

# Comparison of amino acid digestibility and its additivity determined with slaughter or cecectomy method for yellow-feather chicken

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**ABSTRACT** The objective of this experiment was to compare the slaughter and cecectomy methods to determine amino acid (AA) digestibility of corn and soybean meal and their additivity in a corn-soybean meal diet. A completely randomized design was adopted to determine endogenous AA losses (EAAL) and AA digestibility in each of corn, soybean meal, and a corn-soybean meal diet using either slaughter or cecectomy methods. Each treatment contained 6 replicates with 3 chickens per replicate. The endogenous loss (EL) of histidine and glycine was lower and the EL of methionine and phenylalanine was greater when determined by slaughter vs. cecectomy ( $P < 0.05$ ). The EL of arginine, isoleucine, leucine, lysine, methionine, phenylalanine, valine, alanine, aspartic acid, glutamic acid, and serine determined by slaughter were 1.2 to 3.2 times of those from cecectomy. The standard error (SE) of EL of 14 AA (excluding histidine and glycine) obtained by slaughter method was 2.1 to 9.6 times of those by cecectomy method. The apparent and standardized digestibility was not affected by methods for most AA except apparent digestibility of methionine, phenylalanine and glycine, and standardized digestibility of glycine in corn. The apparent and standardized digestibility of most AA except apparent digestibility of glycine and standardized digestibility of lysine, cys-

teine and glycine were less for slaughter versus cecectomy methods in soybean meal ( $P < 0.05$ ). Using slaughter method resulted in reduced apparent digestibility of 15 AA (except glycine) and reduced standardized digestibility of 7 AA (arginine, isoleucine, leucine, valine, aspartic acid, glutamic acid, and proline) relative to cecectomy method ( $P < 0.05$ ), but the standardized digestibility of glycine was greater when determined by slaughter vs. cecectomy methods in corn-soybean meal diet ( $P < 0.05$ ). The mean value of SE of 16 AA digestibility in slaughter method was 2.9 times of that by cecectomy method. The apparent digestibility of 2 and 9 of 16 AA and the standardized digestibility of 15 and 7 of 16 AA were additive when using slaughter and cecectomy determinations, respectively. In conclusion, compared to the slaughter method, cecectomy method had less SE and EAAL but greater apparent digestibility of methionine and phenylalanine in corn, and the apparent digestibility of 15 AA (except glycine) in soybean meal and corn-soybean meal diet. Additivity in apparent and standardized AA digestibility was more inconsistent when determined with slaughter vs. cecectomy methods. These findings suggest that the cecectomy method is more suitable than the slaughter method to determine the digestibility of AA.

**Key words:** additivity, amino acid digestibility, cecectomy method, slaughter method

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## INTRODUCTION

The fundamental function of dietary protein is to provide sufficient amino acid (AA) for animal growth. Therefore, it is important to accurately determine the digestible AA content in feed to meet the protein requirements for poultry (Yu et al., 2021). Recent research employs one of two methods to determine the digestibility of AA, slaughter and cecectomy. Specifically, the slaughter method determined the

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digestibility of AA by measuring concentration of AA and inert index in ileal digesta and diet (Barua et al., 2021; Siegert et al., 2021; Khadour et al., 2022), while cecectomy method determined the AA digestibility by analyzing intake and excreted AA in cecectomized poultry (Rezvani et al., 2008; Cozannet et al., 2011; Zuber et al., 2016b). Kim et al. (2012) reported comparable digestibility of most AA between slaughter method in 21-day-old broilers and cecectomy method in rooster, but the differences in the results of these 2 methods depended on feed ingredients (Al-Marzooq, 2020). Rezvani et al. (2008) observed that the AA digestibility of roasted soybeans and corn gluten meal determined by slaughter method in 27-wk-old laying hens was 4.7% lower than that determined using cecectomy method in 46-wk-old hens. The standard error (SE) of slaughter method was about 3.3 times of cecectomy method. However, the effect of age on AA digestibility confounded comparison of these 2 methods. Previous studies have demonstrated the standardized ileal digestibility (SID) of AA in feed ingredients was additive using slaughter method, but the average difference between the determined and the calculated digestibility of AA in complete diets was  $-2.0\%$  to  $4.7\%$  (Adedokun et al., 2011; Cowieson et al., 2019; Osho et al., 2019). However, few studies focused on the additivity of AA digestibility among feed ingredients determined with cecectomy method. The objective of this study was to compare the difference and additivity between these 2 methods on the AA digestibility of corn and soybean meal in Chinese yellow-feathered chickens with same breed and age. These findings will determine the most suitable method to accurately determine the AA digestibility of feed for yellow-feathered chickens.

## MATERIALS AND METHODS

All experimental procedures were approved by the animal care and welfare committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). The code of ethical inspection was IAS 2020-78.

