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Evaluation of low-ash poultry meal as a protein source in canine foods¹

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ABSTRACT: Eight mature female dogs (18.0 ± 0.2 kg) were used in a replicated 4×4 Latin square experiment to determine the feeding value of low-ash poultry meal (PM) in a complete food fed to dogs. All foods contained graded concentrations of PM (10.4 to 32.5% DM), resulting in foods that were 10, 15, 20, and 25% CP. Daily DMI averaged 284 ± 14 g/d. An increase in PM resulted in an increase in fecal moisture from 44.7 to 55.1% (linear; $P < 0.01$), and fecal DM output increased from 24.8 to 31.6 g/d (linear; $P < 0.05$). Ileal DM flow increased from 27.1 to 40.7 g/d (linear; $P < 0.01$). Small intestinal DM digestibility decreased from 90.4 to 86.1% (linear; $P < 0.01$) and total-tract DM digestibility decreased from 91.2 to 89.4% (linear; $P < 0.01$) as PM increased. Large intestinal DM digestibility

increased from 8.4 to 21.1% with increasing PM (linear; $P < 0.05$). Fecal excretion of CP increased from 5.6 to 10.0 g/d (linear; $P < 0.01$) and ileal flow of CP increased from 6.9 to 15.6 g/d (linear; $P < 0.01$) as PM increased. Small intestinal CP digestibility was unaffected with treatment ($P > 0.05$). Large intestinal CP digestibility increased from 21.6 to 37.1% (linear; $P < 0.05$) with increasing PM. Total-tract CP digestibility increased from 81.0 to 86.6% (linear; $P < 0.01$) as PM increased. Arginine had the highest overall digestibility ranging from 88.5 to 91.3%, whereas cysteine had the lowest digestibility, ranging from 67.1 to 71.4%. These data indicate that PM is a highly digestible protein source for canine foods with inclusions of 10.4 to 32.5% of DM.

Key Words: Amino Acids, Ash, Digestibility, Dogs

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Introduction

Poultry meal (PM) is of considerable value as a protein source in canine foods. Information on the chemical composition and nutrient digestibility of poultry meal indicates that it can be a variable product (Han and Parsons, 1990; Johnson et al., 1998). Two factors believed to affect AA digestibility of animal meals are ash content and processing temperature.

Results of research on apparent digestibility of AA from various sources of PM have been inconsistent. Johnson et al. (1998) found that the low-ash PM had a slightly higher apparent digestibility of the essential AA than the high-ash PM (68.0 vs. 66.9%). However, the low-ash meat and bone meal was significantly lower in apparent digestibility of AA than the high-ash meat and bone meal (approximately 16 percentage units). From this, Johnson et al. (1998) determined that any reduction in protein quality with increased ash content was the result of decreased concentrations of AA per unit of pro-

tein, and not decreased digestibility. The results of other poultry byproduct studies have varied. Zuo et al. (1996) determined that apparent digestibility of essential AA of poultry byproduct meal averaged 69.7% in dogs. Murray et al. (1997) found a higher apparent digestibility of essential AA (81.0%) of poultry byproduct meal, indicating that the protein source was of higher quality when compared with Zuo et al. (1996). Other studies have shown digestibilities ranging from 77 to 89% of CP in poultry based foods (Zuo et al., 1996; Murray et al., 1997). Studies investigating the digestibility of PM in dogs have ranged in concentrations of 7 to 32% of the total food (Zuo et al., 1996; Murray et al., 1997; Johnson et al., 1998). However, no individual study has investigated the effect increasing PM has on apparent small intestinal digestibility of AA in the dog. Therefore, the objective of the present study was to determine the apparent small intestinal digestibility of AA in response to increasing protein from low-ash PM.

