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Standardized Ileal Digestibility of Amino Acids in Feed Ingredients for Broiler Chickens

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ABSTRACT The objective of this experiment was to determine the apparent (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in five feed ingredients for male broiler chickens (Ross 308). The test feed ingredients consisted of corn, wheat, soybean meal (SBM), canola meal (CM), and corn distillers dried grains with solubles (DDGS). The birds were fed a standard starter diet for the first 19 d. On day 19, nine hundred sixty birds were assigned to six dietary treatments with eight replicate cages (20 birds/cage) in a randomized complete block design. All experimental diets contained chromic oxide (0.5% in the diet) as an indigestible index. The diets were offered *ad libitum* for 4 d. At the end of the experiment, all birds were euthanized and ileal digesta samples were collected immediately. The indispensable AA with the least AID and SID were Thr for all the ingredients used in the present study, except for DDGS, in which Lys had the lowest digestibility. For indispensable AA, the AID and SID of Leu, Phe, Arg, and Met were the greatest in corn, DDGS, wheat, SBM, and CM, respectively. The results from the present study showed that there are variations in both the AID and SID of CP and AA among feed ingredients.
(Key words: amino acid, ileal digestibility, nitrogen, feed ingredient, broiler)

INTRODUCTION

Adequate supplies of dietary protein and amino acids (AA) is required to achieve optimum growth performance of commercial broiler chickens. The AA digestibility can vary due to the anti-nutritional factor and chemical composition in feed ingredients. Therefore, the use of digestible AA has been recognized as more appropriate rather than total AA when formulating broiler diets (Lemme et al., 2004). In poultry, the AA digestibility values have been determined using excreta collection method with the cecectomized roosters (NRC 1994). Previous reports (Ravindran et al., 1999; Lemme et al., 2004; Ravindran et al., 2005; Kong and Adeola, 2014) have demonstrated that the risk of deamination by microbiota in the cecum because it leads to inaccuracy of AA digestibility for birds. Therefore, it has been suggested that ileal digestibility is more appropriate value for AA digestibility of broilers than total tract digestibility (Ravindran et al., 1999).

In addition, the digestible AA contents in feed ingredients can be obtained from either apparent (AID) or standardized ileal digestibility (SID). The SID of AA is calculated by the correction of the basal endogenous losses (BEL) of AA for AID. The BEL of AA are related to the dry matter intake and the relative contribution of the BEL of AA to total ileal outflow can be influenced by the AA contents. Therefore, the AID of AA increases as AA intake increase because the relative contribution of BEL of AA to total ileal AA outflow decreases with increasing AA intake. On the other hand, the SID of AA are independent on the AA intake. This may influence additivity which is the fundamental assumption of diet formulation. Feed ingredients used for poultry diets vary CP and AA contents, thus to accurately formulate poultry diet based on digestible AA contents, the use of SID of AA is recommended rather than AID.

A number of studies (Lemme et al., 2004; Ravindran et al., 2005; Huang et al., 2006; Adodokun et al., 2009; Szczurek, 2009; Kong and Adeola, 2013) for determining AA digestibility of corn and soybean meal (SBM) in poultry diets have been reported. However, there is scarce information on standardized ileal AA digestibility of diverse feed ingredients. Therefore,

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the objective of the present study was to determine the AID and SID of CP and AA in five feed ingredients of male broiler chickens.

MATERIALS AND METHODS

The experimental procedure was approved by the Institutional Animal Care and Use Committee at Konkuk University (KU16025).

1. Experimental Diets

Five feed ingredients including corn, wheat, corn distillers dried grains with solubles (DDGS), SBM, canola meal (CM) were used for digestibility study (Table 1). Inclusion rate of test ingredients was adjusted to contain approximately 20% CP in the experimental diet except for corn and wheat. An N-free diet was also prepared to estimate the BEL of CP and AA. All diets contained 1% calcium and 0.45% non-phytate P. Chromic oxide (0.5%) was added in all diets as an indigestible index for the calculation of digestibility. Vitamins and trace minerals were included in all diets to meet or exceed nutrient requirement estimates for broilers (NRC, 1994).

