Evaluation of the pepsin digestibility assay for predicting amino acid digestibility of meat and bone meals

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ABSTRACT Sixteen meat and bone meal (MBM) samples were obtained and selected from various company plants to provide a wide range in pepsin nitrogen digestibility values. Pepsin digestibility was determined using either 0.02 or 0.002% pepsin. Amino acid (AA) digestibility of the 16 MBM samples was then determined using a precision-fed cecectomized rooster assay. The 0.02% pepsin digestibility values were numerically higher than the 0.002% pepsin values. The values varied from 77 to 93% for 0.02% pepsin and from 67 to 91% for 0.002% pepsin. The rooster AA digestibility results showed a wide range of values among MBM samples mostly due to the 4 samples having lowest and highest AA digestibility. A precision-fed broiler chick ileal AA digestibility assay confirmed that there were large differences in AA digestibility among the MBM samples having the lowest and highest rooster digestibility values. Correlation analyses between pepsin and AA digestibility values showed that the correlation values (r) were highly significant ($P < 0.0001$) for all AA when all 16 MBM samples were included in the analysis. However, when the MBM samples with the 2 lowest and the 2 highest rooster digestibility values were not included in the correlation analyses, the correlation coefficient values (r) were generally very low and not significant ($P > 0.05$). The results indicated that the pepsin nitrogen digestibility assay is only useful for detecting large differences in AA digestibility among MBM. There also was no advantage for using 0.02 versus 0.002% pepsin.

Key words: meat and bone meal, pepsin digestibility, amino acid digestibility, poultry

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INTRODUCTION

The composition and digestibility of meat and bone meal (**MBM**) can vary greatly among samples. Differences in the nutrient composition and energy values of MBM are largely due to the rendering of various raw materials (Adedokun and Adeola, 2005; Johnston and Coon, 1979a; Batterham et al., 1986) which makes it difficult to determine nutritive values for feed formulation (Perai et al., 2010). One of the most important concerns of MBM use in poultry and livestock rations is its variability in protein quality (Parsons et al., 1997). It is useful for feed manufacturers and nutritionists to be able to evaluate protein quality of MBM with a rapid, inexpensive, and accurate method in order to produce meals of high nutritional value consistently (Parsons, 1986).

The pepsin nitrogen digestibility assay (AOAC, 1980) has been used by the animal feed industry to monitor quality of MBM, particularly for detecting low-quality samples. It is a moderately simple, inexpensive, and rapid assay, and many samples can be compared at the same time (Ravindran and Bryden, 1999). Research conducted by Parsons et al. (1997) and Johnston and Coon (1979b) showed that the pepsin digestibility assay was useful for detecting differences in protein quality among commercial animal meals if the concentration of pepsin was reduced from 0.2% to 0.02, 0.002, or 0.0002%. To our knowledge, very little additional research has been done with the pepsin assay during the last 20 yr, whereas the processing procedures used at the commercial rendering industry have changed during this time period. The rendering process is energy-intensive. The cost of this energy is a major contributor to operational costs. Consequently, renderers constantly evaluate equipment and practices that permit a reduction in the energy used to process renderable raw materials. One of those variables is the target temperature at which the raw materials are cooked. This target has been reduced over the past 20 yr. Operating temperatures ranging from 245 to 275°F today replace targets of 275 to 300°F in the past (D. Kirstein, Darling International, Inc., Irving, TX; personal communication). The reduction in operating temperatures suggests potential changes in pepsin digestibility and protein/amino acid (**AA**) digestibility.

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The primary objective of the current study herein was to reevaluate the pepsin nitrogen digestibility assay for detecting differences in AA digestibility among MBM produced in current commercial rendering plants. Particular emphasis was on assessing the sensitivity of the pepsin assay for detecting small/moderate differences in AA digestibility vs. large differences among MBM samples. In addition, the AA digestibility of some of the MBM samples was determined in both the precision-fed cecectomized rooster assay and a new precision-fed broiler chicken ileal digestibility assay to determine if values were in agreement between assays.