Materials and Methods

Dogs

Eight ileally cannulated (Walker et al., 1994) mature female mongrel dogs with BW of 18.2 ± 0.2 kg were used to evaluate protein and AA disappearance at the

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Table 1. Crude protein and amino acid composition of protein sources used to formulate the low-ash poultry meal foods^a

Item, % of DM	Low-ash poultry meal	Brewer's rice
Crude protein	69.3	8.3
Arginine	4.37	0.69
Cysteine	1.32	0.19
Glycine	6.53	0.38
Histidine	1.71	0.28
Isoleucine	2.34	0.31
Leucine	4.77	0.70
Lysine	3.11	0.32
Methionine	1.13	0.24
Phenylalanine	2.69	0.42
Threonine	3.02	0.31
Tryptophan	0.52	0.10
Tyrosine	1.84	0.13
Valine	3.16	0.48
Aspartate	5.58	0.78
Serine	4.42	0.43
Glutamate	8.60	1.46
Alanine	4.30	0.55

^aAnalyzed before formulation of foods.

terminal ileum and through the total tract. The dogs were located in the Division of Laboratory Animal Research Facility at the University of Kentucky (Lexington) and were cared for in accordance with Institutional Animal Care and Use Committee protocols. Dogs were housed in an environmentally controlled room at 22°C with a light:dark cycle of 14:10. The kennels measured 1 × 1.5 m, with a slotted floor sitting 0.2 m above ground. Each kennel was cleaned twice daily, following feeding and allowing for 25 min of exercise and socialization with other dogs and people. Water was available ad libitum throughout the experiment.

Feeding and Treatments

The ingredient and chemical compositions of each protein source and food are presented in Tables 1, 2, and 3. Each food was formulated, extruded, and kibbled in accordance with the AAFCO (2000) nutrient guide for dogs and balanced to meet maintenance requirements (Tables 2 and 3); however, the 10 and 15% CP foods were below the AAFCO minimum requirement of 18% CP. Differences between the four foods were based on varying proportions of PM found in the dry food. The source of CP was PM, and foods were 10, 15, 20, and 25% CP. Chromic oxide was added to each food at 0.2% DM to serve as an indigestible marker to determine digestibility. Each day, food was weighed and divided into two equal portions and fed at 0700 and 1700 in stainless steel bowls. Each dog was allowed 20 min to consume the food. Bowls were removed after 20 min, and orts were weighed and recorded. Throughout the experiment, food samples were collected daily and pooled into plastic collection bags for nutrient content analysis.

Sampling

The experiment was designed as a replicated 4 × 4 Latin square. Each experimental period was 14 d in length. During the first 2 d of the period, dogs were fed a 1:1 mixture of their current food and their respective next experimental food in order to avoid meal refusal and gastric problems. Dogs were allowed 6 d for adaptation to each new food.

On the first day of fecal collection (d 7), all feces were removed from the cages and discarded before 0730. Fecal output was collected from this point on for the next 5 d at each mealtime and placed into labeled plastic bags. Samples were frozen as they were collected and pooled by dog within each period.

The ileal sampling period consisted of the 3 d following fecal collection. During ileal collection, Bite-not collars

Table 2. Ingredient composition of low-ash poultry meal foods

Ingredient, %	Crude protein in food, %			
	10	15	20	25
Corn starch ^a	46.6	40.8	35.1	29.5
Low-ash poultry meal	10.4	17.8	25.0	32.5
Rice, brewers	25.0	25.0	25.0	25.0
Grease, choice white	9.5	8.8	8.0	7.0
Cellulose ^b	2.5	2.5	2.5	2.5
Dicalcium phosphate	1.9	1.3	0.7	—
Calcium carbonate	0.4	0.3	0.2	—
Soybean oil	1.0	1.0	1.0	1.0
Palatability enhancer	1.0	1.0	1.0	1.0
Vitamin-trace mineral ^c	1.7	1.5	1.5	1.5

^aBuffalo corn starch from Corn Products International, Westchester, IL.

^bCellulose from J. Rettenmaier USA LP, Schoolcraft, MI.

