Standardized ileal digestible amino acid and metabolizable energy content of wheat from different origins and the effect of exogenous xylanase on their determination in broilers

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ABSTRACT This study was conducted to determine the standardized ileal digestible amino acids (**SID AA**) and nitrogen-corrected apparent metabolizable energy (**AMEn**) contents of 6 wheats from different origins in China and incidentally to investigate the effects of exogenous xylanase addition on SID AA and AMEn determination in broiler chicks. A total of 480 chicks were divided into 48 cages of 10 birds each balanced for body weight and fed 8 types of diets in a completely randomized design (6 replicated cages per diet) from 21 to 26 d of age. The individual wheat constituted the only source of crude protein in a semi-purified experimental diet. A nitrogen-free diet was designed to estimate basal endogenous AA loss and determine the SID AA. Titanium oxide (0.3%) was used as an indigestibility marker, and nutrient digestibility and retention were determined by the substitution method. From day 24 to 26, excreta samples were collected for AMEn determination. On day 26, the birds were euthanized, and ileum contents were obtained for AA digestibility determination. Wheat from Gansu had greater ($P < 0.05$) SID AA contents except Lys, Thr, Phe, and Cys, with a higher ($P < 0.001$) AMEn (11.83 MJ/kg) than the other wheats. The SID content of mean indispensable amino acids and dispensable amino acids were 87.35% and 88.17%, respectively, and the average AMEn value of 6 wheats was 11.14 MJ/kg. Compared with the diet without xylanase, the added xylanase resulted in higher ($P < 0.05$) SID contents of Met, Lys, Trp, Arg, Ile, Leu, Val, Gly, Asp, Glu, Pro, and Ala; the SID AA values were raised by 1.96% (mean of all AA); and the AMEn content was significantly increased (+0.87 MJ/kg) ($P < 0.05$). In conclusion, origins of wheats have significant effects on SID AA and AMEn values which were positively correlated with crude protein content of wheat; exogenous xylanase addition to a wheat-based poultry diet could significantly improve SID AA and AMEn contents for broilers.

Key words: wheat, xylanase, standardized ileal digestible amino acid, energy, broiler

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INTRODUCTION

Feed accounts for 60-70% of poultry production costs (Toghyani et al., 2015), therefore, an economical poultry diet formulation is one of the most important tools for cost reduction and profitability elevation. Amino acid digestibility and metabolizable energy of feed ingredients greatly affect animal performance and the economic benefits of poultry production; to optimize the nutritive value of ingredients in such a way that feed cost is reduced without compromising performance, it is essential for nutritionists to ensure that the digestible amino acid contents and metabolizable energy are considered in the selection of feed ingredients to meet the desired animal specifications. Diet formulation accuracy depends not only on the total nutrient content but also on the digestibility of those nutrients (Ravindran and Bryden, 1999; Lemme et al., 2004). There are differences in the amino acid digestibility and metabolizable energy of feed ingredients from different sources, as was recently underscored by findings from Ravindran et al. (2014) and Adewole et al. (2017) who showed that soybean

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meals and canola meals from different regions of the world differ in nutrient composition, and in the digestible amino acid content when fed to broilers. Standardized ileal digestibility of amino acid (**SID AA**) values are preferred to apparent ileal amino acid digestibility (**AID AA**) values because they account for the contribution of basal ileal amino acids of endogenous origin in the ileal digesta. Feed formulation based on the SID AA values allows diets to be formulated to closely meet animal requirements and minimize nitrogen excretion into the environment. There is a dearth of information on SID AA for many feed ingredients, particularly for a specific target or strain of birds. However, the application of the SID AA concept has only become a hot topic in recent years (Kong and Adeola, 2014; Adeola et al., 2016; Ravindran et al., 2017).

Wheat is an important feed ingredient available to the poultry industry in China, which is the main wheat-producing area in the world. In poultry production, wheat is a common kind of energy ingredient as it includes most of the dietary nutrients. Wheat is a good source of protein and amino acids (**AA**), especially Leu, Arg, Glu, and Pro (Bandegan et al., 2011). However, wheat contains high content of nonstarch polysaccharide (**NSP**) such as arabinoxylans in cell wall fiber–protein matrix, which is considered a main antinutritional factor. Thus, wheat can only be widely used in poultry feed through the exogenous addition of xylanase to decompose the NSP and improve digestibility (Kiarie et al., 2014; Amerah et al., 2017; Liu and Kim, 2017).

