

# Petfood Ingredients and Ingredient Processing Affect Dietary Protein Quality

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Animal by-products have been a major contributor to the growth and expansion of the world's petfood industry and have supplied a majority of the proteins, fats, minerals, and vitamins for pets through the years.<sup>6</sup> Use of plant-based proteins in petfoods is often limited due to the presence of antinutritional factors such as lectins, tannins, and/or complex oligosaccharides. However, plant proteins, when properly processed, can serve as the major protein source in companion animal diets. For example, texturized soy protein, an extruded form of soy flour, is often used in canned petfoods.<sup>12</sup> In companion animal nutrition, protein quality is a most important factor, with both the amino acid (AA) profile of the diet and the bioavailability of those AAs playing critical roles. Because of the variability in raw materials and the processing conditions used to produce proteinaceous ingredients, the nutritional quality of these ingredients is constantly changing. Protein quality of companion animal diets can be assayed using various methods. In this paper we will discuss effects of raw materials and processing conditions on protein quality as well as different methods used to assay protein quality.

## **Animal-Based Protein Sources**

Raw material of animal origin not suitable for human consumption is processed commercially to yield a large quantity of animal protein meals for use by the animal feed industry. A significant amount of this is incorporated into petfoods, especially premium diets. Animal-based protein meals come from by-products of the meat packing, poultry processing, and fish canning industries. Even though animal-based protein meals are variable in quality, they are valuable protein sources because they offer high protein content, energy, and minerals at a competitive cost. The variation in protein quality is due to mixing of animal tis-

ues with bone, fat, guts, heads, hooves, and feathers.<sup>17</sup> Thus the protein quality of animal meals

is dependent on the chemical composition (e.g., ash content) and nutritive value of the raw materials. Processing of raw products from the meat-packing and poultry processing industries differs somewhat from that of the fish canning industry.<sup>9</sup>

## ***By-Products of the Meat Packing and Poultry Processing Industries***

In the United States, 36 billion pounds of raw material are converted to saleable products annually through the rendering process.<sup>9</sup> Rendering is an alternative method of utilizing animal by-products to remove water and minimize bacterial and viral contamination. Through the process of rendering, raw animal by-products are chemically transformed. The process fractionates raw materials into water, fat, and solids. The solid fraction is protein rich and is typically processed into high quality protein meals that serve as an excellent source of dietary protein for companion animals. By-products of this industry vary in protein quality primarily due to mixing of animal tissues and the amounts of bone and fat in the raw material. This is influenced by the degree of trimming fat and the amount of bone introduced from the preparation of primary cuts of meat.

## ***By-Products of the Fish Canning Industry***

Fish meal contains approximately 6% to 12% moisture, 10% to 12% ash, 60% to 72% crude protein (CP), and 2% to 14% fat. High quality fish meal has a low concentration of moisture, fat, and ash and should not have a dark color (resulting from overheating, which can make fish protein less digestible).<sup>1</sup> The process of fish meal manufacturing involves, first, cooking fish followed by pressing to remove

most of the oil and fish solubles. The solubles that contain as much as 20% to 25% of total fish protein are concentrated and added back to the meal. The meal then is dried and ground. Fish meal protein is rich in essential AAs, particularly lysine and the sulfur-containing AAs. Fish meal is incorporated into cat diets at high levels (25% to 33%). However, published literature regarding the bioavailability of fish meal in companion animal diets is not available.

## Plant-Based Protein Sources

Vegetable proteins have been utilized for decades by the petfood industry. During the past several years, the total volume of pet food production has increased substantially while utilization of vegetable protein has remained almost static. This is because protein sources of plant origin may have a number of drawbacks (e.g., the presence of lectins, tannins, trypsin inhibitors, and/or complex oligosaccharides) and are thought by some to be undesirable.<sup>17</sup> Soybean meal (SBM) is the most common protein source of plant origin used in dog diets.<sup>12</sup> The AAs of soybean products is balanced without excesses or major deficiencies. Soybean meal (undehulled) contains approximately 46% CP and 2250 kcal/kg energy (metabolizable energy for poultry).<sup>20</sup> The high CP and energy content and the low crude fiber content of SBM result in its use in high-energy diets. As early as 1942, Koehn reported that 20% SBM (dry matter basis) in the diet was sufficient to meet the protein requirements of the dog in all physiologic states. In general, a dog food that contains high quality animal by-products will have higher digestibility than a plant-based food, but dog foods that contain lower-quality animal by-products may have lower digestibilities than plant-based products with similar nutrient profiles.<sup>5</sup> One problem with feeding SBM is that oligosaccharides (stachyose and raffinose) in SBM have been shown to result in production of gas in rats, dogs, and humans.<sup>21</sup> However, over the years, through genetic manipulation it has been possible to produce varieties of soybeans that contain low levels of oligosaccharides.

