Standardized ileal amino acid digestibility of protein sources for broiler chickens is influenced by the feed form

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ABSTRACT The aim of present study was to determine the influence of the feed form (**FF**) on standardized ileal digestibility (**SID**) of nitrogen (**N**) and amino acids (**AA**) in 3 protein sources (**PS**) for broiler chickens. Six diets were tested in a 3 × 2 factorial arrangement of treatments involving 3 PS (meat and bone meal [**MBM**], soybean meal [**SBM**], and canola meal [**CM**]) in mash and pelleted forms. The basal endogenous N and AA losses were determined by offering a N-free diet in the mash form. From day 1 to 18, the birds were offered a broiler starter diet. The diets and the N-free diet were randomly assigned to 6 replicate cages (8 birds per cage) and fed from day 19 to 23. The ileal digesta were collected on day 23. The SID of N was higher ($P < 0.05$) in SBM followed by MBM and CM. The average SID of AA in SBM and MBM were similar ($P > 0.05$), and greater ($P < 0.05$) than that in CM. The FF had no influence ($P > 0.05$) on the SID of indispensable AA, the only exception being His, which was reduced ($P < 0.05$) by pelleting. Pelleting, however, resulted in reduction ($P < 0.001$) in the SID of all dispensable AA and average of AA. The AA most affected by pelleting was Cys, with a 15.4% decrease in the SID. The standardized ileal digestible contents of protein and the average of indispensable AA and dispensable AA were higher in MBM than in SBM, with CM being the lowest. Pelleting decreased ($P < 0.05$) the digestible protein and total digestible AA contents. These findings reveal that the FF has a substantial impact on AA digestibility estimates of feed ingredients and it must be considered in AA digestibility assays of ingredients with high protein and AA contents.

Key words: Amino acids, Broiler chickens, Digestibility, Feed form, Protein sources

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INTRODUCTION

Intensive selection and breeding programs have resulted in contemporary broilers that are 400% more efficient than the strains grown in 1956 (Zuidhof et al., 2014). Today, broilers can reach a BW of 2.5 kg in 35 d, with a feed conversion ratio of 1.5. Feed is the greatest single-cost item in broiler production, representing about 70% of the total production cost. A precise knowledge of the amino acid (**AA**) digestibility in ingredients is crucial for the formulation of cost-effective and efficient diets. The use of excreta-based digestibility measurements for determining nitrogen (**N**) and AA availability is questionable because of the utilization of undigested dietary protein by hindgut microflora, the addition of microbial proteins to AA excretion in the excreta, and co-voiding of urinary N with feces (Ravindran and Bryden, 1999). In the ileal digestibility assay, these confounding issues are avoided. Ileal AA digestibility values are referred to as either apparent or standardized (or true). The standardized ileal digestibility (**SID**) involves a correction for the inevitable basal endogenous AA (**EAA**) losses from the gastrointestinal tract (Lemme et al., 2004). It has been documented that the SID AA values are more additive than the apparent ileal digestibility (**AID**) values in terms of accuracy of feed formulation (Kong and Adeola, 2013; Cowieson et al., 2019, 2020).

Limited evidence suggests that pelleting influences protein and AA digestibility in broiler chickens (Svihus and Zimonja, 2011; Abdollahi et al., 2010, 2011, 2013). Hydrothermal processing may improve protein digestibility by denaturing and dissociation of the protein structure (Camire, 1991; Thomas et al., 1998; Ludikhuyze et al., 2003). On the contrary, protein denaturation can also delay the digestion of proteins through reduction in protein solubility (Camire, 1991) because solubilization is the essential first step of protein digestion (Cowieson and Ravindran, 2008). A combination

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of high temperature, shear forces, and moisture during hydrothermal processing favors the Maillard product formation (Mauron, 1981; Cheftel, 1986) between free amino groups of certain AA residues, mainly ε-amino group of Lys, and free aldehyde groups of reducing sugars such as glucose and lactose (Martins et al., 2000). Maillard reactions result in enzymatically undegradable end products, leading to a reduction in protein and AA availability. Report indicates that hydrothermal processing may also degrade heat-labile AA including Cys, Lys, Arg, and Thr (Camire et al., 1990).

A large volume of published data exists on the ileal digestible AA content of feed ingredients for broiler chickens (Lemme et al., 2004; Bryden et al., 2009). These evaluations have been accomplished using mash diets because research facilities often do not have access to pelleting equipment. Commercially, however, broiler chickens are fed pelleted diets and the applicability of data generated using mash diets to pelleted diets can be questioned. In addition, available data on the effects of pelleting on protein digestibility have been derived using complete diets rather than a single ingredient. Based on the above data, it was hypothesized that broiler chickens fed the same ingredient, but in a different feed form (**FF**), will show different AA digestibility values. Consequently, the current experiment was designed to evaluate the influence of the FF (mash vs. pellet) on the SID of **N** and AA of 3 protein sources (**PS**), that are, soybean meal (**SBM**), canola meal (**CM**), and meat and bone meal (**MBM**) in broiler chickens.