MATERIALS AND METHODS

Meat and Bone Meals

Sixteen MBM samples were obtained from various commercial rendering plants from Darling International, Inc., Irving, Texas. The samples were selected from plants in a large geographical area and from plants that employed industrial grinders. The samples also represented the entire range of operating temperatures among Darling International, Inc., plants. Samples were not subjected to additional heat after collection. Two of these, Samples 1 and 2, were selected to represent heat-damaged MBM. Pepsin nitrogen digestibility was determined according to the procedure of the Association of Official Analytical Chemists (AOAC, 1980) at the Darling International, Inc., Analytical Laboratory in Ankeny, Iowa, except that the (AOAC, 1980) recommended concentration of 0.2% pepsin was reduced to 0.02 and 0.002%.

Precision-Fed Cecectomized Rooster Assay

Cecectomized Single Comb White Leghorn roosters were utilized in the precision-fed rooster assay according to the procedure of Parsons (1985). All animal care, handling, and euthanasia were approved by the University of Illinois Institutional Animal Care and Use Committee. After 26 h without feed, 4 roosters were tube-fed 30 g MBM sample. The roosters were then placed in a cage with a plastic tray underneath, and the total excreta were collected for 48 h. The excreta samples were frozen and stored at −20°C then freeze-dried, ground, and the MBM and dried excreta were analyzed for CP and AA [AOAC International, 2006; Method 990.03, 982.30 E (a,b,c)] at the Experiment Station Chemical Laboratories, University of Missouri, Columbia. Standardized digestibility of AA was then calculated for each of the 64 roosters using AA excretion of fasted roosters for the endogenous correction.

Precision-Fed Broiler Chicken Ileal Amino Acid Digestibility Assay

The precision-fed ileal AA digestibility assay was conducted using the procedures described by Kim et al. (2011). All animal care, handling, and euthanasia were approved by the University of Illinois Institutional Animal Care and Use Committee. Sixty-four 21-day-old broiler chickens were fasted overnight. Four replicate pens or groups of 4 chickens were then assigned to and tube-fed 10 g one of 4 MBM samples. The 4 MBM samples were the 2 samples that had the lowest and the 2 samples that had the highest AA digestibility values from the rooster assay (Samples 1, 2, 15, and 16). Chromic oxide was added to the MBM samples as an indigestible marker at a level 0.30%. The chickens were then placed in a cage and at 4 h postfeeding, they were euthanized via CO_2 gas, and ileal digesta were collected from the Meckel's diverticulum to the ileal-cecal junction. Ileal contents from the 4 chickens in each replicate pen were pooled, frozen, and stored at −20°C. The samples were later freeze-dried, ground using a mortar and pestle, and the MBM samples and ileal digesta samples were analyzed for AA and chromium (AOAC International, 2006; Method 990.08) at the University of Missouri.

Digestibility Calculations

Standardized AA digestibility values for the precision-fed cecectomized rooster assay were calculated using the following formula. Amino acid digestibility values were standardized using an endogenous correction based on AA excretion by fasted roosters.

$$\begin{aligned}&\text{Standardized AA digestibility, \%}\\&\quad=[(\text{AA consumed, mg} - \text{AA excreted, mg}\\&\qquad+\text{endogenous AA excreted, mg})/\\&\qquad\text{AA consumed, mg}]\times 100.\end{aligned}$$

Standardized AA digestibility values for the precision-fed broiler chicken ileal AA digestibility assay were calculated using the following formula by Moughan et al. (1992). Apparent amino acid digestibility values were standardized using the ileal endogenous AA flow of chickens tube-fed a N-free diet (Kim et al., 2011).

$$\begin{aligned}&\text{Apparent ileal AA digestibility}\\&\quad=[1-(\text{chromium in diet/chromium in digesta})\\&\qquad\times(\text{AA in digesta/AA in diet})]\times 100,\end{aligned}$$

$$\begin{aligned}&\text{Standardized AA digestibility, \%}\\&\quad=\text{apparent digestibility}+[(\text{ileal endogenous}\\&\qquad\text{AA flow, g/kg of DM intake})/\\&\qquad(\text{AA in the diet, g/kg of DM intake})]\times 100.\end{aligned}$$