Zuo et al.<sup>25</sup> investigated digestion responses to conventional and low oligosaccharide SBM incorporation into diets for dogs. Diets (31% CP and 14% fat on a dry matter basis) were corn grain-based containing different levels and types of SBMs (0% SBM, 18.6% conventional SBM,

18.6% low oligosaccharide SBM, 37.1% conventional SBM, and 37.1% low oligosaccharide SBM). The digestibilities of CP and starch at the ileum were higher for dogs fed higher levels of SBM. Ileal AA digestibilities followed the CP response. There were no significant differences in nutrient digestibility between conventional and low oligosaccharide SBM. In addition, oligosaccharide intake was decreased dramatically as a result of substituting the low oligosaccharide SBM for conventional SBM. Also, CP and AA digestibilities in dogs fed SBM-containing diets were higher than in dogs consuming the poultry meal-based control diet.

Bednar et al.<sup>5</sup> measured ileal and total tract digestibilities by dogs fed grain-based diets containing different protein sources. The protein treatments included (1) SBM, (2) poultry meal (PM), (3) poultry by-product meal (PBPM), and (4) beef and bone meal (BBM). Ileal digestibilities of dry matter, organic matter, CP, fat, and total dietary fiber did not differ among the treatments. Total tract digestibility of DM was lower ( $P < .05$ ) for the BBM and SBM diets, while OM digestibility was lower for the SBM diet only. Total tract CP digestibility was similar for BBM, PBPM, and SBM treatments and was higher ( $P < .05$ ) for the PM treatment. As-is fecal excretion was greater ( $P < .05$ ) for the diet containing SBM. Fecal volume on a DM basis was higher ( $P < .05$ ) for the BBM and SBM diets. Fecal scores were higher ( $P < .05$ ) for the SBM treatment as compared to the other treatments. All diets were well utilized by the dog as determined by ileal digestibility, total tract digestibility, and fecal characteristics.

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## Assessment of Protein Quality

Protein quality refers to the amount of available or digestible AA provided per unit of ingredient protein relative to the animal's requirement. Therefore, when assessing protein quality, both AA composition as well as digestibility and availability of the AA must be considered. Protein quality of animal meals is highly variable and is affected by factors such as raw material sources and processing conditions; therefore measuring protein quality is important. Assays such as protein efficiency ratio (PER) determine the quality of protein without singling out the individual factors that dictate quality. However, more advanced methods determine the availability or digestibility

of AAs, which affects overall quality of protein. These methods include the chick growth assay, estimation of biological value (BV) of protein, ileal cannulation digestibility assay, and precision-fed cecectomized rooster digestibility assay.

### ***Protein Efficiency Ratio***

The commonly documented PER assays involve use of weanling male rats or growing chicks. These animals are fed diets consisting of 10% CP with the test feedstuff supplying all of the protein in the diet. The methodology between the rat and chick is generally the same except that the rats are fed the test diet for a period up to 28 days and chicks are fed the experimental diet for 9 to 14 days. In addition to the treatments containing the test feedstuff, a nitrogen-free diet often is fed to correct for maintenance requirements. This allows for the calculation of the net protein ratio (NPR). Weight changes are measured and the PER and NPR are calculated as follows<sup>11</sup>:

PER = Body weight gain (g)/CP intake (g)

NPR = [Body weight gain (g) – Body weight gain (g) of animals fed N-free diet]/CP intake (g)

One criticism of using PER as a measure of protein quality in dog and cat foods is that this test assumes that weight gain in animals is directly related to nitrogen retention. Although this may be true with rats to a large extent, some investigators believe that this may not be a consistent relationship in the growing dog.