^cFormulated to supply (at least) the following (g/kg of food): 0.6 Mg, 1.8 Na, 7.0 K, 7.6 Cl, (mg/kg of food) 211 Fe, 163 Zn, 13 Cu, 13 Mn, 0.4 Se, 1.5 I, (IU/g of food) 18.2 vitamin A, 1.0 vitamin D, 0.18 vitamin E, (mg/kg of food) 0.3 biotin, 1,484 choline, 1.9 folic acid, 62 niacin, 18 pantothenic acid, 8.6 pyridoxine, 8.0 riboflavin, 41 thiamin, and 0.13 vitamin B₁₂.

were placed on the dogs after the morning feeding and removed after the last collection. These collars allowed the dogs to drink water normally and prevented the removal of their collection bags, which were attached to the cannulas during sampling times. Ileal digesta collection began at 0800 on d 12. Plastic, 28-g Whirl-Pak collection bags (Nasco, Fort Atkinson, WI) were placed on the animals' cannulas to collect the ileal digesta. On d 12 and 13, digesta were collected at 0800, 1000, 1200, 1400, and 1600. On d 14, samples were collected at 0900, 1100, 1300, and 1500. At each collection time, samples were weighed, frozen, and added to a pooled sample.

Analyses

After collection, fecal and ileal samples were stored frozen until they were lyophilized using a Dura-Dry MP Freeze-Drier (FTS Systems, Stone Ridge, NY). Dry matter was determined as the difference in sample weight before and after lyophilization. Fecal samples were then ground through a 0.5 mm screen in a Cyclotec 1093 Sample Mill (Tecator, Hoganas, Sweden). Ileal samples were ground using a mortar and pestle. Feed samples were ground using a conventional blender (Hamilton Beach/Proctor Silex, Washington, NC). The dried and

ground samples were then stored in labeled plastic bags at room temperature until further analysis.

Ileal, fecal, and feed samples were dried, ashed, and digested as described by Williams et al. (1962). The solutions were allowed to settle and were analyzed the following day using an ATI Unicam 99 atomic absorption spectrophotometer (Cambridge, U.K.) to determine Cr concentrations in the samples. Protein content ($N \times 6.25$) of the samples were obtained using a Leco CNS2000 (Leco Corp., St. Joseph, MI) N analyzer (AOAC, 1995).

Amino acid analyses of feed and ileal samples were determined according to AOAC (1995). A 10- μ L aliquot was derivatized with 6-aminoquinoly-*N*-hydroxy-succinimidyl carbamate and the AA concentration was determined by reverse-phase liquid chromatography using Millipore Waters AccQ Tag System, as described by Liu et al. (1995).

Calculations and Statistics

Nutrient digestibility was calculated as described by Merchen (1988) using chromium as an indigestible marker. Digesta flows were adjusted for the amount of marker recovered in the feces during the 5-d fecal collection.

Table 3. Chemical composition of low-ash poultry meal foods

Item	Crude protein in food, %			
	10	15	20	25
Dry matter, %	93.6	94.0	93.9	94.2
	Dry matter basis, %			
Organic matter	95.5	95.4	95.2	94.8
Crude protein	10.4	15.1	20.4	25.9
Crude fat ^a	12.5	12.6	12.8	12.8
ME, kcal/kg ^a	3600	3600	3600	3600
Crude fiber ^a	2.2	2.3	2.4	2.5
Calcium ^a	0.8	0.8	0.8	0.8
Phosphorus ^a	0.6	0.6	0.6	0.6
Essential amino acids				
Arginine	0.69	0.97	1.29	1.59
Histidine	0.23	0.31	0.41	0.50
Isoleucine	0.17	0.31	0.49	0.62
Leucine	0.69	0.99	1.36	1.68
Lysine	0.49	0.74	1.05	1.34
Methionine	0.18	0.25	0.34	0.41
Phenylalanine	0.37	0.54	0.74	0.91
Threonine	0.41	0.58	0.81	0.98
Tryptophan	0.17	0.19	0.22	0.24
Valine	0.38	0.56	0.75	0.92
Nonessential amino acids				
Alanine	0.71	0.97	1.26	1.53
Aspartate	0.87	1.24	1.69	2.05
Cysteine	0.13	0.16	0.21	0.24
Glutamate	1.47	2.08	2.75	3.40
Glycine	0.83	1.23	1.68	2.08
Proline	0.63	0.93	1.27	1.54
Serine	0.48	0.69	0.93	1.15
Tyrosine	0.27	0.37	0.50	0.62

^aValues are calculated.