### Experimental Design

A completely randomized design was conducted to compare slaughter and cecectomy methods to determine endogenous AA losses (EAAL) and AA digestibility in corn, soybean meal, and a corn-soybean meal diet. In each of slaughter and cecectomy methods, there were 6 replicates of 3 chickens per replicate for each diet. The calculated AA digestibility of the corn-soybean meal diet was arithmetical summed according to the digestibility and proportion of individual ingredients in the complete diet. The additivity of AA digestibility of corn and soybean meal was tested by

comparing the calculated and determined values in a complete diet.

### Experimental Diets and Birds Management

A nitrogen-free diet was formulated based on the study of Adedokun et al. (2011). The dietary levels of calcium, phosphorus, trace minerals and vitamins met or exceeded the recommended requirements of yellow-feathered chicken in China (NY/T33-2004, 2004; Table 1). Corn and soybean meal were crushed over a 2 mm sieve. The corn or soybean meal diets were formulated with corn or soybean meal as the sole nitrogen source. The corn-soybean meal diet was formulated to test the additivity of AA digestibility in corn and soybean meal. All feed ingredients were evenly mixed then pelleted using a laboratory non-steam press pellet mill (Model SKJ 150, Funong machine Co. Zhengzhou, Henan, China).

The yellow-feathered chickens were individually kept in metabolic cages (0.42 m length  $\times$  0.42 m width  $\times$  0.52 m height) in a temperature-controlled room (25°C) with 12 h of light per day and free access to feed and water. A total of 72 intact and 72 cecectomized roosters (Guangdong Wen's yellow-feathered male chicken 2, 105-day-old) were selected and divided into 6 blocks by initial body weight in intact or cecectomized roosters to determine AA digestibility using slaughter (average body weight of 2.70 kg) or cecectomy methods (average body weight of 2.73 kg), respectively. Within each block, 12 chickens were randomly divided into 4 groups with 3 birds per groups. Each group was randomly fed 1 of 4 diets (nitrogen-free diet, corn diet, soybean meal diet, corn-soybean meal diet) within block. In the slaughter method, a total of 3 digesta samples from each group within block were pooled to provide adequate material for chemical analysis. The determination period lasted 5 d consisting of 4 d of adaption followed by 1 d for ileal digesta after slaughter. Ileal digesta was collected from two-thirds of the distal ileum (the portion of the small intestine from Meckel's diverticulum to approximately 1 cm anterior to the ileocecal junction) by flushing the intact ileal with distilled water, and immediately stored at  $-20^{\circ}\text{C}$  for further chemical analysis according to the procedures described by Osho et al. (2019) and Poursalami et al. (2012). In the cecectomy method, chickens had free access to the respective experimental diet for 55h followed by a 17 h fast. Birds were allowed free access to experimental diets from 09:00 on d 5 to 16:00 on d 8, then fasted from 16:00 on d 8 to 09:00 on d 9. Excreta was collected from 09:00 on d 5 to 9:00 on d 9 and immediately stored at  $-20^{\circ}\text{C}$  for each collection. After the collection, all digesta and excreta samples were freeze-dried (SCIENTZ-50ND, Zhejiang Xinzhi) and ground to a fine powder for further analysis.

**Table 1.** Composition of the experimental diets and amino acid profiles.

Item	Nitrogen free diet	Corn diet	Soybean meal diet	Corn-soybean meal diet
Ingredients, g/kg				
Corn starch	200.5	-	230.5	-
Dextrose	640.0	-	332.1	-
Solka floc	50.0	-	-	-
Corn	-	909.9	-	627.8
Soybean meal	-	-	350.0	285.5
Soybean oil	50.0	50.0	50.0	50.0
Sodium chloride	-	3.0	3.0	3.0
Limestone	13.0	9.0	5.1	10.1
Dicalcium phosphate	19.0	15.6	16.8	11.1
Titanium dioxide	5.0	5.0	5.0	5.0
Premix <sup>1</sup>	5.0	5.0	5.0	5.0
Potassium carbonate	2.6	-	-	-
Magnesium oxide	2.0	-	-	-
Sodium bicarbonate	7.5	-	-	-
Potassium chloride	2.9	-	-	-
Choline chloride	2.5	2.5	2.5	2.5
Analyzed nutrients (%) <sup>2</sup>				
DM	90.47	88.12	90.03	89.59
Calcium	1.00	0.75	0.75	0.83
Total phosphorus	0.36	0.59	0.58	0.58
Indispensable amino acid (%)				
Arginine	-	0.34	1.30	1.30
Histidine	-	0.23	0.49	0.56
Isoleucine	-	0.27	0.84	0.86
Leucine	-	0.96	1.40	1.79
Lysine	-	0.21	0.91	1.02
Methionine	-	0.16	0.24	0.31
Phenylalanine	-	0.37	0.91	1.01
Threonine	-	0.28	0.72	0.77
Valine	-	0.37	0.87	0.95
Dispensable amino acid (%)				
Alanine	-	0.58	0.80	1.04
Aspartic acid	-	0.53	2.09	2.04
Cysteine	-	0.16	0.27	0.32
Glutamic acid	-	1.41	3.26	3.60
Glycine	-	0.29	0.77	0.82
Proline	-	0.67	0.94	1.24
Serine	-	0.38	0.92	1.01
Total amino acid	-	7.22	16.72	18.64

