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Comparison of the Ileal Digestibility of Amino Acids in Meat and Bone Meal for Broiler Chickens and Growing Rats

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Abstract: The apparent ileal amino acid digestibility in 15 meat and bone samples was compared in growing broiler chickens and growing laboratory rat. The animals were given a diet containing meat and bone meal as the sole source of protein. All diets contained chromic oxide as the indigestible marker to calculate the digestibility estimates. Comparison of digestibility data shows that, for all dispensable amino acids, apparent ileal amino acid digestibility was similar ($p>0.05$) between broiler chicken and the growing rat. However, the ileal amino acid digestibility was similar ($p>0.05$) between the two species for only four (histidine, methionine, threonine and valine) of the nine indispensable amino acids. Significant differences ($p<0.01$ to 0.05) were observed between the estimates in the broiler chicken and the rat for arginine, leucine, lysine, phenylalanine and cystine. Differences approaching statistical significance were observed for isoleucine ($p=0.06$) and tyrosine ($p=0.10$). For the amino acids, which differed between the species, the values obtained for broilers were consistently 4 to 7 percentage units lower than those in the rats. The exception was cystine, with digestibility in broilers being 17 percentage units lower than in the rat. The present data do not support the use of the growing rat as a model for growing chickens in the determination of ileal amino acid digestibility.

Key words: Ileal digestibility, amino acids, meat and bone meal, broilers, growing rats

Introduction

Rendered animal by-products such as meat and bone meal (MBM) contain relatively high levels of protein, calcium and available phosphorus, and are commonly used to supply these nutrients in poultry diets in Asia and Australasia. Meat and bone meal typically contains 50 to 60 % protein, but its protein quality may vary greatly both in terms of amino acid composition and digestibility (Skurray, 1974; Parsons, 1991; Parsons *et al.*, 1997; Hendriks *et al.*, 2002).

Because of the variable and modifying effects of hindgut microflora, it is now generally agreed that the analysis of ileal digesta contents rather than excreta is a preferred method for assessing amino acid digestibility in poultry (Ravindran *et al.*, 1999; Ravindran and Bryden, 1999) and pigs (Sauer and Ozimek, 1986). With pigs, the laboratory rat has been shown to be a suitable and relatively inexpensive model for the determination of ileal amino acid digestibility in a range of feed ingredients (Moughan *et al.*, 1984; Donkoh *et al.*, 1994; Pearson *et al.*, 1999). To the authors' knowledge, published data on the correlation between the amino acid digestibility estimates for rat and the broiler chickens are not available. In the present study, 15 MBM samples were assayed to determine the ileal digestibility of amino acids in broiler chickens and the rat. The aim was to examine whether the growing rat can be used as a generalised model animal for growing broiler chickens

in amino acid digestibility assays.

Materials and Methods

Assay diets: Fifteen MBM samples were obtained from commercial sources and evaluated in this study. Each sample was ground to pass through 1-mm mesh and included as the sole source of protein in assay diets. Assay diets were based on maize starch and MBM, and the proportions of maize starch and MBM were varied to obtain a dietary crude protein level of 10 and 16%, respectively, in rat and broiler assays (Table 1). All diets contained 0.3% chromic oxide as an indigestible marker.

Digestibility assays: The digestibility assays for broilers and the growing rats were conducted according to the procedures described by Ravindran *et al.* (2002) and Hendriks *et al.* (2002), respectively. Briefly, a total of 90 broilers and 90 growing rats were used in the study. Each assay diet was fed *ad libitum* to six growing rats (males; 21 days of age; average body weight, 45-55 g) housed in stainless steel metabolism cages or to six broiler chickens (males; 28 days of age; average weight, 1420 g) housed in colony cages. Water was available at all times. After eight (for rats) or three (for broilers) days on the test diet, the animals were euthanased an intracardial injection of sodium pentobarbitone, and the contents of the lower half of the ileum were collected by

Ravindran *et al.*: Ileal Digestibility of Amino Acids in Meat and Bone Meal

Table 1: Composition (g/100 g as fed basis) of assay diets used for broiler and rat trials

Ingredient	Assay diets for broilers	Assay diets for the growing rats
Meat and bone meal	30.00 to 40.00 ¹	15.00 to 20.00 ²
Cellulose	5.00	5.00
Maize oil	3.50	3.50
Trace mineral premix	0.25	5.00
Vitamin premix	0.05	5.00
Chromic oxide	0.30	0.30
Maize starch	to 100	to 100

¹ To supply a protein level of 16% in the assay diet.