It is generally believed that antinutritional factors can lead to a decrease in nutrient digestibility or metabolizable energy content in poultry diets; therefore, it is reasonable to speculate that xylanase addition in the diet may change the SID AA and metabolizable energy of wheat. At present, few data have been published on the SID AA of wheat for broilers (Osho et al., 2019). The objective of this study was to determine the SID AA and AMEn of 6 representative wheats across China, to reveal the wheat origins affect the nutritional value on SID AA and nitrogen-corrected apparent metabolizable energy (**AMEn**), and to investigate the effects of xylanase addition on the determination of SID AA and AMEn for wheat. The information provided by this study will increase data reliability, as well as the confidence level of nutritionists in using this information for dietary formulations.

MATERIALS AND METHODS

Birds and Management

All experimental procedures were reviewed and approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences (FRI-CAAS20181112). Newly hatched one-day-old Ross 308 male broiler chicks were obtained from a local commercial hatchery and raised in the chicken facility of the Institute (Beijing, China). Chicks were reared and housed in an environmentally controlled room at 33°C for the first week after hatching, which was then adjusted by reducing the temperature by 3°C/week until reaching 24°C. Light was provided for 24 h daily throughout the experiment. Feed and water were supplied *ad libitum* in pellet form and via nipple drinkers, respectively. Bird management and supplied nutrients met the recommendations and specifications for the Ross 308 breed (Aviagen, 2014).

Experimental Diets

Six representative samples of wheat from the main wheat-producing areas of China were obtained, and the chemical composition of the samples was analyzed (Table 1). Eight experimental diets were examined in this study (Table 2). Seven wheat diets included wheat as the only source of crude protein (**CP**) in the semi-purified diets; one of the wheat diets was supplemented with xylanase (Xindayang Science and Technology Development Co. Ltd., Beijing, China) produced by a selected strain of *Trichoderma citrinoviride*. Pure enzyme product provided 4.8×10^4 U/g of xylanase. One unit of xylanase was defined as the amount of enzyme that liberated 1 μmol of reducing sugar (xylose equivalent) from xylan substrate (beechwood xylan) per minute at pH 4.8 and 50°C. A semi-purified nitrogen-free diet (**NFD**) was formulated to determine the endogenous amino acid flow (Adeola et al., 2016). All diets were balanced in terms of calcium and phosphorus and supplemented with equal amounts of vitamin and mineral premix. Titanium oxide (0.3%) was included in all experimental diets as an indigestible marker. The analyzed CP and amino acid contents of the diets are presented in Table 3.

Experimental Procedure

From day 1 to 20, chicks were fed conventional corn–soybean meal–based starter and grower diets in pelleted form. On day 21, after 3-h fasting, all chicks were weighed, and 480 healthy chicks within a similar body weight range were randomly divided into 48 cages, with replicates of 10 birds per cage used per diet. Fresh water and feed were available to all chicks *ad libitum* throughout the experimental phase. The birds were fed the 8 diets consecutively for 5 d. From day 24 to 26, excreta samples were obtained daily from each cage and stored at −20°C to determine nutrient retention, and apparent metabolizable energy (**AME**) and AMEn contents. On day 26, all the birds were euthanized with sodium pentobarbital, and the ileum contents (portion of the small intestine from Meckel's diverticulum to approximately 1 cm proximal to the ileocecal junction) were removed, gently flushed with distilled water, pooled for per-replicate cage, and then stored at −20°C until processing.

Table 1. Analyzed composition (on a DM basis) of wheat from different origins used in the study.