Statistical Analysis

Data from both animal assays were subjected to ANOVA (SAS Institute, 2008) for a completely randomized design. Statistical significance of differences among individual treatments in the rooster assay was then determined using the least-significant difference test (Carmer and Walker, 1985) based on the pooled SEM calculated from the ANOVA error mean square. For the statistical comparisons of the cecectomized rooster versus broiler chicken ileal AA digestibility vales, the SEM for the individual treatments were used. Correlations of pepsin nitrogen digestibility with rooster AA digestibility were assessed using Pearson's linear test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The total AA concentrations of the 16 MBM samples are presented in Table 1. These values varied substantially among samples. The variation in AA concentrations among samples was likely primarily due to variation in raw material composition among samples (Adedokun and Adeola, 2005; Johnston and Coon, 1979a; Wang and Parsons, 1998).

Table 1. Dry matter, CP, and total amino acid concentrations (%) in the 16 meat and bone meal samples, as-fed basis.

	MBM Sample Number							
Item	1	2	3	4	5	6	7	8
Dry matter	91.7	94.5	97.5	97.3	96.9	95.5	96.1	96.0
CP	54.1	55.4	48.0	48.5	59.4	51.6	61.3	52.9
Aspartic acid	4.35	4.37	3.85	3.84	4.66	4.08	4.66	4.38
Threonine	1.87	1.88	1.63	1.62	2.12	1.86	2.14	1.97
Serine	1.98	1.71	1.82	1.82	2.54	1.94	2.28	2.30
Glutamic acid	6.72	6.91	6.19	6.17	7.34	6.34	7.27	6.84
Proline	3.75	3.69	4.31	4.27	4.60	3.40	3.96	4.25
Alanine	4.13	4.04	3.81	3.84	4.06	3.59	4.02	3.85
Cysteine	0.34	0.36	0.53	0.52	0.85	0.46	0.64	0.79
Valine	2.76	2.76	2.28	2.27	2.99	2.33	2.78	2.84
Methionine	0.91	0.95	0.67	0.66	0.96	0.87	1.08	0.95
Isoleucine	1.87	1.99	1.63	1.62	2.27	1.71	2.19	2.10
Leucine	3.94	4.00	3.28	3.27	4.06	3.51	4.01	3.86
Tyrosine	1.60	1.56	1.21	1.24	1.66	1.45	1.74	1.60
Phenylalanine	2.21	2.21	1.76	1.77	2.31	1.88	2.21	2.18
Lysine	3.00	3.16	2.64	2.60	3.20	3.13	3.39	3.03
Histidine	1.21	1.26	0.89	0.87	1.19	1.20	1.26	1.10
Arginine	3.59	3.61	3.72	3.70	4.07	3.41	3.95	3.84
Tryptophan	0.27	0.28	0.26	0.27	0.24	0.36	0.42	0.35
	Meat and Bone Meal Sample Number							
Item	9	10	11	12	13	14	15	16
Dry matter	96.1	95.2	95.3	96.0	96.1	96.5	94.2	91.4
CP	58.3	59.8	48.2	46.7	53.1	51.3	50.2	60.0
Aspartic acid	4.41	4.99	3.52	3.36	3.94	4.11	4.05	4.50
Threonine	2.06	2.21	1.48	1.37	1.65	1.79	1.76	2.01
Serine	2.59	2.18	1.69	1.64	1.72	1.83	1.82	1.93
Glutamic acid	6.92	7.69	5.65	5.48	6.25	6.42	6.31	6.83
Proline	4.41	4.01	4.04	3.87	3.91	3.73	3.42	3.57
Alanine	3.97	4.28	3.70	3.53	3.93	3.73	3.60	3.80
Cysteine	0.79	0.61	0.37	0.38	0.34	0.42	0.49	0.51
Valine	2.70	3.01	2.09	1.87	2.46	2.36	2.41	2.68
Methionine	0.91	1.07	0.70	0.64	0.79	0.82	0.85	1.08
Isoleucine	2.07	2.23	1.48	1.33	1.68	1.81	1.78	2.14
Leucine	3.82	4.32	2.88	2.68	3.45	3.43	3.51	3.89
Tyrosine	1.57	1.73	1.07	0.98	1.31	1.37	1.33	1.54
Phenylalanine	2.15	2.31	1.61	1.50	1.92	1.87	1.92	2.11
Lysine	2.98	3.70	2.48	2.36	2.97	2.98	3.14	3.61
Histidine	1.08	1.54	0.81	0.75	1.16	1.17	1.17	1.23
Arginine	3.94	4.03	3.38	3.39	3.47	3.49	3.35	3.71
Tryptophan	0.34	0.37	0.29	0.22	0.32	0.37	0.38	0.40

Table 2. Pepsin nitrogen digestibility values (%) for the 16 meat and bone meal samples[1].

