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# Research Article Comparative Amino Acid Ileal Digestibility of Feed Ingredients Measured with Indigenous and Commercial Strains of Chickens

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# Abstract

**Background and Objective:** The determination of ileal digestibility has become the preferred method for estimating amino acid availability. This study was conducted to determine the apparent ileal digestibility of amino acids in 3 feed ingredients using commercial and indigenous strains of chickens. **Materials and Methods:** Three experimental diets, representing three feed ingredients wheat, barley and sorghum were formulated and evaluated using six replicates of five birds per cage at 19 days of age. On day 23, after adaptation to the experimental diets, feed troughs were removed from every cage for 1 h and then reintroduced for 2 h. Then, the birds were killed to sample the ileal digesta from Meckel's diverticulum to the ileal-cecal-colonic injunction. **Results:** Broiler birds showed significantly (p<0.001) higher digestibility coefficients and digestible amino acid contents for all the cereal grains than local birds. Of the three cereal grains evaluated in this study, sorghum had the lowest amino acid content estimates for wheat were the highest for both breeds (p<0.001). The digestibility coefficient and digestible amino acid content estimates for wheat were the highest for both breeds (p<0.001). **Conclusion:** These data suggest that the classes of chickens significantly influenced the apparent ileal digestibility and digestible amino acid content in the feed ingredients assessed in this study.

Key words: Ileal digestibility, amino acids, breed, grain, broiler, poultry feed

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Data Availability: All relevant data are within the paper and its supporting information files.

# INTRODUCTION

Dietary protein comprises a major element of the cost of poultry feed. In poultry production, it is essential to formulate diets that meet the nutrient requirements of different classes of poultry while minimizing the excretion of excess nutrients into the environment. It is well documented that the contribution of dietary protein to an animal depends not only on its amino acid composition but also on how effectively the amino acids are utilized<sup>1</sup>. Therefore, describing the proteins in dietary ingredients in terms of the digestible amino acid content rather than the total amino acid content better reflects the amino acid amount actually available for maintenance and production purposes<sup>2</sup>. It is well recognized that amino acid digestibility is a sensitive indicator of amino acid availability in dietary ingredients for poultry<sup>3</sup>.

To date, there is a large body of published work on amino acid digestibility coefficients of different feed ingredients<sup>4</sup>. However, the data are variable due to differences in the methodology used, including the site of measurement<sup>5,6</sup>.

The use of digestibility values generated from adult rooster assays of dietary formulations for different genetic strains of chickens, especially those with various growth rates, such as local native chickens, may not be an appropriate practice, as the class of bird can influence the digestibility of amino acids in different feed ingredients<sup>7</sup>. There is a general lack of information on the digestible nutrient contents of local varieties of common cereal grains grown in Oman. Published data on the ileal digestibility of amino acids for these grains are scant. The digestible nutrient contents of these varieties are critical for selection.

All dietary components are important in the formulation of poultry diets but significant attention should be paid to dietary amino acids because controlling the protein status and digestible amino acids has been shown to increase weight gain and feed intake and improve the body composition in broilers<sup>8,9</sup>.

With the expansion of livestock husbandry practices in Oman, local "indigenous" chicken production is becoming widespread. The economic and social value of local chickens in developing countries of the Middle East, such as Oman especially those in rural areas is well recognized; therefore, it is essential to explore opportunities to improve the production of local chickens<sup>10</sup>.

The objective of the current study was to assess the digestibility coefficients of amino acids in feed ingredients using commercial and indigenous strains of chickens.

#### **MATERIALS AND METHODS**

**Ethical approval:** All experimental work was conducted at the Poultry Research Unit at the Agricultural Experiment Station in accordance with the experimental unit policy on animal welfare and the requirements of the procedures involving animals/birds and their care. The study was approved by the Animal Research Ethics Board at Sultan Qaboos University (IG/AGR/ANVS/05/03).

**Ingredients:** The local wheat, barley and sorghum grains used in the current study were developed at the Jimah and Wadi Quriyat research stations in Oman. Various developments have been previously described by Al-Bakri *et al.*<sup>11</sup>.