MATERIALS AND METHODS

The experimental procedure was in accordance with the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing, and teaching, approved by the Massey University Animal Ethics Committee.

Diets and Experimental Design

The experimental design was a 3 × 2 factorial arrangement of treatments involving 3 PS (SBM, MBM, and CM) in 2 FF (mash vs. pellet). The PS ingredients were obtained from a commercial supplier. Three treatment diets containing different inclusion rates of each PS, as the only source of AA in the diet, and corn starch were formulated to contain about 180 g/kg dietary protein (Table 1; Ravindran et al., 2017). After mixing, each diet was divided into 2 equal batches. One batch was offered as mash, and the other was pelleted. For pelleting, the diets were steam-conditioned at 70°C for 30 s using a pellet mill (Richard Size Limited Engineers, Orbit 15, Kingston upon Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring of 3-mm holes and 35-mm thickness.

Basal EAA losses were measured using a N-free diet in the mash form for the calculation of the SID (Table 1). Titanium dioxide (5 g/kg; Merck KGaA, Darmstadt, Germany) was added to all diets as an indigestible marker.

Table 1. Composition of the basal diets (g/kg, as-received basis) used in the ileal amino acid digestibility assay.

Ingredients	MBM	SBM	CM	NFD
Corn starch	546	517	377	842
Test ingredient	383	413	553	-
Soybean oil	30	30	30	50
Solka-Floc (cellulose)	30	-	-	50
Dicalcium phosphate	-	19	19	19
Limestone	-	10	10	13
Dipotassium phosphate	-	-	-	12
Titanium dioxide	5.0	5.0	5.0	5.0
Sodium chloride	2.0	2.0	2.0	2.0
Sodium bicarbonate	2.0	2.0	2.0	2.0
Trace mineral premix[1]	1.0	1.0	1.0	3.0
Vitamin premix[1]	1.0	1.0	1.0	2.0

Abbreviations: CM, canola meal; MBM, meat and bone meal; NFD, nitrogen-free diet; SBM, soybean meal.

[1]Supplied per kg of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 0.2 mg; Zn, 60 mg.

Birds and Housing

A total of 336, one-day-old male broiler chickens (Ross 308) were obtained from a commercial hatchery, raised in floor pens, and fed a commercial broiler starter crumble (AMEn, 3,010 kcal/kg; CP, 230 g/kg) until day 18. On day 18, all birds were weighed and assigned to 42 cages (36 cages for 6 dietary treatments having 6 replicates, and 6 cages for the N-free diet), each housing 8 birds so that the average bird weight per cage was similar. The test diets were offered for 4 d (from day 19–23), and birds had ad libitum access to test diets and water. The feed intake (**FI**) was recorded on a cage basis from day 19 to 23.

The floor pens and grower cages on wire floors were housed in environmentally controlled rooms with 20 h of fluorescent illumination per day. The average temperature was 31°C during the first week and was gradually reduced to 23°C by 21 d of age.

Determination of Ileal Nutrient Digestibility

All birds were euthanized by intravenous injection (0.5 mL per kg BW) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand) on day 23. Digesta were collected from the lower half of the ileum, as described by Ravindran et al. (2005). The ileum was considered as the portion of the small intestine from Meckel's diverticulum to a point about ∼40 mm proximal to the ileocecal junction. The ileal digesta were collected from all birds into a plastic container by gentle flushing with distilled water, pooled within cage, and stored at -20°C until they were freeze-dried (Model 0610, Cuddon Engineering, Blenheim, New Zealand). The diet and freeze-died digesta samples were ground to pass through a 0.5-mm sieve and stored in air-tight plastic containers at 4°C until the analysis of DM, titanium (**Ti**), N, and AA.

Chemical Analysis

The DM was measured using the standard procedure (Method 930.15; AOAC, 2016). The Ti was determined on a UV spectrophotometer following the method of Short et al. (1996). The N was analyzed by combustion (Method 968.06; AOAC, 2016) using a CNS-200 carbon, N, sulfur analyzer (LECO Corporation, St. Joseph, MI) with CP content calculated as N × 6.25. The neutral detergent fiber (**NDF**) was measured (Method 2002.04; AOAC, 2016) by using Tecator Fibertec (FOSS Analytical AB, Höganäs, Sweden). The gross energy was determined by an adiabatic bomb calorimeter (Gallenkamp autobomb, London, UK) standardized with benzoic acid. Crude fat was measured using a Soxhlet extraction procedure (Method 2003.06; AOAC, 2016). Ash was measured by a standard procedure (Method 942.05; AOAC, 2016) using a muffle furnace at 550°C for 16 h. For mineral analysis, the samples were wet-digested in a mixture of nitric and perchloric acids. The concentrations of calcium and phosphorus were measured by inductively coupled plasma-optical emission spectroscopy using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).