Sample Number	0.02% Pepsin	0.002% Pepsin
1	77	67
2	77	71
3	83	72
4	81	73
5	87	78
6	83	80
7	88	80
8	87	81
9	88	82
10	88	84
11	91	84
12	91	85
13	91	85
14	90	86
15	90	89
16	93	91

[1]Values are means of triplicate analyses.

The pepsin nitrogen digestibility values for the 16 MBM samples showed a substantial amount of variation among samples (Table 2). As expected, the values for the 0.02% pepsin nitrogen digestibility were numerically lower than the 0.002% pepsin values. The values varied from 77 to 93% for 0.02% pepsin and from 67 to 91% for 0.002% pepsin.

The standardized AA digestibility values for the 16 MBM samples determined by the precision-fed cecectomized rooster assay are shown in Table 3. As observed above for pepsin digestibility and AA concentrations, there was considerable variation in AA digestibility among MBM samples ($P < 0.05$). These results generally agreed with previous research reported by Parsons et al. (1997). The differences in AA digestibility, particularly cysteine, among samples may be due primarily to processing effects (Baker et al., 1981; Batterham et al., 1986; Wang and Parsons, 1998).

A large part of the variation in AA digestibility among samples was due to the 2 samples that had the lowest AA digestibility values (Samples 1 and 2) and the 2 that had the highest AA digestibility values (Samples 15 and 16). Cysteine exhibited the largest difference in digestibility among MBM samples. The AA digestibility values for the other 12 samples were intermediate and generally did not differ greatly. When all 16 MBM samples were included in the correlation analyses between pepsin digestibility and rooster AA digestibility, the correlation values (r) were significant for all amino acids (Table 4). However, when the 2 samples with the lowest pepsin values and the 2 samples with the highest pepsin values were excluded from the correlation analyses (Table 5), all of the correlation coefficient values (r) were very low and only one of them was significant ($P > 0.05$). Thus, the significant correlation between pepsin digestibility and rooster AA digestibility

Table 3. Standardized amino acid digestibility values (%) for the 16 meat and bone meal samples determined by the precision-fed cecectomized rooster assay[1].