<sup>1</sup>Supplied per kilogram of diets: vitamin A, 2,700 IU; vitamin D<sub>3</sub>, 400 IU; vitamin E, 10.0 IU; vitamin K<sub>3</sub>, 0.5 mg; vitamin B<sub>6</sub>, 3.0 mg; vitamin B<sub>12</sub>, 10 µg; thiamine, 2.0 mg; riboflavin, 5.0 mg; pantothenic acid, 10.0 mg; nicotinic acid, 30 mg; choline, 750 mg; folic acid, 0.5 mg; biotin, 120 µg; Cu (as copper sulfate) 8.0 mg; Fe (as ferrous sulfate) 80 mg; Mn (as manganese sulfate) 80 mg; Zn (as zinc sulfate) 80 mg; I (as calcium iodate) 0.7 mg; Se (as sodium selenite) 0.3 mg.

<sup>2</sup>Values are determined values (DM basis).

## Chemical Analysis

The DM, Ca, and P of experimental diets were determined according to the methods of AOAC (1990). Titanium dioxide (TiO<sub>2</sub>) content of diets and digesta was assessed spectrophotometrically according to the method described by Myers et al. (2004) and Wang and Adeola (2018). Amino acid content was analyzed in accordance with the AOAC 994.12. Samples were oxidized at 0°C in a peroxyformic acid solution for 16 h. Then, cysteine and methionine were oxidized to cysteic acid and methionine sulfone, respectively. Sodium metabisulfite was added to solution to decompose performic acid. Amino acid were hydrolyzed with 6 mol/L HCl for 23 h at 110°C. The hydrolysate was chromatographed with an AA analyzer (Biochrom 30, UK). The separated AA were mixed with ninhydrin and generated a specific color substance (postcolumn derivation)

in the heated reaction coil, which was detected by 570 nm and 440 nm wavelength. The chromatograms of 16 AA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, alanine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine) integrated using OPENLAB software.

## Calculation and Statistical Analysis

Amino acid digestibility determined with slaughter method was calculated according to the following equation:

Apparent ileal digestibility of AA (AID)

$$= \left( 1 - \frac{C_{AA \text{ in digesta}} \times C_{TiO_2 \text{ in diet}}}{C_{AA \text{ in diet}} \times C_{TiO_2 \text{ in digesta}} \right) \times 100\%$$

$$\text{SID} = \text{AID} + \frac{C_{\text{AA in digesta of nitrogen-free diet}} \times C_{\text{TiO}_2 \text{ in nitrogen-free diet}}}{C_{\text{TiO}_2 \text{ in digesta of nitrogen-free diet}} \times C_{\text{AA in diet}}} \times 100\%$$

Where  $C_{\text{AA in digesta}}$  is concentration of AA in digesta,  $C_{\text{AA in diet}}$  is concentration of AA in diet,  $C_{\text{TiO}_2 \text{ in diet}}$  is concentration of  $\text{TiO}_2$  in diet,  $C_{\text{TiO}_2 \text{ in digesta}}$  is concentration of  $\text{TiO}_2$  in digesta,  $C_{\text{AA in digesta of nitrogen-free diet}}$  is concentration of AA in digesta from nitrogen-free diet digesta, and  $C_{\text{TiO}_2 \text{ in nitrogen-free diet}}$  is concentration of  $\text{TiO}_2$  in nitrogen-free diet,  $C_{\text{TiO}_2 \text{ in digesta of nitrogen-free diet}}$  is concentration of  $\text{TiO}_2$  in digesta from nitrogen-free diet.

Amino acid digestibility determined with cecectomy method was calculated according to the following equation:

Apparent AA digestibility

$$= \frac{\text{AA}_{\text{intake}} - \text{AA}_{\text{output}}}{\text{AA}_{\text{intake}}} \times 100\%$$

Standardized AA digestibility

$$= \frac{\text{AA}_{\text{intake}} - \text{AA}_{\text{output}} + \text{EAAL}}{\text{AA}_{\text{intake}}} \times 100\%$$

The calculated value of AA digestibility in diet

$$= \frac{\text{DAA}_{\text{corn}} \times \text{AA}_{\text{corn}} \times \text{P}_{\text{corn}} + \text{DAA}_{\text{SBM}} \times \text{AA}_{\text{SBM}} \times \text{P}_{\text{SBM}}}{\text{AA}_{\text{corn}} \times \text{P}_{\text{corn}} + \text{AA}_{\text{SBM}} \times \text{P}_{\text{SBM}}}$$

Where  $\text{DAA}_{\text{corn}}$  is AA digestibility of corn,  $\text{AA}_{\text{corn}}$  is AA content of corn;  $\text{P}_{\text{corn}}$  is proportion of corn in the diet,  $\text{DAA}_{\text{SBM}}$  is AA digestibility of soybean meal,  $\text{AA}_{\text{SBM}}$  is AA content of soybean meal, and  $\text{P}_{\text{SBM}}$  is proportion of soybean meal in the diet.