Amino acids were determined in an AA analyzer (Biochrom, version 30+, Biochrom Ltd., Cambridge, UK). In brief, samples were hydrolyzed with 6 N HCl (containing phenol) for 24 h at 110 ± 2°C in glass tubes sealed under vacuum. The AA were then detected on a Waters' ion-exchange HPLC system, and the chromatograms were integrated using the manufacturer's software with AA simultaneously detected at 570 and 440 nm. Cys and Met were determined as cysteic acid and methionine sulfone, respectively, by oxidation with performic acid for 16 h at 0°C and neutralization with hydrobromic acid before hydrolysis.

Calculations

Data were expressed on a DM basis for calculations. The AID (%) of nutrients were calculated from the dietary ratio of nutrient to Ti relative to the corresponding ratio in the ileal digesta using the following formula.

$$\text{AID of nutrient} = \left[\left[(\text{nutrient}/\text{Ti})_d - (\text{nutrient}/\text{Ti})_i\right] / (\text{nutrient}/\text{Ti})_d\right] \times 100$$

Where, $(\text{nutrient}/\text{Ti})_d$ = ratio of nutrient to Ti in the diet, and $(\text{nutrient}/\text{Ti})_i$ = ratio of nutrient to Ti in the ileal digesta.

The basal ileal EAA losses from birds fed the N-free diet were calculated as mg of AA flow per kg of the DM intake (Moughan et al., 1992).

$$\text{Basal EAA flow (mg / kg DM intake)} = \text{AA concentration in ileal digesta (mg / kg)} \times [\text{diet Ti (mg / kg) / ileal digesta Ti (mg / kg)}]$$

The apparent digestibility data for N and AA were standardized by using the basal EAA flow.

$$\text{SID (\%)} = \text{AID (\%)} + [\text{basal EAA (mg / kg DM intake) / Ing. AA (mg / kg DM)}]$$

Where, SID = standardized ileal digestibility of the AA; AID = apparent ileal digestibility of the AA; basal EAA = basal endogenous AA loss, and Ing. AA = concentration of the AA in the ingredient.

Data Analysis

Data were analyzed by two-way analysis of variance using the GLM procedure of SAS (version 9.4; 2015; SAS Institute, Cary, NC) to determine the main effects (PS and FF) and their interaction. The cages were the experimental units. Differences were deemed significant when $P \leq 0.05$, and a P-value between 0.05 and 0.10 was considered as a trend. The least significant difference test was used to compare means.

RESULTS

Proximate and Nutrient Compositions

The CP content was higher in MBM (644 g/kg), followed by SBM (484 g/kg) and CM (356 g/kg) (Table 2). The CM contained higher content (250 g/kg) of NDF than SBM (84.6 g/kg). The total AA (**TAA**) content was higher in MBM (537 g/kg) followed by SBM (416 g/kg) and CM (290 g/kg). The contents of total indispensable AA (**IAA**) and total dispensable AA (**DAA**) followed the same pattern, with MBM being the highest content. Among IAA, Arg, Leu, Lys, and Val were present in the greatest concentrations, whereas His, Met, and Trp were the AA with the lowest concentrations, regardless of PS. The major DAA was Glu followed by Gly, Asp, and Pro, with Cys present in the least amount.

FI and DM Digestibility

Pelleting increased the FI of birds during the 4-day assay period by an average of 198 g/bird (527 vs. 329) (Table 3). However, the magnitude of the response to pelleting was greater in SBM (86.3%) than in CM (48.8%) and MBM (50.2%), resulting in a significant ($P < 0.001$) PS × FF interaction. The highest FI was recorded in the pelleted CM diet and the lowest in SBM- and MBM-based mash diets.

There was a significant ($P < 0.001$) main effect of PS on the AID of DM with higher digestibility in birds fed MBM- and SBM-based diets than diets based on the

Table 2. Proximate and amino acid composition of the protein sources (g/kg, as-received basis).

Item	MBM	SBM	CM
DM	945	893	891
Nitrogen (N)	103	77.4	56.9
CP (N × 6.25)	644	484	356
Crude fat	142	12.3	48.5
NDF	-	84.6	250
GE (kcal/kg)	5,064	4,228	4,252
Ash	149	65.4	72.5
Calcium	41.3	3.26	5.78
Phosphorus	23.3	6.61	10.5
Indispensable amino acids (IAA)			
Arg	40.3	33.8	20.4
His	13.3	11.9	8.88
Ile	21.8	20.3	13.5
Leu	44.1	35.2	23.7
Lys	38.7	28.9	19.2
Met	11.8	6.36	6.83
Thr	22.8	18.1	14.6
Trp	6.30	6.36	4.81
Val	30.6	21.9	17.9
Total IAA	230	183	130
Dispensable amino acids (DAA)			
Ala	41.2	20.2	15.1
Asp	49.5	52.7	24.3
Cys[1]	7.0	6.94	7.86
Glu	77.9	83.9	58.9
Gly[1]	61.9	19.5	17.2
Pro	45.4	25.9	22.6
Ser	23.7	23.4	13.9
Total DAA	307	233	160
Total AA[2]	537	416	290

Abbreviations: AA, amino acid; CM, canola meal; GE, gross energy; MBM, meat and bone meal; NDF, neutral detergent fiber; SBM, soybean meal.
[1]Semi-indispensable amino acids for poultry.
[2]Total AA = IAA + DAA.