	Wheat[1]							
Composition	W-1	W-2	W-3	W-4	W-5	W-6	Average	CV
Dry matter, %	90.63	91.04	88.25	88.32	87.71	88.26	89.04	1.59
GE, MJ/kg	16.61	16.79	16.70	16.55	16.28	16.40	16.56	1.14
Crude protein, %	10.48	15.19	13.19	15.41	12.43	12.44	13.19	14.15
Crude fat, %	1.96	1.26	1.81	1.60	1.50	1.72	1.64	15.00
Ash, %	1.58	1.63	1.70	1.65	1.70	1.53	1.63	4.13
Crude fiber, %	2.64	3.21	2.98	3.26	2.37	2.39	2.81	14.16
Nitrogen-free extract, %	73.97	69.75	68.57	66.40	69.71	70.18	69.76	3.55
Indispensable amino acids, g/kg								
Methionine	1.87	2.42	2.11	2.25	2.14	2.05	2.14	8.68
Met + Cys	4.43	5.62	5.63	5.42	5.28	4.97	5.23	8.80
Lysine	3.00	3.96	3.47	3.67	3.32	3.21	3.44	9.95
Threonine	3.11	4.15	3.71	4.01	3.56	3.49	3.67	10.23
Tryptophan	1.37	1.79	1.47	1.77	1.70	1.67	1.63	10.45
Arginine	5.20	7.45	6.00	7.41	6.14	6.16	6.39	13.72
Isoleucine	3.50	5.16	4.52	5.01	4.10	4.21	4.42	13.95
Leucine	6.93	9.72	8.60	9.52	7.98	8.13	8.48	12.26
Valine	4.64	6.44	5.82	6.22	5.38	5.33	5.64	11.69
Histidine	2.53	3.58	3.08	3.50	3.06	2.98	3.12	12.23
Phenylalanine	4.44	6.67	5.62	7.05	5.42	5.72	5.82	16.03
Dispensable amino acids, g/kg								
Glycine	4.34	6.11	5.16	5.95	5.05	4.87	5.25	12.79
Serine	4.90	6.73	6.06	6.80	5.71	5.78	6.00	11.84
Proline	8.78	13.92	13.17	14.01	11.09	10.83	11.97	17.39
Alanine	3.89	5.26	4.58	5.02	4.51	4.37	4.61	10.54
Aspartic acid	5.36	7.66	6.62	7.27	6.39	6.31	6.60	12.19
Glutamic acid	27.83	42.64	38.59	44.10	33.92	34.62	36.95	16.42
Cysteine	2.56	3.20	3.52	3.17	3.14	2.92	3.09	10.41
Total amino acids, g/kg	98.68	142.48	127.73	142.15	117.89	117.62	124.45	13.46

Abbreviations: GE, gross energy.
[1]Origin of 6 wheat samples (W-1=Shandong-Linyi; W-2=Henan-Nanyang; W-3=Anhui-Taihe; W-4=Gansu-Wuwei; W-5=Shaanxi-Xi'an; W-6=Hebei-Handan), CV = coefficient of variation.

Sample Preparation and Analyses

Daily excreta samples were pooled for each cage and oven-dried at 65°C for 72 h, and the ileal digesta samples were freeze-dried after removing from storage in the freezer. Samples of wheats, diets, excreta, and ileal digesta were finely ground using an electric grinder and filtered through a 3-mm screen to ensure a homogeneous mixture for analysis. All samples were analyzed for dry matter (**DM**) and **CP**. The samples were further analyzed as follows: wheats for gross energy (**GE**), ether extract, ash, crude fiber, bulk density, and AA content; ileal digesta and diets for AA and titanium content; and excreta samples for GE and titanium content.

DM was determined by placing duplicate samples in a drying oven at 105°C for 4 h according to the AOAC method (1990; method 930.15), and nitrogen content was determined using an N analyzer (model Kjeltec-8100, FOSS Analytical Co., Ltd. Denmark). The CP was calculated by multiplying the N percentage by 6.25. GE was determined using a Parr-6100 adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Ether extract was determined using ethyl ether as the solvent according to the AOAC (2006; method 920.39). Ash content was determined in accordance with the AOAC method (2006; method 942.05), and crude fiber, in accordance with the AOAC method (2006; method 978.10) using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Bulk density of wheat was determined using a volume-weight instrument (model HGT-1000, Shanghai Dongfang Scales Co., Ltd., Shanghai, China).