The basic statistics were calculated with the MEANS procedure of SAS 9.0 (SAS Inst. Inc., Cary, NC). The 75% (Q3) and 25% (Q1) quantiles of the repeated data in each treatment were calculated with the UNIVARIATE procedure of SAS 9.0. The interquartile range (**IQR**) =  $Q3 - Q1$ . The data outside the range of  $Q1 - 1.5 \times \text{IQR}$  to  $Q3 + 1.5 \times \text{IQR}$  were excluded as outliers. The TTEST procedure of SAS 9.0 was used to analyze the difference between two methods to determine EAAL, AA digestibility of the experimental diets or the difference in calculated and determined AA digestibility of the corn-soybean meal diet.

## RESULTS

### Endogenous Amino Acid Loss

The endogenous loss (**EL**) of histidine (250 vs. 471 mg/kg DM intake) and glycine (619 vs. 1,446 mg/kg DM intake) were less and EL of methionine (218 vs. 68 mg/kg DM intake) and phenylalanine (539 vs. 271 mg/kg DM intake) were greater for the slaughter method than cecectomy method ( $P < 0.05$ ; [Table 2](#)). However, the EL of the remaining 12 AA was not

statistically affected by the methods. The EL of arginine, isoleucine, leucine, lysine, methionine, phenylalanine, valine, alanine, aspartic acid, glutamic acid, serine, and total AA in ileal digesta from slaughtered chickens were 1.2 to 3.2 times of those from cecectomized chickens. The SE of EL of 14 AA and total AA in slaughter method was 2.1 to 9.6 times of these in cecectomy method with the exceptions of histidine and glycine.

### Amino Acid Digestibility

In the corn, the apparent digestibility of methionine and phenylalanine determined by slaughter method was significantly less than determined by cecectomy method ( $P < 0.05$ ), but the apparent digestibility of glycine determined by slaughter method was significantly greater than that determined by cecectomy method ( $P < 0.05$ ; [Table 3](#)). The SE of apparent digestibility of AA determined by slaughter method was 0.8 to 4.4 (mean = 2.6) times of that determined by cecectomy method. In the soybean meal, the apparent digestibility of 15 AA (except glycine) determined by cecectomy were 8.3% to 17.0% greater than that determined by slaughter, moreover, apparent digestibility of total AA determined by cecectomy was 12.1% higher than that determined by slaughter (73.8% vs. 85.9%;  $P < 0.05$ ). The SE of apparent digestibility of 16 AA determined by slaughter method was 0.9 to 5.5 (mean = 3.5) times of that determined by cecectomy method. In the corn-soybean meal diet, apparent digestibility of glycine determined by slaughter method was greater than that determined by cecectomy method (71.5% vs. 56.1%;  $P < 0.05$ ), but apparent digestibility of other 15 AA was significantly less when determined by slaughter vs. cecectomy method ( $P < 0.05$ ). The apparent digestibility of total AA was 5.6% less for determination by slaughter vs. cecectomy method (78.7% vs. 84.3%;  $P < 0.05$ ). The SE of apparent digestibility of 16 AA determined by slaughter was 0.2 to 5.5 (mean = 2.8) times of that determined by cecectomy.

Standardized AA digestibility of glycine was significantly greater when determined by slaughter vs. cecectomy ( $P < 0.05$ ; [Table 4](#)) for corn, and SE of standardized digestibility of 16 AA by slaughter method was 0.8 to 4.4 (mean = 2.8) times when determined by slaughter vs. cecectomy. The standardized digestibility of 13 AA (except lysine, cysteine and glycine) in soybean meal were 5.9% to 11.2% less when determined by slaughter vs. cecectomy ( $P < 0.05$ ), and the standardized digestibility of total AA was 8.0% less when determined by slaughter vs. cecectomy (79.9% vs. 87.9%;  $P < 0.05$ ). The SE of standardized digestibility of 16 AA determined by slaughter method was 0.8 to 5.5 (mean = 3.1) times of that determined by cecectomy method. In the corn-soybean meal diet, the standardized digestibility of arginine, isoleucine, leucine, valine,