2. Animal and Management

Nine hundred sixty Ross 308 male broiler chicks (674±4.5 g) were fed a standard starter diet from d 0 to 19. On d 19, chicks were individually weighed and assigned to 6 treatments with 8 replicate cages (20 birds /cage) in a randomized

Table 1. Ingredients and chemical composition (%) of the experimental diets (as-fed basis)[1]

Item	Corn	Wheat	SBM	CM	DDGS	N-free
Test feed ingredient	94.82	93.03	41.24	52.63	73.00	-
Corn starch	-	-	49.90	39.39	18.67	28.91
Sucrose	-	-	-	-	-	55.00
Soybean oil	-	2.00	4.00	4.00	4.00	4.00
Calcium phosphate	1.78	1.57	1.70	1.37	0.79	2.20
Limestone	2.00	2.00	1.76	1.21	2.14	1.70
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	1.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.40	0.40	0.40	0.40	0.40	-
Cellulose	-	-	-	-	-	5.00
Choline chloride	-	-	-	-	-	0.25
Sodium bicarbonate	-	-	-	-	-	0.75
Potassium chloride	-	-	-	-	-	0.30
Magnesium oxide	-	-	-	-	-	0.09
Potassium carbonate	-	-	-	-	-	0.30
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated composition (%)						
Crude protein	8.1	13.1	20.0	20.0	20.0	-
Apparent metabolizable energy (kcal/kg)	3,176	2,865	3,372	2,991	2,905	3,365
Calcium	1.00	1.00	1.00	1.00	1.00	1.00
Non-phytate phosphorus	0.45	0.45	0.45	0.45	0.45	0.45

Table 1. Continued

Item	Corn	Wheat	SBM	CM	DDGS	N-free
Total amino acids						
Arg	0.4	0.6	1.3	1.1	0.7	-
His	0.2	0.3	0.5	0.5	0.5	-
Ile	0.3	0.4	0.8	0.7	0.7	-
Leu	0.9	0.8	1.4	1.3	1.6	-
Lys	0.2	0.3	1.1	10.2	0.5	-
Met	0.2	0.2	0.3	0.4	0.4	-
Met + Cys	0.3	0.5	0.5	0.8	0.7	-
Phe	0.4	0.6	0.9	0.8	0.9	-
Phe + Tyr	0.6	1.0	1.7	1.3	1.4	-
Thr	0.3	0.4	0.7	0.8	0.7	-
Trp	0.1	0.1	0.3	0.2	0.1	-
Val	0.0	0.5	0.9	0.9	0.9	-

[1] SBM, soybean meal; CM, canola meal; DDGS, corn distillers dried grains with solubles.
[2] Provided per kilogram of diet: vitamin A, 18,000 IU; vitamin D_3, 8,000 IU; vitamin E, 100 IU; vitamin K_3, 5.0 mg; vitamin B_1, 4.0 mg; vitamin B_2, 12.0 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 26 µg; niacin, 100 mg; folic acid, 3 mg; biotin, 0.2 mg; panthothenic acid, 30.0 mg; Mn, 192 mg as manganese sulfate; Zn, 180 mg as zinc sulfate; Fe, 100 mg as ferrous sulfate and ferric oxide; Cu, 48.0 mg as copper sulfate; I, 2.4 mg as calcium iodate; and Se, 0.72 mg as sodium selenite.

complete block design using the Experimental Animal Allotment Program (Kim and Linderman, 2007). Experimental diets and water were offered *ad libitum* from d 19 to 23, and temperature was maintained at 28℃ during this period.

3. Sample Collection

At the end of the experiment (d 23), all birds were euthanized by CO_2 asphyxiation. The ileal digesta from the lower two-thirds of the ileum were collected and rinsed with distilled water into plastic containers. Collected samples were immediately stored at 20℃ until chemical analysis.

4. Chemical Analysis

The test feedstuffs and diets samples were dried at 100℃ for 24 h and ileal digesta samples were freeze-dried. Dried test feedstuffs, diets, and ileal digesta samples were analyzed for N by Kjeldahl procedure using a Kjeltec Auto System (Kjeltec Auto System, Buchi, Flawii, Switzerland). The samples of ingredient, diets, and ileal digesta were analyzed for the AA (AOAC International, 2005; method 982.30).

5. Ileal Digestibility Calculation

Ileal digestibility and BEL of nutrients was calculated described by Kong an Adeola (2014):

$$\text{AID (\%)} = 100 - [(\text{Index}_{\text{feed}} \times \text{N}_{\text{digesta}}) / (\text{Index}_{\text{digesta}} \times \text{N}_{\text{feed}})] \times 100$$

$$\text{BEL (g/kg of DM intake)} = (\text{Index}_{\text{feed}} / \text{Index}_{\text{digesta}}) \times \text{N}_{\text{digesta}}$$

$$\text{SID (\%)} = \text{AID} + [(\text{BEL} / \text{N}_{\text{feed}}) \times 100]$$

where $\text{Index}_{\text{feed}}$ and $\text{Index}_{\text{digesta}}$ are the index concentration of feed and digesta, respectively (g/kg); N_{feed} and $\text{N}_{\text{digesta}}$ are the nutrient concentration of feed and digesta, respectively (g/kg).

6. Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) as a randomized complete block design. The experimental unit was a cage and the fixed effect was treatments. Digestibility values were presented as least squares means and compared using Tukey's test. The significance was set at an alpha-level of 0.05.

RESULTS AND DISCUSSION

Tables 2 and 3 show analyzed CP and AA concentration in 5 test ingredients and experimental diets used in the present study, respectively. The analyzed CP content was the greatest in SBM (45.97%) and least in corn (7.33%). The SBM has the greatest analyzed concentration for most of AA. The analyzed AA concentrations in feed ingredients were within the range of previously reported values (Evonik, 2010; Rostagno et al., 2017). The analyzed CP and AA concentrations in the experimental diets were close to the calculated values.

Table 4 presents the AID of CP and AA for broilers fed experimental diets. The indispensable AA with the least AID was Thr for all the ingredients except for DDGS in which Lys has the least AID. The AID of Leu was greatest indispensable AA in corn and DDGS, Phe in wheat, Arg in SBM, and Met in CM and DDGS. The digestible CP and AA contents in feed ingredients derived from *in vivo* digestibility trial do not always match with total contents derived from chemical analyses (Lemme et al., 2004; Kong et al., 2014; Block and Dekker, 2017). Therefore, the information on the digestible AA content in the feed ingredients is needed for accurate diet formulation. In the present study, 2 cereal grains have similar indispensable AA composition but the AID of AA in corn was greater (P<0.05)

Table 2. Analyzed crude protein (CP) and amino acids (AA) composition (%) in feed ingredients used in this experiment[1]

Item	Corn	Wheat	SBM	CM	DDGS
CP	7.33	9.91	45.97	35.62	27.39
Indispensable amino acids					
Arg	0.41	0.42	3.22	1.88	1.04
His	0.25	0.20	1.18	0.83	0.59
Ile	0.28	0.27	2.06	1.08	0.77
Leu	0.93	0.64	3.47	2.32	2.94
Lys	0.27	0.27	2.72	1.79	0.72
Met	0.18	0.14	0.62	0.62	0.46
Phe	0.38	0.42	2.32	1.26	1.15
Thr	0.31	0.29	1.82	1.47	0.95
Val	0.39	0.35	2.26	1.39	1.05
Dispensable amino acids					
Ala	0.57	0.34	1.96	1.47	1.81
Asp	0.54	0.47	5.10	2.31	1.59
Cys	0.19	0.22	0.63	0.82	0.50
Glu	1.47	2.57	8.04	5.53	4.07
Gly	0.48	0.38	1.89	1.65	1.00
Pro	0.72	0.90	2.27	1.94	1.90
Ser	0.38	0.46	2.26	1.45	1.23
Tyr	0.34	0.25	2.65	0.82	0.84

[1] SBM, soybean meal; CM, canola meal; DDGS, corn distillers dried grains with solubles.

Table 3. Analyzed crude protein (CP) and amino acid (AA) composition (%) of experimental diets

Item	Corn	Wheat	SBM	CM	DDGS
CP	7.11	9.24	20.27	19.69	20.95
Indispensable amino acids					
Arg	0.30	0.40	1.28	0.95	0.75
His	0.20	0.20	0.47	0.44	0.44
Ile	0.19	0.26	0.67	0.53	0.55
Leu	0.82	0.64	1.44	1.21	2.14
Lys	0.20	0.26	1.10	0.90	0.51
Met	0.12	0.12	0.22	0.32	0.32
Phe	0.33	0.44	0.94	0.68	0.86
Thr	0.27	0.30	0.77	0.79	0.74
Val	0.29	0.35	0.71	0.70	0.77
Dispensable amino acids					
Ala	0.48	0.34	0.81	0.77	1.33
Asp	0.44	0.47	2.09	1.22	1.22
Cys	0.17	0.21	0.27	0.45	0.40
Glu	1.23	2.53	3.33	2.96	3.12
Gly	0.26	0.37	0.77	0.86	0.74
Pro	0.66	0.88	0.99	1.10	1.51
Ser	0.38	0.47	1.01	0.81	0.96
Tyr	0.23	0.23	0.59	0.43	0.63

[1] SBM, soybean meal; CM, canola meal; DDGS, corn distillers dried grains with solubles.