Table 3. Influence of the protein source and feed form on the feed intake (g/bird/4 d) and apparent ileal digestibility (%) of DM in broilers.[1]

PS	FF	Feed intake	AID of DM
Meat and bone meal	Mash	283[d]	74.5
	Pellet	425[c]	70.7
Soybean meal	Mash	293[d]	70.9
	Pellet	546[b]	69.8
Canola meal	Mash	410[c]	50.7
	Pellet	610[a]	48.6
Pooled SEM		10.1	2.71
Main effects			
PS			
Meat and bone meal		355	72.6[a]
Soybean meal		419	70.4[a]
Canola meal		510	49.6[b]
Pooled SEM		7.1	1.91
FF			
	Mash	329	65.4
	Pellet	527	63.0
Pooled SEM		5.8	1.56
Probabilities, $P \leq$			
PS		0.001	0.001
FF		0.001	0.292
PS × FF		0.001	0.875

[a-d]Means in a column not sharing a common letter are different ($P < 0.05$).
Abbreviations: AID, apparent ileal digestibility; FF, feed form; PS, protein source.
[1]Each value represents the mean of 6 replicates (8 birds per replicate).

CM. Neither the main effect of FF nor the interaction between PS and FF was significant for AID of DM.

SID of N and AA

No interactions ($P > 0.05$) between PS and FF were observed for the SID of N and AA (Table 4). The effects of PS on the SID of N and all AA, except Met and Cys, were significant ($P < 0.01$ to 0.001). The SID of N was higher in SBM (79.9%; $P < 0.001$) than in the MBM (73.2%) and CM (64.0%). Soybean meal and MBM had similar ($P > 0.05$) average SID values for IAA, which were greater ($P < 0.05$) than CM. The average SID values for DAA was higher ($P < 0.05$) in SBM than in MBM and CM. The average digestibility values for all AA (TAA; IAA + DAA) were similar in SBM and MBM and higher ($P < 0.05$) than CM. Histidine was the only IAA influenced ($P < 0.05$) by the FF, but there were tendencies for the SID of N ($P = 0.060$), Thr ($P = 0.054$), Trp ($P = 0.073$), Val ($P = 0.079$), and the average of IAA ($P = 0.089$) to be reduced by pelleting. Pelleting, however, resulted in reductions ($P < 0.05$) in the SID of all individual DAA, average digestibility of DAA and TAA.

There were no significant ($P > 0.05$) PS × FF interactions for the contents of digestible protein and AA, except Gly ($P < 0.05$; Table 5). Pelleting reduced ($P < 0.05$) the digestible Gly content in MBM compared with mash but had no effect in SBM and CM. The PS had significant ($P < 0.001$) effects on the digestible protein and AA contents, except Cys. The contents of digestible protein, individual IAA (except Ile and Trp), the total of IAA, Ala, Pro, and the total of DAA were higher ($P < 0.05$) in MBM than in SBM. The digestible contents of Asp, Glu, and Ser were greater ($P < 0.05$) in SBM than in MBM. The CM sample had the lowest ($P < 0.05$) digestible contents for protein, IAA, and DAA. The highest and lowest contents of total digestible AA were observed in MBM (397 g/kg) and CM (196 g/kg), respectively, with SBM (333 g/kg) being intermediate. Pelleting significantly ($P < 0.05$) reduced the digestible contents of protein, His and Thr, and tended ($P = 0.051$ to 0.085) to reduce the digestible content of other IAA (except Lys) and the total digestible content of IAA ($P = 0.061$). However, the FF significantly ($P < 0.05$ to 0.001) influenced the digestible contents of all individual DAA, the total of DAA and TAA, regardless of PS, with higher values in mash diets.

DISCUSSION

In general, the ash, calcium, and phosphorus contents of MBM, SBM, and CM were within the range reported in the literature (NRC, 1994; Adedokun et al., 2008, 2009; Woyengo et al., 2010; Ravindran et al., 2014).

The CP content of MBM (644 g/kg) was slightly higher than that in the previous reports (488–600 g/kg; Ravindran et al., 2002; Adedokun et al., 2007). The wide variability in the CP content of MBM may be

Table 4. Influence of the protein source and feed form on the standardized ileal digestibility[1] (%) of nitrogen and amino acids.[2].