**Birds and housing:** Ninety newly hatched chicks of Cobb 500-type broiler chickens and local Omani chickens were obtained from reputable commercial hatcheries at Barka in Muscat. The birds were housed in suspended grower cages. The cages were located in an environmentally controlled metabolic room maintained at 35°C on day 1, with the temperature reduced by 1°C per day to 22°C. The birds had free access to water and feed and lighting was maintained at a photoperiod of 23 h every 24 h. The birds were initially allocated to replicate cages beginning on day 13, with the live weights of birds in replicates differing by less than 10 g. The birds were fed a crumble commercial broiler diet from day one to day 18. The birds were 19 days old at the commencement of the ileal digestibility assay.

**Experimental diets and procedures:** Three test ingredients representing three cereal grains wheat, barley and sorghum were ground using a laboratory hammer mill fitted with a 3 mm screen and then incorporated into semisynthetic diets at one rate of inclusion (500 g kg<sup>-1</sup>, Table 1) as the only component containing protein/amino acids (Table 1). Other raw materials were added sequentially with mixing at slow speed (to ensure the effective homogenization of all ingredients). These diets included the indigestible marker titanium dioxide, a vitamin/mineral premix, vegetable oil and

Table 1: Compositions of experimental diets

Raw material (g kg <sup>-1</sup> )	Wheat	Barley	Sorghum
Barley variety	500	500	500
Vegetable oil	50	50	50
Vitamin and mineral premix*	50	50	50
Titanium dioxide	5	5	5
Starch:glucose (50:50)	395	395	395
Total	1000	1000	1000

\*Designed to meet the requirements of young broiler chickens

a 50:50 mixture of purified maize starch and glucose (in amounts to make the diets 1000 g kg<sup>-1</sup>). Each of the three experimental diets was evaluated in six replicates, with each cage containing 5 birds. Experimental diets were fed ad libitum for four days from 19-23 days of age.

On day 23, the birds were starved for one hour and then fed for 2 h to ensure sufficient gut fill for digesta sample collection. The birds were then killed by an intracardial injection of sodium pentobarbitone. Following dissection of the lower small intestine, the digesta sample was gently flushed with distilled water and collected into a collection vessel. Samples from birds of the same a cage were pooled to provide a large enough sample for chemical analysis following the procedure described by Al-Marzooqi and Wiseman<sup>12</sup>.

**Calculations:** The titanium and amino acid data were used to calculate the coefficient of apparent amino acid digestibility using the following equation, as described by Al-Marzooqi and Wiseman<sup>12</sup>:

$$l = \frac{aa_{dig} \times marker_{diet}}{aa_{diet} \times marker_{dig}}$$

Where:

aa <sub>dig</sub>	:	Amino acid concentration in the digesta
marker <sub>diet</sub>	:	Titanium concentration in the diet
aa <sub>diet</sub>	:	Amino acid concentration in the diet
marker <sub>dig</sub>	:	Titanium concentration in the digesta

From the coefficients and the amino acid contents of the diet, the concentration of ileal apparently digestible amino acids  $kg^{-1}$  was calculated.

**Chemical analyses:** Samples of test ingredients and ileal digesta samples used for laboratory analysis were ground to pass through a 1 mm mesh in a micro-Wiley mill. Samples of ileal contents were freeze dried prior to grinding. Duplicate determinations of the dry matter, crude protein, ether extract, crude fiber and gross energy content were conducted for the test ingredients according to the AOAC<sup>13</sup>. The amino acid contents of duplicate test ingredients and ileal digesta samples were determined at Massey University Analytical Laboratory in New Zealand. The amino acid contents were determined by using a Waters ion-exchange HPLC system, utilizing postcolumn ninhydrin derivatization and fluorescence detection, following hydrolysis in 6 M glass-distilled hydrochloric acid containing 0.1% phenol for 24 h at 110+2°C in evacuated sealed tubes. Lysozyme was used as

an external standard for the amino acid analysis. Titanium (the inert internal marker) was analyzed following the procedure described by Short *et al.*<sup>14</sup>. Chemical analyses were performed in duplicate and repeated if individual data differed by <5%.

**Statistical analysis:** Data were analyzed by analysis of variance using the general linear model procedure of the Statistical Analysis System  $(SAS)^{15}$  (SAS Institute Inc., versions 2 and 6, 2001). The experimental design was a 2×3 factorial with 2 breeds (Cobb 500 and local Omani chicken) and 3 test ingredients (wheat, barley and sorghum). The main parameters tested in the analysis of variance were digestibility coefficients and digestible amino acid contents. Significant differences between treatment means were assessed using the least-significant-difference procedure. Interactions between the treatments were tested using Tukey's multiple comparisons test when significant and excluded from the model when not significant (p>0.05).