**Table 2.** Difference in endogenous amino acid loss determined with slaughter or cecectomy methods (mg/kg DM intake).

Item	Slaughter method <sup>1</sup>	Cececctomy method <sup>1</sup>	Pooled SEM	<i>P</i> -value	Ratio of SE <sup>2</sup>
Indispensable amino acid					
Arginine	546	291	118	0.079	5.0
Histidine	250	471	77	0.017	0.7
Isoleucine	534	341	117	0.130	2.2
Leucine	823	540	193	0.173	2.1
Lysine	572	371	144	0.217	3.6
Met hionine	218	68	48	0.026	9.6
Phenylalanine	539	271	102	0.045	6.7
Threonine	697	678	127	0.886	2.1
Valine	688	392	134	0.073	4.5
Dispensable amino acid					
Alanine	582	396	123	0.185	3.7
Aspartic acid	1,057	664	205	0.106	3.9
Cysteine	292	308	46	0.738	2.5
Glutamic acid	1,401	969	291	0.190	3.7
Glycine	619	1,446	191	0.002	0.7
Proline	710	725	144	0.918	3.6
Serine	666	541	121	0.340	2.7
Total amino acid	10,192	8,470	2,096	0.441	2.8

<sup>1</sup>Values are the means of 6 replicates of 3 roosters in each.

<sup>2</sup>Ratio of standard error in slaughter method to cececctomy method.

aspartic acid, glutamic acid and proline determined by slaughter method was significantly less than by cececctomy method ( $P < 0.05$ ), but the standardized digestibility of glycine was significantly greater when determined by slaughter compared to cececctomy method (79.0% vs. 63.6%;  $P < 0.05$ ). The SE of standardized digestibility of 16 AA determined by slaughter was 0.2 to 4.0 (mean = 2.4) times of that by cececctomy.

### The Additivity of Amino Acid Digestibility

In the slaughter method, the calculated AID values of alanine and cysteine in corn-soybean meal diet were not significantly different from the determined values, but the calculated AID values of the other 14 AA were significantly less than determined values (difference ranged from -13.6% to -2.7%;  $P < 0.05$ ; Table 5). The calculated AID value of total AA was 4.5% less than the determined values ( $P < 0.05$ ). In the cececctomy method, the calculated values of apparent digestibility of leucine, lysine, methionine, threonine, valine, alanine, and aspartic acid in corn-soybean meal diet significantly differed by -8.9% to 1.8% ( $P < 0.05$ ) from the determined values, but there was no significant difference between the calculated and determined value of apparent digestibility of the other 9 AA and total AA.

In the slaughter method, the calculated value of SID of lysine was significantly less than determined value (-9.4%;  $P < 0.05$ ), but the calculated and determined values of SID in each of remaining 15 AA and total AA was not statistically different (difference ranged from -2.9% to 2.3%) for corn-soybean-meal diet. In the cececctomy method, the calculated values of standardized digestibility of isoleucine, leucine, methionine, phenylalanine, valine, alanine, aspartic acid, serine were significantly greater than the determined values (difference ranged from 1.3% to 2.7%;  $P < 0.05$ ) for the corn-soybean meal diet, while the calculated values of

standardized digestibility of lysine was significantly less than the determined value (7.6%;  $P < 0.05$ ). No statistically significant difference was observed between the calculated and determined values of standardized digestibility in each of the other 7 AA and total AA.

## DISCUSSION

Kadim and Moughan (1997) reported that the digesta weight in posterior ileum varied from 0.6 g to 3.9 g per bird collected by slaughter, but the collected dry weight of excreta was 98 g per bird for nitrogen-free diet, 228 g per bird for corn diet, 362 g per bird for soybean meal diet, and 422 g per bird for corn-soybean meal diet in the current cececctomy method. These findings indicate that collection using cececctomy can obtain more undigested matter than collection by slaughter. Our results showed that the EAAL determined by slaughter was not statistically different from these by cececctomy method for 12 out of 16 AA. This result was in accordance with findings by Kadim et al. (2002) who observed that the EAAL in digesta collected from the terminal 15 cm adjacent to the ileo-caecal junction of 30-day-old broilers determined by slaughter was similar to that of excreta collected by total fecal collection. The current values of EAAL determined by slaughter and cececctomy (except glycine) were all within published values for chickens fed nitrogen-free diets (Ravindran, 2021). Greater glycine excretion determined by cececctomy may result from uric acid of excreta. The hydrolysis of excreta samples for AA analysis may produce glycine from uric acid (Zuber et al., 2016a). This reason may lead to reduced digestibility of glycine determined by cececctomy vs. slaughter. Rezvani et al. (2008) observed that the SE of apparent digestibility of 15 AA determined by slaughter was 1.8 to 5.0 (mean = 3.5) times of that determined by cececctomy. Our result showed that SE for EAAL and AA digestibility of diets determined by slaughter was

**Table 3.** Difference between slaughter and cecectomy methods to determine apparent digestibility of amino acid in corn, soybean meal, and corn- soybean meal diet (%).