Amino Acid	Meat and Bone Meal Sample Number								
	1	2	3	4	5	6	7	8	
Threonine	46.2^{e}	47.4^{e}	71.2^{d}	$74.4^{c,d}$	$75.8^{c,d}$	$82.3^{a,b}$	$75.1^{c,d}$	$74.5^{c,d}$	
Cysteine	26.5^{g}	18.8^{g}	$42.8^{e,f}$	$50.6^{c,d,e,f}$	$60.0^{a,b,c}$	$60.0^{a,b,c}$	$53.1^{c,d,e}$	$53.9^{b,c,d,e}$	
Valine	51.5^{e}	51.2^{e}	75.8^{d}	$77.3^{c,d}$	$80.3^{b,c}$	$82.8^{a,b}$	76.1^{d}	$77.6^{c,d}$	
Methionine	53.2^{i}	53.8^{i}	$80.5^{g,h}$	$81.2^{f,g,h}$	$83.4^{d,e,f,g}$	$87.3^{b,c}$	$82.5^{e,f,g,h}$	$83.0^{e,f,g}$	
Isoleucine	52.3^{h}	51.9^{h}	$78.6^{f,g}$	$79.6^{d,e,f,g}$	$82.6^{c,d}$	$84.9^{b,c}$	$78.7^{e,f,g}$	$79.7^{d,e,f,g}$	
Leucine	50.4^{f}	51.3^{f}	$78.6^{d,e}$	$79.5^{c,d,e}$	$82.2^{b,c,d}$	$85.7^{a,b}$	$79.1^{d,e}$	$79.6^{c,d,e}$	
Phenylalanine	56.0^{f}	55.8^{f}	$80.5^{c,d,e}$	$81.4^{c,d,e}$	$83.7^{b,c}$	$85.9^{a,b}$	$80.1^{d,e}$	$80.8^{c,d,e}$	
Lysine	39.7^{f}	39.8^{f}	$69.5^{c,d,e}$	$68.9^{d,e}$	$74.7^{b,c}$	$77.1^{a,b}$	$70.1^{c,d,e}$	$71.3^{c,d}$	
Histidine	47.5^{d}	47.4^{d}	72.4^{c}	71.9^{c}	$74.6^{b,c}$	$78.3^{a,b}$	72.9^{c}	72.7^{c}	
Arginine	61.9^{f}	61.9^{f}	$81.3^{c,d}$	$83.1^{a,b,c}$	$84.6^{a,b,c}$	$85.1^{a,b,c}$	$82.4^{a,b,c}$	$81.6^{b,c,d}$	
Tryptophan	71.0^{c}	76.6^{h}	81.8^{g}	$87.8^{d,e,f}$	82.2^{g}	$91.8^{a,b,c}$	$89.5^{c,d,e}$	$88.2^{c,d,e,f}$	
	Meat and Bone Meal Sample Number								
Amino Acid	9	10	11	12	13	14	15	16	Pooled SEM
Threonine	$75.4^{c,d}$	$78.1^{b,c}$	$78.7^{b,c}$	71.7^{d}	71.7^{d}	$79.0^{b,c}$	84.6^{a}	86.1^{a}	1.9
Cysteine	$54.5^{b,c,d}$	$50.9^{c,d,e}$	$56.5^{a,b,c,d}$	38.2^{f}	$46.9^{d,e,f}$	$58.7^{a,b,c}$	66.7^{a}	$65.0^{a,b}$	4.0
Valine	$76.9^{c,d}$	$80.0^{b,c}$	$80.4^{b,c}$	74.3^{d}	$77.4^{c,d}$	$80.8^{b,c}$	85.8^{a}	86.7^{a}	1.5
Methionine	$81.6^{e,f,g,h}$	$86.0^{c,d}$	$84.0^{d,e,f}$	$81.5^{f,g,h}$	80.0^{h}	$84.5^{c,d,e}$	$89.6^{a,b}$	90.6^{a}	1.1
Isoleucine	$79.9^{d,e,f,g}$	$82.0^{c,d,e}$	$82.1^{c,d,e,f}$	77.9^{g}	$79.5^{d,e,f,g}$	$82.9^{c,d}$	$87.7^{a,b}$	88.9^{a}	1.3
Leucine	$79.5^{c,d,e}$	$82.3^{b,c,d}$	$82.2^{b,c,d}$	77.7^{e}	$79.1^{d,e}$	$83.0^{b,c}$	88.1^{a}	88.2^{a}	3.6
Phenylalanine	$80.4^{c,d,e}$	$83.1^{b,c,d}$	$82.3^{c,d}$	78.8^{e}	$80.0^{d,e}$	$83.8^{b,c}$	87.9^{a}	87.8^{a}	3.0
Lysine	$71.2^{c,d}$	$74.1^{b,c}$	$68.6^{d,e}$	$69.3^{c,d,e}$	65.0^{e}	$73.9^{b,c,d}$	80.7^{a}	$78.3^{a,b}$	2.0
Histidine	72.3^{c}	$74.1^{b,c}$	$74.8^{b,c}$	70.0^{c}	70.0^{c}	72.9^{c}	81.4^{a}	81.3^{a}	6.7
Arginine	$81.3^{c,d}$	$84.9^{a,b}$	$81.6^{b,c,d}$	$79.0^{d,e}$	76.8^{e}	85.9^{a}	$85.2^{a,b}$	$84.5^{a,b,c}$	1.4
Tryptophan	$89.2^{c,d,e,f}$	$88.8^{c,d,e,f}$	$90.8^{b,c,d}$	$86.7^{e,f}$	$85.5^{g,f}$	$90.3^{b,c,d,e}$	$93.7^{a,b}$	94.7^{a}	1.4

[1]Values are the means of 4 cecectomized roosters.