² To supply a protein level of 10% in the assay diet.

gently flushing with distilled water into plastic containers. The digesta were pooled within diets, frozen immediately after collection and subsequently freeze-dried. The ingredient, diet and dried digesta samples were ground to pass through a 0.5 mm sieve and stored in airtight containers at -4°C for chemical analyses.

Chemical analysis: Dry matter was determined by oven drying for 16 h at 105°C (AOAC, 1990). Nitrogen contents of the ingredient samples were determined by the Dumas method using LECO CNS-2000 Carbon, Nitrogen and Sulphur Analyzer. Amino acids were detected on a Waters ion exchange HPLC system according to the procedures described by Hendriks *et al.* (2002). In brief, the chromatograms were integrated using dedicated software (Maxima 820, Waters, Millipore, Milford, MA) with the amino acids identified and quantified using a standard amino acid solution (Pierce, Rockford, IL). Cysteine and methionine were analyzed as cysteic acid and methionine sulphone by oxidation with performic acid for 16 h at 0°C and neutralization with hydrobromic acid prior to hydrolysis. Tryptophan was not determined. The chromium content was measured on an Instrumentation Laboratory atomic absorption spectrophotometer following the method of Costigan and Ellis (1987).

Calculations: Apparent ileal amino acid digestibility coefficients were calculated from the dietary ratio of amino acid to chromium relative to the corresponding ratio in the ileal digesta.

Statistical analysis: The digestibility values for each amino acid were statistically analyzed using the General Linear Models procedure of SAS (1997) for the effects of species. Differences were considered significant at P < 0.05, although probability values up to P < 0.10 are shown in the text if the data suggest a trend.

Results and Discussion

Meat and bone meal is described as the rendered product from mammalian bones and associated tissues such as tendons, ligaments, some skeletal muscle,

lungs and the gastrointestinal tract, but exclusive of any blood, hair, hoof, horn, hide trimmings, rumen contents and manure (AAFCO, 2000). Depending on the proportion of bone to soft tissue used in the rendering process, the finished product is designated as 'meat meal' (containing more than 55 % crude protein and less than 4.4% phosphorus) or 'meat and bone meal' (containing less than 55 % crude protein and more than 4.4 % phosphorus). Although 9 out of the 15 samples evaluated in the present evaluation fall into the category of meat meal, all samples will be termed as MBM for the purpose of this paper.

The data presented in Table 2 highlight the wide variations in the gross contents of crude protein and amino acids among the 15 samples. The crude protein content varied from 38.5 to 67.2% and the variation in lysine content was 1.33 to 4.13%. Such variation in protein and amino acid concentrations is common to this ingredient and consistent with previous published data for MBM produced in different parts of the world, including New Zealand (Skilton *et al.*, 1991; Donkoh *et al.*, 1994; Hendriks *et al.*, 2002), Australia (Skurray, 1974; Skurray and Herbert, 1974), North America (Parsons, 1997; Parsons *et al.*, 1997) and Europe (Rhone-Poulenc, 1995). It has been shown that the observed variations are due largely to differences in the proportion of bones and soft tissues as well as the mammalian species used in the rendering of MBM (Estoe and Long, 1960; Skurray, 1974).

On average, the protein in MBM was found to be a poor source of cystine and histidine, and a rich source of non-essential amino acids (Table 2). In general, the indispensable amino acids and semi-indispensable amino acids (cystine and tyrosine) showed greater variability than dispensable amino acids. Histidine, cystine and tyrosine had the highest variability (coefficients of variation, 29.3 to 32.0%). Glycine and proline had the lowest variability (coefficient of variation, 6.7 to 7.1%).