Item	Corn					Soybean meal					Corn-soybean meal diet				
	Slaughter method <sup>1</sup>	Cececctomy method <sup>1</sup>	Pooled SEM	<i>P</i> -value	Ratio of SE <sup>2</sup>	Slaughter method <sup>1</sup>	Cececctomy method <sup>1</sup>	Pooled SEM	<i>P</i> -value	Ratio of SE <sup>2</sup>	Slaughter method <sup>1</sup>	Cececctomy method <sup>1</sup>	Pooled SEM	<i>P</i> -value	Ratio of SE <sup>2</sup>
Indispensable amino acid															
Arginine	74.7	84.3	4.0	0.056	3.9	80.6	92.2	1.5	<0.001	3.8	84.1	91.0	1.1	<0.001	5.5
Histidine	75.4	75.9	2.9	0.857	2.7	77.5	85.8	1.5	0.002	3.0	81.4	84.6	1.2	0.026	1.3
Isoleucine	67.7	77.0	5.1	0.119	2.8	73.3	89.2	1.9	<0.001	3.6	78.0	86.3	1.2	0.001	4.0
Leucine	83.9	89.2	2.6	0.083	2.8	74.3	89.2	1.8	<0.001	4.5	80.5	88.1	1.1	<0.001	2.5
Lysine	50.7	60.6	8.2	0.274	3.6	69.7	78.6	2.2	0.007	3.0	80.3	84.6	1.3	0.018	4.3
Met hionine	80.5	90.7	3.6	0.032	4.4	74.2	91.2	2.2	0.001	5.5	82.9	89.2	1.7	0.011	5.3
Phenylalanine	78.5	86.0	3.2	0.040	2.2	74.4	88.1	2.0	0.001	4.8	80.1	86.7	1.4	0.001	2.6
Threonine	55.5	61.1	5.2	0.311	2.3	68.9	80.8	1.9	0.001	3.6	70.6	77.4	1.4	0.001	2.6
Valine	70.0	79.8	4.1	0.054	3.0	72.0	87.1	1.9	<0.001	3.0	75.5	83.9	1.2	<0.001	2.2
Dispensable amino acid															
Alanine	81.8	85.3	2.7	0.251	2.9	73.4	85.8	1.9	0.001	3.6	79.2	83.8	1.3	0.001	2.4
Aspartic acid	67.6	74.6	4.7	0.166	2.1	72.5	86.2	1.9	<0.001	4.5	76.0	82.9	1.1	<0.001	2.5
Cysteine	68.3	71.2	2.9	0.339	1.2	65.8	75.5	2.3	0.002	2.3	68.4	76.3	1.5	<0.001	1.3
Glutamic acid	82.7	87.3	2.6	0.127	2.7	76.5	89.1	1.9	0.001	3.0	81.9	88.6	0.9	<0.001	3.0
Glycine	59.2	37.0	7.5	0.014	0.8	69.3	69.4	2.7	0.962	0.9	71.5	56.1	4.7	0.019	0.2
Proline	79.7	82.9	2.2	0.181	1.5	70.5	81.3	2.0	0.002	2.7	77.0	83.0	1.0	<0.001	3.5
Serine	71.0	76.4	3.9	0.193	2.4	73.2	86.1	1.6	<0.001	4.0	77.3	83.7	1.4	0.005	1.3
Total amino acid	75.4	80.0	3.4	0.211	2.5	73.8	85.9	1.8	0.001	3.6	78.7	84.3	1.1	0.001	2.5

<sup>1</sup>Values are the means of 6 replicates of 3 roosters in each.<sup>2</sup>Ratio of standard error in slaughter method to cececctomy method.**Table 4.** Difference between slaughter and cececctomy methods to determine standardized digestibility of amino acid in corn, soybean meal, and corn-soybean meal diet (%).