Samples for AA analysis were prepared by acid hydrolysis in accordance with the AOAC method (1990; method 982.30) as modified by Mills et al. (1989). Briefly, approximately 100 mg of each sample was digested in 4 mL of 6 N HCl for 24 h at 110°C, followed by neutralization with 4 mL of 25% (wt/vol) NaOH, and then cooled to about 25°C. The mixture was then equalized to a 50-mL volume with sodium citrate buffer (pH 2.2) and analyzed using an AA analyzer (model S430, Sykam, Eresing, Germany).

Methionine and cysteine (sulfur-containing AA) were analyzed by performic acid oxidation at 0°C, followed by acid hydrolysis. The AA in the hydrolysate were determined using an AA analyzer (model Biochrom-30, Biochrom Ltd., Cambridge, United Kingdom).

Titanium oxide concentrations were determined in duplicate for samples; samples to be analyzed were ashed and digested in accordance with the procedures described by Lomer et al. (2000) and read on a Varian inductively coupled plasma mass spectrometer (model GC- 450, Varian Inc., Palo Alto, CA).

Calculations and Statistical Analysis

Ileal endogenous AA contents were calculated as milligrams of AA content per kilogram of DM intake using the formula proposed by Moughan et al. (1992):

Table 2. Composition of the experimental diets (on an as-is basis).

	Diet[1,2]							
Ingredient (%)	NFD	W-1	W-2	W-3	W-4	W-5	W-6	W-6+XYL
Corn starch	22.48	-	-	-	-	-	-	-
Dextrose	64.0	-	-	-	-	-	-	-
Cellulose	4.0	-	-	-	-	-	-	-
Wheat	-	91.75	91.75	91.75	91.75	91.75	91.75	91.72
Soybean oil	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Dicalcium phosphate	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Limestone	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Titanium oxide	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium bicarbonate	1.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
NaCl	-	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin–trace mineral premix[3]	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
choline chloride	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
KCl	0.4	-	-	-	-	-	-	-
MgO	0.07	-	-	-	-	-	-	-
Xylanase	-	-	-	-	-	-	-	0.03
Total	100	100	100	100	100	100	100	100
Calculated composition[4]								
ME, MJ/kg	12.62	13.07	13.07	13.07	13.07	13.07	13.07	13.07
CP,%	0.0	9.62	13.94	12.10	14.14	11.40	11.41	11.41
Ca, %	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Available phosphorus, %	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72

[1]NFD = nitrogen-free diet; origin of 6 wheat samples (W-1=Shandong-Linyi; W-2=Henan-Nanyang; W-3=Anhui-Taihe; W-4=Gansu-Wuwei; W-5=Shaanxi-Xi'an; W-6=Hebei-Handan); XYL = xylanase.

[2]Crude protein contents (%) of the 6 feedstuffs were 10.48 (W-1), 15.19 (W-2), 13.19 (W-3), 15.41 (W-4), 12.43 (W-5), and 12.44 (W-6).

[3]Supplied per kg diet: Mn, 125 mg; Zn, 60 mg; Cu, 3 mg; Mo, 0.5 mg; Co, 0.3 mg; I, 1.0 mg; Fe, 25 mg; Se, 200 μg; trans-retinol, 3.33 mg; cholecalciferol, 60 μg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamine, 3.0 mg; riboflavin, 12 mg; calcium pantothenate, 12.8 mg; niacin, 35 mg; pyridoxine, 10 mg; folic acid, 5.2 mg; cyanocobalamin, 0.017 mg; biotin, 0.2 mg; antioxidant, 100 mg.

[4]Calculated composition was based on ingredient composition data from NRC (1994).

$$\text{Ileal amino acid content, mg/kg of DMI} = [\text{amino acid in ileal digesta, mg/kg} \times (\text{TiO}_2 \text{ in diet, mg/kg / TiO}_2 \text{ in digesta, mg/kg})] \quad (1)$$

The AID AA and SID AA values were calculated using the following equations by Adedokun et al. (2008):

$$\text{AID AA, \%} = [1 - (\text{TiO}_2 \text{ in diet / TiO}_2 \text{ in digesta}) \times (\text{amino acid in digesta / amino acid in diet})] \quad (2)$$

Table 3. Analyzed crude protein and amino acid composition (%) of the experimental diets (on an as-is basis).

