier function. There were three measuring areas of the body: left and right pinnae, the inguinal region, and the upper back. The upper back was shaved using standard clipping procedures two days before skin measurements. A total of 3 readings per body area were taken per dog. In addition, the temperature and humidity of the room were monitored and recorded.

Fecal analysis

Fecal score and ATTD

Fecal scores were assessed using a 5-point scale (1= hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured). Total fecal samples were composited by animal and diet and were dried and ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ, USA). Samples were then analyzed for dry matter, ash, CP, acid-hydrolyzed fat, gross energy, and total dietary fiber according to previous methods described for diets and ingredients. Digestibility was calculated for each experimental dietary treatment using the following equation:

$$\text{ATTD of nutrient (\%)} = \frac{\text{Feed intake (g/day) - Fecal output (g/day)}}{\text{Feed intake (g/day)}} \times 100$$

Fecal metabolites

Fresh fecal samples were used for measurement of pH, fecal score, moisture content, and fermentative end-products, including short-chain fatty acids (SCFA), ammonia, branched-chain fatty acids (BCFA), phenols, and indoles. Fecal aliquots for analysis of phenols and indoles were frozen at –20 °C immediately after collection. One aliquot (5 g of feces) was collected and placed in 5 ml of 2*N* hydrochloric acid for ammonia, SCFA, and BCFA analysis. A separate fecal aliquot (2 g of feces) was used to determine phenol and indole concentrations. Fecal SCFA and BCFA concentrations were determined using gas chromatography according to Erwin et al. (1961). Fecal ammonia concentration was determined according to the method of Chaney and Marbach (1962). Fecal phenol and indole concentrations were determined using gas chromatography according to the methods described by Flickinger et al. (2003).

Fecal microbiota

Total DNA was extracted from fresh fecal samples using the MO BIO PowerSoil Kit (MO BIO Laboratories, Carlsbad, CA, USA). Concentration of extracted DNA was quantified using a Qubit® 3.0 Fluorometer (Life Technologies Co., Carlsbad, CA, USA). The quality of extracted DNA was assessed by electrophoresis using agarose gels (Invitrogen™ E-Gel™ EX Double Comb Agarose Gels with SYBR™ Safe DNA Gel Stain 1-2%; Invitrogen, Carlsbad, CA, USA). Full-length 16S PacBio (Pacific Biology, Menlo Park, CA, USA) analysis was performed at the Roy J. Carver Biotechnology Center at the University of Illinois. The 16S amplicons were generated with the barcoded full-length 16S primers from PacBio and the 2× Roche KAPA HiFi Hot Start Ready Mix. The amplicons were concentrated 12-fold with the PacBio Kinnex Concatenation Kit and Kinnex PCR 12-Fold kit to produce 18kb libraries. The concatenated

libraries were sequenced on a PacBio Revio platform using a 30-h movie time. Circular consensus sequence analysis, de-concatenation, and demultiplexing were performed using SMRTlink 13.1 in a read quality greater than 0.999.

For microbial data analysis, PacBio raw sequence amplicons were imported into QIIME2 version 2023.7.0 (Bolyen et al., 2019). DADA2 was used to denoise the reads and generate amplicon sequence variants (Callahan et al., 2016). Samples were rarefied to 26,907 reads. Taxonomic assignment of PacBio amplicon sequence variants was performed using the SILVA database (version 138, 99% operational taxonomic unit full-length sequences) obtained from the QIIME2 data resources (Bokulich et al., 2018). The taxonomic classifications and their quantifications produced by DADA2 were imported into the 'phyloseq' R package in RStudio (version 4.3.0). The rarefied samples were used for alpha and beta diversity analyses. Alpha diversity was assessed by Faith's PD, Fisher, Observed Features, Pielou's evenness, Shannon, and Simpson indexes. For beta diversity, principal coordinate analysis (PCoA) was conducted using Bray-Curtis, weighted UniFrac, and unweighted UniFrac distances. Heatmap correlations between fecal metabolites and microbiota taxa (genus) were performed using the Spearman correlation for each experimental diet using RStudio (version 4.3.0).

Statistical analysis

Normality of residuals was analyzed using PROC UNIVARIATE and the Shapiro-Wilk test was applied to model residuals. When the data were not normally distributed, a logarithmic transformation was applied. Outliers were identified and removed when studentized residuals exceeded ±3 standard deviations. Data were analyzed using SAS (SAS Institute INC., version 9.4, Cary, NC, USA) through PROC MIXED with dog as the random effect and treatment as the fixed effect. Day was used as a repeated measure, when appropriate, to determine the interaction

of treatment by day. Differences among means were determined using pairwise comparisons with Tukey adjustment to control for type-1 experiment-wise error. Results were considered statistically significant at $P < 0.05$, and trends were defined as $0.05 \leq P < 0.10$. Pooled standard errors of the mean were calculated from the MIXED model procedures.

Results

During the study, no statistical differences were observed for biometrical measurements of dogs fed the experimental dietary treatments, and dogs maintained their BW and BCS (Supplementary Table A.1).

ATTD

The fecal output (g/day) in dogs fed the SPHI was lower ($P < 0.05$) compared with the control and SPHC treatments (Table 4.4). The ATTD of dry matter and organic matter was greater ($P < 0.05$) for the SPHI compared with the control treatment, with no differences observed between SPHC and SPHI. The ATTD of CP and gross energy were greater ($P < 0.05$) for both SPH compared with the control treatment. The digestible energy was greater ($P < 0.05$) for the SPHC than in the control treatment, with no differences observed between SPHC and SPHI.

Blood analysis

The concentration of serum MDA was greater for dogs fed the SPHC diet on day 90 compared with days 0 and 45, but that was not the case for the other diets (interaction, $P < 0.05$; Table 4.5). The dogs fed the SPHC diet had less concentration of serum glutathione on day 45 than the control diet on day 90, but on day 90, no differences were observed (interaction, $P <$

0.05). Based on the main effect of treatment, serum concentration of NO was greater ($P < 0.05$) in the dogs fed the SPHI than in dogs fed the control diet.

Inflammatory serum cytokine concentrations were, in general, not statistically different among dietary treatments. At baseline, however, IL-18 was greater in dogs fed the control diet compared with dogs fed both SPH diets (Supplementary Table A.2). Therefore, to account for baseline variation, cytokines were analyzed using changes from baseline (Table 4.6). Using this approach, dogs fed the control diet had a greater ($P < 0.05$) concentration of IL-7 compared with dogs fed the SPHI diet based on the main effect of treatment.

Qualitative and quantitative analysis of coat and skin barrier function

No statistical differences were observed for qualitative hair and skin scores and quantitative coat quality measurements in dogs fed the experimental dietary treatments (Supplementary Table A.3). For skin barrier function parameters, hydration in the inguinal site was greater ($P < 0.05$) in dogs fed the SPHC compared with dogs fed the SPHI diet (Table 4.7). Dogs fed the control and SPHC diets had a reduction in TEWL in the pinnae location over time, but that was not the case for dogs fed the SPHI diet (interaction, $P < 0.05$).

Fecal metabolites

No statistical differences in pH, fecal scores, and SCFA were observed in the fresh feces of dogs fed the experimental dietary treatments (Table 4.8). The indoles in dogs fed the SPHI diet reduced on days 45 and 90 compared with day 0, but that was not the case for dogs fed the other diets (interaction, $P < 0.05$). Based on the main effect of treatment, total phenols and indoles were greater ($P < 0.05$) in dogs fed the control and SPHC diets compared with dogs fed the SPHI diet. Isobutyrate was reduced over time in dogs fed the SPHI diet when comparing day 90 with day 0, but that was not the case for dogs fed the other diets (interaction, $P < 0.05$).

Isovalerate was reduced over time in dogs fed the SPHC diet when comparing day 90 with day 0, but there was an increase in isovalerate in dogs fed the control diet on day 90 compared with day 45 (interaction, $P < 0.05$). There was an increase in valerate and total BCFA for dogs fed the control diet on day 90 compared with days 0 and 45, but that was not the case for dogs fed both SPH, with the exception that there was a decrease in total BCFA for dogs fed the SPHC diet on day 90 compared with day 0 (interaction, $P < 0.05$).

Fecal microbiota

Relative abundance

At phylum level, Firmicutes, Bacteroidota, and Fusobacteriota were the top 3 most abundant phyla (Supplementary Figure A.1); At family level, Fusobacteriaceae, Peptostreptococcaceae, and Lachnospiraceae were the top 3 most abundant families (Supplementary Figure A.2). At genus level, *Fusobacterium*, *Peptoclostridium*, and *Bacteroides* were the top 3 most abundant genus (Figure 4.1). At species level, *Allobaculum stercoricanis*, *Gut metagenome 1*, and *Fusobacterium sp.* were the top 3 most abundant species (Figure 4.2).

At phylum level, there was a greater relative abundance of Fusobacteriota on day 45 than on day 0 in dogs fed the SPHC, but that was not the case for the dogs fed the other diets (Table 4.9; interaction, $P < 0.05$). Based on the main effect of treatment, dogs fed the control diet had greater ($P < 0.05$) relative abundance of Firmicutes compared with dogs fed the SPHC diet. At family level, there was a greater relative abundance of Fusobacteriaceae on day 45 than on day 0 in dogs fed the SPHC, but that was not the case for dogs fed the other diets (interaction, $P < 0.05$). Based on the main effect of treatment, Peptostreptococcaceae concentration was greater ($P < 0.05$) in dogs fed the control diet than in dogs fed the SPHC diet; and dogs fed the SPHC diet had greater ($P < 0.05$) relative abundance of Enterococcaceae compared with dogs fed the SPHI

diet. At genus level, there was a greater ($P < 0.05$) relative abundance of *Fusobacterium* on day 45 in dogs fed the SPHC than the dogs fed the SPHI diet on day 45 and in dogs fed the control and SPHI diets on day 90 (interaction, $P < 0.05$). Based on the main effect of treatment, dogs fed the SPHC diet had a greater ($P < 0.05$) relative abundance of *Alloprevotella* than in dogs fed the control diet, and dogs fed the control diet had a greater ($P < 0.05$) relative abundance of *Peptoclostridium* compared with dogs fed the SPHC diet. There was a greater relative abundance of *Ruminococcus torques* group on day 90 than on day 45 in dogs fed the SPHC diet, but that was not the case for the dogs fed the other diets (interaction, $P < 0.05$). At species level, dogs fed the SPHC diet had a greater ($P < 0.05$) relative abundance of *Fusobacterium sp.* compared with dogs fed the control and SPHI diets based on the main effect of treatment. There was a greater relative abundance of *Gut metagenome* on day 0 than on day 90 in dogs fed the SPHC diet, and on day 0 than on days 45 and 90 in dogs fed the SPHI diet, but that was not the case for the dogs fed the control diet (interaction, $P < 0.05$). There was a greater relative abundance of *Blautia glucerasea* on days 45 and 90 compared with day 0 in dogs fed the SPHI diet, but that was not the case for the dogs fed the other diets (interaction, $P < 0.05$). In addition, based on the main effect of treatment, dogs fed the SPHC diet had a greater ($P < 0.05$) relative abundance of *Bacteroids vulgatus* compared with dogs fed the control diet. On day 0 of dogs fed the control diet and on day 90 of dogs fed the SPHI diet, there was a greater relative abundance of *bacteroids caecigallinarum* than in dogs fed the SPHC diet on day 0 (interaction, $P < 0.05$).

Alpha and beta diversity

Based on alpha diversity indexes, including Faith's PD, Fisher, Observed Features, Pielou's evenness, Shannon, and Simpson, no statistical differences were observed among dogs fed the dietary experimental treatments (Supplementary Figure A.3). Based on beta diversity,

PCoA plots using Bray-Curtis distance and unweighted UniFrac matrix showed that samples from the dietary treatments clustered together, with no statistical differences observed (Supplementary Figures A.4 and A.5). However, PCoA based on the weighted UniFrac distance matrix indicated partial separation among dietary treatments, specifically between dogs fed the control and SPHC diets ($P = 0.019$), and between dogs fed the SPHC and SPHI diets ($P = 0.027$; Figure 4.3a). When evaluated by time point, PCoA showed partial separation between the SPHC and SPHI diets on day 45 ($P = 0.001$), whereas samples from the remaining treatments and time points clustered together (Figure 4.3b).

Heatmap correlation

Overall, fecal metabolites that showed the strongest correlations with microbial taxa at the genus level are described below. The correlations between fecal metabolites and microbiota taxa for dogs fed the control diet are presented in Figure 4.4. Phenols were negatively correlated with *Peptococcus*. Fecal score, acetate, and total SCFA were negatively correlated with *Faecalibacterium*, but positively correlated with *Lactobacillus*. Total BCFA was negatively correlated with *Sutterella* and *Bacteroids*.

The correlations between fecal metabolites and microbiota taxa for dogs fed the SPHC diet are presented in Figure 4.5. *Phascolarctobacterium* and *Lactobacillus* were positively correlated with indoles, butyrate, isobutyrate, isovalerate, total SFCA, and total BCFA but negatively correlated with fecal pH. In addition to that, *Phascolarctobacterium* was positively correlated with fecal score, acetate, and propionate. Fecal score was also positively correlated with Bacteroides. Phenols were positively correlated with *Ruminococcus gnavus group* and *Collinsella*. Acetate and total SFCA were positively correlated with *Alloprevotella*.

Peptostreptococcus was positively correlated with butyrate and valerate. Valerate was also negatively correlated with *Erysipelatoclostridium* but positively associated with *Prevotella*.

The correlations between fecal metabolites and microbiota taxa for dogs fed the SPHI diet are presented in Figure 4.6. Phenols were negatively correlated with *Negativibacillus*, *Eubacterium brachy group*, *Alloprevotella*, and *Prevotella*. Butyrate, isobutyrate, and isovalerate were positively correlated with *Peptostreptococcus*. Isobutyrate was also positively correlated with *Lactobacillus*. Valerate was negatively correlated with *Erysipelatoclostridium*.

Discussion

The pet food market has experienced substantial growth in recent years, driven by increased pet ownership and a shift toward premium ingredients that align with owner expectations for health and product quality (Raditic and Gaylord, 2023). Within this context, fish protein hydrolysates have been increasingly incorporated into companion animal diets to enhance palatability and to serve as functional ingredients that address specific nutritional needs (Folador et al., 2006). However, comprehensive data evaluating the health outcomes when using SPH in canine diets remains limited, demonstrating the need for studies that integrate metabolic, digestive, immunological, and gut microbiota responses.

In the present study, dogs fed diets containing either SPH or chicken meal remained clinically healthy with no adverse health events observed. Overall, the serum chemistry parameters did not differ among dogs fed the different experimental dietary treatments (Supplementary Table A.4). However, dogs fed the SPHI and SPHC had greater concentrations of creatinine and alanine transaminase, respectively, on days 45 and 90 compared with day 0, although values were within the normal reference range for adult dogs. These temporal increases

likely reflect normal metabolic adaptation to dietary protein intake rather than indicators of impaired renal or hepatic function, and the lack of differences between days 45 and 90, together with the absence of clinical abnormalities, supports the interpretation that these changes represent stable physiological adaptation over time. In addition, total cholesterol was reduced over time in dogs fed the SPHC diet by approximately 17%, although values remained within the normal reference range for healthy adult dogs. This reduction may be due to the presence of omega-3 fatty acids in the SPHC, which have been shown to modulate lipid metabolism and reduce circulating cholesterol in dogs (Godoy et al., 2018; Ravić et al., 2022).

Some differences were observed for ATTD among dogs fed the experimental diets. The lower fecal output observed in dogs fed the SPHI diet compared with the control corresponded with greater macronutrient digestibility, particularly dry matter and organic matter. The ATTD of CP and gross energy observed for both SPH agree with data from mink fed these proteins (Tjernsbekk et al., 2017), although the CP digestibility observed in the present study was greater than that reported for white fish hydrolysate fed to dogs (Cabrita et al., 2024). Protein hydrolysates are characterized by lower molecular weight peptides, which may enhance enzymatic accessibility and intestinal absorption, thereby increasing digestibility and reducing fecal mass (Hou et al., 2017). Similar improvements in CP digestibility were reported when SPH was compared with poultry meal and mechanically separated chicken meal (Tjernsbekk et al., 2017).

Serum oxidative stress biomarker analysis demonstrated that dogs fed the SPHC diet presented a greater serum concentration of MDA, a marker of lipid peroxidation, by the end of the experimental period. This can be related to the presence of omega-3 fatty acids in SPHC, which are more susceptible to lipid peroxidation due to their high degree of unsaturation and

vulnerability to reactive oxygen species (Cholewski et al., 2018). Biologically, farmed salmon raised in cold marine environments (e.g., Norway) typically accumulate higher proportions of docosahexaenoic acid (DHA) from algal-based marine food webs, contributing to a high peroxidability index (Nogueira et al., 2019), and potentially greater MDA formation. Importantly, the absence of changes in circulating inflammatory cytokines suggests that the observed increase in lipid peroxidation did not translate into a systemic inflammatory response in dogs fed this diet. Indeed, dogs fed the SPHC diet had lower serum reduced glutathione concentrations compared with the control, likely reflecting increased utilization of endogenous antioxidant defenses in response to elevated oxidative challenge rather than impaired redox regulation. These observations suggest that diets incorporating omega-3-rich SPH as primary protein sources may benefit from the inclusion of additional dietary antioxidants, such as vitamin E. In addition, the increase in serum MDA concentration was observed only at day 90 and not at day 45, demonstrating the importance of longer experimental durations, as oxidative responses may not be detectable in shorter-term feeding studies.

Dogs fed the SPHI diet presented greater serum concentration of NO compared with dogs fed the control diet. However, this increase occurred in the absence of cytokine-driven inflammation. This is likely due to the high concentrations of arginine (~ 6% of CP) and non-protein nitrogen (~ 19% of CP) that were analyzed for this ingredient, which may increase substrate availability for NO synthesis via circulating arginine (Evora et al., 2003) or through bioactive peptides that modulate NO metabolism (Chakrabarti and Wu, 2016). Previous studies also reported high concentrations of non-protein nitrogen in fish protein hydrolysates (Folador et al., 2006; Zinn et al., 2009). In contrast, dogs fed the control diet had an increase in IL-7 from baseline, but accompanied by reductions in both neutrophil percentage and absolute neutrophil

counts over time (Supplementary Table A.5), supporting a compensatory, homeostatic response involving altered leukocyte kinetics rather than cytokine-driven inflammation (Chen et al., 2021). Consistent with this interpretation, previous studies using chicken-based products in extruded diets for dogs have also reported no evidence of inflammatory responses (Zinn et al., 2009; Hsu et al., 2024).

Skin barrier function was improved in dogs fed the SPHC diet, as indicated by increased hydration at the inguinal location and reduced TEWL at the pinnae. The TWEL is a marker of epidermal barrier integrity, with greater values reflecting increased permeability and risk of skin dryness and irritation (Lau-Gillard et al., 2010). Dogs fed the SPHC diet had reduced TEWL on day 45, whereas a similar reduction in dogs fed the control diet was only evident by day 90, indicating a faster barrier response in dogs fed the SPHC. This earlier response may be related to greater bioavailability of omega-3 fatty acids (Cabrita et al., 2024), which are recognized modulators of TEWL through their anti-inflammatory and barrier-supporting effects (Parke et al., 2021). Improvements observed in both the SPHC and control diets, but not in SPHI, are likely associated with the provision of essential fatty acids from salmon and chicken, which are critical components of epidermal phospholipids and ceramides involved in regulating water permeability (Parke et al., 2021). To our knowledge, this is the first study to directly evaluate the effects of SPH on canine skin barrier function using biophysical measurements. Lewis et al. (2025) reported improved dermatological clinical signs in dogs fed a SPH-based diet, whereas no changes in TEWL or skin hydration were observed in dogs fed a chicken hydrolyzed diet for 28 days (Hsu et al., 2024), suggesting that both ingredient composition and study length are critical determinants of dietary effects on canine skin barrier function.

Fecal metabolite differences were primarily associated with protein-derived fermentation

and their correlations with microbial taxa, whereas SCFA concentrations were unaffected, likely due to similar fiber content and sources across dietary treatments. Total phenols and indoles were the lowest in dogs fed the SPHI diet and decreased further over time. This observation is consistent with previous studies demonstrating that highly digestible hydrolyzed proteins provide less substrate for hindgut protein fermentation (Pinto et al., 2023; Hsu et al., 2024), which aligns with the greater CP digestibility observed for both SPH diets compared with the control. However, despite similar ATTD of CP between SPHC and SPHI, dogs fed the SPHC diet had concentrations of total phenols and indoles comparable to those of dogs fed the control diet. Ingredient amino acid analysis showed substantially greater concentrations of tryptophan and tyrosine in SPHC (38% and 50% greater, respectively) than in SPHI. As these aromatic amino acids are primary precursors for indole and phenolic compounds, their greater dietary availability may have increased the amount of fermentable substrate reaching the hindgut (Phillips et al., 2003; Pilla and Suchodolski, 2020). This interpretation is further supported by the greater relative abundance of *Fusobacterium* in dogs fed the SPHC diet, a genus commonly associated with proteolytic activity (Pilla and Suchodolski, 2020). In contrast, dogs fed the SPHI diet showed a greater relative abundance of *Blautia glucerasea*, which increased over time and was moderately negatively correlated with fecal phenols and indoles, consistent with its primary role in carbohydrate fermentation rather than proteolysis (Liu et al., 2021). However, despite this increase, the low relative abundance of this species within the overall microbiota suggests that amino acid substrate availability played a more influential role than microbial composition in phenol and indole production.

Total fecal BCFA, isovalerate, and valerate, fermentative end-products of branched-chain amino acid metabolism, increased over time in dogs fed the control diet, consistent with its lower

CP digestibility and the greater relative abundance of *Peptoclostridium*, a genus from the family Peptostreptococcaceae, associated with protein degradation in the canine gut (Pilla and Suchodolski, 2020). These results indicate a shift toward proteolytic fermentation, characterized by increased fecal BCFA as well as phenol and indole production, metabolites associated with luminal toxicity, fecal odor, and gastrointestinal disease (Nyangale et al., 2012). In contrast, despite the presence of proteolytic taxa, dogs fed the SPHC diet had reductions in fecal isovalerate and total BCFA over time, suggesting altered microbial metabolic activity rather than a reduction in protein fermentation per se, as metabolite production can shift with microbial function and substrate availability (Nyangale et al., 2012). In this context, it should be acknowledged that fecal metabolite concentrations are an indirect measure of microbial activity and may not accurately reflect *in vivo* production rates or fully represent true substrate availability and fermentation dynamics within the gut (Rose et al., 2007; Hsu et al., 2024). Nevertheless, the reduced fecal BCFA in dogs fed the SPHC diet may reflect a greater contribution of carbohydrate-fermenting taxa, such as the increased relative abundance of the genus *Alloprevotella*, which positively correlated with fecal SCFA, thereby limiting BCFA formation through substrate competition. However, although SPHC reduced fecal BCFA over time, it was associated with greater fecal phenol and indole concentrations similar to the control diet, whereas SPHI more effectively suppressed fecal isobutyrate over time and lowered valerate concentrations, as well as phenol and indoles, suggesting reduced formation of metabolites often linked to microbial protein fermentation, with potential beneficial effects including contributions to epithelial energy metabolism and intestinal barrier regulation (Nyangale et al., 2012).