Table 4. Dry matter digestibility in dogs fed increasing concentrations of low-ash poultry meal

Item	Crude protein in food, %				SEM ^a	Contrasts ^b	
	10	15	20	25		Linear	Quadratic
Body weight, kg	17.8	18.0	18.2	18.1	0.2	NS	NS
DMI, g/d	278.8	269.4	294.5	295.1	7.6	$P < 0.05$	NS
Fecal moisture, %	44.7	49.6	51.3	55.1	1.1	$P < 0.01$	NS
Feces, g of DM/d	24.8	25.2	28.6	31.6	2.1	$P < 0.05$	NS
Ileal flow, g of DM/d	27.1	27.9	35.6	40.7	3.1	$P < 0.01$	NS
DM digestibility							
Small intestine, %	90.4	89.9	87.8	86.1	1.1	$P < 0.01$	NS
Large intestine, % ^c	8.4	8.8	15.5	21.1	4.6	$P < 0.05$	NS
Total tract, %	91.2	90.8	90.3	89.4	0.6	NS	NS

^aStandard error of mean, $n = 8$.

^bProbability of a greater F -value.

^cPercentage of ileal flow.

NS = nonsignificant, $P > 0.05$.

Data were analyzed as a replicated 4×4 Latin square using the GLM and regression procedures of SAS (SAS Inst., Inc., Cary, NC). The experimental unit was dog, the model included square, treatment, period (square), and dog (square), and the error was residual error mean square. Means were separated using polynomial contrasts for linear, quadratic, and cubic effects of PM inclusion. Differences were considered significant when $P < 0.05$.

Results

All dogs remained healthy throughout the experiment. There were no differences in BW during the experiment ($P > 0.05$, Table 4). No differences in BW were expected since foods were adjusted to supply the proper amount of energy required for maintenance of BW each period. Despite these attempts at equalizing intake, a linear increase in intake ($P < 0.05$) with increasing PM occurred. Fecal moisture increased linearly ($P < 0.01$), as did fecal DM output and ileal DM flow ($P < 0.05$ and P

< 0.01 , respectively), in response to increasing the PM in the foods. Small intestinal DM decreased as PM increased ($P < 0.01$). Large intestinal DM digestibility increased linearly ($P < 0.05$) as PM increased. Total-tract DM digestibility was not affected ($P > 0.05$) by increasing PM.

Table 5 depicts CP ($N \times 6.25$) digestibilities. As expected, CP intake increased ($P < 0.01$) with increasing CP. There was also a linear increase ($P < 0.01$) in fecal CP excretion and ileal flow of CP ($P < 0.01$). Small intestinal CP digestibility was not affected ($P > 0.05$) by increased concentrations of PM, whereas large intestinal CP digestibility increased linearly ($P < 0.05$). Total-tract CP digestibility increased linearly ($P < 0.01$) as PM increased.

Amino acid disappearance as a percentage of intake is shown in Table 6. Digestibilities for all AA were not affected by increased PM, with the exception of isoleucine and tryptophan. Arginine had the highest digestibility, ranging from 88.5 to 91.3%. Cysteine had the lowest overall digestibility, ranging from 67.1 to 71.4%.

Table 5. Crude protein digestibility in dogs fed increasing concentration of low-ash poultry meal

Item	Crude protein in food, %				SEM ^a	Contrasts ^b	
	10	15	20	25		Linear	Quadratic
CP intake, g/d	28.8	40.6	60.0	73.8	0.2	$P < 0.01$	NS
Feces, g of CP/d	5.6	6.3	8.1	10.0	0.1	$P < 0.01$	NS
Ileal flow, g of CP/d	6.9	8.8	12.5	15.6	0.2	$P < 0.01$	NS
CP Digestibility							
Small intestine, %	76.3	79.3	79.4	78.1	2.3	NS	NS
Large intestine, % ^c	21.6	24.1	27.4	37.0	4.0	$P < 0.05$	NS
Total tract, %	81.0	84.5	86.3	86.6	1.3	$P < 0.01$	NS

^aStandard error of mean, $n = 8$.