The ileal digestibility of amino acids, determined for broiler chickens and growing rats, also varied substantially (Table 3). The current data, in common with previous reports for rats (Skilton *et al.*, 1991; Donkoh *et al.*, 1994; Hendriks *et al.*, 2002) and chickens (Parsons, 1991; Rhone-Poulenc, 1995; Karakas *et al.*, 2001), confirm that the digestibility of amino acids in MBM varies substantially among commercially available samples. The coefficient of variation for the digestibility of amino acids in broiler chickens ranged from 11.3 (for valine) to 24.9% (for cystine) and those in growing rats ranged from 13.6 (for phenylalanine) to 39.5% (for aspartic acid). The apparent ileal digestibility of lysine in the 15 meat and bone meal samples for broilers and rats ranged from 43 to 82% and 48 to 85%, respectively. The corresponding ranges for methionine, cystine, threonine and amino acid N were: 55 to 84% and 53 to

Ravindran *et al.*: Ileal Digestibility of Amino Acids in Meat and Bone Meal

Table 2: Variation in amino acid concentrations (g/100 g, as received) of 15 samples of meat and bone meal

Parameter	Range	Mean	SD	CV, %
Dry matter	91.6-98.1	95.5	1.72	1.80
Crude protein (N x 6.25)	38.5-67.2	54.0	6.25	11.6
Indispensable amino acids				
Arginine	2.68-4.58	3.69	0.47	12.8
Histidine	0.28-1.47	0.85	0.26	30.3
Isoleucine	0.73-2.26	1.43	0.35	24.8
Leucine	1.67-5.12	3.26	0.77	23.7
Lysine	1.33-4.13	2.63	0.66	25.1
Methionine	0.51-1.54	0.96	0.23	24.1
Phenylalanine	0.90-2.66	1.67	0.38	22.8
Threonine	0.91-2.85	1.83	0.45	24.4
Valine	1.12-3.26	2.12	0.46	21.8
Semi-indispensable amino acids				
Cystine	0.15-0.58	0.38	0.12	32.0
Tyrosine	0.51-2.02	1.17	0.34	29.3
Dispensable amino acids				
Alanine	3.02-4.53	3.83	0.36	9.3
Aspartic acid	2.47-5.85	3.99	0.75	18.7
Glycine	6.25-8.12	6.95	0.50	7.1
Glutamic acid	4.16-8.78	6.49	1.09	16.8
Proline	3.91-4.81	4.32	0.29	6.7
Serine	1.26-2.84	2.07	0.38	18.5
Amino acid N	4.92-9.06	6.99	0.94	13.5

Table 3: Variation in ileal amino acid digestibility (%) of 15 samples of meat and bone meal for broiler chickens and growing rats

Parameter	Broiler chickens			Growing rats		
	Range	SD	CV, %	Range	SD	CV, %
Indispensable amino acids						
Arginine	41.6-78.5	10.8	15.6	61.3-88.5	13.9	18.1
Histidine	45.3-78.1	10.6	17.3	37.1-79.0	17.9	29.8
Isoleucine	53.8-78.1	8.0	11.5	50.0-81.5	11.1	15.1
Leucine	50.6-81.4	8.1	11.6	57.1-88.0	11.3	15.1
Lysine	42.5-80.4	11.4	16.5	47.6-84.9	11.9	15.8
Methionine	55.9-86.2	8.3	11.3	53.4-87.3	11.8	15.3
Phenylalanine	57.8-82.6	9.1	12.5	51.5-87.4	10.5	13.6
Threonine	48.8-71.5	8.0	12.9	40.3-82.4	14.4	23.7
Valine	52.4-77.3	7.7	11.3	58.8-84.8	12.0	16.7
Semi-indispensable amino acids						
Cystine	26.7-55.0	9.7	24.9	35.9-78.9	11.8	15.3
Tyrosine	54.9-80.2	8.2	11.8	53.3-85.1	11.5	15.7
Dispensable amino acids						
Alanine	42.6-78.2	10.7	15.6	54.6-82.9	13.2	18.6
Aspartic acid	20.9-68.9	11.5	24.4	10.1-71.8	18.5	39.5
Glycine	36.6-76.1	11.0	17.4	32.9-75.8	13.8	22.1
Glutamic acid	56.8-73.0	8.8	13.2	50.6-82.3	13.1	19.5
Proline	38.0-73.5	11.0	18.2	30.9-78.0	14.2	23.3
Serine	43.6-70.9	7.9	13.5	30.4-77.0	15.1	26.9
Amino acid N	57.2-77.8	9.1	14.0	52.1-80.8	12.9	19.2