#### RESULTS

The chemical compositions of wheat, barley and sorghum are summarized in Table 2. The crude protein was higher in wheat than those of barley and sorghum by 6.2 and 21.9%, respectively. The fiber contents of barley, wheat and sorghum

Table 2:	Chemical	compositions	and	amino	acid	contents	of	different cereal
	arains							

grains			
Chemical composition	Wheat	Barley	Sorghum
Dry matter (g kg <sup>-1</sup> DM)	931.5	928.7	910.0
Crude protein (g kg <sup>-1</sup> DM)	141.3	132.5	110.3
Crude fiber (g kg <sup>-1</sup> DM)	29.3	68.2	49.1
Ether extract (g kg <sup>-1</sup> DM)	15.6	18.4	26.1
Gross energy (MJ g <sup>-1</sup> DM)	16.4	15.7	15.6
Essential amino acids (g kg <sup>-1</sup> DM)			
Threonine	3.6	3.7	2.9
Valine	5.7	5.9	4.7
Methionine	1.9	1.8	1.4
Isoleucine	4.2	3.9	3.6
Leucine	8.3	7.7	11.9
Phenylalanine	5.8	5.6	4.9
Histidine	3.4	2.9	2.4
Lysine	3.8	4.1	2.1
Arginine	6.3	5.3	3.5
Nonessential amino acids (g kg <sup>-1</sup> DM)			
Aspartic acid	8.0	8.1	6.8
Tyrosine	3.9	3.2	3.4
Serine	5.3	4.3	3.8
Glutamic acid	38.9	28.1	19.5
Proline	12.8	12.0	7.6
Glycine	5.5	4.7	2.9
Alanine	4.9	4.8	8.6
Total	122.3	106.0	89.8

were 68.2, 29.3 and 49.1 g kg<sup>-1</sup>, respectively, which indicates that barley had more fiber than wheat and sorghum by 57 and 28%, respectively. The gross energy content was 4.3% higher in wheat than in the other grains. Sorghum had a higher fat content, as determined by the ether extract, than those of wheat and barley by 40.2 and 29.5%, respectively. The gross energy contents of the three grains were similar.

The amino acid contents of wheat, barley and sorghum are presented in Table 2. Sorghum had lower levels of sulfur-containing amino acids than the other two grains. For the most essential amino acids for poultry performance, wheat and barley contained 26.3 and 22.1% more methionine (1.9 and 1.8 vs. 1.4 g kg<sup>-1</sup> DM); 19.4 and 21.6% more threonine (3.6 and 3.7 vs. 2.9 g kg<sup>-1</sup> DM) and 44.7 and 48.8% more lysine (3.8 and 4.1 vs. 2.1 g kg<sup>-1</sup> DM) than sorghum, respectively. The variations in the amino acid concentrations of the feed ingredients evaluated in this study were largely related to the protein level in the grain. In these grains, the amino acid concentrations generally increased with increasing protein levels. Wheat had higher amino acid concentrations than did barley and sorghum (Table 2).

The mean apparent ileal digestibility coefficients and digestible essential and nonessential amino acid contents determined in the ileum for wheat, barley and sorghum are shown in Table 3 and 4, respectively. For the three feed ingredients evaluated, in comparison to the other two grains, sorghum had the lowest digestibility coefficients and digestible contents (p<0.001) in both breeds of chicken. In general, the class of chickens had a significant effect on the digestibility and digestible amino acid contents (p<0.001). The average digestibility coefficients and the digestible amino acid contents in wheat, barley and sorghum for broilers were higher (p<0.001) than those for local Omani chickens. For broiler birds, the overall mean amino acid digestibility coefficients (and ranges across grains) were 0.84 in wheat (0.71-0.94), 0.72 in barley (0.53-0.92) and 0.67 in sorghum (0.48-0.86), whereas for local birds, the overall amino acid digestibility coefficients (and range across grains) were 0.72 in wheat (0.53-0.89), 0.60 in barley (0.39-0.82) and 0.47 in sorghum (0.21-0.68).