^bProbability of a greater F -value.

^cPercentage of ileal flow.

NS = non-significant, $P > 0.05$.

Table 6. Disappearance of amino acids (percent of intake) in cannulated dogs fed increasing concentrations of low-ash poultry meal

Item	Crude protein in food, %				SEM ^a	Contrasts ^b	
	10	15	20	25		Linear	Quadratic
Essential amino acids							
Arginine	88.5	91.3	90.7	89.9	1.3	NS	NS
Histidine	81.7	86.5	84.4	83.0	2.2	NS	NS
Isoleucine	53.7	71.4	74.9	75.4	4.6	<i>P</i> < 0.01	NS
Leucine	78.3	83.5	83.5	82.6	2.3	NS	NS
Lysine	77.1	83.0	83.0	82.0	2.6	NS	NS
Methionine	86.3	89.0	90.3	89.0	1.6	NS	NS
Phenylalanine	78.7	83.7	83.8	83.2	2.2	NS	NS
Threonine	70.2	77.3	76.8	75.5	3.2	NS	NS
Tryptophan	86.6	84.7	80.8	77.2	2.0	<i>P</i> < 0.01	NS
Valine	72.8	79.3	78.8	77.7	2.9	NS	NS
Nonessential amino acids							
Alanine	81.1	84.6	84.2	82.4	2.2	NS	NS
Aspartate	68.9	76.0	72.8	69.4	4.0	NS	NS
Cysteine	67.1	71.4	71.1	69.0	4.0	NS	NS
Glutamate	80.2	84.5	83.3	81.8	2.3	NS	NS
Glycine	80.3	85.6	83.8	81.7	2.4	NS	NS
Proline	78.2	83.8	83.2	81.3	2.5	NS	NS
Serine	70.1	77.6	76.2	75.3	3.2	NS	NS
Tyrosine	75.6	80.0	80.0	78.7	2.7	NS	NS

^aStandard error of mean, *n* = 8.

^bProbability of greater *F*-value.

NS = nonsignificant, *P* > 0.05.

Discussion

The purpose of this study was to determine the apparent small intestinal digestibility of protein and AA in response to increasing protein from low-ash PM. Total-tract and small intestinal digestibility have been shown to vary because of differences in processing conditions and poultry byproduct sources (Muir et al., 1996; Zuo et al., 1996; Murray et al., 1997; Johnson et al., 1998). The results obtained in the present study are dissimilar to the Johnson et al. (1998) study, which investigated low-ash poultry byproduct meal as a protein source in canine foods. Johnson et al. (1998) reported that DM small intestinal digestibility was 76% and CP small intestinal digestibility was 68% for PM. Our values for small intestinal and total-tract DM digestibility were approximately 7% higher (Table 4), indicating that the poultry meal used in the present study may be of higher quality.

In another study, Zuo et al. (1996) fed an extruded and kibbled food containing 32% poultry byproduct meal to ileally cannulated dogs. The CP digestibilities were approximately 10 percentage units lower (66% ileal digestibility and 77% total tract) when compared with our study. This lower digestibility suggests that the poultry byproduct meal used in their study was lower quality reinforcing the fact that poultry byproduct meal can be highly variable.

The results from our canine AA digestibility study are in agreement with Muir et al. (1996) and Murray et al. (1997), who reported that poultry byproduct meal had apparent digestibilities of AA ranging from 66 (aspartate) to 89% (arginine) when poultry byproduct meals

were fed to dogs. Of the AA studied, arginine had the highest small intestinal digestibility, whereas cysteine, aspartate and isoleucine had the lowest. The small intestinal digestibilities of AA observed by Muir et al. (1996), Murray et al. (1997), and in the present study, are higher than the digestibilities observed by Zuo et al. (1996) and Johnson et al. (1998).