			Indispensable amino acids										Dispensable amino acids								
PS	FF	N	Arg	His	Ile	Leu	Lys	Met	Thr	Trp	Val	IAA	Ala	Asp	Cys[3]	Glu	Gly[3]	Pro	Ser	DAA	TAA
MBM	Mash	76.0	83.0	78.8	77.9	80.7	81.7	81.2	76.2	74.9	78.5	79.2	77.9	72.5	65.9	79.1	71.6	73.1	75.5	73.7	76.7
	Pellet	70.3	77.8	75.7	74.9	78.1	78.5	78.4	71.8	72.1	74.7	75.8	71.3	65.9	56.8	73.8	59.6	62.7	69.9	65.7	71.3
SBM	Mash	82.5	88.7	84.6	82.5	83.2	84.3	85.4	77.3	80.7	82.0	83.2	81.9	80.5	74.6	86.5	77.9	83.2	83.2	81.1	82.1
	Pellet	77.3	85.7	78.6	76.8	78.1	81.8	79.9	68.9	72.8	76.0	77.6	75.6	72.7	58.9	81.2	69.9	76.0	76.2	72.9	75.2
CM	Mash	64.8	77.1	73.8	63.4	70.0	64.3	77.4	58.5	63.9	64.2	68.1	68.3	60.7	63.7	79.1	63.9	63.8	63.4	66.1	67.4
	Pellet	63.3	78.2	71.4	62.1	68.2	63.2	76.3	56.1	62.7	62.8	66.8	66.1	57.1	57.2	77.1	60.4	61.3	60.1	62.8	65.1
Pooled SEM		2.59	1.93	2.13	2.54	2.33	2.39	2.26	3.09	2.59	2.53	2.38	2.71	2.76	3.46	1.85	3.08	2.72	2.68	2.70	2.52
Main effects																					
PS																					
MBM		73.2^{b}	80.4^{b}	77.2^{b}	76.5^{a}	79.4^{a}	80.1^{a}	79.8	73.9^{a}	73.5^{a}	76.6^{a}	77.5^{a}	74.6^{a}	69.2^{b}	61.4	76.5^{b}	65.6^{b}	67.9^{b}	72.7^{b}	69.7^{b}	74.0^{a}
SBM		79.9^{a}	87.2^{a}	81.6^{a}	79.7^{a}	80.7^{a}	83.1^{a}	82.6	73.1^{a}	76.8^{a}	79.0^{a}	80.4^{a}	78.8^{a}	76.6^{a}	66.8	83.9^{a}	73.9^{a}	79.6^{a}	79.7^{a}	77.0^{a}	78.7^{a}
CM		64.0^{c}	77.7^{b}	72.9^{b}	62.8^{b}	69.1^{b}	63.8^{b}	76.8	57.3^{b}	63.3^{b}	63.5^{b}	67.5^{b}	67.2^{b}	58.8^{c}	60.4	78.1^{b}	62.2^{b}	62.6^{b}	61.8^{c}	64.4^{b}	66.3^{b}
Pooled SEM		1.83	1.37	1.51	1.79	1.65	1.69	1.59	2.19	1.83	1.79	1.68	1.91	1.95	2.44	1.31	2.18	1.92	1.89	1.91	1.78
FF																					
	Mash	74.4	82.9	79.1^{a}	74.6	77.9	76.8	81.3	70.7	73.1	74.9	76.8	76.0^{a}	71.2^{a}	68.1^{a}	81.6^{a}	71.2^{a}	73.4^{a}	73.9^{a}	73.6^{a}	75.4^{a}
	Pellet	70.3	80.6	75.4^{b}	71.3	74.8	74.5	78.2	65.6	69.2	71.2	73.4	70.9^{b}	65.2^{b}	57.6^{b}	77.4^{b}	63.3^{b}	66.7^{b}	68.8^{b}	67.1^{b}	70.5^{b}
Pooled SEM		1.49	1.11	1.23	1.47	1.35	1.39	1.30	1.79	1.49	1.46	1.37	1.56	1.59	1.99	1.07	1.78	1.57	1.55	1.56	1.45
Probabilities, $P \leq$																					
PS		0.001	0.001	0.001	0.001	0.001	0.001	0.051	0.001	0.001	0.001	0.001	0.001	0.001	0.156	0.001	0.002	0.001	0.001	0.001	0.001
FF		0.060	0.148	0.044	0.117	0.104	0.255	0.101	0.054	0.073	0.079	0.089	0.030	0.013	0.001	0.009	0.004	0.005	0.023	0.006	0.025
PS × FF		0.681	0.269	0.606	0.703	0.773	0.906	0.621	0.634	0.421	0.672	0.669	0.664	0.741	0.408	0.589	0.399	0.356	0.797	0.612	0.648

[a-c]Means in a column not sharing a common letter are different ($P < 0.05$).

Abbreviations: CM, canola meal; MBM, meat and bone meal; SBM, soybean meal; FF, feed form; PS, protein source; N, nitrogen; IAA, average digestibility of indispensable amino acids; DAA, average digestibility of dispensable amino acids; TAA, average digestibility of all amino acids.

[1]Apparent digestibility values were standardized using the following basal ileal endogenous flow values (g/kg DM intake), determined by feeding a N-free diet: N, 2.8; Arg, 0.59; His, 0.27; Ile, 0.54; Leu, 0.87; Lys, 0.57; Met, 0.22; Thr, 1.04; Trp, 0.19; Val, 0.72; Ala, 0.65; Asp, 1.15; Cys, 0.42; Glu, 1.42; Gly, 0.68; Pro, 0.83; and Ser, 0.86.

[2]Each value represents the mean of 6 replicates (8 birds per replicate).

[3]Semi-indispensable amino acids for poultry.

Table 5. Influence of the protein source and feed form on the standardized digestible protein (CP) and amino acid contents[1] (g/kg, as-received basis).