### ***Chick Growth Assay***

A commonly used method of assessing protein quality based on AA availability is the chick or pig growth assay, usually designed as a slope-ratio assay.<sup>2</sup> This assay is designed to determine the ability of a feedstuff protein to provide a deficient AA in the diet. The standard procedure involves using a basal diet deficient in the test AA and supplementing it with graded levels of the crystalline test AA or the test feedstuff to yield a linear growth response curve. The AA availability then is calculated using the ratio of the slopes from the growth response lines for the test AA and test feedstuff. Unfortunately, only one AA can be test-

ed at a time. Therefore the chick growth assay is both time consuming and expensive.<sup>19</sup>

### ***Biological Value***

Estimation of biological value (BV) of protein may provide more accurate measures of protein quality than PER, but assays are time consuming and labor intensive. Biological value is defined as the percentage of absorbed protein retained by the body. Nitrogen balance studies are conducted in which food, fecal, and urinary nitrogen are collected and measured. True BV is determined by first accounting for fecal and urinary losses of endogenous nitrogen when the animal is consuming a protein-free diet. One problem with using BV as a measurement of protein quality is that it does not account for protein digestibility. For example, if the small portion of very indigestible protein that is absorbed is used efficiently by the body, it could still have a very high BV.

### ***Ileal Cannulation Digestibility Assay***

This procedure has been widely accepted as the best way to assess AA digestibility in pigs.<sup>24</sup> Prior to ileal cannulation the fecal analysis method developed by Kuiken and Lyman<sup>16</sup> was widely used and accepted. In this method the differences between the amount of AA consumed and the amount of AA excreted in feces were measured. However, a basic problem with the fecal analysis method is microbial fermentation in the large intestine, which leads to 62% to 76% of the total nitrogen excreted in the feces consisting of bacterial nitrogen. The latter indicates extensive metabolism of protein by the microbes in the hindgut, resulting in AA digestibilities of the test source often being overestimated. Therefore the ileal cannulation method is more accurate. This technique used in dogs includes insertion of a T-type cannula constructed of polyvinyl chloride pipe into the terminal ileum approximately 10 cm proximal to the ileocecal junction.<sup>22</sup> This allows for collection of the digesta before microbial fermentation in the hindgut can occur. Several studies with ileal-cannulated dogs have shown this method to be more sensitive than the fecal analysis method for detecting differences in AA digestibilities among feedstuffs.<sup>5,13,18,25</sup> Some drawbacks to this technique may include the cost of animal maintenance, the labor-intensive nature

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of the assay, and the need of using a marker to estimate digesta flow. In spite of these drawbacks, this is probably the most accurate method currently available to estimate protein quality in companion animal diets.

### *Cecec-tomized Rooster Assay*

A more rapid and simple method of assessing AA digestibility is the precision-fed cecec-tomized rooster assay. This involves crop-intubating approximately 30 g of the test product into roosters after a 24 hour fast. Quantitative excreta collections then are made over the next 48 hours. In addition, excreta collections are taken from fasted roosters to allow measurement of endogenous AA losses. Ceca make up most of the hindgut in poultry, and bacterial breakdown of AA can be greatly reduced by using cecec-tomized roosters rather than conventional roosters. Using cecec-tomized roosters is similar to using ileal-cannulated pigs or dogs. However, the surgical procedure for removing the ceca is much simpler than the ileal cannulation procedure. In addition, the birds are easier to maintain after surgery and the ability to use quantitative excreta collections eliminates the problems of obtaining a representative sample and using a marker.