The overall mean amino acid digestible content in g kg<sup>-1</sup> DM (and range across grains) for broiler birds was 3.23 in wheat (0.82-17.75), 2.41 in barley (0.78-11.50) and 1.92 in sorghum (0.61-7.18), whereas for local birds, the overall mean amino acid digestible content in g kg<sup>-1</sup> DM (and range across grains) was 2.88 in wheat (0.79-16.78), 2.10 in barley (0.69-10.78) and 1.30 in sorghum (0.30-5.50).

Table 3: Apparent ileal digestibility coefficients of amino acids in the test ingredients for commercial broiler and local Omani chick	kens
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	Breed									
	Broiler			Local Omani						
	Grain			Grain						
Amino acid	Wheat	Barley	Sorghum	Wheat	Barley	Sorghum	SEM	Breed	Grain	B*G
Essential amino acids										
Threonine	0.71ª	0.53 <sup>b</sup>	0.48 <sup>b</sup>	0.53 <sup>b</sup>	0.39 <sup>b</sup>	0.21 <sup>c</sup>	0.085	***	***	NS
Valine	0.82ª	0.72 <sup>ab</sup>	0.66 <sup>bc</sup>	0.68 <sup>bc</sup>	0.59°	0.45 <sup>d</sup>	0.029	**	**	NS
Methionine	0.91ª	0.92ª	0.86 <sup>ab</sup>	0.89ª	0.82 <sup>ab</sup>	0.68 <sup>b</sup>	0.025	***	***	NS
Isoleucine	0.84ª	0.71 <sup>b</sup>	0.68 <sup>b</sup>	0.70 <sup>b</sup>	0.59 <sup>bc</sup>	0.48 <sup>c</sup>	0.032	***	***	NS
Leucine	0.87ª	0.76 <sup>ab</sup>	0.75 <sup>abc</sup>	0.74 <sup>bc</sup>	0.64 <sup>cd</sup>	0.57 <sup>d</sup>	0.030	***	***	NS
Phenylalanine	0.88ª	0.75 <sup>b</sup>	0.74 <sup>b</sup>	0.77 <sup>b</sup>	0.68 <sup>bc</sup>	0.56°	0.027	***	***	NS
Histidine	0.82ª	0.71 <sup>b</sup>	0.60 <sup>bc</sup>	0.71 <sup>b</sup>	0.56°	0.35 <sup>d</sup>	0.032	***	***	NS
Lysine	0.84ª	0.75 <sup>ab</sup>	0.72 <sup>ab</sup>	0.70 <sup>b</sup>	0.59 <sup>cd</sup>	0.49 <sup>d</sup>	0.027	***	***	NS
Arginine	0.85ª	0.72 <sup>b</sup>	0.74 <sup>ab</sup>	0.73 <sup>b</sup>	0.60 <sup>c</sup>	0.56°	0.027	***	***	NS
Nonessential amino acids								***	***	
Aspartic acid	0.79ª	0.65 <sup>b</sup>	0.63 <sup>b</sup>	0.66 <sup>b</sup>	0.50 <sup>c</sup>	0.44 <sup>c</sup>	0.025	***	***	NS
Serine	0.81ª	0.61 <sup>b</sup>	0.57 <sup>bc</sup>	0.66 <sup>b</sup>	0.48 <sup>cd</sup>	0.35 <sup>d</sup>	0.033	***	***	NS
Glutamic acid	0.94ª	0.84 <sup>bc</sup>	0.74 <sup>d</sup>	0.88 <sup>ab</sup>	0.78 <sup>cd</sup>	0.56 <sup>e</sup>	0.019	***	***	*
Proline	0.92ª	0.81 <sup>bc</sup>	0.63 <sup>d</sup>	0.86 <sup>ab</sup>	0.76 <sup>c</sup>	0.39 <sup>e</sup>	0.025	***	***	**
Glycine	0.78ª	0.60 <sup>bc</sup>	0.48 <sup>cd</sup>	0.66 <sup>b</sup>	0.45 <sup>d</sup>	0.26 <sup>e</sup>	0.031	***	***	NS
Alanine	0.81ª	0.69 <sup>b</sup>	0.74 <sup>ab</sup>	0.67 <sup>b</sup>	0.53°	0.58 <sup>bc</sup>	0.031	***	***	NS
Tyrosine	0.85ª	0.69 <sup>bc</sup>	0.70 <sup>bc</sup>	0.73 <sup>b</sup>	0.60 <sup>cd</sup>	0.52 <sup>d</sup>	0.026	***	***	NS
AVG	0.84	0.72	0.67	0.72	0.60	0.47				