The protein sources evaluated in this study did not affect alpha diversity but resulted in shifts in beta diversity, consistent with previous studies using chicken meal and protein

hydrolysates (Pinto et al., 2023; Hsu et al., 2024). Because alpha diversity is commonly used as an indicator of microbial ecosystem resilience, its maintenance across treatments suggests that the experimental diets supported a stable gut microbiome (Suchodolski, 2011). As expected for healthy dogs, Firmicutes, Bacteroidota, and Fusobacteriota were the dominant phyla across all treatments (Pilla and Suchodolski, 2020). Beta diversity based on Bray-Curtis and unweighted UniFrac distances showed overlap among treatments, whereas partial separation observed with weighted UniFrac indicates that diet- and time-related effects were primarily driven by shifts in the relative abundance of phylogenetically related taxa. Specifically, partial separation between dogs fed the control and SPHC diets corresponded with greater relative abundance of *Fusobacterium* and *Alloprevotella* in SPHC and greater relative abundance of *Peptoclostridium* in the control diet, indicating a shift toward a distinct, protein-fermenting microbial community. Partial separation between dogs fed the SPHC and SPHI further corresponded with greater relative abundance of Fusobacteriota and Fusobacteriaceae in SPHC, indicating lineage-wide shifts likely reflecting differences in raw material composition and processing methods, which can affect the microbiome (Barko et al., 2018). In addition, the partial separation between SPHC and SPHI on day 45 was consistent with the greater relative abundance of *Fusobacterium* in dogs fed SPHC at that time point. Overall, these results indicate that the dietary treatments modulated microbial community structure at functional and compositional levels without altering overall microbial diversity.

Conclusions

Inclusion of SPH in extruded canine diets resulted in greater ATTD of most nutrients compared with the control diet, with SPHI also reducing fecal output. Blood parameters

remained within reference ranges across treatments, although dogs fed SPHC had reduced total cholesterol over time. The use of SPHC strengthened skin barrier function, as evidenced by reduced TEWL and increased hydration, with a faster response than the control diet. Although serum MDA concentration was increased in dogs fed SPHC, likely due to the oxidative susceptibility of omega-3 fatty acids, no systemic inflammatory responses were observed. From a gastrointestinal perspective, dogs fed SPHI had lower fecal concentrations of protein-fermentative metabolites, whereas the control diet and SPHC showed more proteolytic fermentation through distinct microbial pathways. Differences in beta diversity between SPHC and SPHI further demonstrate that SPH processing characteristics influence gut microbiome structure. These results support the hypothesis that SPH provides improved digestibility and health outcomes relative to chicken meal and demonstrate the importance for pet food manufacturers to define the functional target of their formulations when selecting among protein hydrolysates due to distinct physiological and microbial effects.

Tables

Table 4.1. Chemical composition of experimental diets on as-is basis[1]

Item, %	Control	SPHC	SPHI
Test ingredient	-	29.50	21.74
Chicken meal	30.48	-	-
Brewer's rice	38.84	38.88	39.91
Chicken fat	8.50	8.50	12.00
Corn gluten meal	8.00	8.00	8.00
Corn yellow dent	5.00	5.00	5.00
Cellulose	4.00	4.00	4.00
Spray-dried whole egg	2.00	2.00	2.00
Sugar beet pulp	1.00	1.00	1.00
Palatant[2]	1.00	1.00	1.00
Dicalcium phosphate	0.00	0.50	3.00
Limestone	0.00	0.50	1.50
Potassium chloride	0.47	0.42	0.19
Sodium chloride	0.20	0.20	0.20
Mineral premix[3]	0.18	0.18	0.18
Vitamin premix[4]	0.18	0.18	0.18
Choline chloride	0.15	0.14	0.10

[1]SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

Table 4.1 (cont.)

[2]Dog palatability enhancer designed for pet food products, composed of water, hydrolyzed pork liver, and mixed tocopherols (St. Charles, MO 63304, U.S.A).

[3]The mineral premix provided the following quantities of micro minerals per kg of complete diet: Cu, 18 mg as cupric carbonate; I, 1.8 mg as potassium iodate; Fe, 135 mg as ferric citrate; Mn, 18 mg as manganous carbonate; Se, 396 mg as sodium selenite; Zn, 180 mg as zinc carbonate; Co, 3.8 mg as cobalt sulfate (New Brunswick, NJ 08901, U.S.A).

[4]The vitamin premix provided the following quantities of vitamins per kg of complete diet: Vitamin A, 18000 IU as retinyl acetate; Vitamin D_3, 2700 IU as cholecalciferol; Vitamin E, 144 IU as alpha-tocopheryl acetate; Vitamin K, 2.16 mg as menadione sodium bisulfite; Biotin, 0.108 mg; Vitamin B_{12}, 115 ug; Folic acid, 1.08 mg; Nicotinic acid, 124.2 mg; D-pantothenic acid, 50.4 mg as calcium pantothenate; Pyridoxine, 30.6 mg as Pyridoxine-HCL; Riboflavin, 30.6 mg; Thiamin, 30.66 mg as thiamin-HCL (New Brunswick, NJ 08901, U.S.A).

Table 4.2. Analyzed chemical composition of experimental diets[1]

Item, %	Control	SPHC	SPHI
Dry matter	95.84	95.38	93.88
		Dry matter basis	
Organic matter	92.19	92.93	92.56
Ash	7.81	7.07	7.44
Crude protein	32.78	32.61	31.15
Acid-hydrolyzed fat	15.42	14.89	14.74
Total dietary fiber	10.09	10.06	10.87
Insoluble dietary fiber	7.36	7.65	7.43
Soluble dietary fiber	2.73	2.41	3.44
Nitrogen-free extract[2]	33.56	35.03	35.31
Gross energy, kcal/g	5.10	5.15	4.99
Metabolizable energy[3], kcal/g	3.63	3.63	3.58

[1]SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Nitrogen-free extract was calculated as 100 – (% crude protein + % acid-hydrolyzed fat + % total dietary fiber + % ash + % moisture).

[3]Metabolizable energy was calculated as (3.5 × crude protein) + (8.5 × acid-hydrolyzed fat) + (3.5 × nitrogen-free extract).

Table 4.3. Analyzed chemical composition of experimental ingredients[1]

Item, %	SPHC	SPHI
Dry matter	96.65	96.31
	Dry matter basis	
Organic matter	84.89	89.35
Ash	15.11	10.26
Crude protein[2]	71.49	95.65
Acid-hydrolyzed fat	16.22	2.19
Docosahexaenoic acid[3]	1.30	-
Eicosapentaenoic acid[3]	0.60	-
Docosapentaenoic Acid[3]	0.20	-
Total dietary fiber	2.69	0.10
Insoluble dietary fiber	2.59	0.10
Soluble dietary fiber	0.10	ND[4]
Gross energy, kcal/g	5.35	4.72

[1]SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Crude protein was calculated using a nitrogen conversion factor of 6.25. For SPHI2, if a nitrogen conversion factor of 5.60 is used, crude protein content is 82.54%.

[3]Values provided by the manufacturer.

[4]ND = not detected.

Table 4.4. Fecal score and apparent total tract digestibility (ATTD) of dogs fed the experimental diets[1]

Item, on dry matter basis	Control	SPHC	SPHI	SEM	*P*-value
Feed intake, g/d	151.6	162.2	154.9	5.408	0.3779
Fecal output, g/d	29.6[a]	26.9[ab]	23.1[b]	1.402	0.0108
Fecal score[2]	2.4	2.3	2.3	0.109	0.6278
ATTD, %					
Dry matter	80.6[b]	83.2[ab]	85.1[a]	0.795	0.0018
		Dry matter basis			
Organic matter	86.0[b]	88.0[ab]	89.2[a]	0.580	0.0023
Crude protein	81.8[b]	88.0[a]	89.2[a]	0.769	<0.0001
Acid-hydrolyzed fat	93.7	94.3	94.7	0.353	0.1227
Total dietary fiber[3]	30.5	31.8	40.1	3.163	0.0814
Gross energy	86.6[b]	88.7[a]	89.8[a]	0.558	0.0015
Digestible energy, kcal/g	4.4[b]	4.6[a]	4.5[ab]	0.028	0.0054

[a-b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

[1]SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Fecal scores were determined using a 5-point scale (1= hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured).

[3]Tendency ($0.05 \leq P < 0.10$) to be greater in SPHI compared with control.

Table 4.5. Serum oxidative stress biomarkers of dogs in the experimental diets[1]

	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
Item	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
SOD, ng/ml	0.5	0.5	0.6	0.4	0.4	0.5	0.5	0.5	0.4	0.045	0.8201	0.0008	0.3656
MDA, nmol/ml	6.5^{ab}	5.3^{b}	7.4^{ab}	6.2^{ab}	5.4^{b}	7.4^{ab}	7.5^{ab}	8.3^{a}	7.7^{a}	0.493	0.0659	<0.0001	0.0106
Glutathione, mmol/L	7.7^{ab}	5.7^{ab}	6.4^{ab}	5.5^{ab}	4.4^{b}	7.1^{ab}	8.0^{a}	5.7^{ab}	5.5^{ab}	0.736	0.0286	0.2317	0.0486
Cortisol, ng/ml	47.3	36.9	37.6	51.91	58.27	42.58	64.70	63.15	58.64	5.878	0.2414	0.0002	0.5713
Catalase, U/ml	10.4	7.8	11.3	8.9	8.5	10.1	10.6	12.6	12.5	1.303	0.3188	0.0358	0.4772
TBARS, nmol/ml	6.5	5.7	5.4	5.8	6.4	6.0	5.9	6.5	6.8	0.610	0.9816	0.4386	0.4081
Nitric oxide[2], U/L	4.4	5.6	7.0	6.1	7.1	7.7	5.6	6.8	7.2	0.786	0.0412	0.0953	0.9454

[a-b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

[1]MDA, malondialdehyde; SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive species.

[2]Greater ($P < 0.05$) in SPHI compared with control.

Table 4.6. Changes from baseline in inflammatory cytokines of dogs fed the experimental diets[1]

	D45			D90			SEM	Type 3 fixed effects *P*-value		
Item, pg/ml	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
IL-7[2]	20.5	15.2	-22.8	70.9	11.0	-17.7	15.464	0.0147	0.0669	0.0751
IL-8	37.1	-32.2	-77.4	-105.3	170.7	267.1	137.900	0.6427	0.0374	0.4980
IL-18	55.6	42.7	3.0	18.2	28.5	16.8	22.373	0.4005	0.7787	0.9682
MCP-1	-2.9	2.2	-0.2	0.3	30.0	41.2	28.301	0.8764	0.1647	0.7046
KC-like	-1.5	-2.3	-0.6	2.3	4.3	5.7	7.085	0.9683	0.1722	0.9537
IP-10	0.4	-0.1	0.2	0.3	0.1	0.2	0.232	0.3649	0.9465	0.8637

[1]IL, interleukin; IP, interferon ϒ-induced protein 10 kDa; KC, keratinocyte chemoattractant; MCP, monocyte chemoattractant protein; SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Greater ($P < 0.05$) in control compared with SPHI. Tendency ($0.05 \leq P < 0.10$) to have an interaction between treatment and day.

Table 4.7. Skin barrier function of dogs fed the experimental diets[1]

	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
Item	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Hydration, AU													
Pinnae[2]	33.6	34.9	36.0	20.3	21.5	24.6	13.6	15.0	18.6	2.980	0.2415	<0.0001	0.3925
Inguinal[3]	10.5	16.2	7.6	4.1	4.3	2.1	1.6	4.7	0.9	1.507	0.0094	<0.0001	0.1862
Upper back	0.8	1.5	0.3	0.7	0.6	0.6	0.8	0.5	0.3	0.436	0.2958	0.4610	0.3271
Sebum, µg/cm													
Pinnae[2,4]	55.6	69.3	72.1	33.6	34.2	45.4	43.7	46.3	50.0	6.494	0.0806	<0.0001	0.3383
Inguinal	1.9	3.4	1.1	1.1	1.2	0.9	0.9	0.7	1.3	0.440	0.3179	0.0082	0.1037
Upper back	2.7	4.9	5.8	5.2	7.3	4.9	4.6	7.7	3.4	1.440	0.1543	0.4592	0.1389
TEWL, g/m^2/h													
Pinnae[2]	9.8^{ab}	11.5^{a}	9.2^{abc}	10.0^{ab}	8.7^{bc}	7.7^{bc}	7.1^{c}	7.5^{bc}	7.6^{bc}	0.551	0.0630	<0.0001	0.0339
Inguinal	9.2	7.9	11.9	9.5	8.9	9.3	6.1	6.7	6.6	0.989	0.2042	0.0004	0.3045
Upper back	14.6	11.7	12.5	14.1	12.3	12.2	9.9	8.2	9.8	1.316	0.1719	0.0009	0.9241

[a-b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Table 4.7 (cont.)

[1]AU, arbitrary units; SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate; TEWL, transepidermal water loss.

[2]Mean of left and right pinnae.

[3]Greater ($P < 0.05$) in SPHC compared with SPHI.

[4]Tendency ($0.05 \leq P < 0.10$) to be greater in SPHI compared with control.

Table 4.8. Fecal score, pH, and fecal metabolites of dogs fed the experimental diets[1]

	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
Item	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Fecal score[2]	3.0	3.2	3.2	2.9	2.7	2.7	2.9	2.4	2.7	0.195	0.5422	0.0037	0.2376
pH	6.6	6.5	6.5	6.7	6.9	7.0	6.8	7.1	6.9	0.120	0.3311	<0.0001	0.2050
						µmol/g dry matter basis							
Phenols & Indoles													
Phenols	0.8	0.7	0.8	0.8	0.9	0.6	0.6	0.8	0.6	0.128	0.5508	0.3674	0.1579
Indoles	3.3[a]	3.5[a]	3.3[a]	2.6[abc]	3.3[a]	2.0[bc]	3.3[a]	3.0[ab]	1.7[c]	0.247	0.0017	<0.0001	0.0018
Total[3]	4.0	4.1	3.8	3.1	4.0	2.6	3.9	3.7	2.2	0.319	0.0024	0.0096	0.0551
SCFA													
Acetate	213.0	205.6	224.3	190.6	210.8	209.3	198.4	187.4	188.7	16.616	0.9121	0.1329	0.6908
Butyrate	48.0	57.9	46.4	43.1	48.9	43.1	52.2	44.3	43.9	4.874	0.4365	0.2845	0.3548
Propionate	88.7	95.9	96.7	82.4	101.3	96.4	94.4	91.3	85.8	6.831	0.5310	0.7659	0.2992
Total	349.7	350.0	367.3	316.1	360.9	348.7	345.0	322.9	318.4	25.825	0.9471	0.3012	0.4812
BCFA													
Isobutyrate	12.0[ab]	13.8[ab]	14.1[a]	10.0[ab]	14.1[a]	10.6[ab]	13.4[ab]	10.2[ab]	9.5[b]	0.970	0.2480	0.0180	0.0024

Table 4.8 (cont.)

	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
Item	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Isovalerate	20.9^{abc}	27.2^{a}	23.1^{abc}	18.4^{c}	22.7^{abc}	18.9^{bc}	26.3^{ab}	18.5^{bc}	20.3^{abc}	1.651	0.3683	0.0229	0.0015
Valerate	1.3^{c}	2.0^{bc}	1.7^{bc}	1.1^{c}	4.9^{ab}	1.4^{c}	8.4^{a}	2.2^{bc}	1.4^{c}	0.738	0.0207	0.0004	<0.0001
Total	31.8^{bc}	42.2^{ab}	34.8^{abc}	29.3^{bc}	34.9^{abc}	30.5^{bc}	46.2^{a}	28.3^{c}	29.7^{bc}	2.522	0.2582	0.0824	<0.0001
Ammonia[4]	68.4	83.0	78.9	86.1	81.3	95.1	58.5	69.1	75.1	5.787	0.0750	0.0003	0.3782

[a-c]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

[1]BCFA, branched chain fatty acids; SEM, standard error of the mean; SCFA, short chain fatty acids; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Determined using a 5-point scale (1= hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured).

[3]Greater ($P < 0.05$) in control and SPHC compared with SPHI.

[4]Tendency ($0.05 \leq P < 0.10$) to be greater in SPHI compared with control.

Table 4.9. Relative abundance of fecal microbiota taxa (phylum to species level) in dogs fed the experimental diets[1]

	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
Item, %	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Phylum													
Firmicutes[2]	53.9	46.4	46.7	52.6	40.6	52.8	58.2	52.3	54.2	3.313	0.0487	0.0173	0.2949
Fusobacteriota	18.0^{b}	22.0^{b}	22.7^{ab}	22.0^{ab}	30.4^{a}	18.2^{b}	17.9^{b}	23.9^{ab}	19.0^{b}	1.947	0.0108	0.0344	0.0047
Family													
Fusobacteriaceae	18.0^{b}	22.0^{b}	22.7^{ab}	22.0^{ab}	30.4^{a}	18.2^{b}	17.9^{b}	23.9^{ab}	19.0^{b}	1.947	0.0108	0.0344	0.0047
Peptostreptococcaceae[2]	22.6	18.2	19.1	22.6	12.9	19.2	23.9	19.1	21.0	1.119	0.0265	0.0859	0.1467
Acidaminococcaceae[3]	2.1	2.3	2.0	1.4	1.7	2.1	1.4	1.1	1.2	0.317	0.8130	<0.0001	0.0640
Enterococcaceae[4]	0.2	0.1	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.053	0.0296	0.9585	0.9200
Genus													
Fusobacterium	18.0^{b}	22.0^{ab}	22.3^{ab}	22.0^{ab}	28.9^{a}	18.2^{b}	17.9^{b}	23.1^{ab}	18.8^{b}	2.004	0.0401	0.0505	0.0117
Peptoclostridium[2]	20.3	16.0	16.8	19.5	10.9	16.5	22.7	17.3	20.2	2.294	0.0373	0.0062	0.2593
Alloprevotella[5]	4.9	8.8	6.7	4.8	10.4	7.0	4.8	7.6	4.6	1.275	0.0231	0.0815	0.5956
[Ruminococcus]torques	2.9^{ab}	2.8^{ab}	2.4^{ab}	4.2^{a}	1.5^{b}	3.3^{ab}	4.7^{a}	3.7^{a}	3.1^{ab}	0.586	0.1638	0.0560	0.0131
Phascolarctobacterium[3]	2.1	2.3	2.0	1.4	1.7	2.1	1.4	1.3	1.4	0.317	0.813	<0.0001	0.0640
Species													
Fusobacterium sp.[6]	0.0	1.4	0.0	0.3	2.0	0.0	0.0	1.1	0.0	0.588	0.0056	0.7767	0.9994

Table 4.9 (cont.)

Item, %	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Gut metagenome	1.7^{ab}	1.3^{ab}	2.0^{a}	1.7^{ab}	0.7^{bc}	0.9^{bc}	0.9^{bc}	0.6^{c}	0.8^{bc}	0.232	0.1015	<0.0001	0.0173
Blautia glucerasea	0.6^{b}	0.4^{b}	0.6^{b}	0.5^{b}	0.3^{b}	1.5^{a}	0.7^{ab}	0.6^{ab}	1.4^{a}	0.149	0.0001	0.0030	0.0042
Bacteroides vulgatus[5]	0.1	0.2	0.2	0.2	1.1	0.4	0.4	0.9	0.4	0.152	0.0100	<0.0001	0.2132
Bacteroides caecigallinarum	0.9^{a}	0.0^{b}	0.5^{ab}	0.4^{ab}	0.1^{ab}	0.3^{ab}	0.4^{ab}	0.1^{ab}	0.7^{a}	0.140	0.0185	0.4096	0.0095

[a-b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

[1]SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Greater ($P < 0.05$) in control compared with SPHC.

[3]Tendency ($0.05 \leq P < 0.10$) to have an interaction between treatment and day.

[4]Greater ($P < 0.05$) in SPHC compared with SPHI.

[5]Greater ($P < 0.05$) in SPHC compared with control.

[6]Greater ($P < 0.05$) in SPHC compared with control and SPHI.

Figures

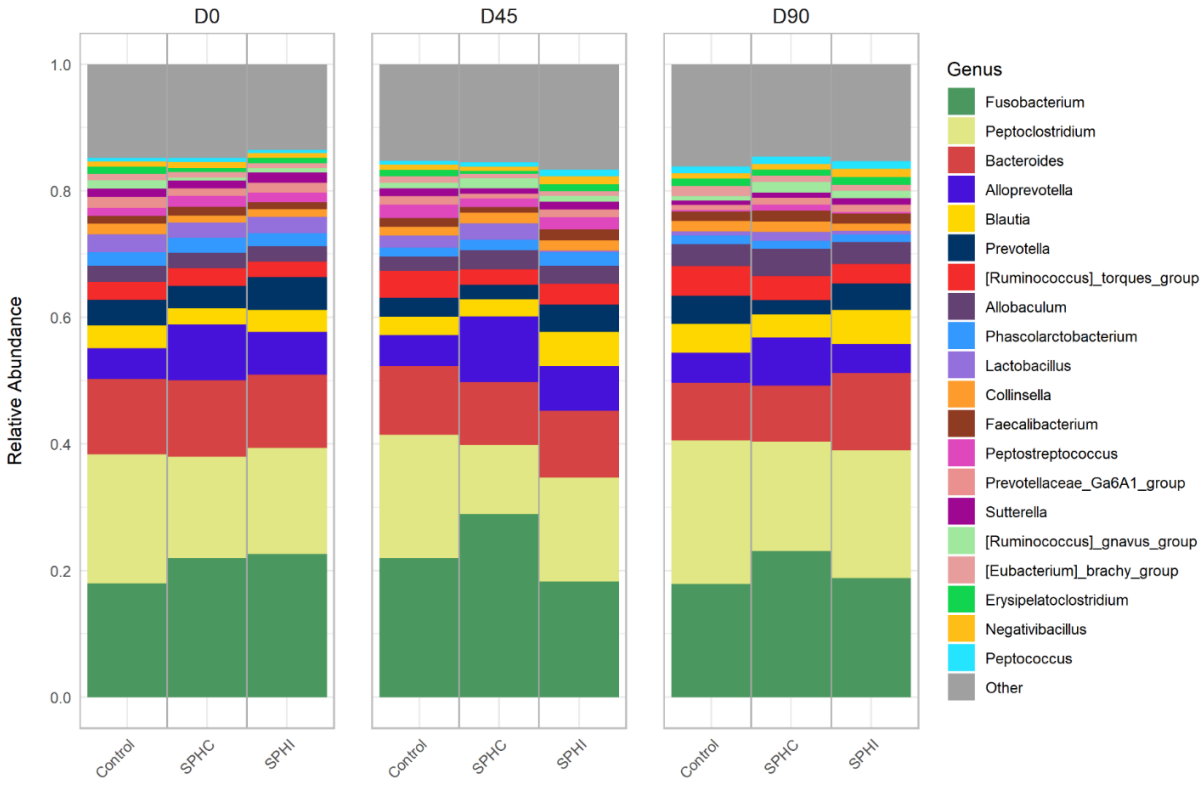

Figure 4.1. Genus composition of fecal microbiota from dogs fed the experimental diets.