Item	Corn					Soybean meal					Corn-soybean meal diet				
	Slaughter method <sup>1</sup>	Cececctomy method <sup>1</sup>	Pooled SEM	<i>P</i> -value	Ratio of SE <sup>2</sup>	Slaughter method <sup>1</sup>	Cececctomy method <sup>1</sup>	Pooled SEM	<i>P</i> -value	Ratio of SE <sup>2</sup>	Slaughter method <sup>1</sup>	Cececctomy method <sup>1</sup>	Pooled SEM	<i>P</i> -value	Ratio of SE <sup>2</sup>
Indispensable amino acid															
Arginine	90.5	88.7	4.0	0.665	3.9	84.9	93.1	1.5	0.002	3.8	88.4	91.9	1.1	0.021	3.7
Histidine	86.1	86.5	2.9	0.893	3.0	82.6	89.6	1.6	0.002	2.1	85.8	88.2	1.3	0.097	1.3
Isoleucine	87.3	83.6	5.0	0.493	3.4	79.7	90.9	1.9	0.001	3.0	84.2	88.0	1.3	0.022	3.0
Leucine	92.4	92.2	2.6	0.945	3.6	80.2	90.7	1.9	0.002	3.6	85.1	89.3	1.1	0.004	2.5
Lysine	78.2	69.8	8.2	0.348	3.3	76.0	80.2	2.2	0.113	2.6	85.8	86.1	1.4	0.832	3.3
Met hionine	93.9	92.8	3.6	0.775	4.4	83.3	92.2	2.2	0.009	5.5	89.9	90.0	1.7	0.954	4.0
Phenylalanine	93.2	89.7	3.2	0.297	2.1	80.3	89.2	2.0	0.005	4.8	85.5	87.8	1.4	0.146	2.2
Threonine	80.6	73.7	5.0	0.219	3.2	78.6	84.5	1.9	0.022	3.0	79.7	81.1	1.4	0.360	2.2
Valine	88.6	85.2	4.1	0.438	3.0	79.9	88.8	1.9	0.003	3.0	82.8	85.6	1.3	0.047	2.4
Dispensable amino acid															
Alanine	91.8	88.7	2.7	0.300	3.1	80.7	87.7	1.9	0.010	3.0	84.8	85.4	1.3	0.668	2.4
Aspartic acid	87.5	81.0	4.7	0.196	2.1	77.6	87.4	1.9	0.002	3.6	81.2	84.2	1.1	0.018	2.5
Cysteine	86.2	80.7	2.8	0.077	1.3	76.6	79.9	2.4	0.196	1.9	77.4	80.2	1.5	0.093	1.3
Glutamic acid	92.6	90.8	2.6	0.507	2.7	80.8	90.3	1.9	0.002	3.0	85.8	89.7	1.0	0.002	3.0
Glycine	80.3	62.9	7.5	0.042	0.8	77.3	76.9	2.8	0.890	0.8	79.0	63.6	4.7	0.019	0.2
Proline	90.4	88.4	2.2	0.400	1.5	78.1	84.3	2.0	0.012	2.4	82.8	85.4	1.0	0.030	1.3
Serine	88.6	83.8	3.8	0.243	3.0	80.4	88.4	1.7	0.003	3.2	83.9	85.9	1.4	0.212	3.5
Total amino acid	89.5	86.1	3.4	0.331	2.7	79.9	87.9	1.9	0.005	3.0	84.2	86.2	1.2	0.111	2.2

<sup>1</sup>Values are the means of 6 replicates of 3 roosters each.<sup>2</sup>Ratio of standard error in slaughter method to cececctomy method.

**Table 5.** Difference between calculated and determined values of AA digestibility in corn-soybean meal diet determined with slaughter or cecectomy method (%).

Item	Difference in apparent digestibility				Difference in standardized digestibility			
	Slaughter method <sup>1</sup>	<i>P</i> -value <sup>2</sup>	Cecectomy method <sup>3</sup>	<i>P</i> -Value <sup>4</sup>	Slaughter method <sup>1</sup>	<i>P</i> -value <sup>2</sup>	Cecectomy method <sup>3</sup>	<i>P</i> -value <sup>4</sup>
Indispensable amino acid								
Arginine	-4.5	0.009	-0.2	0.408	-2.5	0.072	0.4	0.244
Histidine	-4.5	0.006	-1.6	0.090	-2.2	0.073	0.5	0.556
Isoleucine	-5.9	0.004	0.3	0.407	-2.9	0.057	1.4	0.020
Leucine	-2.7	0.050	1.1	0.035	-0.5	0.654	1.9	0.007
Lysine	-13.6	<0.001	-8.9	<0.001	-9.4	0.001	-7.6	<0.001
Met hionine	-6.5	0.011	1.8	0.003	-2.8	0.145	2.4	0.001
Phenylalanine	-4.7	0.016	0.9	0.148	-2.0	0.201	1.5	0.040
Threonine	-4.9	0.012	-1.4	0.047	-0.6	0.674	0.8	0.197
Valine	-4.0	0.018	1.3	0.032	-0.6	0.654	2.3	0.005
Dispensable amino acid								
Alanine	-2.7	0.075	1.8	0.014	0.0	1.000	2.7	0.003
Aspartic acid	-4.3	0.006	1.3	0.028	-1.9	0.108	2.1	0.005
Cysteine	-1.8	0.180	-2.2	0.054	2.3	0.109	0.0	0.957
Glutamic acid	-3.8	0.008	0.0	0.961	-1.9	0.090	0.7	0.111
Glycine	-4.6	0.009	5.6	0.277	-1.0	0.401	10.0	0.081
Proline	-3.1	0.013	-1.1	0.142	-0.2	0.831	0.4	0.577
Serine	-4.7	0.018	-0.1	0.823	-1.4	0.340	1.3	0.022
Total amino acid	-4.5	0.008	0.1	0.793	-1.8	0.153	1.2	0.050

<sup>1</sup>Values are the calculated minus determined AA digestibility in corn-soybean meal diet determined with slaughter method.