AVG: Average digestibility of all amino acids. \*p<0.05, \*\*p<0.001, \*\*\*p<0.0001, NS: Not significant. \*\* Within each analysis, means not sharing a common superscript are significantly different (p<0.05). B\*G: Breed\*Grain

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Table 4: Apparent ileal digestible amino acid contents in the test ingredients for commercial broiler and local Omani chickens

	Breed									
	Broiler			Local Omani						
	Grain			Grain						
Amino acid	Wheat	Barley	Sorghum	Wheat	Barley	Sorghum	SEM	Breed	Grain	B*G
Essential amino acids										
Threonine	1.24ª	0.96 <sup>b</sup>	0.68 <sup>bc</sup>	0.93 <sup>b</sup>	0.72 <sup>b</sup>	0.30 <sup>c</sup>	0.010	***	***	NS
Valine	2.28ª	2.06 <sup>ab</sup>	1.51 <sup>cd</sup>	1.90 <sup>bc</sup>	1.71 <sup>bc</sup>	1.04 <sup>d</sup>	0.010	***	***	NS
Methionine	0.82ª	0.78 <sup>ab</sup>	0.61 <sup>bc</sup>	0.79ª	0.69 <sup>ab</sup>	0.48 <sup>c</sup>	0.004	*	***	NS
Isoleucine	1.72ª	1.37 <sup>b</sup>	1.20 <sup>bc</sup>	1.44 <sup>ab</sup>	1.15 <sup>bc</sup>	0.85°	0.008	***	***	NS
Leucine	3.47 <sup>b</sup>	2.84 <sup>cd</sup>	4.41ª	2.98 <sup>bcd</sup>	2.42 <sup>d</sup>	3.41 <sup>bc</sup>	0.016	***	***	NS
Phenylalanine	2.45ª	2.04 <sup>b</sup>	1.78 <sup>bc</sup>	2.16 <sup>ab</sup>	1.86 <sup>bc</sup>	1.37 <sup>c</sup>	0.011	***	***	NS
Histidine	1.33ª	0.99 <sup>bc</sup>	0.71 <sup>de</sup>	1.15 <sup>ab</sup>	0.79 <sup>cd</sup>	0.41 <sup>e</sup>	0.005	***	***	NS
Lysine	1.52ª	1.49ª	0.76 <sup>c</sup>	1.26 <sup>b</sup>	1.19 <sup>b</sup>	0.53 <sup>c</sup>	0.005	***	***	NS
Arginine	2.59ª	1.85°	1.27 <sup>de</sup>	2.23 <sup>b</sup>	1.53 <sup>cd</sup>	0.97 <sup>e</sup>	0.009	***	***	NS
Nonessential amino acids										
Aspartic acid	3.07ª	2.56 <sup>b</sup>	2.12 <sup>bc</sup>	2.59 <sup>b</sup>	2.00 <sup>c</sup>	1.50°	0.011	***	***	NS
Serine	2.06ª	1.28 <sup>c</sup>	1.07 <sup>cd</sup>	1.68 <sup>b</sup>	1.02 <sup>cd</sup>	0.66 <sup>d</sup>	0.009	***	***	NS
Glutamic acid	17.75ª	11.50 <sup>b</sup>	7.18 <sup>c</sup>	16.78ª	10.78 <sup>b</sup>	5.50°	0.065	*	***	NS
Proline	5.70ª	4.74 <sup>ab</sup>	2.37 <sup>c</sup>	5.39 <sup>ab</sup>	4.48 <sup>b</sup>	1.48°	0.026	*	***	NS
Glycine	2.12ª	1.37 <sup>c</sup>	0.69 <sup>de</sup>	1.78 <sup>b</sup>	1.02 <sup>d</sup>	0.38 <sup>e</sup>	0.008	***	***	NS
Alanine	1.94°	1.62 <sup>cd</sup>	3.15ª	1.60 <sup>d</sup>	1.26 <sup>d</sup>	2.47 <sup>b</sup>	0.010	***	***	NS
Tyrosine	1.63ª	1.08 <sup>c</sup>	1.19 <sup>bc</sup>	1.40 <sup>b</sup>	0.94 <sup>c</sup>	0.88 <sup>c</sup>	0.006	***	***	NS
AVG	3.23	2.41	1.92	2.88	2.10	1.39				