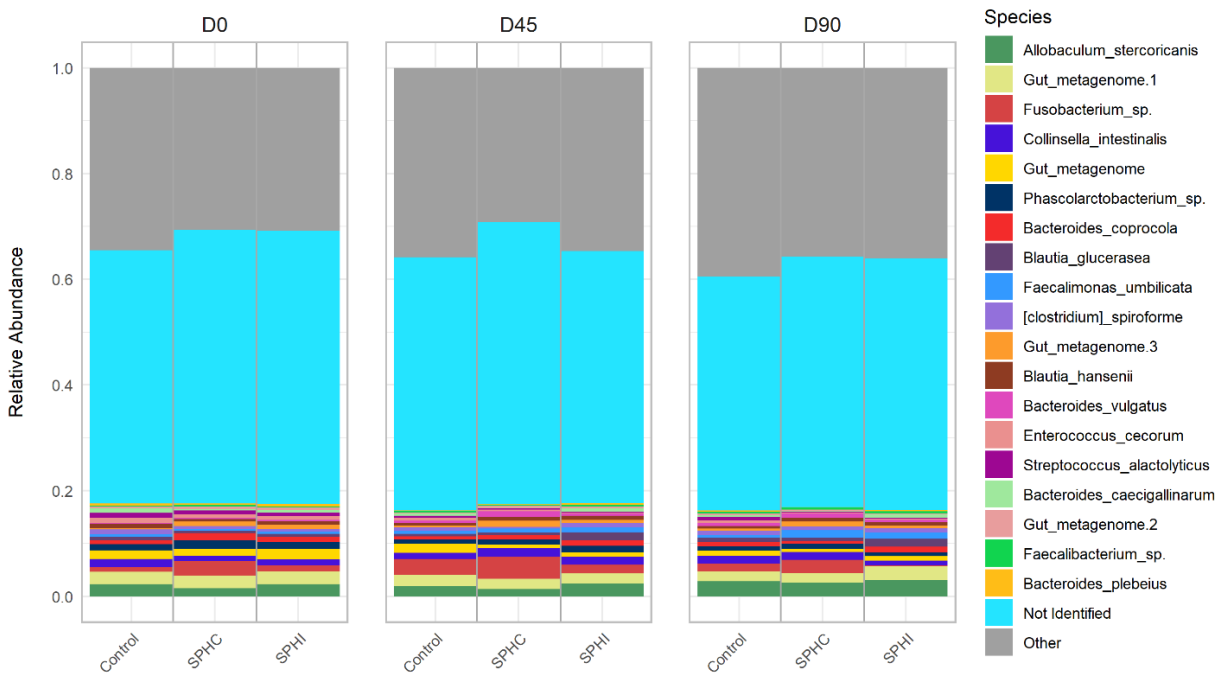

Figure 4.2. Species composition of fecal microbiota from dogs fed the experimental diets.

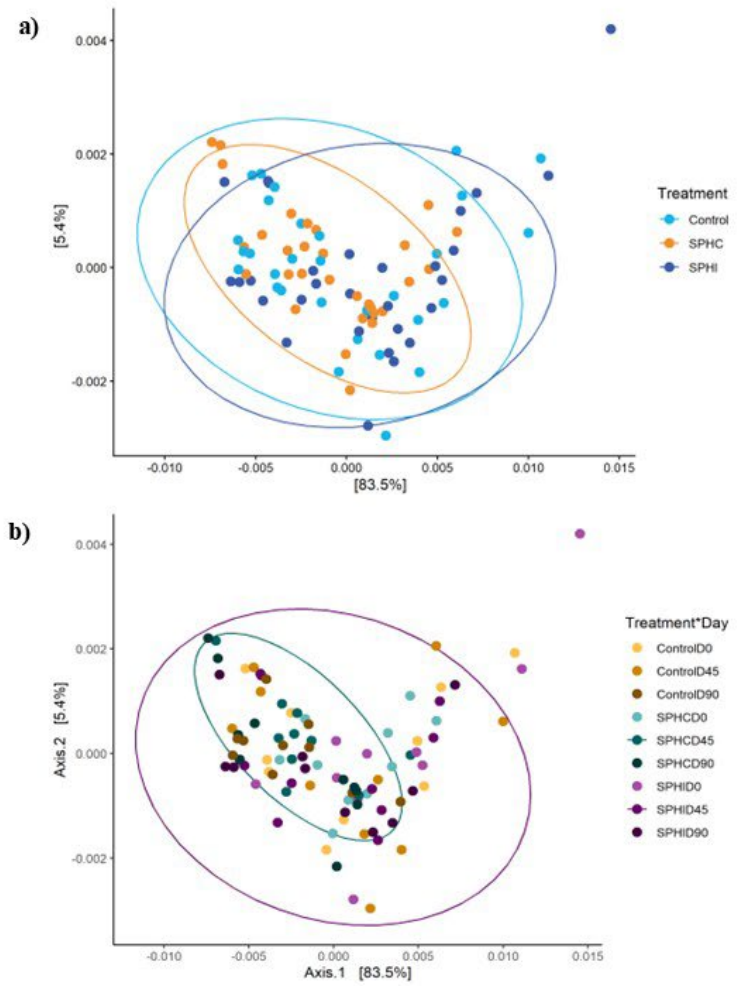

Figure 4.3. Beta diversity of fecal microbiota from dogs fed the experimental diets using principal coordinate analysis (PCoA) based on weighted Unifrac matrix. Control vs. SPHC ($P = 0.019$); SPHC vs. SPHI ($P = 0.027$); SPHCD45 vs. SPHID45 ($P = 0.001$). Control: Chicken meal-based diet; SPHC: salmon protein hydrolyzed concentrate diet; SPHI: salmon protein hydrolyzed isolate diet.

Figure 4.4. Spearman correlation of fecal metabolites and microbiota taxa in dogs fed the control diet. BCFA: branched-chain fatty acids; PI: phenols and indoles; SCFA: short-chain fatty acids.

Figure 4.5. Spearman correlation of fecal metabolites and microbiota taxa in dogs fed the salmon protein hydrolysate concentrate (SPHC) diet. BCFA: branched-chain fatty acids; PI: phenols and indoles; SCFA: short-chain fatty acids.

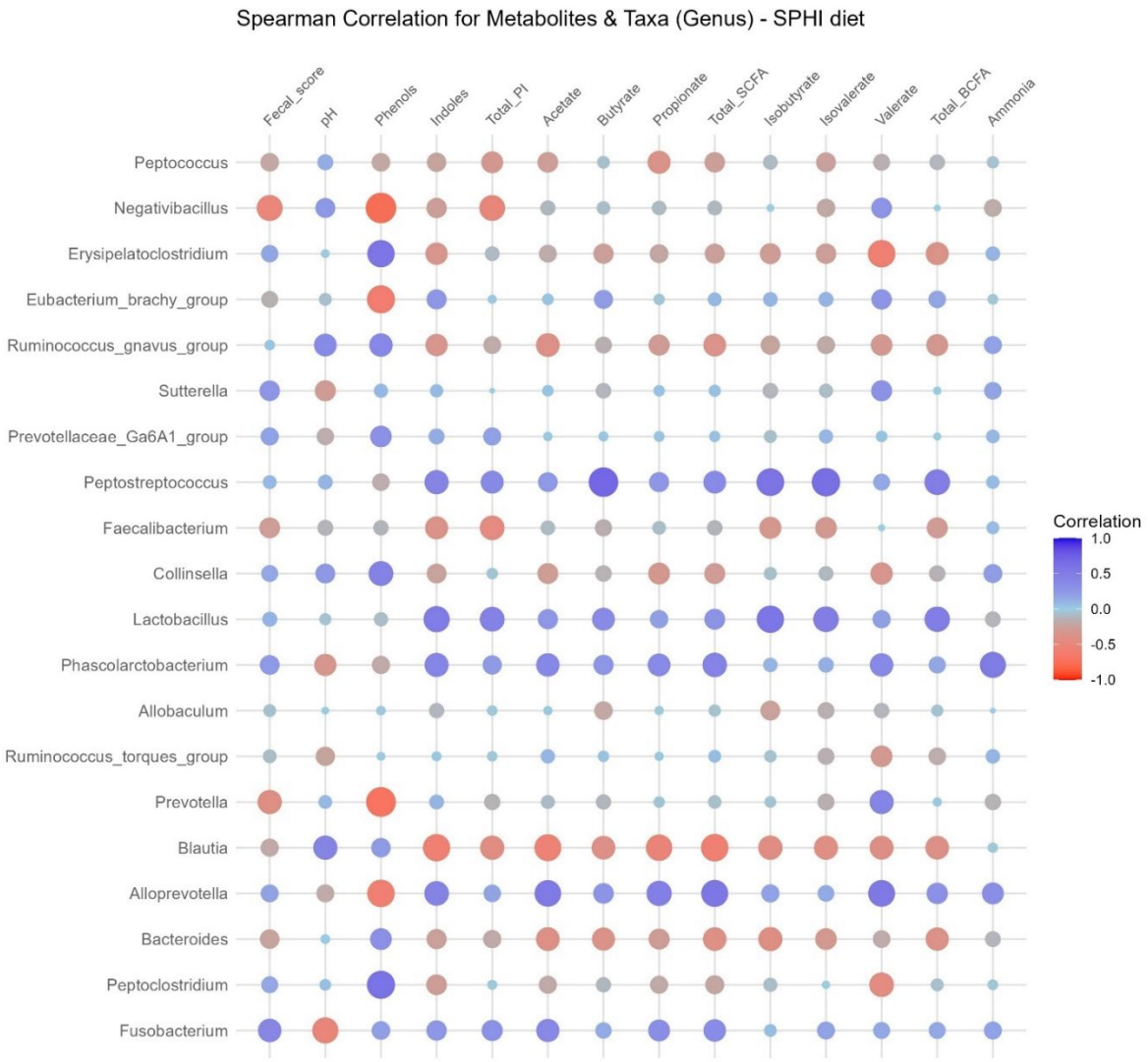

Figure 4.6. Spearman correlation of fecal metabolites and microbiota taxa in dogs fed the salmon protein hydrolysate isolate (SPHI) diet. BCFA: branched-chain fatty acids; PI: phenols and indoles; SCFA: short-chain fatty acids.

Literature Cited

AOAC Int. 2019. Official Methods of Analysis of AOAC. 21st ed., Association of Official Analytical Chemists. Rockville, MD

Association of American Feed Control Officials (AAFCO). 2023. Official publication. Champaign, IL

Barko, P. C., McMichael, M. A., Swanson, K. S., and Williams, D. A. 2018. The gastrointestinal microbiome: a review. J. Vet. Intern. Med. 32:9-25. doi:10.1111/jvim.14875

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., and Caporaso, J. G. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90. doi:10.1186/s40168-018-0470-z

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F. 2019. Reproducible, interactive, scalable, and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852-857. doi:10.1038/s41587-019-0209-9

Cabrita, A. R., Maia, M. R., Alves, A. P., Aires, T., Rosa, A., Almeida, A., and Fonseca, A. J. 2024. Protein hydrolysate and oil from fish waste reveal potential as dog food ingredients. Front. Vet. Sci. 11:1372023. doi:10.3389/fvets.2024.1372023

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13:581-583. doi:10.1038/nmeth.3869

Chakrabarti, S., and Wu, J. 2016. Bioactive peptides on endothelial function. Food Sci. Hum. Wellness 5:1-7. doi:10.1016/j.fshw.2015.11.004

Chaney, A. L., and Marbach, E. P. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132. doi:10.1093/clinchem/8.2.130

Chen, D., Tang, T. X., Deng, H., Yang, X. P., and Tang, Z. H. 2021. Interleukin-7 biology and its effects on immune cells: mediator of generation, differentiation, survival, and homeostasis. Front. Immunol. 12:747324. doi:10.3389/fimmu.2021.747324

Cholewski, M., Tomczykowa, M., and Tomczyk, M. 2018. A comprehensive review of chemistry, sources and bioavailability of omega-3 fatty acids. Nutrients 10:1662. doi:10.3390/nu10111662

De Godoy, M. R. C., McLeod, K. R., and Harmon, D. L. 2018. Influence of feeding a fish oil-containing diet to mature, overweight dogs: effects on lipid metabolites, postprandial glycaemia and body weight. J. Anim. Physiol. Anim. Nutr. 102:e155-e165. doi:10.1111/jpn.12723

Erwin, E. S., Marco, G. J., and Emery, E. M. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1771. doi:10.3168/jds.S0022-0302(61)89956-6

Evora, P. R. B., Pearson, P. J., Rodrigues, A. J., Viaro, F., and Schaff, H. V. 2003. Effect of arginine vasopressin on the canine epicardial coronary artery: experiments on V1-receptor-mediated production of nitric oxide. Arq. Bras. Cardiol. 80:489-494. doi:10.1590/S0066-782X2003000500002

Flickinger, E. A., Schreijen, E. M. W. C., Patil, A. R., Hussein, H. S., Grieshop, C. M., Merchen, N. R., and Fahey, G. C. Jr. 2003. Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. J. Anim. Sci. 81:2008-2018. doi:10.2527/2003.8182008x

Folador, J. F., Karr-Lilienthal, L. K., Parsons, C. M., Bauer, L. L., Utterback, P. L., Schasteen, C. S., Bechtel, P. J., and Fahey, G. C. 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.2005-636

Hou, Y., Wu, Z., Dai, Z., Wang, G., and Wu, G. 2017. Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. J. Anim. Sci. Biotechnol. 8:209-232. doi:10.1186/s40104-017-0153-9

Hsu, C., Marx, F., Guldenpfennig, R., Valizadegan, N., and de Godoy, M. R. 2024. The effects of hydrolyzed protein on macronutrient digestibility, fecal metabolites and microbiota, oxidative stress and inflammatory biomarkers, and skin and coat quality in adult dogs. J. Anim. Sci. 102:skae057. doi:10.1093/jas/skae057

Kandyliari, A., Mallouchos, A., Papandroulakis, N., Golla, J. P., Lam, T. T., Sakellari, A., et al. 2020. Nutrient composition and fatty acid and protein profiles of selected fish by-products. Food Secur. 9:190. doi:10.3390/foods9020190

Kristinsson, H. G., and Rasco, B. A. 2000. Fish protein hydrolysates: production, biochemical, and functional properties. Crit. Rev. Food Sci. Nutr. 40:43-81. doi:10.1080/10408690091189266

Laflamme, D. 1997. Development and validation of a body condition score system for dogs. Canine pract. 22:10-15. ISSN: 1057-6622

Lau-Gillard, P. J., Hill, P. B., Chesney, C. J., Budleigh, C., and Immonen, A. 2010. Evaluation of a hand-held evaporimeter (VapoMeter®) for the measurement of transepidermal water loss in healthy dogs. Vet. Dermatol. 21:136-145. doi:10.1111/j.1365-3164.2009.00738.x

Lewis, T. P., Moore, G. E., Laporte, C., Daristotle, L., and Frantz, N. Z. 2025. Evaluation of hydrolyzed salmon and hydrolyzed poultry feather diets in restrictive diet trials for diagnosis of food allergies in pruritic dogs. Front. Vet. Sci. 12:1560806. doi:10.3389/fvets.2025.1560806

Liu, X., Mao, B., Gu, J., Wu, J., Cui, S., Wang, G., and Chen, W. 2021. Blautia—a new functional genus with potential probiotic properties?. Gut Microbes 13:1875796. doi:10.1080/19490976.2021.1875796

Marsh, K. A., Ruedisueli, F. L., Coe, S. L., and Watson, T. G. D. 2000. Effects of zinc and linoleic acid supplementation on the skin and coat quality of dogs receiving a complete and balanced diet. Vet. Dermatol. 11:277-284. doi:10.1046/j.1365-3164.2000.00202.x

Nogueira, M. S., Scolaro, B., Milne, G. L., and Castro, I. A. 2019. Oxidation products from omega-3 and omega-6 fatty acids during a simulated shelf life of edible oils. LWT 101:113-122. doi:10.1016/j.lwt.2018.11.044

Nyangale, E. P., Mottram, D. S., and Gibson, G. R. 2012. Gut microbial activity, implications for health and disease: the potential role of metabolite analysis. J. Proteome Res. 11:5573-5585. doi:10.1021/pr300637d

NRC. 2006. Nutrient requirements for dogs and cats. 6th rev. ed. Natl. Acad. Press, Washington, DC

Parke, M. A., Perez-Sanchez, A., Zamil, D. H., and Katta, R. 2021. Diet and skin barrier: the role of dietary interventions on skin barrier function. Dermatol. Pract. Concept 11:e2021132. doi:10.5826/dpc.1101a132

Pilla, R., and Suchodolski, J. S. 2020. The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. Front. Vet. Sci. 6:502799. doi:10.3389/fvets.2019.00498

Phillips, R. S., Demidkina, T. V., and Faleev, N. G. 2003. Structure and mechanism of tryptophan indole-lyase and tyrosine phenol-lyase. Biochim. Biophys. Acta Proteins Proteom. 1647:167-172. doi:10.1016/S1570-9639(03)00089-X

Pinto, C. F. D., Sezerotto, P. P., Barcellos, J. F., Bortolo, M., Guldenpfennig, R., Marx, F. R., and Trevizan, L. 2023. Effects of hydrolyzed chicken liver on digestibility, fecal and urinary characteristics, and fecal metabolites of adult dogs. J. Anim. Sci. 101:skad366. doi:10.1093/jas/skad366

Raditic, D., and Gaylord, L. 2023. Novel trends in nutrition: pet food categorization, owner perception and current marketing. Integr. Vet. Med. 85-93. doi:10.1002/9781119823551.ch11

Ramakrishnan, V. V., Hossain, A., Dave, D., and Shahidi, F. 2024. Salmon processing discards: a potential source of bioactive peptides–a review. Food Prod. Process. Nutr. 6:22. doi:10.1186/s43014-024-00144-9

Ravić, B., Debeljak-Martacić, J., Pokimica, B., Vidović, N., Ranković, S., Glibetić, M., and Popović, T. 2022. The effect of fish oil-based foods on lipid and oxidative status parameters in police dogs. Biomolecules 12:1092. doi:10.3390/biom12081092

Rees, C. A., Bauer, J. E., Burkholder, W. J., Kennis, R. A., Dunbar, B. L., and Bigley, C. E. 2001. Effects of dietary flax seed and sunflower seed supplementation on normal canine serum polyunsaturated fatty acids and skin and hair coat condition scores. Vet. Dermatol. 12:111-117. doi:10.1046/j.1365-3164.2001.00234.x

Rose, D. J., DeMeo, M. T., Keshavarzian, A., and Hamaker, B. R. 2007. Influence of dietary fiber on inflammatory bowel disease and colon cancer: importance of fermentation pattern. Nutr. Rev. 65:51-62. doi:10.1111/j.1753-4887.2007.tb00282.x

Sieja, K. M., Oba, P. M., Applegate, C. C., Pendlebury, C., Kelly, J., and Swanson, K. S. 2023. Evaluation of high-protein diets differing in protein source in healthy adult dogs. J. Anim. Sci. 101:skad057. doi:10.1093/jas/skad057

Suchodolski, J. S. 2011. Companion animals symposium: microbes and gastrointestinal health of dogs and cats. J. Anim. Sci. 89:1520-1530. doi:10.2527/jas.2010-3377.

Tjernsbekk, M. T., Tauson, A. H., Kraugerud, O. F., and Ahlstrøm, Ø. 2017. Raw mechanically separated chicken meat and salmon protein hydrolysate as protein sources in extruded dog food: effect on protein and amino acid digestibility. J. Anim. Physiol. Anim. Nutr. 101:e323-e331. doi:10.1111/jpn.12608

Zinn, K. E., Hernot, D. C., Fastinger, N. D., Karr-Lilienthal, L. K., Bechtel, P. J., Swanson, K. S., and Fahey, G. C., Jr. 2009. Fish protein substrates can substitute effectively for poultry by-product meal when incorporated in high-quality senior dog diets. J. Anim. Physiol. Anim. Nutr. 93:447-455. doi:10.1111/j.1439-0396.2008.00826.x

Chapter 5: Protein Quality of Seaweeds and The Effects of Seaweed Polysaccharides on Amino Acid Digestibility

Abstract

The hypothesis that protein quality of 3 seaweed species (i.e., *Ulva lactuca*, *Porphyra umbilicalis*, and *Saccharina latissima*) is less than in casein and whey protein isolate (WPI) as indicated by the Digestible Indispensable Amino Acid Score (DIAAS) method was tested. The second hypothesis was that seaweed polysaccharides (i.e., carrageenan, agar, alginate, and cellulose) decrease amino acid (AA) digestibility of high-quality proteins, including casein and WPI. Twelve growing pigs with a T-cannula installed in the distal ileum were used. The seaweeds, as well as polysaccharide-protein blends, were included in 12 experimental diets. Each diet was fed to 6 pigs and ileal digesta were collected from the cannulas and analyzed for AA. The standardized ileal digestibility (SID) of AA was determined, and DIAAS for children from 6 months to 3 years old and individuals older than 3 years was calculated. Results demonstrated that SID of most AA in the 3 seaweeds was less than 80%, and DIAAS was less than 75 for all seaweeds. The SID of AA in casein or WPI was not reduced when carrageenan, agar, or cellulose was added to the diet, but the SID of most indispensable AA was reduced when alginate was added to the casein diet, indicating a reduction in intestinal absorption. In conclusion, no claims regarding protein quality can be made for seaweeds because of the low DIAAS, which is likely a result of native cell wall structures reducing AA digestibility. However, the soluble fibers in seaweed polysaccharides did not reduce AA digestibility in casein or WPHI, except for alginate, which reduced AA digestibility in casein.

Keywords: algae; fibers; protein quality; amino acid digestibility; milk proteins; phycocolloids

Introduction

Alternative proteins are expected to be consumed in increased quantities in the future due to rising global demand for protein (Van der Spiegel et al., 2013), and seaweeds represent a diverse group of macroalgae that may be used in human nutrition (Boyd et al., 2022). Edible seaweeds are rich sources of some amino acids (AA), minerals, and specific undigestible polysaccharides (i.e., phycocolloids) that may be utilized as functional ingredients in food preparations (Costa et al., 2021). More than 150 species of seaweed have been incorporated into human diets in different cultures (Boyd et al., 2022). Among the most widely consumed species are *Ulva spp.* (commonly known as sea lettuce, which is a green seaweed), *Porphyra spp.* (commonly known as nori, red seaweed), and *Saccharina spp.* (commonly known as sugar kelp, brown seaweed), and each species is valued for its unique nutritional and functional properties (Costa et al., 2021).

Digestibility of nutrients in seaweeds depends on the species, chemical composition, structural characteristics, biomass treatment, and functional use (Juul et al., 2022). The high concentration of polysaccharides along with anti-nutritional factors in seaweeds, as well as the complex structure and composition of their cell walls, reduce protein digestibility *in vitro* (De Bhowmick and Hayes, 2022; Tibbetts et al., 2016; Cebrián-Lloret et al., 2024). Seaweed polysaccharides may also delay digestion and reduce *in vitro* digestibility of AA in high-quality proteins, such as whey protein isolate (WPI) and casein (Díaz-Piñero et al., 2025). However, values for standardized ileal digestibility (SID) of AA in seaweeds or in high-quality proteins consumed together with seaweed polysaccharides have not been determined *in vivo*.

The Digestible Indispensable Amino Acid Score (DIAAS) has been recommended by the Food and Agriculture Organization (FAO) to determine protein quality in human foods, and

because the SID of AA determined in pigs can be directly translated to humans due to digestive and anatomical similarities (Hodgkinson et al., 2022), measuring SID of AA in pigs enables calculation of DIAAS in food proteins for human consumption (FAO, 2014). Determination of SID requires correction of ileal outflow of AA for basal endogenous losses of AA (Stein et al., 2007), a method that has not been applied in previous experiments using seaweeds and seaweed polysaccharides. Therefore, this experiment was conducted to test the hypothesis that protein quality of 3 seaweed species is less than in casein and WPI as measured by the DIAAS method, and that seaweed polysaccharides (i.e., carrageenan, agar, alginate, and cellulose) decrease AA digestibility in casein and WPI.

Material and Methods

Experimental ingredients and diets

Three seaweeds [i.e., Ulva (*Ulva lactuca*), Nori (*Porphyra umbilicalis*), and Saccharina (*Saccharina latissima*)] were purchased from VitaminSea Seaweed (Buxton, ME, USA). Casein powder in the form of micellar casein and WPI powder were purchased from Ingredia S.A. (Arras, France). Polysaccharides, including agar, alginate, carrageenan, and carboxymethylcellulose (a soluble derivative of cellulose; hereafter referred to as cellulose) were purchased in powder form from Industrias Roko S.A. (Asturias, Spain). All ingredients were delivered to the University of Illinois (Urbana, IL, USA), and their chemical composition was determined (Table 5.1).