			Indispensable amino acids										Dispensable amino acids								
PS	FF	CP	Arg	His	Ile	Leu	Lys	Met	Thr	Trp	Val	IAA	Ala	Asp	Cys[2]	Glu	Gly[2]	Pro	Ser	DAA	Total AA
Meat and bone meal	Mash	484	33.4	10.4	16.9	35.6	31.6	9.57	17.4	4.68	24.0	184	32.1	35.9	4.59	61.7	44.4[a]	33.2	17.9	230	413
	Pellet	448	31.3	10.0	16.3	34.4	30.4	9.24	16.4	4.51	22.9	175	29.4	32.6	3.96	57.6	36.9[b]	28.5	16.6	206	381
Soybean meal	Mash	393	29.9	10.1	16.7	29.2	24.3	5.43	14.0	5.13	17.9	153	16.5	42.4	5.18	72.6	15.2[c]	21.6	19.5	193	346
	Pellet	368	28.9	9.33	15.6	27.5	23.6	5.08	12.5	4.63	16.6	144	15.3	38.3	4.09	68.1	13.6[c,d]	19.8	17.9	177	321
Canola meal	Mash	229	15.8	6.55	8.58	16.6	12.3	5.28	8.51	3.07	11.5	88.1	10.3	14.8	5.01	46.6	10.9[d]	14.4	8.86	111	199
	Pellet	224	15.9	6.39	8.39	16.2	12.1	5.21	8.16	3.02	11.2	86.6	9.99	13.9	4.49	45.4	10.4[d]	13.9	8.39	106	193
Pooled SEM		12.08	0.59	0.235	0.439	0.74	0.60	0.173	0.539	0.147	0.55	3.94	0.684	1.09	0.251	1.36	1.25	0.90	0.515	5.81	9.66
Main effects																					
PS																					
Meat and bone meal		466[a]	32.4[a]	10.2[a]	16.7[a]	35.0[a]	30.9[a]	9.41[a]	16.9[a]	4.59[a]	23.5[a]	180[a]	30.7[a]	34.2[b]	4.28	59.6[b]	40.6	30.8[a]	17.3[b]	218[a]	397[a]
Soybean meal		380[b]	29.5[b]	9.69[b]	16.2[a]	28.3[b]	23.9[b]	5.26[b]	13.3[b]	4.88[a]	17.3[b]	148[b]	15.9[b]	40.3[a]	4.64	70.3[a]	14.4	20.7[b]	18.7[a]	185[b]	333[b]
Canola meal		226[c]	15.9[c]	6.47[c]	8.48[b]	16.4[c]	12.2[c]	5.25[b]	8.33[c]	3.04[b]	11.3[c]	87.4[c]	10.2[c]	14.3[c]	4.75	45.9[c]	10.7	14.2[c]	8.62[c]	109[c]	196[c]
Pooled SEM		8.54	0.42	0.166	0.310	0.52	0.43	0.122	0.381	0.104	0.39	2.79	0.48	0.78	0.178	0.96	0.89	0.63	0.364	4.11	6.83
FF																					
	Mash	368[a]	26.4	9.01[a]	14.1	27.2	22.8	6.76	13.3[a]	4.29	17.8	142	19.6[a]	30.9[a]	4.93[a]	60.3[a]	23.5	23.1[a]	15.4[a]	178[a]	319[a]
	Pellet	346[b]	25.4	8.58[b]	13.4	26.0	22.1	6.51	12.3[b]	4.05	16.9	135	18.2[b]	28.3[b]	4.18[b]	57.0[b]	20.3	20.7[b]	14.3[b]	163[b]	298[b]
Pooled SEM		6.97	0.34	0.136	0.25	0.43	0.35	0.099	0.31	0.085	0.32	2.28	0.39	0.63	0.145	0.78	0.72	0.52	0.29	3.36	5.58
Probabilities, $P \leq$																					
PS		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.167	0.001	0.001	0.001	0.001	0.001	0.001
FF		0.033	0.059	0.032	0.074	0.070	0.151	0.085	0.037	0.051	0.053	0.061	0.015	0.005	0.001	0.006	0.004	0.003	0.012	0.004	0.012
PS × FF		0.443	0.167	0.505	0.558	0.662	0.693	0.670	0.567	0.313	0.593	0.582	0.225	0.329	0.495	0.419	0.022	0.078	0.517	0.252	0.382

[a-d]Means in a column not sharing a common letter are significantly different ($P < 0.05$).

Standardized digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardized ileal digestibility (%)]/100.

Abbreviations: FF, feed form; PS, protein source; IAA, total digestible indispensable amino acid contents; DAA, total digestible dispensable amino acid contents; total AA, total digestible content of all amino acids.

[1]Each value represents the mean of 6 replicates (8 birds per replicate).