### **Processing Methods and Protein Quality**

In addition to the variability in raw materials, quality of animal protein meals also is influenced by processing conditions. The raw products undergo a process called rendering, which involves thermal processing, hydrolysis, separation, extraction, filtration, and drying. Rendering is categorized into two primary types: dry (e.g., batch or continuous cooking) or wet (continuous cooking at low temperature).<sup>9</sup> In batch cooking the batch is charged and discharged within a certain period. The amount of time that the material is in the cooker may vary greatly depending on when the cooker is closed and when it is opened to discharge. Tissues are heated to temperatures of 70° to 150°C to allow cells to release their fat content. The material may be in the cooker for an extended amount of time (a few hours). The solid portion that remains at the end of processing is sold as meat or meat and bone meal. In continuous dry rendering the raw material is fed continuously into the cooker and the cooked material is discharged at a constant rate, with much shorter

average residence times.<sup>8</sup> However, in continuous dry rendering, no pressure is applied to the system. Since no pressure is used, this system requires even higher temperatures in order to render by-products properly. Also, sterilization or hydrolysis of by-products such as wool or feathers cannot be achieved.<sup>7</sup> Wet rendering uses water, pressure, and steam to achieve temperatures around 70° to 100°C to remove the fat.<sup>10</sup> Although this process may require several hours, maintaining low temperatures in the system is important in producing proteins with acceptable AA digestibility and availability values.<sup>4</sup> Higher temperatures with low moisture content can damage the protein quality, partially due to the Maillard reaction between reducing sugars and free amino groups of AAs. The availability of all essential AAs has been found to decline as the processing temperature increases, with lysine, histidine, methionine, and arginine being the most severely affected.<sup>15</sup> Batterham<sup>2</sup> reported that when the processing temperature for meat and bone meal was increased from 125° to 150°C, lysine availability for chicks decreased from 85% to 35%.

Murray et al.<sup>18</sup> investigated the effects of raw vs. rendered animal by-products incorporated into dogs' diets on nutrient digestion at the ileum and in the total tract. Diets contained dehydrated egg as the primary protein source and were supplemented with animal by-products (rendered meat and bone meal, fresh beef, poultry by-product meal, and fresh poultry). Ileal digestibility of organic matter, CP, fat, gross energy, and all AAs (except cystine) were higher ( $P < .07$ ) for diets containing fresh poultry than for diets containing rendered poultry by-products. However, there

were no differences in ileal digestibility of nutrients when dogs consumed diets containing fresh or rendered beef. This led to the conclusion that rendering of poultry, but not beef, had a slight negative effect on nutrient digestibility in the small intestine, possibly as a result of the rendering process or quality of raw materials that constituted the poultry by-product meal.

Wang<sup>23</sup> reported the effects of processing and raw materials on protein quality of 32 meat and bone meals and 12 poultry by-product meals. In this study cecec-tomized roosters were used to determine true AA digestibility. Meat and bone meal samples were produced at different processing temperatures ranging from 96° to 152°C using seven different commercial cooking systems. An increase in

*Quality of animal protein meals also is influenced by processing conditions.*



temperature during processing generally decreased true digestibility of AA in meat and bone meal and poultry by-product meal. The poultry by-product meals used in this study were prepared from different raw materials and were produced at temperatures ranging from 118° to 149°C. Again, digestibilities of AA were consistently lower for meals that were processed at higher temperatures.

Johnson et al.<sup>15</sup> studied digestibilities of nine animal by-product meals using cecectomized roosters and ileal-cannulated dogs. The true digestibility of total AAs by roosters averaged 76% for the nine meals fed alone, with the low temperature meat and bone meal being highest (84%) and the low ash lamb meal being lowest (66%). Digestibilities of AAs were higher in the meat and bone meal processed at low temperature as compared to the meal processed at high temperature. The ileal-cannulated dog assay yielded AA digestibilities that were similar to those of the rooster assay.

## Conclusions

High quality protein sources, whether of animal or plant origin, are key ingredients in companion animal diets. Despite the fact that a knowledge of bioavailability of individual nutrients in feed ingredients is essential for accurate formulation of diets, there is very little information available in this regards in companion animal nutrition. Protein quality can be assayed using various methods such as PER, chick growth assay, ileal cannulation digestibility assay, and precision-fed cecectomized rooster digestibility assay. The ileal cannulation digestibility assay appears to be the most precise method to estimate protein quality in companion animal diets. The overall protein quality of an animal meal is influenced by the raw materials and the processing conditions used to produce the product. It is essential that standards be established with regards to the raw materials and conditions under which these raw materials are processed. Furthermore, these standards should be based on the protein quality of the processed product. The protein quality (i.e., AA digestibility and availability) of companion animal dietary ingredients should be established using one of the techniques mentioned above, preferably the digestibility assays.

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