	Diet[1]							
Item	NFD	W-1	W-2	W-3	W-4	W-5	W-6	W-6+XYL
Crude protein	0.31	10.01	13.82	12.68	14.34	12.64	11.67	12.07
Indispensable amino acids								
Methionine	0.00	0.16	0.21	0.20	0.20	0.19	0.18	0.19
Met + Cys	<0.01	0.39	0.50	0.51	0.49	0.47	0.43	0.47
Lysine	<0.01	0.29	0.37	0.34	0.36	0.33	0.30	0.33
Threonine	0.00	0.29	0.38	0.35	0.39	0.35	0.33	0.35
Tryptophan	<0.02	0.12	0.16	0.14	0.16	0.13	0.15	0.16
Arginine	0.00	0.47	0.64	0.59	0.69	0.57	0.55	0.59
Isoleucine	<0.01	0.31	0.45	0.41	0.46	0.41	0.37	0.39
Leucine	<0.02	0.64	0.86	0.80	0.88	0.78	0.73	0.77
Valine	<0.03	0.42	0.56	0.53	0.57	0.52	0.48	0.51
Histidine	<0.02	0.23	0.30	0.28	0.33	0.28	0.27	0.28
Phenylalanine	0.00	0.42	0.60	0.55	0.65	0.54	0.53	0.53
Dispensable amino acids								
Glycine	<0.01	0.40	0.54	0.50	0.56	0.49	0.45	0.49
Serine	<0.01	0.47	0.65	0.56	0.68	0.59	0.56	0.57
Proline	0.00	0.85	1.37	1.19	1.43	1.25	1.12	1.19
Alanine	<0.02	0.36	0.48	0.44	0.48	0.44	0.41	0.44
Aspartic acid	0.01	0.50	0.70	0.62	0.71	0.63	0.60	0.64
Glutamic acid	<0.03	2.72	3.80	3.64	4.07	3.45	3.15	3.27
Cysteine	<0.01	0.23	0.29	0.31	0.29	0.27	0.26	0.27

[1]NFD = nitrogen-free diet; origin of 6 wheat samples (W-1=Shandong-Linyi; W-2=Henan-Nanyang; W-3=Anhui-Taihe; W-4=Gansu-Wuwei; W-5=Shaanxi-Xi'an; W-6=Hebei-Handan); XYL = xylanase.

$$\text{SID AA, \%} = \text{AID AA, \%} + [(\text{IEAA content, g / kgof DMI}) / (\text{AA content of the raw material, g / kgof DM})] \times 100 \quad (3)$$

where IEAA content is the endogenous ileal AA content calculated using Eq. (1) from the ileal digesta of chicks fed an NFD.

The AME and AMEn for wheats were calculated by TiO_2 marker using the following equations according to Roza et al. (2018):

$$\text{Indigestibility factor(IF)} = TiO_2 \text{ in diet} / TiO_2 \text{ in excreta} \quad (4)$$

$$\text{AME, MJ / kg} = \text{GE in diet} - (\text{GE in excreta} \times \text{IF}) \quad (5)$$

$$\text{AMEn, MJ / kg} = \text{AMEn} + 8.22 \times [\text{N in diet} - (\text{N in excreta} \times \text{IF})] \quad (6)$$

$$\text{Apparent retention of gross energy(GE), \%} = \text{AME / GE of the raw material} \times 100 \quad (7)$$

where GE is the gross energy and N is nitrogen content, which is calculated by CP (crude content) = N content × 6.25.