[2]Semi-indispensable amino acids for poultry.

attributed to the variation in the source of raw materials (Karakas et al., 2001; Adedokun et al., 2007). In the case of SBM, the CP content (484 g/kg) was similar to the value of 485 g/kg documented by NRC (1994) and close to the range (464–482 g/kg) reported by Ravindran et al. (2014). A number of factors including production method, hull inclusion, and growing conditions influence the nutrient content of SBM (Mateos et al., 2018). The CP content of CM (356 g/kg) was within the range (266 to 394 g/kg) reported by Bryden et al. (2009). The contents of IAA, DAA, and TAA in the 3 PS assayed in the present study approximated the published values (Ravindran et al., 2005, 2014; Bryden et al., 2009; Woyengo et al., 2010; Kim et al., 2012). The AA composition of ingredients of the plant origin can be influenced by the country of origin, variation in the planting season, cultivar, and harvesting practices (Evers et al., 1999).

Feeding pelleted diets increases the FI, which is the major driver of weight gain in broiler chickens (Svihus et al., 2004; Abdollahi et al., 2018a,b). However, there is a wide variation in improvement of the FI because of pelleting, with increases ranging from 2.8% (Serrano et al., 2012) to 64% (Amerah et al., 2007) being reported. Predictably, in the present study, birds offered pelleted diets showed different extents of increased FI in the 3 PS compared with those fed mash diets, with a significant interaction between the PS and FF.

The higher SID of N in SBM than in MBM and CM in the present study agrees with the findings of Lemme et al. (2004), who reported higher SID of CP in SBM (90.0%) than in CM (76.0%) and MBM (65.0%) in broiler chickens. The MBM evaluated in the current work was rendered from slaughter by-products of cattle and sheep, but the average SID of AA for MBM (74.0%) of this sample was higher than the value (63.2%) reported by Adedokun et al. (2007), with MBM of cattle origin. The findings in the current work were consistent with the wide variation (62.0–82.0%) reported for the SID AA of MBM in the literature (Adedokun et al., 2009, 2014). The average SID of AA for SBM (78.7%) in the present study was comparable to previous findings (79.2–85.0%, Ravindran et al., 2014; 79.9%, Cowieson et al., 2019) and the value for CM (66.3%) was lower than that (79.0%) reported by Adedokun et al. (2008) and Kim et al. (2012).

The higher average SID of all AA in MBM and SBM than in CM in the present study was expected. Among plant-based PS, SBM is the preferred choice in poultry feed formulations as it has high CP (485 g/kg), more consistent AA profile, and a high AA digestibility (Ravindran et al., 2014). Meat and bone meal also contains higher amount of CP (about 550 g/kg), although a wide variation in terms of protein quality (AA composition and digestibility) exists in it because of the differences in rendering conditions and methods, and variation in raw material inputs, such as species and proportion of different offal components (Skurray, 1974; Parsons et al., 1997; Ravindran et al., 2002). Adedokun et al. (2008), in a study with 21-day-old broiler chickens, reported a higher SID AA for SBM (87.3%) than for CM (79.8%), which is coherent with present findings. In another study by Ravindran et al. (2005), a higher AID was reported for SBM (82.0%), with a lower value for CM (78.0%).

Lower AA digestibility in CM can be attributed to several reasons and the primary factor being its high fiber content (Janssen and Carré, 1985; Bell, 1993). The content of fiber in canola seeds is higher than in soybean seeds (159 vs. 60 g/kg; Sauer et al., 1982), and this is reflected in the fiber content of CM (Newkirk, 2009). The content of NDF in CM ranges from 220 to 300 g/kg (Maison, 2013), and the NDF (250 g/kg) content of CM used in the present study was within this range. Canola meal contains a higher content (4.0–7.78 g/kg) of phytate P than SBM (3.54–4.53 g/kg; Selle and Ravindran, 2007). The negative influence of phytate on protein digestion and absorption is now well documented (Selle and Ravindran, 2007). In addition to forming indigestible complexes with protein, phytate can influence the gut capacity for transportation of Na-dependent nutrients including AA by changing Na partitioning (Cowieson et al., 2009). Canola meal also contains a higher content of lignin with associated polyphenols (Khajali and Slominski, 2012), which can bind with proteins and lower digestibility. According to Newkirk and Classen (2002), the removal of some AA during the desolventization and toasting stage of prepress solvent extraction of canola seeds may also have contributed to the lower digestibility.

A high conditioning temperature during pelleting process has been shown to reduce the digestibility of CP in wheat SBM– and corn SBM–based diets (Abdollahi et al., 2011, 2013). Loar et al. (2014) reported 3 to 5% reduction in the digestibility of some AA in a corn SBM–based diet when the conditioning temperature increased from 74 to 85 and 96°C. Despite a moderate conditioning temperature (70°C) in the present study, among IAA, pelleting reduced the SID of His compared with mash and tended to reduce those of N, Thr, Trp, Val, and average of IAA. Pelleting also reduced the SID of all DAA, average DAA by 8.8%, and average TAA by 6.5% compared with mash. Cysteine was the most affected DAA, with 15.4% reduction in the SID because of pelleting, followed by 11.1% in Gly, 9.1% in Pro, 8.4% in Asp, 6.9% in Ser, and 5.1% in Glu. Heat susceptibility of Cys has long been known (Evans and McGinnis, 1948). Papadopulos (1989) found that the most heat-labile AA was Cys followed by Lys, Arg, Thr, and Ser after autoclaving at 110 to 130°C. The present findings confirm that Cys digestibility is deteriorated during the pelleting process. Shirley and Parsons (2000) found that the processing pressure applied during or after the rendering process caused the greatest negative influence on the digestibility of Cys, compared with other AA, in MBM. It has been documented that, in the absence of reducing substances, degradation of Cys during heat processing can form dehydroalanine by the breaking of disulfide bonds. Dehydroalanine can form either lanthionine by reacting with rest of Cys or lysinoalanine by reacting with ε-amino groups of Lys (Bohak, 1964; Papadopulos, 1989). Amino acid derivatives such as lysinoalanine and lanthionine formed during heating process have been reported to be poorly digestible (Gilani et al., 2005).