Twelve diets were formulated to contain 10% crude protein on a dry matter basis as recommended (Tables 5.2 and 5.3; FAO, 2014). Three diets contained each of the 3 seaweeds mixed with casein. Two additional diets contained casein or WPI as the only source of AA. The

remaining diets were a mixture of casein or WPI with one of the four polysaccharides at different ratios (casein:agar 1:3, 1:1, or 3:1; casein:carrageenan 1:3; casein:alginate 1:3; casein: cellulose 1:3; and WPI:agar 1:3), forming different protein-polysaccharide blends. A nitrogen-free diet was also used to measure the basal endogenous losses of AA. Vitamins and minerals were included in all diets to meet or exceed current nutrient requirement estimates for growing pigs [National Research Council (NRC, 2012)]. All diets contained 0.40% titanium dioxide as an indigestible marker. A sample of each diet was collected at the time of diet mixing and used for chemical analysis.

Experimental design

Twelve growing gilts with an average initial body weight of 25.7 ± 1.4 kg had a T-cannula installed in the distal ileum (Stein et al., 1998). Pigs were allotted to a 12 × 6 Youden square design with twelve protein-containing diets and six periods in each square. All diets were fed to one pig in each period, and no pig received the same diet more than once during the experiment. There were, therefore, 6 observations per treatment. Pigs were fed the nitrogen-free diet in the middle of the experiment to enable calculation of SID of AA by using each pig as its own control (FAO, 2014; Hodgkinson et al., 2020).

Pigs were housed in individual pens (1.5 × 2.5 m) in an environmentally controlled room. Each pen had smooth sides, partially slatted floors, a feeder, and a nipple drinker. All pigs were fed their assigned diets in a daily amount equivalent to 8% of body weight $^{0.75}$ calculated on a dry matter basis. Daily feed allotments were divided into 2 equal meals provided every day at 0700 and 1600 h. Water was available at all times, and the amount of feed supplied each day was recorded. Pig weights were recorded at the beginning of each experimental period to calculate feed allowance for the following period.

The initial 5 days of each period were considered an adaptation period to the diet, and ileal digesta were collected for 9 hours after feeding the first meal on days 6 and 7 according to standard procedures (Hodgkinson et al., 2020; Stein et al., 1998). In short, a plastic bag was attached to the cannula barrel, and the digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta or at least once every 30 minutes and immediately frozen at – 20 ºC to prevent bacterial degradation of the AA in the digesta. At the conclusion of the experiment, pigs had an average final body weight of 35.9 ± 2.8 kg.

Chemical analysis

At the end of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. Ileal digesta samples were lyophilized and finely ground prior to chemical analysis. Samples of all ingredients, diets, and ileal digesta were analyzed for dry matter (Method 930.15; AOAC Int., 2019), and AA were analyzed [Method 982.30 E(a, b, c); AOAC Int., 2019] on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA, USA). Nitrogen was analyzed by combustion (Method 990.03; AOAC Int., 2019) using a LECO FP628 Nitrogen analyzer (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as nitrogen × 6.25, but for the seaweeds and milk proteins, a Jones nitrogen conversion factor of 5.00 and 6.38, respectively, was also used (Mariotti et al., 2008; Angell et al., 2016). In addition, diets and ingredients were analyzed for insoluble and soluble dietary fiber (Method 991.43; AOAC Int., 2019) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA), and total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber. Ingredients and diet samples were analyzed for ash (Method 942.05; AOAC Int., 2019), and diets and ileal digesta samples were also analyzed for titanium (Myers et al., 2004).

Calculations

Values for apparent ileal digestibility (AID) and SID of AA in all diets were calculated using the direct procedure according to Stein et al. (2007). The AID and SID of AA in each of the 3 seaweeds were subsequently calculated using the difference procedure by subtracting the contribution from casein to obtain the AID and SID of AA in each of the 3 casein-seaweed diets (Kong and Adeola, 2014). The DIAAS reference ratio was calculated using the following equation (FAO, 2013):

Digestible indispensable AA reference ratio = digestible indispensable AA content in 1 g protein of food (mg) / mg of the same dietary indispensable AA in 1g of the reference protein.

The DIAAS values in the seaweeds and in the milk proteins were calculated for two different age groups (i.e., children from 6 months to 3 years and individuals older than 3 years, including older children, adolescents, and adults; FAO, 2013):

DIAAS (%) = 100 × lowest value of the digestible indispensable AA reference ratio.

The DIAAS for all ingredients was calculated using the Jones factor of 6.25 as recommended (FAO, 2013), but for the seaweeds, DIAAS values were also calculated using a Jones factor of 5.0 (Angell et al., 2016), and for casein and WPI, a Jones factor of 6.38 (Mariotti et al., 2008) was also used.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS 9.4 version, Inst. Inc., Cary, NC, USA) with the pig being the experimental unit. Normality of residuals and homogeneity of variances were confirmed using the UNIVARIATE procedure of SAS. Outliers were identified as observations that deviated from the 1st or 3rd quartiles by ± 3 times the interquartile range. The model included diet as the fixed effect and pig and period as random

effects. Treatment means were calculated using the LSMEANS statement in SAS, and if significant, means were separated using the PDIFF option in the MIXED procedure with Tukey adjustment. Results were considered significant at $P < 0.05$.

Results

Digestibility of AA in seaweeds and protein-polysaccharide blends

Values for AID of most AA were greater ($P < 0.05$) in casein, WPI, and all protein-polysaccharide blends than in the 3 seaweeds (Table 5.4). There was no difference in the AID of arginine between any of the ingredients and the protein-polysaccharide blends. Likewise, there were no differences between WPI and WPI:agar 1:3 for the AID of any of the AA. Among all protein-polysaccharide blends, the casein-alginate blend had a reduced ($P < 0.05$) AID of histidine, lysine, methionine, threonine, tryptophan, valine, aspartic acid, cysteine, and serine compared with casein. Likewise, when comparing casein with other blends, the AID of histidine was reduced ($P < 0.05$) in casein:carrageenan 1:3 and casein:agar 1:3, the AID of lysine and methionine was reduced ($P < 0.05$) in casein:carrageenan 1:3, the AID of serine was reduced ($P < 0.05$) in casein:agar: 1:3, and the AID of tyrosine was reduced ($P < 0.05$) in casein:cellulose 1:3.

The SID of most AA were greater ($P < 0.05$) in casein, WPI, and all protein-polysaccharide blends than in the 3 seaweeds (Table 5.5). There were no differences between WPI and WPI:Agar 1:3, whereas for casein blends, the SID of isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine, and aspartic acid was reduced ($P < 0.05$) in casein:alginate 1:3 compared with casein. In addition, the SID of tyrosine was less ($P < 0.05$) in

casein:cellulose 1:3 than in casein, but the SID of arginine was less in casein ($P < 0.05$) than in casein:cellulose 1:3.

DIAAS in seaweeds and milk proteins

The DIAAS for children from 6 months to 3 years and individuals older than 3 years was greater ($P < 0.05$) in casein than in WPI and the 3 seaweeds (Table 5.6), but DIAAS in WPI was greater ($P < 0.05$) than in the 3 seaweeds. There was no limiting AA for casein and WPI (DIAAS ≥ 100), but histidine was the limiting AA for the three seaweeds for both age groups.

Discussion

Protein quality of seaweeds

The use of seaweeds as food ingredients in human diets aligns with efforts to diversify protein supply for humans (Boyd et al., 2022). Seaweeds are classified into 3 main groups, according to their pigments: green, red, and brown (Samarasinghe et al., 2021), and provide limited energy due to their low concentrations of lipids and digestible carbohydrates. Whereas the protein levels differ among species, brown seaweeds generally contain less crude protein on a dry matter basis (3 to 16%) than green (11 to 26%) or red (11 to 33%) varieties (Holdt and Kraan, 2011; García-Vaquero and Hayes, 2016). The crude protein analyzed in the seaweeds used in this experiment were within the above ranges, and *Saccharina latissima* contained less protein than *Ulva lactuca* and *Porphyra umbilicalis* seaweeds. The most abundant indispensable AA in the 3 seaweeds were leucine and valine, whereas histidine and tryptophan were the least abundant indispensable AA, which also agrees with published data (Sharma et al., 2018; Samarasinghe et al., 2021). All seaweeds had high concentrations of ash and total dietary fiber, with the fiber fraction consisting of approximately equal proportions of soluble and insoluble

components. The concentration of fiber in *Ulva lactuca* and Porphyra umbilicalis was less than previously reported for these species (Yaich et al., 2011; Ferreira et al., 2025), whereas *Saccharina latissima* had greater fiber concentrations than reported by Samarasinghe et al. (2021), but within the range observed for this species when harvested at different times (Lafeuille et al., 2023). These differences are likely due to variations in environmental factors and harvest time, which result in variability in chemical composition even within the same species (García-Vaquero and Hayes, 2016; Lafeuille et al., 2023).

Protein digestibility in previous experiments have been reported to be between 25.0 and 79.0% for green seaweeds (Azizi et al., 2021; De Bhowmick & Hayes, 2022; Juul et al., 2022; Molina-Gilarranz et al., 2025), between 49.0 and 79.0% for red seaweeds (Cian et al., 2014; De Bhowmick & Hayes, 2022; Cebrián-Lloret et al., 2024), and between 58.8 and 82.0% for brown seaweeds (Tibbetts et al., 2016; Azizi et al., 2021; De Bhowmick & Hayes, 2022; Juul et al., 2022). The AID of most AA in the seaweeds was below 70%, which is also in agreement with published values *in vivo* (Azizi et al., 2021). The reason the SID of most AA in the 3 seaweeds was below 80% is likely due to the high fiber concentration and the presence of antinutritional factors in seaweeds, which contribute to an increase in specific endogenous losses of AA in the small intestine (Stein et al., 2007; De Oliveira et al., 2009). The reduced SID of AA in *Saccharina latissima* compared with *Ulva lactuca* and *Porphyra umbilicalis* seaweeds may be related to the highly recalcitrant structure of cell walls in *Saccharina latissima* that remained intact during digestion, preventing digestion of the protein trapped in the fiber matrix (Souto-Prieto et al., 2024; Correa et al., 2026). Digestibility of the protein fraction in seaweeds may also be reduced by interactions with cell wall polysaccharides and phenolic compounds, which can

form complexes resistant to enzymatic digestion in the small intestine (Holdt and Kraan, 2011; Cebrián-Lloret et al., 2024).

The reason the SID of arginine for the seaweeds was close to or greater than 100% is likely because of the high basal endogenous losses measured in pigs fed the nitrogen-free diet in this experiment. Although overestimation of basal endogenous losses for some AA can occur when using a nitrogen-free diet (Stein et al., 2007), the basal endogenous losses of AA observed in this experiment was substantially greater than published average values (Lee and Stein, 2022). It is possible that compensatory mobilization of endogenous protein to support indispensable AA supply increased endogenous losses in this experiment compared with previous experiments because pigs had been consuming high-fiber diets before the nitrogen-free diet was fed (Leterme et al., 2000; Ten Have et al., 2012). This observation highlights a limitation of *in vitro* experiments, which do not account for physiological endogenous AA losses that occur *in vivo*, but additional research is needed to fully understand how seaweed impacts basal endogenous losses of AA. Nevertheless, the SID of AA in casein and WPI was in agreement with published values (NRC, 2012; Mathai et al., 2017), indicating that the experimental approach worked as intended.

The protein quality of the 3 seaweeds assessed by the DIAAS method demonstrated that no protein quality claims can be made because DIAAS values were below 75 for individuals older than 6 months (FAO, 2013), even when a Jones factor of 5.0 was used. Histidine was the first limiting AA for the 3 seaweeds due to its low concentration and low digestibility. Therefore, to meet AA requirements in a meal, consumption of additional food with a high concentration of digestible histidine is needed. These observations demonstrate the need for future research to

identify high-histidine ingredients that can be used to complement seaweed proteins to provide a meal that is balanced in all indispensable AA needed by humans.

Effects of seaweed polysaccharides on AA digestibility of high-quality proteins

The composition of cell-wall polysaccharides differs among seaweed species, with green seaweeds containing mainly cellulose, mannans, and xylans, brown seaweeds characterized by alginate, laminarin, and fucoidan, and red seaweeds being rich in sulfated galactans such as agar and carrageenan (Costa et al., 2021). Therefore, the use of agar, carrageenan, alginate, and cellulose in the current experiment allowed for a comparative evaluation of distinct classes of polysaccharides that are representative of seaweed diversity. To our knowledge, this is the first time these extracted polysaccharides have been analyzed for total dietary fiber using the enzymatic-gravimetric method, which uses sequential digestion followed by separation of insoluble and soluble dietary fibers.

To determine the effect of seaweed polysaccharides on AA digestibility of high-quality protein sources, the ratios of casein or WPI to polysaccharides in the experimental diets were designed to reflect the natural composition of seaweeds, which typically contain between 40 and 70% polysaccharides and between 3 and 33% protein (García-Vaquero and Hayes, 2016). Therefore, a ratio of casein:polysaccharide (1:3) was selected for all polysaccharides. For agar, two additional casein:agar ratios (1:1 and 3:1) were used to assess potential dose-response effects on AA digestibility, and a WPI:agar ratio of 1:3 was also used. Agar was selected due to its structural relevance in red seaweeds, its widespread use as a gelling agent in food products (Cebrián-Lloret et al., 2024), and its capacity to delay the digestion process of high-quality proteins *in vitro* (Fontes-Candia et al., 2022). Whereas it would have been ideal to test multiple ratios for all polysaccharides, budget constraints related to the number of diets limited this

possibility. In addition, by maintaining the same inclusion levels of casein or WPI in both the single diets and in the blends, the specific effect of each polysaccharide on AA digestibility could be determined.

The observations that agar, carrageenan, and cellulose did not affect digestibility of AA in casein or WPI and that variation in agar inclusion had no measurable effect on AA digestibility in casein indicate that the polysaccharides had minimal influence on AA digestibility in casein or WPI. Soluble dietary fiber is characterized by its water-holding capacity and gel-forming properties (García-Vaquero and Hayes, 2016), which may limit digestibility by reducing access of proteolytic enzymes through increased viscosity (Cervantes-Pahm et al., 2014). However, most inhibitory effects reported were demonstrated *in vitro*, whereas *in vivo* data indicate that soluble dietary fiber may modify digesta characteristics and fermentation, but have minimal influence on AA digestibility or on epithelial transport mechanisms (Middelbos et al., 2007; Jha et al., 2008; Cervantes-Pahm et al., 2014). In addition, SDS-PAGE and peptidomics analysis of ileal digesta from pigs fed the protein-polysaccharide blends demonstrated that the majority of protein in the digest was endogenous pancreatic enzymes, which is in agreement with the high basal endogenous losses of AA observed in this experiment (Correa et al., 2026).

The observation that the SID of most indispensable AA was reduced in casein when alginate was added to the diet indicates that alginate has different properties in the small intestine than other polysaccharides. Alginate is an anionic, carboxylated polysaccharide that may chelate calcium, which is essential for maintaining the integrity of casein micelles. Alginate may also interact with cationic regions of proteins, resulting in formation of insoluble protein-alginate aggregates that are less accessible to digestive enzymes under gastrointestinal pH conditions

compared with proteins not associated with alginate (Guo et al., 2020). Alginate reduced *in vitro* protein hydrolysis rate (Díaz-Piñero et al., 2025), and rats fed alginate-containing meals had decreased proteolytic accessibility of proteins and reduced postprandial plasma AA concentrations compared with rats not fed alginate (Guo et al., 2020). The alginate-containing diets used in this experiment also had a greater negative net charge compared with diets containing other polysaccharides, which may have contributed to the reduction in AA digestibility of casein in the casein-alginate blend (Correa et al., 2026).

Conclusions

Results for DIAAS in the 3 seaweeds indicated that no claims regarding protein quality can be made for seaweeds, and further advancements in processing are required to supply digestible indispensable AA to meet requirements for humans. In contrast, DIAAS for casein and WPI was greater than 100 for children from 6 months to 3 years and also for individuals older than 3 years, and casein and WPI were, therefore, classified as excellent proteins. The hypothesis that protein quality in seaweed is less than in casein and WPI was, therefore, confirmed. Seaweed-derived polysaccharides such as carrageenan, agar, and cellulose did not negatively impact the SID of AA in casein or WPI, but alginate reduced the digestibility of most indispensable AA in casein. These results indicate that soluble fibers may or may not reduce AA digestibility of high-quality proteins, and their effects are fiber-specific dependent. Therefore, the hypothesis that polysaccharides from seaweed reduce AA digestibility was only partially accepted.

Tables

Table 5.1. Nutrient composition of experimental ingredients (as-fed basis)[1]

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Agar	Carrageenan	Cellulose[2]	Alginate
Dry matter	84.65	86.81	86.19	94.78	95.12	93.62	90.22	93.91	85.66
Crude protein[3]	16.85 [13.48]	23.25 [18.60]	10.52 [8.41]	79.62 [81.27]	85.02 [86.79]	0.09	0.05	ND	ND
Total dietary fiber	36.80	33.80	42.10	5.90	3.70	88.20	76.70	77.30	63.80
Soluble fiber	17.90	17.10	17.40	3.30	ND	81.80	76.00	77.30	62.50
Insoluble fiber	18.90	16.70	24.60	2.60	3.70	6.40	0.70	ND	1.30
Ash	21.04	28.28	29.66	7.88	2.68	1.37	21.22	17.86	20.27
Indispensable AA									
Histidine	0.20	0.27	0.18	2.39	1.91	-	-	-	-
Isoleucine	0.67	0.97	0.53	4.47	5.42	-	-	-	-
Leucine	1.06	1.74	0.87	7.85	11.59	-	-	-	-
Lysine	0.69	1.26	0.60	6.55	9.22	-	-	-	-

Table 5.1 (cont.)

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Agar	Carrageenan	Cellulose[2]	Alginate
Methionine	0.27	0.43	0.26	2.30	2.25	-	-	-	-
Phenylalanine	0.84	1.03	0.57	4.19	3.51	-	-	-	-
Threonine	0.64	1.29	0.62	3.44	4.44	-	-	-	-
Tryptophan	0.18	0.23	0.11	0.97	2.01	-	-	-	-
Valine	0.93	1.62	0.70	5.50	5.25	-	-	-	-
Dispensable AA						-	-	-	-
Alanine	1.30	2.38	1.28	2.57	4.55	-	-	-	-
Arginine	0.69	1.38	0.56	2.92	2.51	-	-	-	-
Aspartic acid	1.59	2.21	1.18	5.89	10.12	-	-	-	-
Cysteine	0.40	0.52	0.35	0.40	2.59	-	-	-	-
Glutamic Acid	1.51	2.18	1.47	18.24	16.82	-	-	-	-
Glycine	0.87	1.50	0.61	1.49	1.64	-	-	-	-
Proline	0.65	1.04	0.57	8.81	4.95	-	-	-	-

Table 5.1 (cont.)

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Agar	Carrageenan	Cellulose[2]	Alginate
Serine	0.61	0.94	0.47	3.80	3.37	-	-	-	-
Tyrosine	0.42	0.74	0.29	4.46	3.58	-	-	-	-

[1]ND, not detected; WPI, whey protein isolate.

[2]Carboxymethylcellulose.

[3]Values in brackets for the 3 seaweeds and both milk products represent crude protein values calculated using a Jones nitrogen conversion factor for crude protein of 5.00 and 6.38, respectively. All other values were calculated using a Jones factor of 6.25.

Table 5.2. Ingredient composition of experimental diets (as-fed basis)[1]

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Casein: Carrageenan	Casein: Cellulose[2]	Casein: Alginate	Casein: Agar			WPI: Agar	Nitrogen-free
						1:3	1:3	1:3	1:3	1:1	3:1	1:3	
Seaweed	25.00	25.00	25.00	-	-	-	-	-	-	-	-	-	-
Casein	7.50	6.00	9.20	12.00	-	12.00	12.00	12.00	12.00	12.00	12.00	-	-
WPI	-	-	-	-	11.20	-	-	-	-	-	-	11.20	-
Polysaccharide	-	-	-	-	-	36.00	36.00	36.00	36.00	12.00	4.00	33.60	-
Corn starch	46.30	47.55	44.80	65.85	66.70	30.60	29.90	30.90	29.90	53.90	61.90	33.10	77.60
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Canola oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Solka floc	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.40	1.65	1.50	1.60	1.80	1.30	1.30	1.50	1.50	1.50	1.50	1.80	2.10
Limestone	0.50	0.50	0.20	0.75	0.50	0.30	-	0.80	0.80	0.80	0.80	0.50	0.50
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium Oxide	-	-	-	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Potassium carbonate	-	-	-	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vit-min. premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Table 5.2 (cont.)

[1]WPI, whey protein isolate. All diets, except the nitrogen-free diet, were formulated to contain approximately 10% of crude protein (dry matter basis).

[2]Carboxymethylcellulose.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D3 as cholecalciferol, 1,660 IU; vitamin E as DL alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 5.3. Nutrient composition of experimental diets (as-fed basis)[1]

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Casein: Carrageenan	Casein: Cellulose[2]	Casein: Alginate	Casein: Agar			WPI: Agar	Nitrogen-free
						1:3	1:3	1:3	1:3	1:1	3:1	1:3	
Dry matter	89.60	90.97	91.27	91.47	92.04	91.85	92.46	89.78	90.98	92.02	92.16	90.60	91.20
Crude protein	9.76	9.28	10.13	9.39	9.70	8.84	8.31	8.35	9.27	9.34	9.42	9.27	0.28
Total dietary fiber	12.40	11.60	13.90	3.50	3.20	30.90	31.30	26.50	35.30	14.10	7.00	32.90	2.80
Soluble fiber	4.70	4.50	4.70	0.10	ND	27.50	28.20	22.90	29.90	10.20	3.60	27.50	ND
Insoluble fiber	7.70	7.10	9.20	3.40	3.20	3.40	3.10	3.60	5.40	3.90	3.40	5.40	2.80
Ash	7.26	9.49	8.81	3.38	3.08	9.03	9.45	10.04	3.48	3.20	3.23	2.55	1.72
Indispensable AA													
Histidine	0.23	0.20	0.27	0.29	0.22	0.29	0.30	0.29	0.29	0.29	0.30	0.22	0.00
Isoleucine	0.52	0.46	0.56	0.54	0.62	0.55	0.59	0.55	0.55	0.55	0.56	0.64	0.02
Leucine	0.88	0.81	0.97	0.96	1.33	0.97	1.00	0.96	0.97	0.97	0.99	1.32	0.03
Lysine	0.69	0.64	0.78	0.80	1.05	0.82	0.84	0.80	0.84	0.82	0.83	1.08	0.02
Methionine	0.25	0.20	0.24	0.27	0.24	0.26	0.28	0.26	0.27	0.27	0.28	0.24	0.01
Phenylalanine	0.54	0.45	0.54	0.51	0.41	0.52	0.53	0.51	0.52	0.51	0.53	0.41	0.02
Threonine	0.44	0.45	0.47	0.42	0.50	0.39	0.42	0.41	0.42	0.42	0.43	0.49	0.01
Tryptophan	0.10	0.08	0.09	0.10	0.22	0.12	0.11	0.09	0.11	0.11	0.12	0.22	0.02
Valine	0.66	0.62	0.69	0.66	0.60	0.67	0.70	0.67	0.67	0.67	0.69	0.61	0.01
Dispensable AA													
Alanine	0.54	0.58	0.53	0.32	0.53	0.32	0.33	0.32	0.32	0.73	0.33	0.53	0.02

Table 5.3 (cont.)