<sup>2</sup>Values are the *P*-values of T-test for the calculated value compared with determined value of AA digestibility in corn-soybean meal diet determined with slaughter method.

<sup>3</sup>Values are the calculated minus determined AA digestibility in corn-soybean meal diet determined with cecectomy method.

<sup>4</sup>Values are the *P*-values of T-test for the calculated value compared with determined values of AA digestibility in corn-soybean meal diet determined with cecectomy method.

0.7 to 9.6 (mean = 3.5) and 0.2 to 5.5 (mean = 2.9) times of that obtained by cecectomy. This phenomenon may be due to the fact that the slaughter method assumes that the index marker (e.g., TiO<sub>2</sub>) was distributed evenly in the digesta and synchronously transits to the posterior part of the digestive tract (Bryan et al., 2019). However, fact, the determined values of AA digestibility varied depending on ileal digesta samples collected at different times (Kim et al., 2017). These findings indicate that the uniformity of ileal digesta has a great influence on the variation of AA digestibility (Jallier et al., 2003). In cecectomy method, excreta from each bird was collected and mixed for more homogeneity, thereby reducing the effect of sample uniformity on variation of AA digestibility. For these reasons, the variation of digestibility determined by slaughter was greater than that obtained by cecectomy method.

Values for apparent digestibility were calculated by subtracting the total excretion of AA from the quantity ingested by the chicken. The total excretion of AA contained EAAL and undigested feed protein, thus greater EAAL reduced the apparent digestibility of AA (Stein et al., 2007). In the present study, the apparent digestibility of majority of AA determined by slaughter method was less than that by cecectomy method. This may be associated with higher EAAL for slaughter compared to cecectomy method. However, Ravindran et al. (1999) reported no difference in the apparent digestibility of AA in grain and plant protein feed ingredients obtained by slaughter method or excreta analysis for finishing broilers. These inconsistencies may result from the total excreta collection used in the current study, while Ravindran et al. (1999) used acid-insoluble ash to estimate the amount of excreta. Acid-insoluble ash generally

underestimates digestibility due to recovery below 100% (Sales and Janssens, 2003; Kim et al., 2020). The standardized digestibility of AA determined by slaughter and cecectomy were relatively similar in corn and corn-soybean meal diet, while the digestibility determined by slaughter was about 8% lower than that by cecectomy in soybean meal. Our results were in accordance with these presented in previous studies. Rezvani et al. (2008) reported that the true AA digestibility of corn gluten meal and toasted soybean determined by slaughter was 5 % (2–7%) less than that by cecectomy in 27-wk-old hens. Kadim et al. (2002) observed that the true AA digestibility of protein feed ingredients was less when determined by slaughter compared to excreta collection method.

Angkanaporn et al. (1996) observed an additivity of the SID in soybean meal, sunflower meal, and meat bone meal determined by slaughter in broilers, but the determined digestibility was 2.0% to 4.7% greater than calculated value in 4 diets composed of 2 or 3 feed ingredients. Cowieson et al. (2019) indicated that the SID was additive for corn and soybean meal determined by slaughter method. Osho et al. (2019) reported that the SID determined by slaughter was 1.2% greater than calculated values in a wheat-rape seed meal diet, but 2.0% less than calculated values in a wheat-rape seed meal-sorghum DDGS diet, however, the digestibility of some AA was not additive in these 2 diets. In the present study, the calculated values of the apparent and standardized digestibility of majority of AA was less than the determined values determined by slaughter. Statistical analysis indicates the apparent AA digestibility is not additive, but the standard digestibility (except lysine) is additive. However, when determined by cecectomy, the apparent digestibility of 8 of 16 AA and the standardized digestibility of 7 of 16 AA

were additive. The calculated values of the apparent and standardized AA digestibility were relatively close to determined values. From a statistical point of view, the same difference between the calculated and determined values of dietary AA digestibility was more likely to be significant in the cecectomy method than the slaughter method because of less SE in the cecectomy method. Therefore, similar absolute values in difference between the calculated and determined digestibility means the additivity of SID determined using slaughter was similar to determination with cecectomy.

## CONCLUSION

In summary, the slaughter method had greater SE and EAAL but reduced apparent digestibility of methionine and phenylalanine in corn, and the apparent digestibility of 15 AA (except glycine) in soybean meal and corn-soybean meal diet compared to cecectomy method. The additivity of apparent AA digestibility obtained by slaughter method was less than those by cecectomy. The additivity of SID obtained by cecectomy was close to those from slaughter because of similar absolute values in difference between the calculated and determined digestibility. Therefore, these findings suggest that cecectomy method is more suitable than slaughter to determine AA digestibility of roosters.

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## DISCLOSURES

The authors declare no conflict of interest in this study.

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