Variation in protein quality and AA availability among major protein ingredients and the response to varying levels of inclusion are of special concern to the pet food industry. Because of differences in raw material source and processing conditions, AA composition and availability may be variable among animal meals. Therefore, studies focusing on protein quality and AA availability of individual ingredients and different dietary concentrations of these individual ingredients are needed.

Increased processing time has been shown to decrease digestibility and AA availability (Johnson et al. 1998). Poultry byproduct normally contains only dry rendered parts of the chicken, such as heads, feet, undeveloped eggs, gizzards, and intestines, but not feathers, except for the few that may be included from collection processes. Renderers that provide poultry byproducts for the pet food industry have different methods for determining what will be utilized for byproducts. The differences in sorting methods can lead to varying concentrations of byproducts, resulting in a highly variable product (Murray et al., 1997). This variability can affect digestibility and AA profiles, making it difficult to determine the quality of the protein used to formulate canine foods.

This study indicates that PM fed at an inclusion level of 10.4% (10% CP) of the food can supply adequate amounts of digestible leucine, histidine, arginine, threonine, tryptophan, and lysine when compared to NRC (1985) requirements for canine maintenance. However inadequate amounts of digestible methionine, isoleucine, phenylalanine, and valine were supplied by the 10% CP food. The requirements for threonine and valine were not met until PM was included as 17.8% (15% CP) of the food. Isoleucine and phenylalanine requirements were not met until PM was included as 25.0% (20% CP) of the food. Therefore, foods containing less than 25.0% PM as a supplemental protein source would not meet the minimum AA requirements for maintenance in the dog. To be able to effectively utilize PM in lower protein foods (<20% CP), the food would need to include a phenylalanine and isoleucine supplement or utilize another protein source (plant protein source combined with animal protein source) to effectively supply all the essential AA needed for canine maintenance. Brewer's rice was included as 25% of the DM in each food and contributed from 8 to 20% of total protein. It is believed that the contribution of brewer's rice to total protein did not affect digestibility in this study; thus, PM digestibility was maintained across a wide range of inclusions.

These data show the value of ileal-cannulated dogs to determine digestibility. Total-tract CP digestibility increased with increasing PM; however, no differences in small intestinal CP digestibility were observed. This would result in large changes of AA utilization in the large intestine. Further, microbial transformation or production of AA in the large intestine can also lead to erroneous estimates of digestion and absorption (Williams, 1995). Crude protein or AA present in the feces can be of dietary, endogenous, or microbial origin, making it impossible to differentiate AA origin in the feces.

The general assumption in the pet food industry is that animal protein usually has a higher digestibility and is of higher quality when compared to plant protein sources. Proteins of plant origin usually have a lower digestibility than animal proteins because plant fiber and carbohydrates lower digestion, due to a reduced degradation rate of nutrients in the gut and increased bacterial activity (Meyer, 1984; Neirinck et al., 1991; Murray et al., 1997). The digestibility of PM in the present study was investigated by adding increasing concentrations of PM into the foods. A ranking of dietary treatments according to apparent digestibility showed that ileal digestibility of the 10% PM food for dogs tended to be lower than that of 25% PM food. However, the impact of endogenous protein and AA on apparent digestibility would have been greatest for the 10% CP diet, and the differences among digestibilities of all treatments were small. These results show that PM is digested efficiently at a wide range of concentrations in the dog.

Implications

For dogs, a high-quality protein source should contain all the essential amino acids in proper amounts and be readily bioavailable. The results of this study imply that poultry meal in dietary inclusions of 10 to 32.5% of the food can be an excellent source of protein for dogs; however, adequate amounts of digestible amino acids were not supplied until poultry meal was incorporated as 32.5% (25% crude protein) of the dry matter. Complementary sources of protein may be needed to meet amino acid requirements at lower protein intakes.

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