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Casein: Carrageenan	Casein: Cellulose[2]	Casein: Alginate	Casein: Agar			WPI: Agar	Nitrogen-free
						1:3	1:3	1:3	1:3	1:1	3:1	1:3	
Arginine	0.39	0.41	0.39	0.34	0.27	0.33	0.33	0.31	0.33	0.32	0.35	0.26	0.01
Aspartic acid	0.86	0.80	0.87	0.72	1.15	0.73	0.74	0.71	0.74	0.05	0.75	1.15	0.02
Cysteine	0.13	0.12	0.12	0.05	0.29	0.30	0.06	0.06	0.06	2.26	0.05	0.29	0.01
Glutamic Acid	1.82	1.62	2.17	2.25	1.96	2.25	2.27	2.23	2.27	0.19	2.32	1.94	0.03
Glycine	0.34	0.37	0.29	0.19	0.19	0.19	0.19	0.18	0.19	1.07	0.19	0.19	0.01
Proline	0.84	0.75	0.98	1.06	0.57	1.08	1.10	1.05	1.06	0.50	1.10	0.56	0.02
Serine	0.47	0.44	0.49	0.50	0.41	0.41	0.45	0.45	0.48	0.37	0.51	0.38	0.01
Tyrosine	0.35	0.32	0.38	0.38	0.28	0.37	0.33	0.34	0.35	0.32	0.39	0.23	0.01

[1]AA, amino acids; ND, not detected; WPI, whey protein isolate. Values for protein in diets were calculated using a Jones factor of 6.25.

[2]Carboxymethylcellulose.

Table 5.4. Apparent ileal digestibility (AID) of amino acids (AA) in individual ingredients and protein-polysaccharide blends[1]

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Casein: Carrageenan 1:3	Casein: Cellulose[2] 1:3	Casein: Alginate 1:3	Casein: Agar 1:3	Casein: Agar 1:1	Casein: Agar 3:1	WPI: Agar 1:3	SEM	*P*-value
Indispensable AA														
Histidine	20.5^{e}	14.2^{e}	9.0^{e}	87.7^{ab}	82.7^{cd}	81.3^{cd}	86.0^{bc}	77.1^{d}	82.7^{cd}	87.1^{ab}	88.2^{a}	78.8^{d}	3.11	<0.001
Isoleucine	48.5^{c}	58.5^{c}	51.4^{c}	87.5^{ab}	89.0^{a}	80.7^{ab}	86.9^{ab}	78.2^{b}	82.2^{ab}	85.6^{ab}	88.1^{a}	88.5^{a}	1.92	<0.001
Leucine	57.7^{cd}	64.0^{c}	51.4^{d}	91.0^{ab}	92.6^{a}	84.3^{ab}	89.9^{ab}	83.0^{b}	86.9^{ab}	90.3^{ab}	91.9^{a}	91.2^{ab}	1.77	<0.001
Lysine	51.2^{d}	41.2^{d}	37.1^{d}	83.8^{a}	84.5^{a}	70.4^{c}	84.0^{a}	72.1^{bc}	77.8^{abc}	81.6^{a}	84.3^{a}	80.8^{ab}	3.57	<0.001
Methionine	67.0^{d}	60.3^{e}	42.5^{f}	93.9^{a}	93.4^{ab}	90.7^{bc}	93.2^{ab}	88.3^{c}	92.9^{ab}	93.7^{a}	94.9^{a}	92.6^{ab}	1.47	<0.001
Phenylalanine	57.3^{c}	55.3^{c}	48.4^{c}	90.3^{ab}	86.9^{ab}	83.5^{ab}	89.2^{ab}	81.6^{b}	87.4^{ab}	90.5^{ab}	92.0^{a}	84.4^{ab}	2.02	<0.001
Threonine	41.2^{c}	44.2^{c}	36.8^{c}	78.2^{a}	80.4^{a}	70.6^{ab}	74.9^{a}	60.0^{b}	71.0^{ab}	77.3^{a}	79.8^{a}	77.2^{a}	2.73	<0.001
Tryptophan	67.0^{bc}	60.5^{c}	-1.7^{d}	84.5^{ab}	92.4^{a}	76.4^{abc}	78.4^{abc}	61.1^{c}	77.1^{abc}	85.1^{ab}	87.8^{a}	90.3^{a}	4.21	<0.001
Valine	49.9^{d}	61.5^{c}	50.6^{d}	87.8^{a}	86.8^{a}	79.8^{ab}	86.4^{ab}	77.2^{b}	82.8^{ab}	86.2^{ab}	88.7^{a}	84.8^{ab}	2.01	<0.001
Dispensable AA														
Alanine	52.8^{abc}	67.1^{abc}	71.4^{ab}	49.8^{abc}	75.7^{a}	46.1^{abc}	67.0^{abc}	44.0^{bc}	38.5^{c}	56.6^{abc}	57.5^{abc}	73.4^{ab}	7.02	<0.001
Arginine	64.3	51.8	66.1	41.5	45.9	68.7	78.5	72.3	37.6	55.7	50.9	55.6	11.78	0.260
Aspartic acid	51.2^{f}	57.1^{ef}	53.7^{f}	82.4^{abc}	87.1^{a}	71.9^{cd}	80.3^{abc}	67.3^{de}	74.5^{bcd}	81.7^{abc}	83.7^{ab}	84.8^{ab}	2.55	<0.001
Cysteine	43.7^{abc}	16.0^{c}	32.0^{bc}	32.9^{bc}	85.7^{a}	54.8^{bc}	17.2^{c}	-41.5^{d}	7.5^{cd}	35.7^{bc}	37.2^{bc}	74.7^{ab}	9.65	<0.001
Glutamic Acid	43.5^{c}	59.9^{b}	58.8^{b}	90.4^{a}	89.8^{a}	85.5^{a}	90.1^{a}	80.7^{a}	85.9^{a}	88.2^{a}	90.1^{a}	88.3^{a}	2.15	<0.001
Serine	6.7^{f}	50.4^{de}	39.9^{e}	80.4^{a}	76.8^{ab}	81.0^{ab}	79.2^{ab}	63.1^{cd}	66.7^{bc}	74.4^{abc}	78.3^{ab}	73.3^{abc}	3.23	<0.001
Tyrosine	30.9^{d}	52.3^{c}	40.7^{cd}	89.2^{a}	84.0^{ab}	85.1^{ab}	74.2^{b}	82.2^{ab}	86.8^{a}	89.8^{a}	91.2^{a}	81.4^{ab}	2.39	<0.001

[a-f]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 5.4 (cont.)

[1]WPI, whey protein isolate. Data are means of 6 observations per treatment. However, due to statistical outliers, casein, casein:agar 1:1, and WPI:agar 1:3 have means of 5 observations per treatment, and the 3 seaweeds, casein:agar 1:3, casein:carrageenan 1:3, casein:cellulose 1:3, and casein:alginate 1:3 have means of 4 observations per treatment.

[2]Carboxymethylcellulose.

Table 5.5. Standardized ileal digestibility (SID) of amino acids (AA) in individual ingredients and protein-polysaccharide blends[1,2]

Item, %	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	Casein: Carrageenan 1:3	Casein: Cellulose[3] 1:3	Casein: Alginate 1:3	Casein: Agar 1:3	Casein: Agar 1:1	Casein: Agar 3:1	WPI: Agar 1:3	SEM	*P*-value
Indispensable AA														
Histidine	54.8^{b}	54.9^{b}	40.4^{c}	101.4^{a}	101.1^{a}	95.7^{a}	99.8^{a}	91.2^{a}	96.1^{a}	100.8^{a}	102.0^{a}	96.7^{a}	2.69	<0.001
Isoleucine	62.4^{d}	75.5^{c}	64.8^{d}	97.1^{a}	98.0^{a}	91.2^{ab}	97.2^{a}	87.8^{b}	92.1^{ab}	94.9^{a}	98.0^{a}	96.8^{ab}	1.84	<0.001
Leucine	72.6^{de}	79.5^{d}	64.6^{e}	100.6^{ab}	99.7^{ab}	93.9^{bc}	99.5^{abc}	92.5^{c}	96.3^{abc}	99.8^{abc}	101.4^{a}	98.1^{abc}	1.74	<0.001
Lysine	89.3^{abc}	71.6^{bc}	63.3^{c}	96.4^{ab}	94.4^{ab}	84.0^{abc}	97.2^{ab}	85.2^{abc}	89.4^{abc}	94.1^{abc}	97.9^{a}	90.3^{abc}	5.27	0.003
Methionine	76.7^{c}	71.8^{c}	52.5^{d}	99.3^{ab}	99.5^{ab}	96.9^{ab}	98.6^{ab}	93.9^{b}	98.3^{ab}	99.2^{ab}	100.4^{a}	98.6^{ab}	1.31	<0.001
Phenylalanine	68.7^{de}	71.4^{d}	61.0^{e}	100.7^{ab}	100.6^{ab}	94.6^{bc}	100.0^{abc}	92.4^{c}	97.6^{abc}	100.9^{ab}	102.7^{a}	97.6^{abc}	1.67	<0.001
Threonine	64.1^{c}	66.7^{c}	57.3^{c}	100.4^{a}	99.9^{a}	94.4^{ab}	98.9^{a}	82.5^{bc}	93.7^{ab}	99.1^{a}	101.8^{a}	96.3^{ab}	3.65	<0.001
Tryptophan	83.4^{ab}	89.0^{ab}	32.0^{c}	99.3^{a}	99.6^{a}	89.7^{ab}	93.9^{ab}	78.1^{b}	91.5^{ab}	98.3^{a}	100.7^{a}	97.2^{a}	3.89	<0.001
Valine	64.7^{d}	76.5^{c}	64.8^{d}	98.7^{a}	99.4^{a}	91.3^{ab}	97.6^{ab}	88.1^{b}	93.7^{ab}	96.8^{a}	99.8^{a}	96.8^{ab}	1.98	<0.001
Dispensable AA														
Alanine	73.7^{c}	92.6^{abc}	100.8^{abc}	92.6^{abc}	106.3^{ab}	93.4^{abc}	118.6^{a}	93.4^{abc}	88.1^{bc}	100.2^{ab}	102.6^{ab}	101.2^{abc}	7.20	0.002
Arginine	122.6^{abc}	104.0^{bc}	157.8^{a}	95.2^{c}	120.5^{abc}	124.2^{abc}	142.4^{ab}	136.9^{abc}	92.0^{c}	110.5^{abc}	110.7^{bc}	127.0^{abc}	12.50	0.001
Aspartic acid	65.6^{d}	75.4^{cd}	69.6^{d}	99.4^{a}	98.9^{a}	90.8^{ab}	100.4^{a}	85.3^{bc}	91.9^{ab}	97.8^{a}	101.9^{a}	96.0^{ab}	2.44	<0.001
Cysteine	78.6^{c}	59.7^{c}	58.3^{c}	145.4^{abc}	107.5^{bc}	73.4^{c}	126.0^{abc}	59.0^{c}	111.8^{abc}	158.1^{ab}	166.1^{a}	96.4^{c}	14.39	<0.001
Glutamic Acid	62.5^{e}	80.2^{cd}	74.6^{d}	97.2^{ab}	98.3^{a}	93.3^{ab}	98.5^{ab}	88.0^{bc}	93.0^{ab}	94.9^{ab}	97.4^{a}	96.4^{ab}	2.02	<0.001
Serine	31.0^{f}	76.6^{de}	68.2^{e}	97.5^{abc}	99.3^{ab}	104.3^{a}	101.0^{ab}	83.5^{cd}	86.0^{bcd}	91.6^{abcd}	96.5^{abc}	96.3^{abc}	3.50	<0.001
Tyrosine	50.5^{c}	73.8^{c}	64.0^{c}	101.5^{a}	100.9^{a}	97.2^{ab}	88.4^{b}	95.7^{ab}	99.7^{a}	102.5^{a}	103.4^{a}	101.2^{a}	2.63	<0.001

[a-e]Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 5.5 (cont.)

[1]WPI, whey protein isolate. Data are means of 6 observations per treatment. However, due to statistical outliers, casein, casein:agar 1:1, and WPI:agar 1:3 have means of 5 observations per treatment, and the 3 seaweeds, casein:agar 1:3, casein:carrageenan 1:3, casein:cellulose 1:3, and casein:alginate 1:3 have means of 4 observations per treatment.

[2]The standardized ileal digestibility values were calculated by correcting values for basal ileal endogenous losses for each pig as its own control. Nevertheless, average values of basal ileal endogenous losses (g/kg dry matter intake) were calculated as follows: Histidine 0.51, Isoleucine 0.76, Leucine 1.30, Lysine 1.46, Methionine 0.21, Phenylalanine 0.78, Threonine 1.34, Tryptophan 0.23, Valine 1.06, Arginine 2.17, Alanine 1.84, Aspartic acid 1.79, Cysteine 1.03, Glutamic acid 2.21, Serine 1.22, Tyrosine 0.65.

[3]Carboxymethylcellulose.

Table 5.6. Reference ratios and digestible indispensable amino acid score (DIAAS) in ingredients[1,2]

Item	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	SEM	*P*-value
			Child reference ratio[3]				
Histidine	0.34 [0.42]	0.31 [0.38]	0.32 [0.41]	1.52 [1.49]	1.12 [1.10]		
Isoleucine	0.80 [1.00]	0.95 [1.19]	1.00 [1.25]	1.72 [1.69]	1.93 [1.89]		
Leucine	0.70 [0.87]	0.89 [1.11]	0.80 [1.00]	1.52 [1.50]	2.05 [2.01]		
Lysine	0.65 [0.80]	0.60 [0.77]	0.60 [0.75]	1.44 [1.43]	1.78 [1.74]		
SAA	1.11 [1.39]	0.96 [1.23]	1.12 [1.39]	1.36 [1.33]	2.20 [2.16]		
AAA	0.92 [1.14]	1.04 [1.30]	0.96 [1.20]	2.13 [2.10]	1.60 [1.57]		
Threonine	0.79 [0.98]	1.18 [1.47]	1.06 [1.32]	1.44 [1.43]	1.67 [1.63]		
Tryptophan	1.05 [1.31]	1.04 [1.30]	0.39 [0.49]	1.43 [1.40]	2.77 [2.71]		
Valine	0.85 [1.05]	1.22 [1.53]	0.98 [1.23]	1.61 [1.58]	1.41 [1.38]		
DIAAS, %	34^{c} [42] (Histidine)	31^{c} [38] (Histidine)	32^{c} [41] (Histidine)	136^{a} [133]	112^{b} [110]	2.45	0.0001
			Older child, adolescent, adult reference ratio[4]				
Histidine	0.42 [0.52]	0.38 [0.47]	0.41 [0.50]	1.90 [1.86]	1.41 [1.37]		
Isoleucine	0.86 [1.06]	1.02 [1.27]	1.06 [1.33]	1.84 [1.81]	2.06 [2.02]		

Table 5.6 (cont.)

Item	Ulva seaweed	Nori seaweed	Saccharina seaweed	Casein	WPI	SEM	*P*-value
Leucine	0.76 [0.94]	0.96 [1.20]	0.86 [1.08]	1.65 [1.62]	2.22 [2.17]		
Lysine	0.77 [0.96]	0.72 [0.91]	0.71 [0.90]	1.71 [1.70]	2.11 [2.07]		
SAA	1.31 [1.63]	1.13 [1.41]	1.31 [1.63]	1.58 [1.58]	2.58 [2.53]		
AAA	1.17 [1.45]	1.32 [1.65]	1.21 [1.52]	2.71 [2.66]	2.03 [1.99]		
Threonine	0.98 [1.22]	1.46 [1.82]	1.31 [1.64]	1.79 [1.78]	2.07 [2.02]		
Tryptophan	1.36 [1.69]	1.34 [1.67]	0.50 [0.63]	1.84 [1.81]	3.57 [3.49]		
Valine	0.91 [1.13]	1.31 [1.64]	1.06 [1.32]	1.73 [1.70]	1.52 [1.49]		
DIAAS, %	42^{c} [52] (Histidine)	38^{c} [47] (Histidine)	41^{c} [50] (Histidine)	158^{a} [158]	141^{b} [137]	2.92	<0.001

[a-c]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]AAA, aromatic amino acids (phenylalanine + tyrosine); SAA, sulfur amino acids (methionine + cysteine); SEM, standard error of the mean; WPI, whey protein isolate. First-limiting AA is in parentheses.

[2]Values in brackets for the three seaweeds and both milk products represent DIAAS values calculated using a Jones nitrogen conversion factor for crude protein of 5.00 and 6.38, respectively.

Table 5.6 (cont.)

[3]The DIAAS were calculated using the recommended AA scoring pattern for a child (6 months to 3 years). The indispensable AA reference patterns are expressed as mg AA/g protein: Histidine, 20; Isoleucine, 32; Leucine, 66; Lysine, 57; Sulfur AA, 27; Aromatic AA, 52; Threonine, 31; Tryptophan, 8.5; Valine, 43.2 (FAO, 2013).

[4]The DIAAS were calculated using the recommended AA scoring pattern for an older child, adolescent, and adult (older than 3 years). The indispensable AA reference patterns are expressed as mg AA/g protein: Histidine, 16; Isoleucine, 30; Leucine, 61; Lysine, 48; Sulfur AA, 23; Aromatic AA, 41; Threonine, 25; Tryptophan, 6.6; Valine, 40.2 (FAO, 2013).

Literature Cited

Angell, A. R., Angell, S. F., de Nys, R., and Paul, N. A. 2016. Seaweed as a protein source for mono-gastric livestock. Trends Food Sci. Technol. 54:74-84. doi:10.1016/j.tifs.2016.05.014

AOAC Int. 2019. Official Methods of Analysis of AOAC. 21st ed., Association of Official Analytical Chemists. Rockville, MD

Azizi, M. N., Loh, T. C., Foo, H. L., Akit, H., Izuddin, W. I., Shazali, N., and Samsudin, A. A. 2021. Chemical compositions of brown and green seaweed, and effects on nutrient digestibility in broiler chickens. Animals 11:2147. doi:10.3390/ani11072147

Boyd, C. E., McNevin, A. A., and Davis, R. P. 2022. The contribution of fisheries and aquaculture to the global protein supply. Food Secur. 14:805-827. doi:10.1007/s12571-021-01246-9

Cebrián-Lloret, V., Martínez-Abad, A., Recio, I., López-Rubio, A., and Martínez-Sanz, M. 2024. In vitro digestibility of proteins from red seaweeds: Impact of cell wall structure and processing methods. Food Res. Int. 178:113990. doi:10.1016/j.foodres.2024.113990

Cervantes-Pahm, S. K., Liu, C. M., and Stein, H. H. 2014. Effect of novel fiber ingredients on ileal and total tract digestibility of energy and nutrients in growing pigs. J. Anim. Sci. 92:575-583. doi:10.2527/jas.2013-6883

Cian, R. E., Fajardo, M. A., Alaiz, M., Vioque, J., González, R. J., and Drago, S. R. 2014. Chemical composition, nutritional and antioxidant properties of the red edible seaweed Porphyra columbina. Int. J. Food Sci. Nutr. 65:299-305. doi:10.3109/09637486.2013.854746

Correa, Y., Fanelli, N. S., Martinez, J. C., Jiménez-Holgado, C., Recio, I., Stein, H. H., and Martinez-Sanz, M. 2026. Unravelling the multi-scale structural organisation of in vivo ileal digesta from diets containing protein–seaweed polysaccharide blends. Food Res. Int. (Submitted for publication).

Costa, M., Cardoso, C., Afonso, C., Bandarra, N. M., and Prates, J. A. 2021. Current knowledge and future perspectives of the use of seaweeds for livestock production and meat quality: A systematic review. J. Anim. Physiol. Anim. Nutr. 105:1075-1102. doi:10.1111/jpn.13509

De Bhowmick, G., and Hayes, M. 2022. In vitro protein digestibility of selected seaweeds. Foods 11:289. doi:10.3390/foods11030289.

de Oliveira, M. N., Freitas, A. L. P., Carvalho, A. F. U., Sampaio, T. M. T., Farias, D. F., Teixeira, D. I. A., Gouveia, S. T., and Pereira, J. G. 2009. Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. Food Chem. 115:254-259. doi:10.1016/j.foodchem.2008.11.081

Díaz-Piñero, L., Correa, Y., Navas, A. C., Martínez, J. C., Recio, I., and Martínez-Sanz, M. 2025. Investigating the impact of different dietary fibres on the gastrointestinal digestion of food proteins. Food Hydrocoll. 112182. doi:10.1016/j.foodhyd.2025.112182

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation #92. Rome

FAO. 2014. Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO expert working group. Rome

Ferreira, J., Trigo, M., Aubourg, S. P., Prego, R., Ferreira, L. M., Pacheco, M., and Gaivão, I. 2025. Nutritional profiling of red seaweeds Grateloupia turuturu and Porphyra umbilicalis: literature-based insights into their potential for novel applications and partial replacement of conventional agricultural crops. Eur. Food Res. Technol. 1-13. doi:10.1007/s00217-025-04496-0

Fontes-Candia, C., Jiménez-Barrios, P., Miralles, B., Recio, I., López-Rubio, A., and Martínez-Sanz, M. 2022. Development of polysaccharide-casein gel-like structures resistant to in vitro gastric digestion. Food Hydrocoll. 127:107505. doi:10.1016/j.foodhyd.2022.107505

García-Vaquero, M., and Hayes, M. 2016. Red and green macroalgae for fish and animal feed and human functional food development. Food Rev. Int. 32:15-45. doi:10.1080/87559129.2015.1035760

Guo, L., Goff, H. D., Xu, F., Liu, F., Ma, J., Chen, M., and Zhong, F. 2020. The effect of sodium alginate on nutrient digestion and metabolic responses during both in vitro and in vivo digestion process. Food Hydrocoll. 107:105304. doi:10.1016/j.foodhyd.2019.105304

Hodgkinson, S. M., Stein, H. H., de Vries, S., Hendriks, W. H., and Moughan, P. J. 2020. Determination of true ileal amino acid digestibility in the growing pig for calculation of digestible indispensable amino acid score (DIAAS). J. Nutr. 150:2621-2623. doi:10.1093/jn/nxaa210

Hodgkinson, S. M., Stroebinger, N., Van Der Wielen, N., Mensink, M., Montoya, C., Hendriks, W. H., De Vries, S., Stein, H. H., and Moughan, P. J. 2022. Comparison of true ileal amino acid digestibility between adult humans and growing pigs. J. Nutr. 152:1635-1646. doi:10.1093/jn/nxac077

Holdt, S. L., and Kraan, S. 2011. Bioactive compounds in seaweed: functional food applications and legislation. J. Appl. Phycol. 23:543-597. doi:10.1007/s10811-010-9632-5

Jha, R., Leterme, J. D., and Sève, B. 2008. Nutrient utilisation and intestinal fermentation are differentially affected by the consumption of resistant starch varieties and conventional fibres in pigs. Br. J. Nutr. 100:930-938. doi:10.1017/S0007114508921713

Juul, L., Stødkilde, L., Ingerslev, A. K., Bruhn, A., Jensen, S. K., and Dalsgaard, T. K. 2022. Digestibility of seaweed protein from *Ulva* sp. and *Saccharina latissima* in rats. Algal Res. 63:102644. doi:10.1016/j.algal.2022.102644

Kong, C., and Adeola, O. 2014. Evaluation of amino acid and energy utilization in feedstuffs for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917-925 doi:10.5713/ajas.2014.r.02

Lafeuille, B., Tamigneaux, É., Berger, K., Provencher, V., and Beaulieu, L. 2023. Variation of the nutritional composition and bioactive potential in edible macroalga Saccharina latissima cultivated from Atlantic Canada subjected to different growth and processing conditions. Foods 12:1736. doi:10.3390/foods12081736