[a–i]Values within a row with no common superscripts are significantly different ($P < 0.05$).

Table 4. Correlation of pepsin nitrogen digestibility with rooster amino acid digestibility when all 16 meat and bone meal samples are included in the correlation analysis.

Amino Acid	0.02% Pepsin Digestibility		0.002% Pepsin Digestibility	
	r	*P* value	r	*P* value
Threonine	0.74	<0.0001	0.76	<0.0001
Cysteine	0.63	<0.0001	0.64	<0.0001
Valine	0.75	<0.0001	0.75	<0.0001
Methionine	0.77	<0.0001	0.76	<0.0001
Isoleucine	0.77	<0.0001	0.75	<0.0001
Leucine	0.76	<0.0001	0.74	<0.0001
Phenylalanine	0.74	<0.0001	0.72	<0.0001
Lysine	0.70	<0.0001	0.70	<0.0001
Histidine	0.71	<0.0001	0.70	<0.0001
Arginine	0.64	<0.0001	0.62	<0.0001
Tryptophan	0.71	<0.0001	0.78	<0.0001

Table 5. Correlation of pepsin nitrogen digestibility with rooster amino acid digestibility when meat and bone meal Samples 1, 2, 15, and 16 are excluded from the correlation analysis.

Amino Acid	0.02% Pepsin Digestibility		0.002% Pepsin Digestibility	
	r	*P* value	r	*P* value
Threonine	−0.02	0.90	0.19	0.20
Cysteine	−0.02	0.91	0.08	0.58
Valine	−0.02	0.87	0.14	0.35
Methionine	−0.02	0.88	0.20	0.17
Isoleucine	−0.03	0.84	0.14	0.35
Leucine	−0.06	0.67	0.12	0.43
Phenylalanine	−0.16	0.29	0.01	0.96
Lysine	−0.17	0.24	−0.01	0.96
Histidine	−0.18	0.23	−0.06	0.69
Arginine	−0.26	0.07	−0.12	0.41
Tryptophan	0.14	0.33	0.40	0.01

was mostly due to the 2 lowest digestibility and the 2 highest digestibility samples. These results suggest that the pepsin N digestibility assay is sensitive for detecting large differences in AA digestibility among samples, but not small or moderate differences.

When comparing standardized AA digestibility in cecectomized roosters to ileal AA digestibility in broiler chickens for the 2 lowest pepsin digestibility MBM samples, all AA digestibility values except for cysteine and lysine were significantly higher for roosters than broiler chickens for Sample 1 (Table 6). However, for Sample 2, all but 3 AA digestibility values were not significantly different between roosters and broiler chickens, and when significant differences did occur, the broiler chickens values were higher than the rooster values for 2 of the 3 AA. A comparison of the rooster and broiler chickens standardized AA digestibility values for the 2 highest pepsin digestibility MBM samples (Samples 15 and 16) are presented in Table 7. For Sample 15, digestibility values were significantly different between roosters and broiler chickens for only 3 AA. In contrast, for Sample 16, most AA digestibility values were significantly higher for roosters than broiler chickens. Similar inconsistent differences between precision-fed roosters and broiler chickens as those observed herein were also reported by Kim et al. (2012). However, Kim et al. (2012) did report that AA digestibility values were higher for roosters than broiler chickens for

Table 6. Standardized amino acid digestibility (%) for the 2 lowest pepsin digestibility meat and bone meal samples determined by the precision-fed cecectomized rooster assay (PFR) and precision-fed broiler chicken ileal assay (PFC).