Table 4. Apparent ileal digestibility (%) of crude protein and amino acids in the experimental diets[1]

Item	Experimental diets					SEM[2]	*P*-value
	Corn	Wheat	SBM	CM	DDGS		
Crude protein	78.1^{b}	73.1^{c}	84.0^{a}	73.7^{c}	72.9^{c}	1.0	<0.01
Indispensable amino acids							
Arg	87.0^{b}	74.4^{d}	91.4^{a}	84.9^{bc}	81.0^{c}	1.0	<0.01
His	86.3^{a}	78.7^{c}	87.7^{a}	82.1^{b}	75.8^{c}	0.7	<0.01
Ile	76.8^{b}	74.1^{b}	84.5^{a}	73.1^{b}	73.0^{b}	1.5	<0.01
Leu	87.9^{a}	77.6^{b}	85.3^{a}	77.9^{b}	85.0^{a}	0.9	<0.01
Lys	76.0^{b}	70.0^{c}	89.4^{a}	76.6^{b}	62.7^{d}	1.5	<0.01
Met	87.7^{ab}	79.9^{c}	88.9^{a}	86.6^{ab}	85.0^{b}	1.4	<0.01
Phe	84.8^{ab}	81.6^{b}	87.3^{a}	81.2^{b}	81.3^{b}	1.1	<0.01

Table 4. Continued

Item	Experimental diets					SEM[2]	*P*-value
	Corn	Wheat	SBM	CM	DDGS		
Thr	66.8[b]	60.5[c]	79.3[a]	67.0[b]	65.3[bc]	1.4	<0.01
Val	80.1[a]	73.4[b]	83.2[a]	72.3[b]	74.0[b]	1.3	<0.01
Dispensable amino acids							
Ala	86.8[a]	68.2[d]	84.5[ab]	77.3[c]	82.5[b]	1.1	<0.01
Asp	77.1[ab]	65.2[cd]	83.4[a]	70.5[bc]	62.6[d]	1.8	<0.01
Cys	78.1[a]	75.2[ab]	73.5[bc]	70.4[cd]	68.6[d]	1.7	<0.01
Glu	88.4[a]	90.6[a]	88.8[a]	84.6[b]	81.8[b]	0.7	<0.01
Gly	73.6[b]	70.7[b]	81.6[a]	73.7[b]	66.0[c]	1.0	<0.01
Pro	84.8[a]	86.7[a]	83.4[a]	70.6[c]	78.1[b]	1.0	<0.01
Ser	77.7[b]	73.8[bc]	84.2[a]	70.6[c]	73.7[bc]	1.2	<0.01
Tyr	85.5[ab]	77.2[c]	88.6[a]	77.8[c]	82.9[b]	1.0	<0.01

[a-d] Means in a row different superscripts are significantly different ($P<0.05$).
[1] SBM, soybean meal; CM, canola meal; DDGS, corn distillers dried grains with solubles.
[2] SEM, standard error of the means.

than those in wheat except for Ile and Phe. Ravindran et al. (2005) reported that average AID coefficient of AA in corn (0.81) was less than that of wheat (0.83). This discrepancy may be caused by the different wheat cultivar and age of chickens used between studies. In the cereal grains and cereal byproduct evaluated, Thr and Lys were the least digestible indispensable AA and the most digestible AA was Phe in wheat, Leu in corn, and Leu and Met in DDGS. This result is in agreement with those reported by Ravindran et al. (2005). In addition, Thr was also the least digestible indispensable AA in SBM and CM. Threonine is a major AA in endogenous protein which is included as the output of protein for the AID calculation, consequently this may decrease AID of Thr (Ravindran et al., 2005; Kong and Adeola, 2013; Osho et al., 2019).

Table 5 shows the BEL and SID of CP and AA for broilers fed experimental diets. The estimated BEL of CP and AA was lower than the previously reported value (Lemme et al., 2004). The differences in estimates may be attributed to the method used to estimate BEL. Lemme et al. (2004) reported average BEL derived from five experiments using enzymatically hydrolyzed casein (EHC) method. Although the EHC method remove the uncertainty concerning the 100% digestibility of casein protein, the EHC method may increase BEL estimate when incomplete separation of EHC from endogenous protein occurs (Hodgkinson et al., 2000).