Lee, S. A., and Stein, H. H. 2022. Digestibility and availability of nutrients in feed ingredients. In: Sustainable swine nutrition. 2nd ed. (Lee, I. Chiba Ed.). 493-546. https://doi.org/10.1002/9781119583998.ch19

Leterme, P., Froidmont, E., Rossi, F., and Théwis, A. 2000. The high water-holding capacity of pea inner fibers affects the ileal flow of endogenous amino acids in pigs. J. Nutr. 130:2491-2496. doi:10.1093/jn/130.10.2491

Mariotti, F., Tomé, D., and Mirand, P. P. 2008. Converting nitrogen into protein—beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. Nutr. 48:177-184. doi:10.1080/10408390701279749

Mathai, J. K., Liu, Y., and Stein, H. H. 2017. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br. J. Nutr. 117:490-499. doi:10.1017/S0007114517000125

Middelbos, I. S., Fastinger, N. D., and Fahey, G. C., Jr. 2007. Evaluation of fermentable oligosaccharides in diets fed to dogs in comparison to fiber standards. J. Anim. Sci. 85:3033-3044. doi:10.2527/jas.2006-800

Myers, W. D., Ludden, P. A., Nayigihugu, V., and Hess, B. W. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179-183. doi:10.2527/2004.821179x

Molina-Gilarranz, I., Cebrian-Lloret, V., Recio, I., and Martinez-Sanz, M. 2025. Impact of structure and composition on the digestibility and nutritional quality of alternative protein-rich extracts from the green seaweed Ulva lacinulata. Food Res. Int. 201:115646. doi:10.1016/j.foodres.2024.115646

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

Samarasinghe, M. B., Van Der Heide, M. E., Weisbjerg, M. R., Sehested, J., Sloth, J. J., Bruhn, A., and Hernández-Castellano, L. E. 2021. A descriptive chemical analysis of seaweeds, Ulva sp., Saccharina latissima, and Ascophyllum nodosum harvested from Danish and Icelandic waters. Anim. Feed Sci. Technol. 278:115005. doi:10.1016/j.anifeedsci.2021.115005

Sharma, S., Neves, L., Funderud, J., Mydland, L. T., Øverland, M., and Horn, S. J. 2018. Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. Algal Res. 32:107-112. doi:10.1016/j.algal.2018.03.001

Souto-Prieto, A., Martinez-Sanz, M., Ferreiro, T., Parada-Pena, P., Abuin-Arias, L., Cobos, A., and Lopez-Sanchez, P. 2024. Insights into the structuring ability of two brown seaweeds (Laminaria digitata and *Saccharina latissima*) for applications as natural texturisers. Algal Res. 80:103548. doi:10.1016/j.algal.2024.103548

Stein, H. H., Sève, B., Fuller, M. F., Moughan, P. J., and De Lange, C. F. 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

Stein, H. H., Shipley, C. F., and Easter, R. A. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436. doi:10.2527/1998.7651433x

Ten Have, G. A., Engelen, M. P., Soeters, P. B., and Deutz, N. E. 2012. Absence of post-prandial gut anabolism after intake of a low quality protein meal. Clin. Nutr. 31:273-282. doi:10.1016/j.clnu.2011.10.007

Tibbetts, S. M., Milley, J. E., and Lall, S. P. 2016. Nutritional quality of some wild and cultivated seaweeds: nutrient composition, total phenolic content, and in vitro digestibility. J. Appl. Phycol. 28:3575-3585. doi:10.1007/s10811-016-0863-y

Van der Spiegel, M., Noordam, M. Y., and Van der Fels-Klerx, H. J. 2013. Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Compr. Rev. Food Sci. Food Saf. 12:662-678. doi:10.1111/1541-4337.12032

Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., and Attia, H. 2011. Chemical composition and functional properties of Ulva lactuca seaweed collected in Tunisia. Food Chem. 128:895-901. doi:10.1016/j.foodchem.2011.03.114

Chapter 6: Standardized Ileal Digestibility of Amino Acids in Four Egg Products Fed to Weanling Pigs

Abstract

An experiment was conducted to test the hypothesis that a) the standardized ileal digestibility (SID) of amino acids (AA) in pasteurized egg products is sufficient for these ingredients to serve as a high-quality protein source in weanling pig diets; and b) the SID of AA in trypsin-inhibitor-free egg protein does not differ from that of casein, and is greater than the SID of AA in egg products containing residual trypsin inhibitor activity (TIA). Five diets that included raw egg powder (REP), dry-pasteurized egg powder (DPE), liquid pasteurized egg powder (LPE), trypsin inhibitor-free egg powder (TFE), or casein as the only source of AA were formulated. A nitrogen-free diet was also formulated to determine basal endogenous losses of crude protein (CP) and AA. Twelve weanling pigs (initial body weight: 11.99 ± 0.53 kg) with a T-cannula in the distal ileum were allotted to a replicated 6 × 6 Latin square design with six diets and six periods of 7 days. Ileal digesta samples were collected on days 6 and 7 of each period and were analyzed for CP and AA. Results indicated that no differences were observed in SID of CP or AA between REP and DPE or LPE, except that LPE had greater ($P < 0.05$) SID of Lys than REP. The SID of most AA was greater ($P < 0.05$) in TFE and casein compared with REP, DPE, and LPE, indicating that the residual TIA in REP, DPE, and LPE reduced AA digestibility. However, the SID of Lys, Cys, and Ser was not different between TFE and LPE. The SID of His, Lys, Asp, and Ser was less ($P < 0.05$) in TFE than in casein whereas the SID of other AA and CP in TFE was not different from that in casein. In conclusion, all egg products had excellent SID of CP (> 82.0 %) and AA (> 80.0 %), but only when TIA were almost completely eliminated, as in TFE, were the SID of AA in egg protein not different from the SID of AA in casein. Overall, egg products can serve as effective sources of AA in diets for young pigs.

Keywords: amino acids; digestibility; egg protein; heat treatment; trypsin inhibitor activity

Introduction

Weanling pigs experience significant physiological stress during the transition from milk to solid feed, in part due to an underdeveloped gastrointestinal tract. At weaning, digestive enzyme secretion and absorptive capacity are still immature, which can reduce the efficiency of nutrient absorption (Pluske et al., 1996; Lallès et al., 2007). Therefore, inclusion of highly digestible protein sources in diets for young pigs is critical to support optimal growth and development (Lallès et al., 2007). Feeding poorly digestible proteins may result in an excess of undigested nitrogen reaching the hindgut, which can promote the proliferation of pathogenic bacteria and increase the risk of post-weaning diarrhea (Goodband et al., 2014). To minimize this risk and enhance nutrient absorption, it is essential to formulate diets based on protein sources with high digestibility and low antigenicity (Song et al., 2010).

Animal proteins generally provide high-quality protein with a balanced amino acid (AA) profile, but some animal proteins, such as meat and fish meals, may contain connective tissue, high concentrations of minerals, variability in protein quality due to processing, or an imbalance among specific AA, which results in low digestibility of AA (Gottlob et al., 2006; Kong et al., 2014). However, egg proteins have a favorable AA profile and have high digestibility of AA (Fanelli et al., 2024) and may be beneficial in diets for young animals (Ruxton et al., 2010). Various egg products are available for feed use, differing in ingredient composition and processing methods, which may influence nutrient composition and AA digestibility. Raw whole eggs typically contain 1,510 kcal/kg gross energy, 12.5% protein, 11.2% fat, and several vitamins and minerals (Ruxton et al., 2010). Approximately 80% of the egg albumen is composed of proteins that contribute to the physicochemical properties of the egg. Some of these proteins include ovomucoid, ovostatin, ovoinhibitor, and cystatin (Miranda et al., 2015). These proteins may inhibit proteases, including trypsin and chymotrypsin in humans and pigs (Imondi et al.,

1973; Mine and Yang, 2008). The structural characteristics of albumen proteins are expected to reduce protein digestibility in raw eggs, but heat denaturation alters the conformation of these proteins, preventing the inhibition of digestive enzymes. Indeed, heat treatment increased in vitro protein digestibility of egg albumen from 40% to 80% (Schmidt et al., 2007), and the standardized ileal digestibility (SID) of AA in heat-treated or cooked eggs is between 80% and 97% (Heo et al., 2012; Fanelli et al., 2024).

Casein is a milk protein that does not contain antinutritional factors, has a well-characterized AA profile, and SID values for most indispensable AA that approach 100% (Stein et al., 2007; Park et al., 2018). Therefore, casein can serve as a comparative protein for evaluating the digestibility of experimental ingredients such as egg products subjected to thermal processing. To our knowledge, however, there is no published information about the SID of AA in pasteurized egg protein devoid of trypsin inhibitor activity (TIA) and other pasteurized heat-treated egg products compared with raw eggs or casein when fed to weanling pigs, and it is not known to which extent mild heat processing or removal of trypsin inhibitors will increase the SID of AA in eggs. Therefore, the objectives of this experiment were to test the hypothesis that a) pasteurized egg products are high-quality proteins when fed to weanling pigs; and b) the SID of AA in trypsin-inhibitor-free egg protein does not differ from the SID of AA in casein, and is greater than the SID of AA in egg products containing residual TIA.

Material and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment before animal work was initiated. Pigs were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Dietary treatments

Four different egg products (Symrise Pet Food North America, Hodges, SC, USA) including raw egg powder (REP), dry-pasteurized egg powder (DPE), liquid pasteurized egg powder (LPE), and trypsin inhibitor-free egg powder (TFE) were prepared (Table 6.1). All egg powders were spray-dried at Dahmes Stainless Inc. (New London, MN, USA) using a Bustle style spray-dryer and Spraying Systems Co. SPRAYDRY NOZZLES = SK Serious 54/21 = 54 orifice 21 core. To produce DPE, samples were stored in a heated room at 60 °C for 168 hours after drying. To produce LPE, liquid whole eggs were heated to 60 °C for 30 minutes before drying, and TFE was produced by heating liquid whole eggs to 60 °C with pH raised to 9 for 2 hours. The level of TIA in egg products was as follows (mg/g): REP, 22.6; DPE, 16.7; LPE, 13.5; and TFE, 2.3, with TIA in TFE reduced by approximately 90.0 % compared with REP.

Six experimental diets were prepared (Tables 6.2 and 6.3). Five diets included each source of egg product or casein as the sole source of crude protein (CP) and AA. A nitrogen-free diet was also formulated to determine basal endogenous losses of CP and AA. All diets also contained cornstarch, lactose, sucrose, and soybean oil, and 0.40% chromium oxide. Minerals and vitamins were included in all diets to meet or exceed the estimated nutrient requirements of young pigs (NRC, 2012). A sample of each diet was collected at the time of diet mixing.

Animals, housing, and sample collection

A total of 12 weanling pigs (initial average body weight: 11.99 ± 0.53 kg) that had a T-cannula installed at the distal ileum (Stein et al., 1998) were used. Pigs were allotted to experimental diets using a replicated 6 × 6 Latin square design with six diets and six periods of 7 days. Therefore, there were a total of 12 observations per treatment. Pigs were housed individually in 1.2 × 1.5 m pens equipped with a self-feeder, a nipple waterer, and a fully slatted tribar floor composed of parallel triangle-shaped steel bars designed to ease the flow of manure.

Pigs were fed their respective diets daily at 0700 h, and all pigs were fed their assigned diets at 4% of body weight. Water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The initial 5 days of each experimental period were considered the adaptation period, but ileal digesta were collected on days 6 and 7 from 0700 to 1600 h using standard procedures (Stein et al., 1998). Cannulas were opened at the beginning of collection and a 225-mL plastic bag was attached to the cannula barrel using a cable tie. Digesta flowing into the bag were collected and bags were replaced whenever they were full or at least once every 30 min. All samples were stored at –20 °C after collection. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized and finely ground (Lagos and Stein, 2019).

Chemical analysis

Ingredient, diet, and ileal digesta samples were analyzed for dry matter (method 930.15; AOAC Int., 2019) and for nitrogen using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA; method 990.03; AOAC Int., 2019). Crude protein was calculated as analyzed nitrogen × 6.25. Ingredient and diet samples were analyzed for ash (method 942.05; AOAC Int., 2019) and for acid-hydrolyzed ether extract by acid-hydrolysis using 3 *N* HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology, Macedon, NY, USA). Amino acids in ingredient, diet, and ileal digesta samples were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110 °C [method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E(b); AOAC Int., 2019].

Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC Int., 2019]. Diet and ileal digesta samples were also analyzed for chromium using Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC Int., 2019), and samples were prepared using nitric acid-perchloric acid [method 968.08D(b); AOAC Int., 2019]. All egg products were also analyzed for TIA (method 22-40; AACC Int., 2006) at the University of Missouri, MO, USA.

Calculations

Values for apparent ileal digestibility (**AID**) of CP and AA in each diet were calculated using the following equation (Stein et al., 2007):

AID = [1 – [(D / F) × (Cr_f / Cr_d)] × 100, where AID is the AID value of CP or AA (%) in egg protein, D is the concentration of CP or AA in the ileal digesta (dry matter basis), F is the CP or AA concentration in the feed (dry matter basis), Cr_f is the chromium concentration in the feed (dry matter basis), and Cr_d is the chromium concentration in the ileal digesta (dry matter basis).

The basal endogenous flow to the distal ileum of CP and each AA was determined based on the flow obtained after feeding the nitrogen-free diet using the following equation (Stein et al., 2007):

Basal endogenous loss = D × (Cr_f / Cr_d), where the basal endogenous loss of CP or each AA is determined in mg per kg of dry matter intake.

By correcting the AID for the basal endogenous loss of CP or AA, SID was calculated using the following equation (Stein et al., 2007):

SID = AID + [(basal endogenous loss / F) × 100], where SID is the SID value of CP or AA (%) in egg protein.

Statistical analysis

The normality of residuals and homogeneity of variances were verified. When these assumptions were not met, data were transformed using the BOXCOX procedure in SAS (version 9.4; SAS Inst. Inc.,

Cary, NC, USA). The transformation was applied to all variables that did not meet the normality assumption, using a λ value of 3, and assumptions were subsequently re-checked. Outliers were identified and removed after calculation of AID and SID values and prior to ANOVA as values that deviated from the first or third quartiles by ± 3 times the interquartile range using internally studentized residuals obtained from the PROC MIXED influence diagnostics. As a result, the number of replicates used in the analysis ranged from 9 to 11. Data were analyzed using the PROC MIXED in SAS using the pig as the experimental unit. The statistical model included diet as the fixed effect and pig, period, and square as random effects. Least square means were calculated, and means were separated with the PDIFF option using Tukey's adjustment if the model *P*-value was significant. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

The AID of CP, His, Lys, Asp, Glu, Ser, and Tyr was greater ($P < 0.05$) in casein than in all egg products, but the AID of CP, His, and Tyr was greater ($P < 0.05$) in TFE compared with REP, DPE, and LPE, and the AID of Lys, Asp, Glu, and Ser was greater ($P < 0.05$) in TFE than in REP and DPE (Table 6.4). The AID for all other AA was greater ($P < 0.05$) in TFE and casein when compared with the other egg products, with the exception that the AID of Cys was greater ($P < 0.05$) in TFE than in REP, DPE, and casein.

The SID of CP, Arg, Ile, Leu, Met, Phe, Thr, Trp, Val, Ala, Glu, and Tyr was greater ($P < 0.05$) in TFE and casein compared with the other egg products, and TFE and casein also had greater ($P < 0.05$) SID of Cys than REP and DPE (Table 6.5). The SID of His, Lys, Asp, and Ser was greater ($P < 0.05$) in casein than in all egg products, but TFE had greater ($P < 0.05$) SID of His and Asp compared with REP, DPE, and LPE, and also greater ($P < 0.05$) SID of Lys and Ser than REP and DPE.

Discussion

In swine diet formulation, egg products may be considered specialty protein ingredients and can be appropriately compared with specialty soy ingredients and plasma-based proteins, but egg proteins are usually priced lower than plasma proteins. Although this experiment focused on AA digestibility, egg products also have a high concentration of fat, and therefore, have a greater energy concentration than other specialty proteins. Therefore, inclusion of egg products in diets for pigs will also increase diet energy concentration, which may result in increased feed efficiency.

The analyzed egg-based ingredients varied in CP and total AA concentrations (47.0 to 57.0 %), which may be a result of the processing methods used to manufacture each ingredient. Acid-hydrolyzed ether extract was greater in LPE and TFE compared with REP and DPE, which may reflect the differences in liquid pasteurization processes or raw material sourcing and separation prior to drying. The greater ash concentration in TFE may be due to processing steps intended to inactivate TIA, which involved thermal and pH treatment with inorganic salts (Vagadia et al., 2017).

Among indispensable AA, REP generally had the greatest concentrations among the egg products, likely due to the absence of processing, although concentrations were less than those in casein. The observation that Lys was greater in REP (4.3 %) compared with the heated egg products (3.7 to 4.1 %) likely reflects protein denaturation and Maillard reactions that can occur during prolonged temperature processing, particularly in DPE, LPE, and TFE (Teodorowicz et al., 2017). However, the Lys:CP ratio among REP, DPE, and LPE was similar (7.7 to 8.0%), whereas in TFE, the Lys:CP ratio was reduced (7.0%). Similarly, dispensable AA such as Ala, Asp, and Ser followed the same trend, with REP having greater concentrations than LPE and TFE. Nevertheless, despite the greater AA

concentrations observed in REP, heat treatment is required to eliminate pathogens, and thus the REP used in this experiment does not represent a commercially available ingredient.

The SID of CP and all AA in casein was in agreement with reported values (> 98.0%), confirming the validity of the current results and supporting its use as a comparative protein (Deglaire et al., 2009; Cervantes-Pahm and Stein, 2010; NRC, 2012; Park et al., 2018). The SID of AA (from 76.0 to 87.0 %) in REP was in agreement with values reported by Sung et al. (2020). The SID for CP and all AA in DPE, LPE, and TFE were either not different from or greater than in most common animal protein sources (NRC, 2012). Specifically, the average SID of AA in LPE (approximately 84.0 %) was in agreement with the SID of AA in spray-dried plasma protein, which is considered a high-quality protein for weanling pigs, and the average SID of AA in TFE (approximately 92.0 %) was greater than in spray-dried plasma protein (Mateo and Stein, 2007; Bailey et al., 2025). This confirms that these pasteurized egg products are high-quality proteins that may be used in diets for weanling pigs, providing digestibility values that are greater than 80.0 % for most AA.

The observation that the SID of all AA in DPE or LPE was less than the SID of AA in fried, boiled, or scrambled eggs (Fanelli et al., 2024) is likely due to the fact that the egg products used in this experiment were pasteurized and not cooked. Pasteurization applies a relatively mild heat treatment to reduce microbial load while preserving the functional properties of eggs, whereas cooking subjects eggs to higher temperatures that induce greater protein denaturation. This enhanced denaturation reduces TIA and exposes peptide bonds, thereby facilitating enzyme access and improving AA digestibility (Hodgkinson et al., 2018). The observation that the SID of all AA in TFE were in agreement with values for fried, boiled, or scrambled eggs (Fanelli et al., 2024) further indicates that reduction in TIA (from 22.6 to 2.3 mg/g) increases SID of AA. Similarly, heat treatment at 110 °C for 10 minutes in egg by-

products was more effective in increasing protein digestibility in rats than spray-drying (Schmidt et al., 2007).

The heat processing used to produce DPE or LPE did not affect the SID of CP or AA when compared with REP (average range of 80.0 to 84.0 %). This is in agreement with data demonstrating that spray-dried technical albumen that was stored at 70 °C for 3 days and then fed to weaned pigs had AID of AA that was not different from egg by-products (Schmidt et al., 2003). This may be because the temperature used to produce DPE or LPE was not high enough or was applied for too short time to inactivate a significant part of the trypsin inhibitors. Although it is well established that heat treatment reduces TIA, the temperature and duration of heating at which inactivation begins remain unclear. In eggs, the primary trypsin inhibitor is ovomucoid, which differs structurally and functionally from the Kunitz and Bowman–Birk inhibitors in soybeans and soybean co-products, and therefore may respond differently to thermal processing. This distinction is important when considering effects on AA digestibility, as the extent of ovomucoid inactivation likely contributes to the improvement observed with different thermal processes (Plancken et al., 2004). This hypothesis is supported by the fact that when TIA was reduced in TFE, the SID of CP and all AA increased and was greater than in the other three egg products and not different from casein.

In the present experiment, the egg products were included as the sole protein source in diets for weanling pigs to ensure requirements for protein were met while determining differences in AA digestibility. However, inclusion rates exceeding 30% are not representative of practical feeding scenarios. Under commercial conditions, pasteurized egg products would more likely be included at levels of 3 to 5% of the diet for young pigs. At such inclusion rates, the contribution of TIA from DPE and LPE would likely be comparable to that in the diet containing TFE in the current experiment (Supplementary Table B.1), and SID of AA in DPE and LPE may, therefore, be greater than measured

in this experiment and closer to the SID of AA determined for TFE. Conversely, if egg powders were included at levels greater than 5%, lower AA digestibility, as observed with DPE and LPE in the present study, would need to be considered in diet formulation. These observations support the potential use of pasteurized egg products as a high-quality protein source in practical diets for weanling pigs, but further research is needed to validate this hypothesis under commercial feeding conditions, particularly given that TFE is not yet commercially available.

Conclusions

In general, all egg products used in this experiment had high SID of CP and AA. However, only when egg TIA was reduced, were the SID of AA comparable with values for casein. This was not the case for commercially available products such as DPE and LEP that had lower SID of AA than casein, which likely was due to the residual TIA. Results indicate that targeted reduction of TIA is more effective in improving the SID of AA than mild thermal treatments alone. Regardless, egg products can be used as a source of AA in diets for young pigs.

Tables

Table 6.1. Analyzed nutrient composition of ingredients (as-fed basis)[1]

Item	REP	DPE	LPE	TFE	Casein
Dry matter, %	96.16	94.79	90.02	95.89	89.43
Crude protein, %	56.58	51.75	47.12	52.21	86.52
Acid-hydrolyzed ether extract, %	31.85	31.20	35.44	33.10	0.69
Ash, %	4.99	5.87	4.75	7.90	1.62
Trypsin inhibitor activity, mg/g	22.60	16.70	13.50	2.31	-
Indispensable amino acids, %					
Arginine	3.45	3.20	2.84	3.04	3.25
Histidine	1.55	1.43	1.28	1.33	2.63
Isoleucine	3.26	3.05	2.67	2.77	4.81
Leucine	4.96	4.56	4.08	4.26	8.29
Lysine	4.34	4.13	3.66	3.68	7.13
Methionine	1.98	1.81	1.56	1.60	2.48
Phenylalanine	3.33	3.02	2.64	2.74	4.57
Threonine	2.70	2.42	2.22	2.37	3.66
Tryptophan	0.86	0.80	0.67	0.77	1.20
Valine	4.03	3.66	3.23	3.40	5.87
Dispensable amino acids, %					
Alanine	3.32	3.03	2.74	2.81	2.71
Aspartic acid	5.75	5.11	4.62	4.92	6.32
Cysteine	1.48	1.17	1.06	1.08	0.35

Table 6.1 (cont.)

Item	REP	DPE	LPE	TFE	Casein
Glutamic acid	6.97	6.37	5.70	5.84	19.86
Glycine	2.05	1.86	1.67	1.74	1.65
Proline	2.21	2.04	1.88	1.91	9.31
Serine	3.70	3.45	3.08	3.25	4.19
Tyrosine	2.26	2.12	1.88	2.00	5.09

[1]REP = raw egg powder; DPE = dry-pasteurized egg powder; LPE = liquid pasteurized egg powder; TFE = free trypsin inhibitor activity egg powder.