	Meat and Bone Meal Sample Number							
	1				2			
Amino Acid	PFR[1]	SEM	PFC[2]	SEM	PFR	SEM	PFC	SEM
Threonine	46.2[a]	0.9	31.0[b]	3.2	47.4	0.9	49.4	2.9
Cysteine	26.5	1.7	22.8	4.6	18.8[b]	2.0	46.0[a]	3.8
Valine	51.5[a]	1.1	39.0[b]	1.9	51.2	1.2	56.7	2.9
Methionine	53.2[a]	0.8	36.1[b]	1.2	53.8	1.2	56.0	3.4
Isoleucine	52.3[a]	1.3	38.2[a]	1.6	51.9	1.3	57.6	3.2
Leucine	50.4[a]	0.9	37.0[b]	1.8	51.3	1.0	55.0	2.9
Phenylalanine	56.0[a]	1.0	41.9[b]	1.8	55.8	0.8	58.0	2.7
Lysine	39.7	1.7	35.0	1.6	39.8[b]	1.3	55.2[a]	3.4
Histidine	47.4[a]	1.3	30.9[b]	2.1	47.4	1.4	51.1	3.2
Arginine	61.9[a]	0.9	49.7[b]	1.3	61.9	0.9	64.1	2.6
Tryptophan	71.3[a]	1.6	39.3[b]	3.9	76.6[a]	2.0	52.3[b]	3.0

[1]Mean of 4 roosters.

[2]Mean of 4 replicate pens of 4 chicks.

[a,b]Means within a row within sample number with no common superscripts are significantly different ($P < 0.05$).

Table 7. Standardized amino acid digestibility (%) for the 2 highest pepsin digestibility meat and bone meal samples determined by the precision-fed cecectomized rooster assay (PFR) and precision-fed broiler chicken ileal assay (PFC).

	Meat and Bone Meal Sample Number							
	15				16			
Amino Acid	PFR[1]	SEM	PFC[2]	SEM	PFR	SEM	PFC	SEM
Threonine	84.6	1.2	84.2	1.8	86.1[a]	1.4	79.8[b]	1.1
Cysteine	66.7	1.8	71.5	3.9	65.0	2.7	66.0	1.9
Valine	85.8	1.0	85.3	1.4	86.7[a]	1.0	81.5[b]	0.9
Methionine	89.6	0.7	89.2	0.7	90.6[a]	0.7	84.4[b]	1.1
Isoleucine	87.7	1.0	87.7	1.1	88.9[a]	0.8	83.5[b]	1.0
Leucine	88.1	0.9	87.5	1.1	88.3[a]	0.9	82.6[b]	1.0
Phenylalanine	87.9	0.9	87.3	1.0	88.1[a]	0.8	82.8[b]	1.0
Lysine	80.7[b]	1.8	87.3[a]	1.1	78.3	3.9	82.6	1.0
Histidine	81.4[b]	0.8	85.6[a]	1.1	81.6	2.1	79.7	1.0
Arginine	85.2	1.9	88.7	1.3	84.5	0.8	84.4	1.0
Tryptophan	93.7[a]	1.9	88.2[b]	0.9	94.7[a]	1.1	80.2[b]	1.1

[1]Mean of 4 roosters.

[2]Mean of 4 replicate pens of 4 chicks.

[a,b]Means within a row within sample number with no common superscripts are significantly different ($P < 0.05$).

several feed ingredient samples. It was observed in the current study that the rooster values were higher than the broiler chickens values for 2 of the 4 MBM samples. As discussed by Kim et al. (2012), the lower values for broiler chickens may be due, at least partly, to collecting ileal digesta from the entire ileal section of the small intestine. Some previous studies have shown that there is AA disappearance from the intestine as digesta moves through the ileum; thus, collecting digesta from the entire ileum may overestimate undigested AA and result in underestimation of AA digestibility (Kadim and Moughan, 1997; Kluth et al., 2005; Rezvani et al., 2008).

When comparing the AA digestibility values for all 4 MBM samples in Tables 6 and 7, both the rooster and broiler chicken ileal assay yielded much lower values for Samples 1 and 2 than Samples 15 and 16. Thus, both assays were sensitive and in agreement for detecting large differences in AA digestibility among MBM samples.

The results of this study show that the pepsin digestibility assay is valuable only for detecting large differences in protein quality among MBM when levels of 0.02 or 0.002% pepsin are used. The pepsin assay is most useful for detecting very poor-quality samples and to a lesser extent very high-quality samples. Both the precision-fed cecectomized rooster and precision-fed ileal broiler chicken assays are acceptable methods for determining and detecting differences in AA digestibility among MBM samples.

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