87%; 28 to 56% and 39 to 73%; 49 to 73% and 47 to 83%; 43 to 76% and 52 to 84%, respectively. The observed variability in digestibility is likely to reflect both

processing damage and the relative amounts of muscle protein and collagen in raw materials (Aitkinson and Carpenter, 1970; Skurray and Herbert, 1974; Wang and

Ravindran *et al.*: Ileal Digestibility of Amino Acids in Meat and Bone Meal

Table 4: Comparison of apparent ileal digestibility of amino acids¹ from meat and bone meal for broiler chickens and rats

Amino acid	Broilers	Rats	Pooled SEM	Significance, p ≤
Indispensable amino acids				
Arginine	69.4	76.7	1.49	**
Histidine	61.6	60.1	3.30	NS
Isoleucine	69.4	73.8	1.49	0.06
Leucine	70.1	74.7	1.38	*
Lysine	69.2	75.0	1.28	**
Methionine	74.0	76.9	1.31	NS
Phenylalanine	72.4	77.3	1.17	*
Threonine	61.8	60.7	2.32	NS
Valine	68.3	72.0	1.68	NS
Semi-indispensable amino acids				
Cystine	38.9	56.1	2.91	**
Tyrosine	69.6	73.3	1.50	0.10
Dispensable amino acids				
Alanine	68.1	70.7	1.67	NS
Aspartic acid	47.2	46.8	3.50	NS
Glycine	63.6	62.7	2.02	NS
Glutamic acid	66.6	67.3	1.75	NS
Proline	60.6	60.9	2.00	NS
Serine	58.2	56.1	2.49	NS
Amino acid N	64.9	67.2	1.81	NS

NS, not significant; * p<0.05; ** p<0.01. ¹ Each mean represents values from 15 samples

Parsons, 1998). Collagen is the major protein in bone, connective tissues, cartilage and tendon. Estoe and Long (1960) found that the collagen is deficient in most indispensable amino acids and also poorly digested owing to its poor amino acid balance.

The average ileal amino acid digestibility of the 15 meat and bone meal samples in the broiler and the rat are summarized in Table 4. Comparison of digestibility data shows that for all six dispensable amino acids, apparent ileal amino acid digestibility was similar (p>0.05) between the growing broiler chicken and the growing rat. However, the ileal amino acid digestibility was similar (p>0.05) between the two species for only four (histidine, methionine, threonine and valine) of the nine indispensable amino acids. Significant differences (p<0.01 to 0.05) were observed between the estimates in the broiler chicken and the rat for arginine, leucine, lysine, phenylalanine and cystine. Differences approaching statistical significance were observed for isoleucine (p=0.06) and tyrosine (p=0.10). For these amino acids, which differed between the species, the values obtained for broilers were consistently lower than those in the rats. The species differences in digestibility were generally 4 to 7 percentage units in favour of the rat. The exception was cystine, with digestibility in broilers being 17 percentage units lower than in the rat. The reasons for or the significance of this observation is unclear. In both species, the poorly digested amino acids were cystine and aspartic acid. It has been shown that cystine is the amino acid that is affected most by processing temperature (Wang and Parsons, 1998) and

processing pressure (Shirley and Parsons, 2000).

To the authors' knowledge, the current study is the first published report comparing the amino acid digestibility in a feed ingredient for broiler chickens and growing rats. The present findings suggest that the digestibility values generated with the growing rat for the majority of essential amino acids (arginine, leucine, lysine, phenylalanine and isoleucine) and the two semi-indispensable amino acids (cystine and tyrosine) in MBM are not directly transferable to the broiler chicken. These data are in contrast to those reported in studies with pigs and growing rats, wherein the rat was found to be a good animal model for the determination of amino acid digestibility in feed ingredients for growing pigs (Moughan *et al.*, 1984; Donkoh *et al.*, 1994; Pearson *et al.*, 1999). The present data appear to suggest that there are intrinsic differences in the manner that chickens and rats digest proteins, which is consistent with the differences between the two species in terms of the anatomy and physiology of the gastrointestinal tract.

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Ravindran et al.: Ileal Digestibility of Amino Acids in Meat and Bone Meal

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