Table 6.2. Ingredient composition of experimental diets (as-fed basis)[1]

Item, %	REP	HEP	LPE	TFE	Casein	Nitrogen-free
Cornstarch	30.60	31.60	25.60	31.60	43.60	54.64
Test protein	35.00	34.00	40.00	34.00	21.00	-
Lactose	12.00	12.00	12.00	12.00	12.00	12.00
Sucrose	15.00	15.00	15.00	15.00	10.00	20.00
Soybean oil	-	-	-	-	6.00	5.00
Ground limestone	0.30	0.30	0.30	0.30	1.00	0.40
Dicalcium phosphate	1.80	1.80	1.80	1.80	1.10	2.16
Cellulose	4.00	4.00	4.00	4.00	4.00	4.00
Magnesium oxide	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	0.40
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50

[1]REP, raw egg powder; HEP, hotboxed egg powder; LPE, liquid pasteurized egg powder; TFE, free trypsin inhibitor activity egg powder.

[2]The vitamin-micromineral premix provides the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron

Table 6.2 (cont.)

sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 6.3. Analyzed nutrient composition of experimental diets (as-fed basis)[1]

Item, %	REP	HEP	LPE	TFE	Casein	Nitrogen-free
Dry matter	94.98	94.27	93.04	94.27	93.09	93.95
Crude protein	19.03	17.34	18.48	16.56	18.79	0.47
Acid-hydrolyzed ether extract	8.41	9.28	13.94	9.79	6.02	3.22
Ash	4.64	4.20	4.57	4.49	2.81	3.37
Indispensable amino acids						
Arginine	1.13	1.11	1.11	0.93	0.65	0.01
Histidine	0.47	0.46	0.47	0.39	0.54	0.01
Isoleucine	1.06	1.06	1.04	0.85	1.00	0.01
Leucine	1.65	1.64	1.64	1.36	1.72	0.02
Lysine	1.46	1.47	1.47	1.15	1.51	0.02
Methionine	0.64	0.67	0.63	0.53	0.50	0.00
Phenylalanine	1.06	1.06	1.03	0.85	0.93	0.01
Threonine	0.86	0.85	0.87	0.73	0.77	0.01
Tryptophan	0.29	0.28	0.27	0.27	0.25	0.02
Valine	1.28	1.27	1.26	1.04	1.21	0.01
Dispensable amino acids						
Alanine	1.12	1.11	1.12	0.91	0.57	0.01
Aspartic acid	1.90	1.88	1.89	1.60	1.33	0.01
Cysteine	0.44	0.43	0.44	0.36	0.09	0.00
Glutamic acid	2.51	2.48	2.45	2.04	4.18	0.03
Glycine	0.65	0.64	0.65	0.54	0.35	0.01

Table 6.3 (cont.)

Item, %	REP	HEP	LPE	TFE	Casein	Nitrogen-free
Proline	0.70	0.70	0.70	0.59	1.95	0.01
Serine	1.21	1.21	1.19	1.00	0.93	0.01
Tyrosine	0.66	0.67	0.68	0.55	0.88	0.01

[1]REP, raw egg powder; HEP, hotboxed egg powder; LPE, liquid pasteurized egg powder; TFE, free trypsin inhibitor activity egg powder.

Table 6.4. Apparent ileal digestibility of crude protein and amino acids in protein sources[1]

Item, %	REP	DPE	LPE	TFE	Casein	SEM	*P*-value
n	9	10	10	11	11	-	-
Crude protein	72.7^{c}	71.2^{c}	75.3^{c}	82.1^{b}	89.5^{a}	1.70	<0.0001
Indispensable amino acids							
Arginine	80.3^{b}	80.9^{b}	84.8^{b}	90.3^{a}	92.0^{a}	1.48	<0.0001
Histidine	71.3^{c}	72.6^{c}	77.6^{c}	85.1^{b}	94.6^{a}	1.63	<0.0001
Isoleucine	76.8^{b}	77.9^{b}	81.7^{b}	90.5^{a}	94.4^{a}	1.62	<0.0001
Leucine	77.7^{b}	78.7^{b}	82.7^{b}	91.5^{a}	95.1^{a}	1.46	<0.0001
Lysine	74.3^{d}	77.2^{cd}	81.2^{bc}	83.9^{b}	94.8^{a}	1.46	<0.0001
Methionine	81.2^{b}	82.0^{b}	86.3^{b}	94.9^{a}	96.9^{a}	1.75	<0.0001
Phenylalanine	76.7^{b}	77.5^{b}	82.0^{b}	91.6^{a}	94.7^{a}	1.76	<0.0001
Threonine	69.8^{b}	71.8^{b}	73.6^{b}	80.4^{a}	86.2^{a}	1.66	<0.0001
Tryptophan	74.8^{b}	75.2^{b}	80.9^{b}	92.2^{a}	94.3^{a}	1.51	<0.0001
Valine	75.4^{b}	76.7^{b}	80.8^{b}	89.8^{a}	93.8^{a}	1.58	<0.0001
Dispensable amino acids							
Alanine	74.6^{b}	76.2^{b}	79.8^{b}	87.3^{a}	86.1^{a}	1.65	<0.0001
Aspartic acid	73.5^{c}	74.5^{c}	75.8^{bc}	81.2^{b}	91.2^{a}	1.59	<0.0001

Table 6.4 (cont.)

Item, %	REP	DPE	LPE	TFE	Casein	SEM	*P*-value
Cysteine	66.4^{bc}	70.0^{bc}	74.2^{ab}	79.9^{a}	63.1^{c}	2.33	<0.0001
Glutamic acid	75.4^{c}	77.3^{c}	81.5^{bc}	87.8^{b}	95.7^{a}	1.64	<0.0001
Serine	71.7^{c}	72.6^{c}	74.8^{bc}	79.0^{b}	92.0^{a}	1.33	<0.0001
Tyrosine	76.7^{c}	78.2^{c}	81.8^{c}	89.9^{b}	95.3^{a}	1.38	<0.0001

[a-d]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]REP = raw egg powder; DPE = dry-pasteurized egg powder; LPE = liquid pasteurized egg powder; SEM = standard error of the mean; TFE = free trypsin inhibitor activity egg powder.

Table 6.5. Standardized ileal digestibility of crude protein and amino acids in protein sources[1,2]

Item, %	REP	DPE	LPE	TFE	Casein	SEM	*P*-value
n	9	10	10	11	11	-	-
Crude protein	82.5^{b}	81.8^{b}	85.1^{b}	93.2^{a}	99.1^{a}	1.70	<0.0001
Indispensable amino acids							
Arginine	86.9^{b}	87.6^{b}	91.4^{b}	98.3^{a}	103.3^{a}	1.48	<0.0001
Histidine	76.3^{c}	77.7^{c}	82.5^{c}	91.1^{b}	98.9^{a}	1.63	<0.0001
Isoleucine	80.2^{b}	81.2^{b}	85.1^{b}	94.7^{a}	97.9^{a}	1.62	<0.0001
Leucine	81.2^{b}	82.2^{b}	86.1^{b}	95.7^{a}	98.4^{a}	1.46	<0.0001
Lysine	77.9^{d}	80.7^{cd}	84.7^{bc}	88.4^{b}	98.2^{a}	1.46	<0.0001
Methionine	82.6^{b}	83.3^{b}	87.7^{b}	96.6^{a}	98.7^{a}	1.75	<0.0001
Phenylalanine	80.0^{b}	80.8^{b}	85.3^{b}	95.7^{a}	98.3^{a}	1.76	<0.0001
Threonine	78.0^{b}	80.0^{b}	81.5^{b}	89.8^{a}	95.1^{a}	1.66	<0.0001
Tryptophan	78.4^{b}	78.9^{b}	84.7^{b}	96.1^{a}	98.5^{a}	1.51	<0.0001
Valine	79.0^{b}	80.3^{b}	84.3^{b}	94.2^{a}	97.5^{a}	1.58	<0.0001
Dispensable amino acids							
Alanine	80.3^{b}	81.9^{b}	85.4^{b}	94.2^{a}	97.0^{a}	1.65	<0.0001
Aspartic acid	77.6^{c}	78.7^{c}	79.9^{c}	83.1^{b}	97.1^{a}	1.59	<0.0001

Table 6.5 (cont.)

Item, %	REP	DPE	LPE	TFE	Casein	SEM	*P*-value
Cysteine	73.4[c]	77.2[c]	81.1[bc]	88.5[ab]	97.0[a]	2.33	<0.0001
Glutamic acid	79.1[b]	81.0[b]	85.2[b]	92.3[a]	97.9[a]	1.64	<0.0001
Serine	76.6[c]	77.4[c]	79.7[bc]	84.9[b]	98.2[a]	1.33	<0.0001
Tyrosine	81.4[b]	82.7[b]	86.1[b]	95.4[a]	98.7[a]	1.38	<0.0001

[a-d]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]REP, raw egg powder; DPE, dry-pasteurized egg powder; LPE, liquid pasteurized egg powder; SEM, standard error of the mean; TFE, free trypsin inhibitor activity egg powder.

[2]Average values of basal ileal endogenous losses (g/kg dry matter intake) were as follows: Crude protein 19.48; Arginine 0.79; Histidine 0.25; Isoleucine 0.37; Leucine 0.61; Lysine 0.54; Methionine 0.10; Phenylalanine 0.37; Threonine 0.74; Tryptophan 0.11; Valine 0.48; Alanine 0.67; Aspartic acid 0.84; Cysteine 0.33; Glutamic acid 0.97; Serine 0.62; and Tyrosine 0.32.

Literature Cited

AACC Int. 2006. Approved methods of the AAC. 10th ed., American Association of Cereal Chemists. St. Paul, MN

AOAC Int. 2019. Official Methods of Analysis of AOAC. 21st ed., Association of Official Analytical Chemists. Rockville, MD

Bailey, H. M., Ibagon, J. A., Campbell, J. M., and Stein, H. H. 2025. Inclusion of spray dried plasma in diets fed to young pigs increases the ileal digestibility of crude protein and amino acids of other ingredients in the diet. Anim. Biosci. (Epub ahead of print). doi:10.5713/ab.25.0621

Cervantes-Pahm, S. K., and Stein, H. H. 2010. Ileal digestibility of amino acids in conventional and fermented soybean meal, fish meal, and casein fed to weanling pigs. J. Anim. Sci. 88:2674–2683. doi:10.2527/jas.2009-2563

Deglaire, A., Moughan, S. M., and Moughan, P. J. 2009. True ileal amino acid digestibility of a protein-rich breakfast meal and of individual dietary proteins in the growing pig. Br. J. Nutr. 102:1661–1670. doi:10.1017/S0007114509991080

Fanelli, N. S., Martins, J. C. F. R., and Stein, H. H. 2024. The digestible indispensable amino acid score (DIAAS) in eggs and egg-containing breakfast meals is greater than in toast breads or hash browns served without eggs. J. Nutr. Sci. 13:e68. doi:10.1017/jns.2024.71

Goodband, R. D., Tokach, M. D., Dritz, S. S., DeRouchey, J. M., and Woodworth, J. C. 2014. Practical starter pig amino acid requirements in relation to immunity, gut health, and growth performance. J. Anim. Sci. Biotechnol. 5:12. doi:10.1186/2049-1891-5-12

Gottlob, R. O., DeRouchey, J. M., Tokach, M. D., Goodband, R. D., Dritz, S. S., Nelssen, J. L., Hastad, C. W., and Knabe, D. A. 2006. Amino acid and energy digestibility of protein sources for growing pigs. J. Anim. Sci. 84:1396–1402. doi:10.2527/jas.2005-549

Heo, J. M., Kiarie, E., Kahindi, R. K., Maiti, P. K., Woyengo, T. A., and Nyachoti, C. M. 2012. Standardized ileal amino acid digestibility in egg from hyperimmunized hens fed to weaned pigs. J. Anim. Sci. 90:239–241. doi:10.2527/jas.53983

Hodgkinson, S. M., Montoya, C. A., Scholten, M., Stroebinger, N., Van Der Wielen, N., Mensink, M., and Hendriks, W. H. 2018. Cooking conditions affect the true ileal digestible amino acid content and digestible indispensable amino acid score (DIAAS) of bovine meat as determined in pigs. J. Anim. Sci. 96:2695–2706. doi:10.1093/jas/sky086

Imondi, A. R., and Bird, P. J. 1973. The site of absorption of amino acids in the small intestine of the growing pig. Br. J. Nutr. 29:71–77. doi:10.1079/BJN19730009

Kong, C., Kang, H. G., Kim, B. G., and Kim, K. H. 2014. Ileal digestibility of amino acids in meat meal and soybean meal fed to growing pigs. Asian-Australas. J. Anim. Sci. 27:990–995. doi:10.5713/ajas.2014.14217

Lagos, L. V., and Stein, H. H. 2019. Oven drying of ileal digesta from growing pigs reduces the concentration of amino acids compared with freeze drying and results in reduced calculated values for endogenous losses and elevated estimates for ileal digestibility of amino acids. J. Anim. Sci. 97:820–828. doi:10.1093/jas/sky454

Lallès, J. P., Bosi, P., Smidt, D., and Seve, H. J. M. G. 2007. Nutritional management of gut health in pigs around weaning. Proc. Nutr. Soc. 66:260–268. doi:10.1017/S002966510700548X

Mateo, C. D., and Stein, H. H. 2007. Apparent and standardized ileal digestibility of amino acids in yeast extract and spray dried plasma protein by weanling pigs. Can. J. Anim. Sci. 87:381–383. doi:10.4141/CJAS06039

Mine, Y., and Yang, M. 2008. Recent advances in the understanding of egg allergens: basic, industrial, and clinical perspectives. J. Agric. Food Chem. 56:4874–4900. doi:10.1021/jf073505n

Miranda, J. M., Anton, X., Redondo-Valbuena, C., Roca-Saavedra, P., Rodriguez, J. A., Lamas, A., Franco, C. M., and Cepeda, A. 2015. Egg and egg-derived foods: effects on human health and use as functional foods. Nutrients 7:706–729. doi:10.3390/nu7010706

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

Park, J. C., Kim, B. G., and Kim, I. H. 2018. Standardized ileal digestibility of amino acids in distillers dried grains with solubles and casein fed to growing pigs. Anim. Feed Sci. Technol. 240:24–29. doi:10.1016/j.anifeedsci.2018.03.003

Plancken, I. V. D., Van Remoortere, M., Van Loey, A., and Hendrickx, M. E. 2004. Trypsin inhibition activity of heat-denatured ovomucoid: a kinetic study. Biotechnol. Prog. 20:82–86. doi:10.1021/bp034126m

Pluske, J. R., Hampson, D. J., and Williams, I. H. 1996. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51:215–236. doi:10.1016/S0301-6226(97)00057-2

Ruxton, C., Derbyshire, H., and Gibson, E. 2010. The nutritional properties and health benefits of eggs. Nutr. Food Sci. 40:263–279. doi:10.1108/00346651011043961

Schmidt, L. D., Blank, G., Boros, D., and Slominski, B. A. 2007. The nutritive value of egg by-products and their potential bactericidal activity: in vitro and in vivo studies. J. Sci. Food Agric. 87:378–387. doi:10.1002/jsfa.2685

Schmidt, M., Goodband, R. D., Tokach, M. D., Nelssen, J. L., Dritz, S. S., James, B. W., and Woodworth, J. C. 2003. Nutritional evaluation of egg by-products in diets for weanling pigs. J. Anim. Sci. 81:2270–2278. doi:10.2527/2003.8192270x

Stein, H. H., Shipley, C. F., and Easter, R. A. 1998. Technical note: a technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433–1436. doi:10.2527/1998.7651433x

Stein, H. H., Seve, B., Fuller, M. F., Moughan, P. J., and de Lange, C. F. M. 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

Song, Y. S., Pérez, V. G., Pettigrew, J. E., Martínez-Villaluenga, C., and de Mejia, E. G. 2010. Fermentation of soybean meal and its inclusion in diets for newly weaned pigs reduced diarrhea and measures of immunoreactivity in the plasma. Anim. Feed Sci. Technol. 159:41–49. doi:10.1016/j.anifeedsci.2010.04.011

Sung, J. Y., Ji, S. Y., and Kim, B. G. 2020. Amino acid and calcium digestibility in hatchery byproducts fed to nursery pigs. Anim. Feed Sci. Technol. 270:114703. doi:10.1016/j.anifeedsci.2020.114703

Teodorowicz, M., van Neerven, J., and Savelkoul, H. 2017. Food processing: the influence of the Maillard reaction on immunogenicity and allergenicity of food proteins. Nutrients 9:835. doi:10.3390/nu9080835

Vagadia, B. H., Vanga, S. K., and Raghavan, V. 2017. Inactivation methods of soybean trypsin inhibitor – a review. Trends Food Sci. Technol. 64:115–125. doi:10.1016/j.tifs.2017.02.003

Chapter 7: Concluding Remarks

Proteins have long been evaluated for their capacity to serve as a nutrient source in diets for humans and animals. Protein quality is influenced by multiple factors, including ingredient source, chemical composition, processing conditions, amino acid (AA) digestibility, and the physiological responses of the species consuming the protein.

The results obtained with salmon protein hydrolysates demonstrated that not all hydrolyzed proteins provide the same nutritional value. Differences in raw materials, chemical composition, and processing methods demonstrated variability in protein quality and health parameters for humans and dogs, respectively, indicating that some hydrolysates may provide good nutritional value, whereas others may not contribute similarly. These observations indicate that food and feed manufacturers should clearly define the functional and nutritional targets of formulations when selecting among hydrolyzed protein products.

The experiment conducted with seaweeds and seaweed-derived co-products provided insights into the protein quality of alternative proteins. Although seaweeds contain bioactive components, their AA profile and digestibility currently limit their ability to serve as primary protein sources for human nutrition without additional processing or adjustments. However, seaweed polysaccharides, which are sources of dietary fiber, may influence the AA digestibility of dietary proteins, but their effects depend on the specific type of polysaccharide and its physicochemical characteristics; therefore, additional *in vivo* studies are needed.

The results obtained with egg proteins fed to weanling pigs demonstrated their high nutritional value but also indicated that pasteurization alone may not be sufficient to reduce trypsin inhibitor activity in egg products and that more extensive heat treatment or longer time may be required to minimize the effects of these inhibitors on protein digestion. These

observations emphasize the importance of considering processing methods when producing egg-derived ingredients intended for animal nutrition.

Results from these experiments demonstrate the importance of evaluating proteins beyond protein concentration alone when assessing emerging or existing protein ingredients and highlight the need for comprehensive evaluation to better understand their nutritional potential and limitations for use in human foods and animal diets.

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Appendix A: Supplementary Tables and Figures from Chapter 4

Tables

Supplementary Table A.1. Biometrical measurements of dogs fed experimental diets[1]

	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
Item	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Body weight, kg	9.4	9.1	9.4	9.8	9.6	9.8	9.5	9.5	9.6	0.352	0.8972	<0.0001	0.7771
BCS[2]	5.2	5.2	5.6	5.4	5.2	5.5	5.2	5.2	5.5	0.139	0.1519	0.4082	0.5295

[1]SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Body condition score, 9-point scale (Laflamme, 1997).

Supplementary Table A.2. Baseline inflammatory cytokine concentrations (day 0) in dogs fed the experimental diets[1,2]

Item, pg/ml	Control	SPHC	SPHI	SEM
IL-7	66.3	47.6	46.9	29.147
IL-8	1,377.3	680.2	981.6	209.390
IL-18	321.9	71.9	69.3	47.728
MCP-1	311.1	194.1	209.3	30.425
KC-like	71.6	48.4	49.4	8.277
IP-10	0.5	0.5	0.7	0.124

[1]IL, interleukin; IP, interferon ϒ-induced protein 10 kDa; KC, keratinocyte chemoattractant; MCP, monocyte chemoattractant protein; SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Values are presented as LSmeans ± SEM. Baseline values are shown for descriptive purposes only; statistical inference regarding dietary effects was performed using changes from baseline.

Supplementary Table A.3. Qualitative hair and skin scores and quantitative coat quality of dogs fed the experimental diets[1]

	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
Item	Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Hair condition score[2]	3.1	3.1	3.2	3.2	3.1	3.0	2.9	3.0	2.9	0.135	0.8756	0.1143	0.5977
Skin condition score[3]	3.1	3.1	2.9	3.0	3.0	3.2	2.9	2.9	2.9	0.127	0.9465	0.1097	0.3758
Coat quality													
Glossiness[4]	3.2	3.1	3.4	3.4	3.6	3.5	3.5	3.5	3.4	0.195	0.9197	0.1522	0.6654
Softness[5]	3.0	3.2	3.4	3.1	3.4	3.3	3.5	3.3	3.6	0.195	0.6202	0.0422	0.3763
Greasiness[6]	3.3	3.4	3.5	2.9	3.0	3.2	3.6	3.5	3.2	0.159	0.9997	0.0007	0.0832
Scale[7]	2.3	2.3	2.3	3.7	4.1	4.0	4.3	4.2	4.3	0.153	0.8231	<0.0001	0.1799

[1]SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]Scores classified as: 1. Dull, coarse, dry, 2. Poorly reflective, non-soft, 3. Medium reflective, medium soft, 4. Highly reflective, very soft, 5. Greasy (Rees et al., 2001).

[3]Scores classified as: 1. Dry, 2. Slightly dry, 3. Normal, 4. Slightly greasy, 5. Greasy (Rees et al., 2001).

[4]Scores classified as: 1 = very dull; 5 = very shiny (Marsh et al., 2000).

[5]Scores classified as: 1 = very brittle; 5 = very soft (Marsh et al., 2000).

Supplementary Table A.3 (cont.)

[6]Scores classified as: 1 = very greasy; 5 = not greasy (Marsh et al., 2000). Tendency ($0.05 \leq P < 0.10$) to have an interaction between treatment and day.

[7]Scores classified as: excessive scale; 5= no scale (Marsh et al., 2000).

Supplementary Table A.4. Serum chemistry of dogs fed experimental diets[1]

Item	Reference range[2]	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
		Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
Creatinine, mg/dL	0.5-1.5	0.7^{ab}	0.6^{ab}	0.6^{b}	0.7^{ab}	0.7^{ab}	0.7^{a}	0.7^{ab}	0.6^{ab}	0.7^{a}	0.041	0.7537	<0.0001	0.0234
BUN, mg/dL	6-30	17.0	15.3	16.4	16.4	15.1	14.9	16.1	14.7	13.5	1.094	0.4911	0.0070	0.2312
Total protein, g/dL	5.1-7.0	6.0	6.0	6.1	5.8	6.1	6.0	5.8	5.9	6.0	0.079	0.1357	0.0080	0.1492
Albumin, g/dL	2.5-3.8	3.3	3.3	3.2	3.3	3.4	3.3	3.3	3.3	3.3	0.058	0.5963	0.0649	0.1835
Globulin, g/dL	2.7-4.4	2.7	2.7	2.9	2.5	2.7	2.7	2.5	2.6	2.7	0.084	0.1539	0.0003	0.5692
Albumin:globulin	0.6-1.1	1.3	1.2	1.1	1.4	1.3	1.2	1.3	1.3	1.2	0.051	0.2164	0.0005	0.9554
Ca, mg/dL	7.6-11.4	9.9	10.0	9.8	10.1	10.2	10.1	10.0	10.0	10.04	0.090	0.6388	<0.0001	0.2499
P, mg/dL	2.7-5.2	3.5	3.9	3.9	3.8	3.7	3.9	3.9	3.8	4.1	0.165	0.4935	0.2280	0.2218
Na, mmol/L	141-152	145.0	144.3	144.8	146.9	147.3	147.5	146.5	146.6	147.2	0.394	0.5168	<0.0001	0.4814
K, mmol/L	3.9-5.5	4.3	4.3	4.3	4.2	4.2	4.2	4.2	4.2	4.4	0.069	0.6075	0.2522	0.4043
Na:K	28-36	33.8	33.8	33.9	35.3	34.9	35.0	34.8	34.9	33.7	0.568	0.7447	0.0097	0.5664
Cl, mmol/L	107-118	110.9	110.3	111.4	112.5	112.7	113.2	111.3	112.6	112.5	0.470	0.3237	<0.0001	0.1228
Glucose, mg/dL	68-126	81.5	82.1	84.3	85.4	82.8	87.8	75.3	82.1	81.8	3.779	0.7321	0.0014	0.1329
APT, U/L	7-92	42.4	51.7	53.4	38.6	50.8	54.0	33.6	44.4	50.7	6.694	0.2031	0.0002	0.6042
CALP, U/L	0-40	1.6	2.3	1.7	1.9	2.3	2.4	2.2	2.3	2.3	0.3867	0.6492	0.0528	0.3744

Supplementary Table A.4 (cont.)