Similar to the results in the AID, the determined SID of CP and AA varied ($P<0.01$) among test ingredients. The SID of Leu was greatest indispensable AA in corn and DDGS, Phe in wheat, Arg in SBM, and Met in CM. The indispensable AA with the least SID was Thr for corn, wheat, SBM, and CM, whereas Lys was the least SID indispensable AA in DDGS. The low Lys digestibility in DDGS may be attributed to the Maillard reaction during the drying process in DDGS production. In this reaction, the free amino group of Lys can be bound to the reducing sugars resulting in decrease in the digestibility of Lys in DDGS (Fastinger et al., 2006).

The SID of AA is slightly less in corn used in present study than those reported by Adedokun et al. (2008). However, comparable SID for SBM were observed between studies. The SID of indispensable AA in DDGS in the present study are comparable with values in light-DDGS reported by Adedokun et al. (2008) in 21-d-old broilers. However, Osho et al. (2019) reported less SID of indispensable AA in DDGS. Theses discrepancies may be attributed to the difference in processing

Table 5. Standardized ileal digestibility (%) of crude protein and amino acids in the experimental diets[1,3]

Item	Experimental diets					SEM[2]	*P*-value
	Corn	Wheat	SBM	CM	DDGS		
Crude protein	84.5[a]	78.0[b]	86.3[a]	76.0[b]	75.1[b]	1.0	<0.01
Indispensable amino acids							
Arg	90.3[a]	76.8[c]	92.2[a]	85.9[b]	82.3[b]	1.0	<0.01
His	88.8[a]	81.1[b]	88.7[a]	83.2[b]	76.9[c]	0.7	<0.01
Ile	84.1[ab]	79.4[bc]	86.6[a]	75.7[c]	75.5[c]	1.5	<0.01
Leu	91.1[a]	81.6[c]	87.1[b]	80.0[c]	86.2[b]	0.9	<0.01
Lys	79.7[b]	72.9[c]	90.1[a]	77.4[bc]	64.2[d]	1.5	<0.01
Met	90.8[a]	82.8[c]	90.6[a]	87.8[ab]	86.1[bc]	1.2	<0.01
Phe	88.6[a]	84.5[b]	88.6[a]	83.0[b]	82.8[b]	1.1	<0.01
Thr	78.7[a]	71.3[b]	83.6[a]	71.2[b]	69.7[b]	1.4	<0.01
Val	85.5[a]	77.8[b]	85.4[a]	74.6[b]	76.1[b]	1.3	<0.01
Dispensable amino acids							
Ala	89.7[a]	72.4[d]	86.2[b]	79.1[c]	83.6[b]	1.1	<0.01
Asp	83.6[a]	71.2[bc]	84.8[a]	72.8[b]	64.9[c]	1.8	<0.01
Cys	85.0[a]	80.9[ab]	78.0[b]	73.1[c]	71.6[c]	1.6	<0.01
Glu	91.1[a]	91.9[a]	89.8[a]	85.7[b]	82.9[b]	0.7	<0.01
Gly	79.4[ab]	74.9[c]	83.6[a]	75.5[bc]	68.0[d]	1.1	<0.01
Pro	88.4[ab]	89.4[a]	85.8[b]	72.7[d]	79.7[c]	1.0	<0.01
Ser	85.0[a]	79.7[b]	86.9[a]	74.0[c]	76.5[bc]	1.2	<0.01
Tyr	89.4[a]	81.2[bc]	90.1[a]	79.9[c]	84.4[b]	1.0	<0.01

[a-d] Means in a row different superscripts are significantly different ($P<0.05$).
[1] SBM, soybean meal; CM, canola meal; DDGS, distillers dried grains with solubles.
[2] SEM, standard error of the means.
[3] Standardized ileal digestibility was calculated using the following basal endogenous losses (mg/kg DM intake): Crude protein, 4,535; Arg, 97; His, 49; Ile, 136; Leu, 258; Lys, 74; Met, 36; Phe, 126; Thr, 324; Val, 157; Ala, 139; Asp, 285; Cys, 121; Glu, 333; Gly, 154; Pro, 237; Ser, 275; Tyr, 91.

condition of DDGS samples and details in methods employed for digestibility determination.

The differences between AID and SID of CP ranged from 2.2 (DDGS) to 6.4 (corn) percentage points indicating that relative significance of BEL on total ileal outflow is greater for corn than for DDGS (An et al., 2020). The correction of AID for BEL can reduce this influence and consequently increase an additivity of digestibility for diet formulation (Kong and Adeola, 2013).

In conclusion, the results of the present study showed that there are variations in both AID and SID of CP and AA among feed ingredients. It is also confirmed that the digestible CP and AA contents in feed ingredients determined by using animals do not always match with total contents from chemical analyses.

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