The lower SID of AA in pelleted diets in the present study might also be the result of a higher FI. Overconsumption followed by overload of starch in birds fed pelleted diets have been shown to reduce the digestibility of starch (Abdollahi et al., 2013, 2018b) through a faster passage rate; this might also apply to AA in high-protein ingredients. Engberg et al. (2002) observed lower pancreas weights and lower activities (units/100 g BW) of amylase, lipase, trypsin, and chymotrypsin in pellet-fed birds than those fed mash diet. A decreased SID AA in pellet-fed birds in the present study may also be explained by the reduced activities of proteolytic enzymes. In addition, the lower digestive organ weights induced by feeding pelleted diets might shorten the digesta retention time, compromising the digestion and absorption of nutrients (Frikha et al., 2009; Naderinejad et al., 2016; Abdollahi et al., 2018b).

The gizzard pH is variable depending on the FI, retention time, and the chemical characteristics of feed (Svihus, 2011). The proteolytic enzyme pepsin is secreted as a precursor called pepsinogen by the chief cells in the proventriculus. Pepsin is autoactivated in the acidic environment and then initiates the breakdown of dietary proteins into peptides. Because poultry feeds have a pH close to neutral, a higher FI may result in elevation of the gizzard pH if the gastric juice is not secreted in accordance. Previous studies have documented a higher gizzard pH with the pelleted diet than with the mash diet (Engberg et al., 2002; Naderinejad et al., 2016), with potential deleterious effects on protein digestion.

Pelleting reduced the digestible CP contents by 6.0% (346 vs. 368 g/kg) compared with mash. Protein solubilization in the gastrointestinal tract is the initial step in protein digestion (Cowieson and Ravindran, 2008). Generally, the intact protein in feed ingredients is in the stable form with a 3-dimensional configuration (Davis and Williams, 1998). Although the hydrothermal processing induces some degree of protein denaturation and may enhance protein digestibility, the conditions such as the pressure, moisture added during thermal treatment, temperature, and time of processing determine the eventual impact (Svihus and Zimonja, 2011). The hydrophobic AA that are inward oriented in their native form are turned outward during the denaturation, which might reduce protein solubilization (Araba and Dale, 1990; Camire, 1991) and compromise protein digestion. Several factors are associated with the reduction of digestible AA contents in the pelleted diet. Maillard reaction during feed processing might be a possible factor contributing to reduced AA availability. As discussed by Cheftel (1986), favorable conditions for Maillard reaction are high temperature (>180°C) and low moisture (<15%) combined with >100 rpm shear. However, pelleting involves conditioning of dry feed by adding steam that increases the moisture level by up to 3 to 4% units and a temperature rise to 85°C to 90°C. Subsequently the feed is passed through die holes and friction in these holes and the friction by rolls which force the material into the holes produce a further rise in the temperature (Svihus et al., 2004). Svihus and Zimonja (2011), however, speculated that the conditions of commercial pelleting are too mild to cause Maillard reactions. Lysine is the AA most affected by the Maillard reaction, but the SID of Lys was unaffected by pelleting in the present study.

Hydrothermal treatment of the feed has also been suggested to degrade Cys, Lys, Arg, Thr, and Ser, reducing their digestibility (Camire et al., 1990). These observations are in line with the present findings of reductions or tendency to reduce the digestible contents of most IAA (except Lys), all DAA, and TAA in pelleted PS compared with mash.

CONCLUSIONS

This is the first report demonstrating that the FF has a substantial impact on digestibility estimates of AA, particularly DAA, in high-protein feed ingredients for broiler chickens, and therefore, it should be considered in AA digestibility assays. The reduction of AA digestibility in pelleted assay diets may be due partly to the impact of hydrothermal processing. However, other factors induced by pellet-feeding such as feed overconsumption, AA overload, shorter digesta retention time, and an elevated gizzard pH might be even more influential. The present findings question the validity of using AA digestibility data that have been derived using mash diets for commercial broiler chickens' formulations where feed is generally offered in the pelleted form. Application of AA digestibility data generated using mash diets can overestimate the availability of AA in PS for broiler chickens, considerably affecting the precision of feed formulation and consequent growth performance.

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DISCLOSURES

The authors declare no conflicts of interest.

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