Item	Reference range[2]	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
		Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
ALT, U/L	8-65	27.8^{b}	27.5^{b}	23.9^{b}	30.9^{ab}	38.4^{a}	27.6^{b}	30.9^{ab}	38.8^{a}	27.9^{b}	2.277	0.0252	<0.0001	0.0026
GGT, U/L	0-7	3.1	3.4	3.1	2.9	2.8	3.2	3.1	3.0	3.3	0.231	0.8239	0.1290	0.1423
Total bilirubin, mg/dL	0.1-0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.018	0.6449	0.1663	1.0000
CPK, U/L	26-310	147.8	122.8	141.3	128.0	140.5	130.7	144.6	133.3	151.4	15.125	0.9040	0.2130	0.2380
Cholesterol total, mg/dL	129-297	183.6^{ab}	200.5^{a}	188.3^{ab}	189.4^{ab}	174.4^{b}	181.2^{ab}	186.8^{ab}	166.6^{b}	175.9^{ab}	9.655	0.8868	<0.0001	<0.0001
Triglycerides, mg/dL	32-154	40.3	41.9	41.9	39.3	41.3	42.9	39.1	38.5	42.7	2.665	0.5376	0.7139	0.8021
Bicarbonate, mmol/L	16-24	22.1	21.5	21.0	21.9	21.4	21.7	22.1	21.5	21.8	0.409	0.3954	0.5689	0.5186
Anion gap	8-25	16.2	16.8	16.5	16.6	17.3	16.9	17.2	16.5	17.2	0.480	0.8966	0.2365	0.3089

[a-b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

[1]ALT, alanine transaminase; APT, Alkaline phosphatase; BUN, blood urea nitrogen; CALP, calcium-linked alkaline phosphatase; CPK, creatine phosphokinase; GGT, gamma-glutamyl transferase; SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate.

[2]University of Illinois Veterinary Diagnostic Laboratory reference ranges.

Supplementary Table A.5. Complete blood count (CBC) of dogs fed the experimental diets[1]

Item	Reference range[2]	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
		Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
RBC, M/uL	5.50-8.50	7.0	7.0	7.1	7.1	7.1	7.3	7.0	6.8	7.3	0.150	0.3777	0.2289	0.3652
Reticulocytes count, %		0.6	0.6	0.6	0.4	0.4	0.5	0.4	0.4	0.5	0.052	0.2030	0.0002	0.9828
A reticulocytes count, u/L		38790	39635	47469	29390	30375	37384	26250	29642	34151	4208.021	0.1450	0.0002	0.9966
Hemoglobin, g/dL	12.0-18.0	16.0	15.6	16.1	16.3	16.2	17.0	16.3	15.7	17.0	0.355	0.1166	0.0146	0.4912
Hematocrit, %	35.0-52.0	46.7	45.7	46.9	47.4	47.5	49.0	47.1	45.3	48.6	0.977	0.2233	0.0153	0.2893
Mean cell volume, fl	58.0-76.0	66.5	65.4	66.3	67.2	66.6	67.2	66.7	66.4	66.8	0.527	0.5076	0.0004	0.6610
MCH, pg	20.0-25.0	22.7	22.3	22.8	23.2	22.7	23.3	23.1	23.0	23.4	0.226	0.2315	<0.0001	0.3913
MCHC, g/dL	33.0-38.6	34.2	34.1	34.4	34.5	34.1	34.7	34.7	34.6	35.0	0.219	0.2491	0.0009	0.7775
Platelets, K/uL	200-700	313.9	315.3	300.3	270.4	326.0	294.4	267.1	294.5	282.8	22.657	0.5782	0.0664	0.4460
Mean platelet volume, fl		10.4	10.2	10.7	10.9	10.3	10.8	10.4	10.0	10.4	0.287	0.5056	<0.0001	0.1910
WBCC, K/uL	6.0-17.0	7.9	8.6	8.0	7.3	7.7	7.9	6.8	7.4	7.4	0.655	0.8103	0.0092	0.8994
Neutrophils, %		66.4^{a}	62.6^{ab}	61.6^{ab}	59.9^{b}	68.2^{a}	63.9^{ab}	61.9^{b}	62.4^{ab}	65.3^{ab}	2.749	0.8967	0.8080	0.0040
Lymphocytes, %		24.9	27.7	26.8	28.4	26.9	25.9	30.4	30.0	27.5	2.058	0.6624	0.0856	0.5518
Monocytes, %		4.7	4.8	5.4	6.6	5.8	5.5	4.9	4.7	4.2	0.682	0.7412	0.0557	0.7037
Eosinophils[3], %		2.3	3.7	3.2	2.2	3.0	3.6	2.5	4.1	3.1	0.564	0.0778	0.5439	0.6771
A neutrophils, K/uL	3.0-11.5	5.2^{a}	5.2^{ab}	4.5^{ab}	3.9^{b}	7.1^{a}	4.9^{ab}	4.3^{b}	4.6^{ab}	5.3^{ab}	0.604	0.7311	0.3782	0.0139

Supplementary Tabel A.5 (cont.)

Item	Reference range[2]	D0			D45			D90			SEM	Type 3 fixed effects *P*-value		
		Control	SPHC	SPHI	Control	SPHC	SPHI	Control	SPHC	SPHI	Trt*Day	Trt	Day	Trt*Day
A lymphocytes, K/uL	1.0-4.8	1.9	2.3	2.1	2.0	2.2	2.0	2.0	2.2	2.1	0.191	0.6129	0.9990	0.8062
A monocytes, K/uL	0.2-1.4	0.4	0.4	0.4	0.5	0.4	0.5	0.3	0.4	0.3	0.061	0.9856	0.0186	0.8523
A eosinophils[4], K/uL	0.1-1.0	0.2	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.058	0.0630	0.8098	0.9515

[a-b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

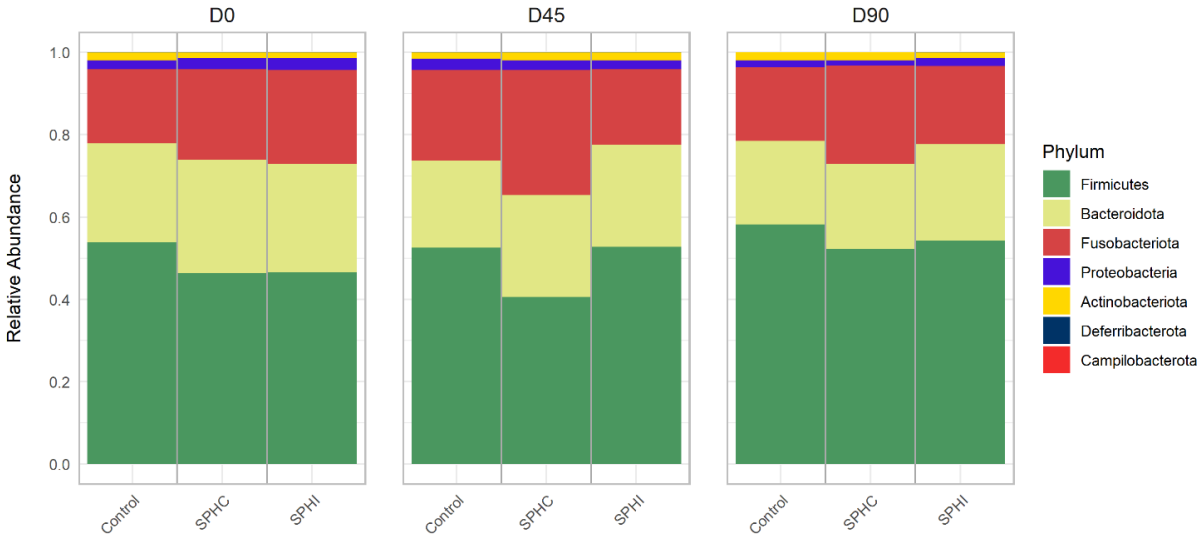
[1]MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cells; SEM, standard error of the mean; SPHC, salmon protein hydrolysate concentrate; SPHI, salmon protein hydrolysate isolate; WBCC, white blood cells count.

[2]University of Illinois Veterinary Diagnostic Laboratory reference ranges.

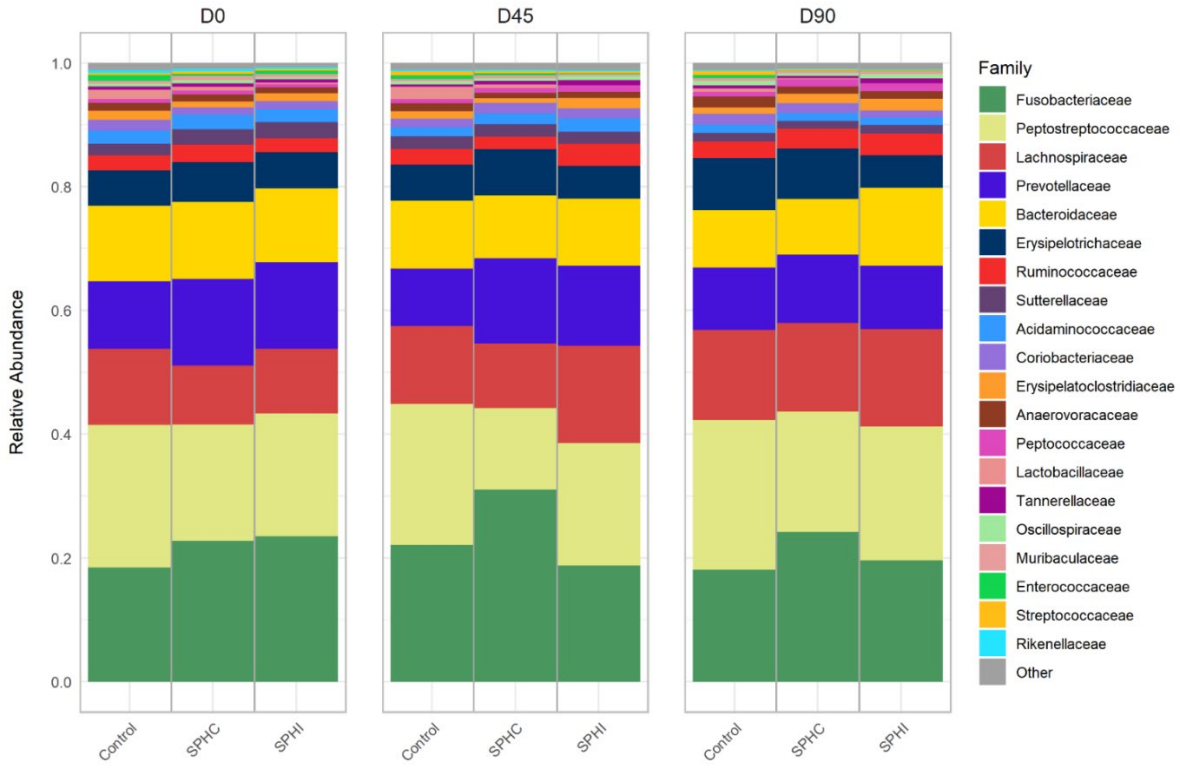
[3]Tendency ($0.05 \leq P < 0.10$) to be greater in SPHC compared with control.

[4]Tendency ($0.05 \leq P < 0.10$) to be greater in SPHC and SPHI compared with control.

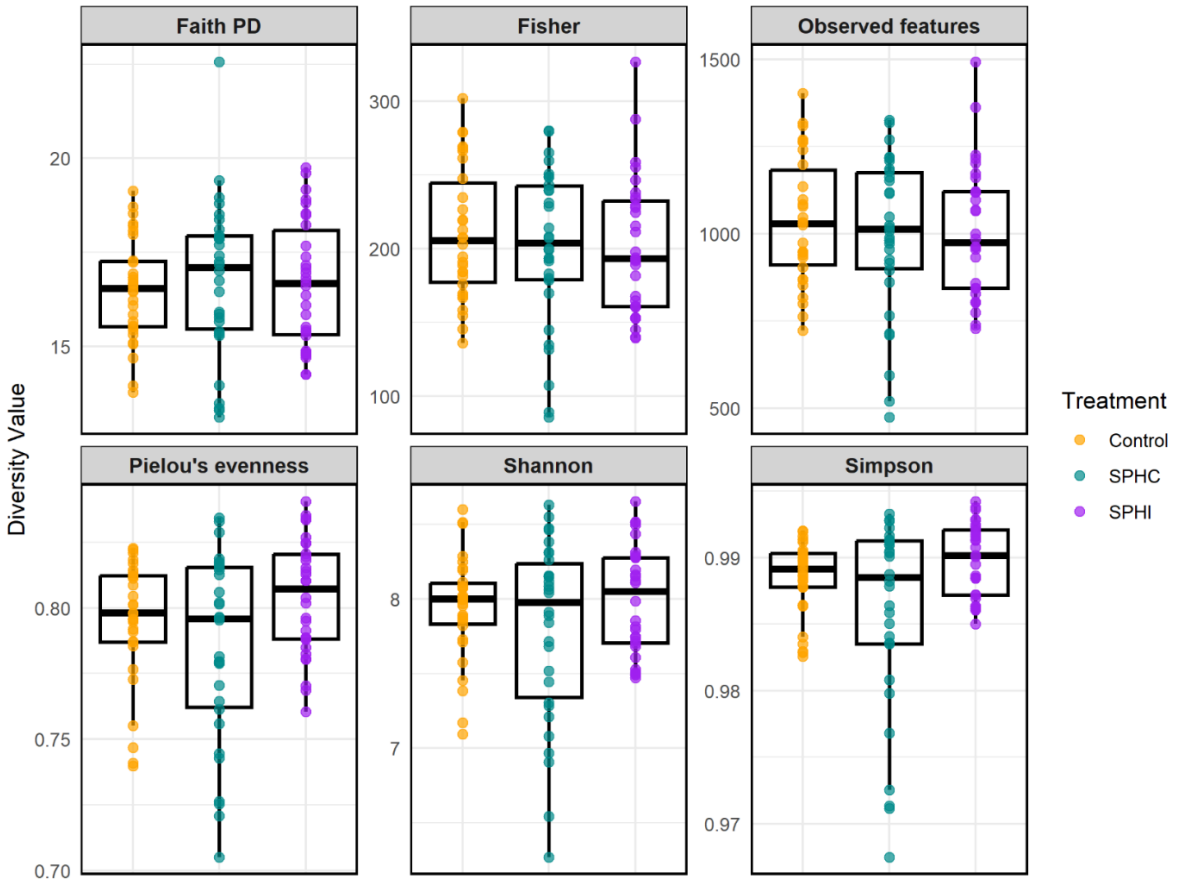
Figures



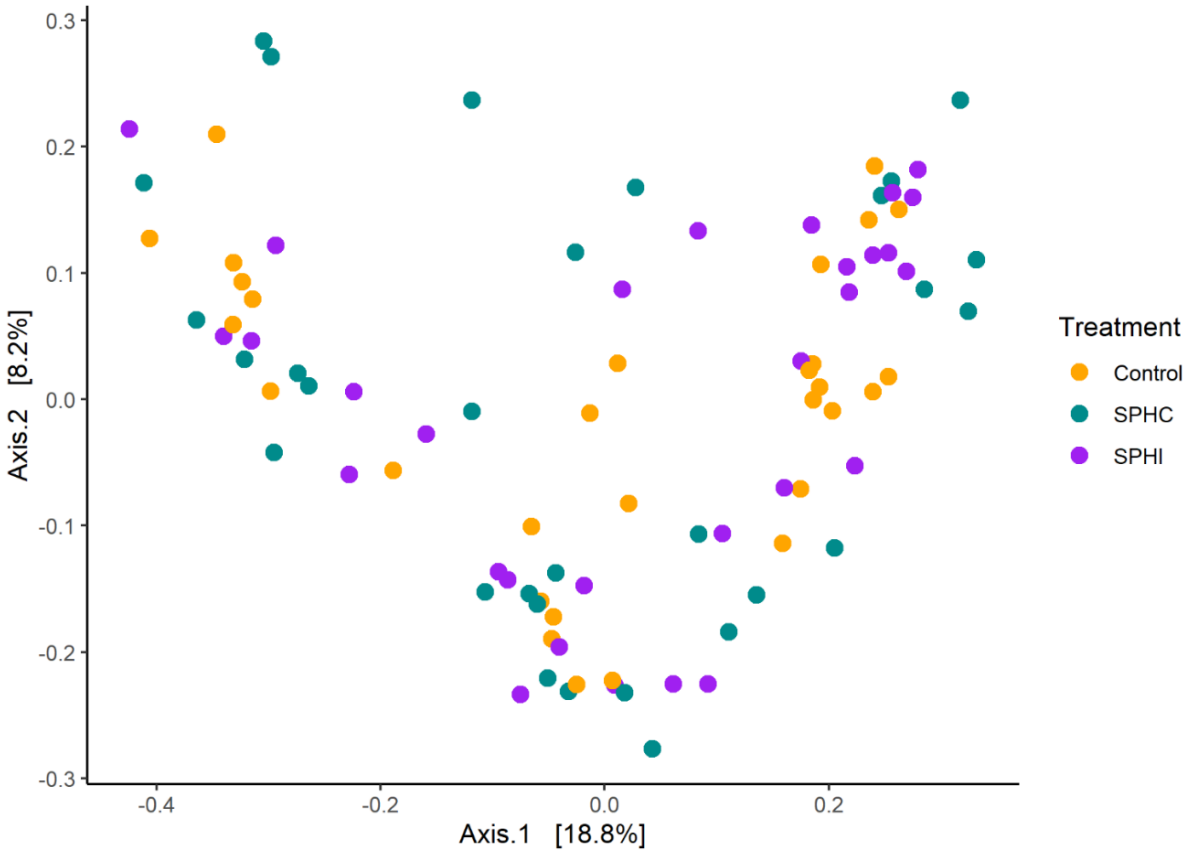
Supplementary Figure A.1. Phylum composition of fecal microbiota from dogs fed the experimental diets.



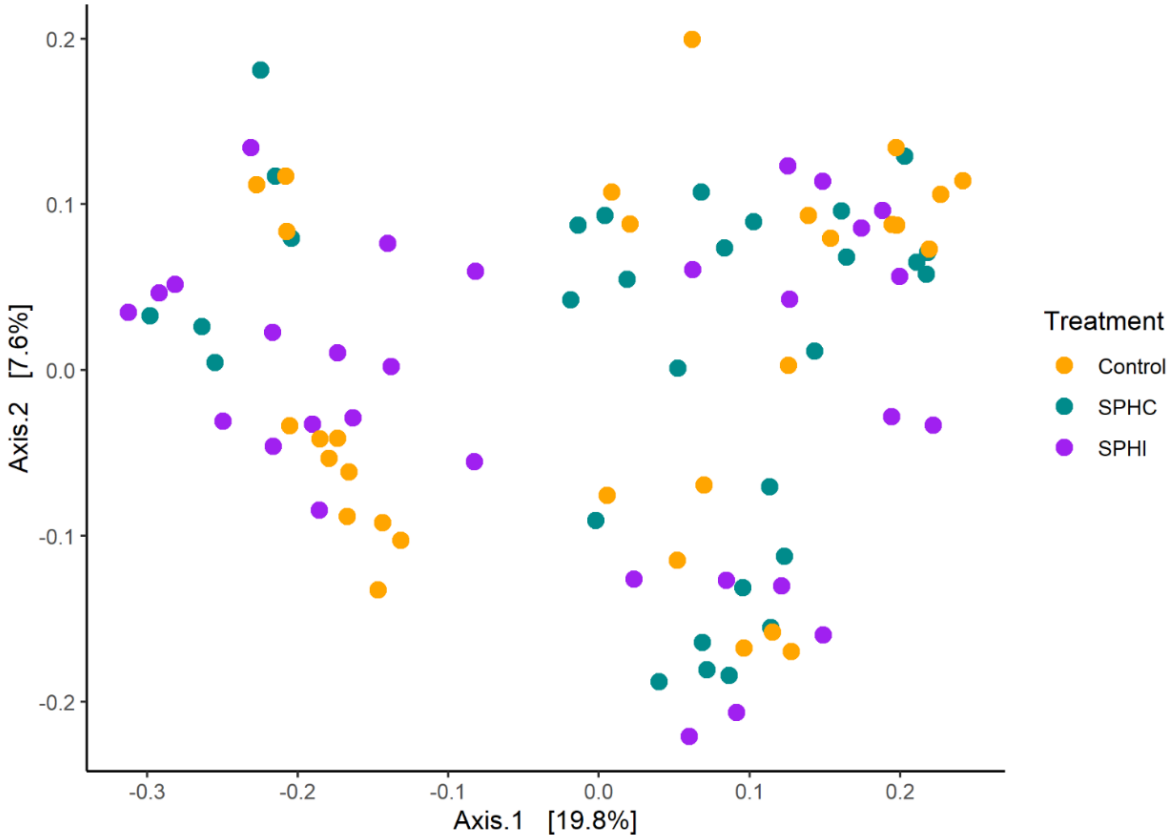
Supplementary Figure A.2. Family composition of fecal microbiota from dogs fed the experimental diets.



Supplementary Figure A.3. Alpha diversity of fecal microbiota from dogs fed the experimental diets. Control: Chicken meal-based diet; SPHC: salmon protein hydrolyzed concentrate diet; SPHI: salmon protein hydrolyzed isolate diet.



Supplementary Figure A.4. Beta diversity of fecal microbiota from dogs fed the experimental diets using Bray-Curtis distance. Control: Chicken meal-based diet; SPHC: salmon protein hydrolyzed concentrate diet; SPHI: salmon protein hydrolyzed isolate diet.



Supplementary Figure A.5. Beta diversity of fecal microbiota from dogs fed the experimental diets using unweighted Unifrac matrix. Control: Chicken meal-based diet; SPHC: salmon protein hydrolyzed concentrate diet; SPHI: salmon protein hydrolyzed isolate diet.

Appendix B: Supplementary Table from Chapter 6

Table

Supplementary Table B.1. Calculated levels of trypsin inhibitor activity (TIA) at different diet inclusion rates (as-fed basis)[1]

Item	DPE	LPE	TFE
Present study			
Inclusion in diet, %	34.00	40.00	34.00
TIA in diet, mg/g	5.68	5.40	0.68
Commercial conditions			
TIA in diet with 3% inclusion, mg/g	0.50	0.41	-
TIA in diet with 4% inclusion, mg/g	0.67	0.54	-
TIA in diet with 5% inclusion, mg/g	0.84	0.68	-

[1]DPE = dry-pasteurized egg powder; LPE = liquid pasteurized egg powder; TFE = free trypsin inhibitor activity egg powder.