AVG: Average digestibility of all amino acids. \*p<0.05, \*\*p<0.001, \*\*\*p<0.0001, NS: Not significant. \*\* Within each analysis, means not sharing a common superscript are significantly different (p<0.05). B\*G: Breed\*Grain

#### DISCUSSION

The selective breeding and development of new strains has been very effective in increasing the market weights of commercial broilers. Although it is recognized that the utilization of nutrients by broilers is influenced by genotype<sup>16</sup>, published information on the effects of the genotype on amino acid digestibility among different strains of chickens, especially those with differing growth rates, such as indigenous chickens, is scarce. Thus, the comparison of values obtained in the present study with published data is difficult. For the ingredients tested in the current study, broilers had a higher digestibility of amino acids than did local Omani chickens. One explanation for the improved digestion in broiler strains may be that modern fast-growing broilers have more nutrient transport capacity and greater intestinal mass than other breeds of chickens<sup>17,18</sup>. It was suggested by Ravindran *et al.*<sup>5</sup> that the innately different nutrient utilization observed between breeds could be due to differences in the structure of the gastrointestinal tract, which relate to changes in digestive enzyme output, absorptive capacity and digesta transit time. Villus growth in young chicks is stimulated by the presence of feed and the expansion of surface area that occurs with villus growth and has been used to explain increased absorptive capacity<sup>19,20</sup>. It may be assumed that such increased

feed intake and efficient feed utilization have also altered the intestinal function, which could influence the intestinal morphology<sup>21</sup>.

Uni *et al.*<sup>19</sup> reported that Arbor Acres broilers had greater villus heights than Lohman laying chickens. Al-Marzooqi *et al.*<sup>10</sup> reported similar findings, with Cobb500 broilers having greater villus heights in the jejunum and ileum than local chickens. From a morphological point of view, it could be expected that the longer villi in the broiler chickens resulted in an increased surface area that allowed greater absorption of available nutrients<sup>22</sup>.

The development of the absorptive structure of the intestine was found to be correlated with changes in the digestion and absorption of feed<sup>19</sup>. A significant positive correlation between the apparent excreta amino acid digestibility and the intestinal weight:length ratio in broilers was reported by Maisonnier *et al.*<sup>23</sup>. The lower apparent digestibility values of most of the amino acids obtained for local Omani chickens might be attributed to their relatively lower intake<sup>24</sup>. One problem encountered with local Omani chickens is that they tend to have behaviors similar to those of scavenger birds, such as wasting feed by scratching, even though the feeders are adjusted to prevent further feed loss.

Part of the detected differences in apparent ileal digestibility between the two breeds of chickens for the number of essential amino acids can be attributed to the differences in endogenous losses<sup>25</sup>. These endogenous secretions include large quantities of mucus. The main component of mucus is mucins, which are known to be rich in threonine<sup>26</sup>. Mucin also has high concentrations of glutamic acid, aspartic acid, serine and glycine<sup>27</sup> and differences in the apparent digestibility of these nonessential amino acids may mirror differences in mucin secretion between chicken strains. Moreover, it must be acknowledged that factors such as the passage rate and physiological statuses pertaining to the growth rate, maintenance and feed consumption may affect apparent digestibility measurements<sup>27,28</sup> and such discrepancies can at least partially explain the differences observed between the test ingredients for the two breeds.

# CONCLUSION

In general, the influence of the strain of chicken on the digestibility and digestible amino acid content assessed in this study were highly significant. Overall, the present study suggests that the practice of using amino acid digestibility values generated with commercial strains for indigenous chickens may not be applicable. Future studies will need to generate additional data that will help nutritionists formulate diets that more closely match the indigenous birds' requirements and minimize the excretion of excess nutrients into the environment.

#### SIGNIFICANCE STATEMENT

This study revealed that accurate information on the amino acid digestibility in feed ingredients is required for diet formulation for various classes of poultry. This study will help researchers understand how to enhance the productive performance of indigenous birds and reduce environmental pollution because of efficient nutrient utilization.

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