All analyses were performed using SPSS, version 18.0, for Windows (SPSS, Chicago, IL). Data were analyzed using one-way ANOVA, and means were compared using the Duncan multiple range test. The effect of xylanase on wheat AMEn and SID AA values was determined, treatment means (W-6 vs. W-6 + xylanase) were compared using the student t test procedure, and differences were considered statistically significant at $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

AA Composition of Wheats From Different Origins

The chemical and AA composition of wheat samples from different origins is presented in Table 1. The average DM of samples was 89.04% (coefficient of variation [CV] = 1.59%). The GE content ranged from 16.28 to 16.79 MJ/kg (DM basis), and the CV was 1.14%. The CP content of samples was consistent among the 6 wheat types, with an average of 13.19%, a range of 10.48 to 15.41%, and the variation was considered large (CV = 14.15%). The average crude fat and fiber contents of samples were 1.64% and 2.81%, respectively. The results implied that variation in location, agronomic practices, and environment substantially affected wheat composition. Based on chemical analyses, the W-2 (Henan-Nanyang) and W-4 (Gansu-Wuwei) samples appeared to be of better quality than others, with high reactive AA content. The overall chemical composition of the samples ranged within those previously reported for wheat (Bryden et al., 2009; Bandegan et al., 2011). Among the indispensable AA, Met, Trp, and His contents were the lowest, whereas Arg and Leu contents were the highest. The lowest and most abundant dispensable AA were Cys and Glu, respectively. It is worth noting that the Glu content was 3 to 8 times to that of the other AA; this characteristic is related to the protein composition in wheat. This corroborated findings for wheat storage proteins, which have a higher proportion of Glu and Pro, but differed in that Lys existed in a smaller proportion than previously reported (Osho et al., 2019). The overall AA profile of the wheat samples showed no difference with NRC (1994) values for these feedstuffs.

Endogenous AA Flow in Broilers

The analyzed CP and AA composition of the experimental diets is shown in Table 3. Tested ingredients (wheats) were formulated to be the only source of dietary CP because when CP is below 16.5%, SID AA is dependent on the dietary AA level (Stein et al., 2007). To optimize the dietary formulation and reduce nutrient excretion, it is very important to determine the raw material SID AA (Adedokun et al., 2007; Ravindran et al., 2009). This requires an accurate assessment of the contribution of AA endogenous losses through NFD. The CP content of the NFD in this study was only 0.31%, and the AA composition was very low. As illustrated in Table 4, the ileal endogenous CP and AA flows for all AA in broilers at 26 D were determined, the lowest flow in Met and Trp (86.80 and

Table 4. Ileal endogenous amino acid flow (mg/kg of DM feed intake) in broiler chicks fed a nitrogen-free diet (NFD) at 26 d of age[1].

Item	Average	SD
CP	7813.24	924.62
Indispensable amino acids		
Met	86.80	12.83
Met + Cys	260.52	23.60
Lys	259.45	57.21
Thr	422.03	35.44
Trp	97.99	10.96
Arg	275.50	37.99
Ile	247.06	33.54
Leu	394.55	47.32
Val	336.30	28.43
His	152.76	10.39
Phe	320.11	12.41
Dispensable amino acids		
Cys	173.72	12.17
Gly	335.19	31.90
Ser	422.79	40.02
Pro	363.48	31.36
Ala	291.39	32.17
Asp	570.17	59.26
Glu	689.90	81.91

[1]Data are means of 6 pens of broilers with 10 broilers per pen.

Table 5. Standardized ileal amino acid digestibility in broilers fed wheats from different origins[1].

Item (%)	W-1	W-2	W-3	W-4	W-5	W-6	SEM	*P*-value	Average	CV
CP	84.39	83.13	84.43	87.19	85.32	86.15	0.51	0.26	85.10	1.69
Indispensable amino acids										
Methionine	90.36^{d}	91.00^{c,d}	91.78^{b,c}	92.85^{a}	91.58^{b,c}	92.28^{a,b}	0.17	<0.001	91.64	0.97
Met + Cys	89.55^{a,b}	88.07^{b}	89.89^{a}	90.95^{a}	90.13^{a}	90.10^{a}	0.26	0.035	89.78	1.07
Lysine	80.81	81.07	82.84	84.49	81.75	82.71	0.41	0.093	82.28	1.66
Threonine	80.40	79.32	80.19	84.88	82.38	82.51	0.84	0.448	81.61	2.50
Tryptophan	82.31^{a,b,c}	79.75^{c}	81.30^{b,c}	85.08^{a}	78.68^{c}	84.04^{a,b}	0.58	0.004	81.86	3.00
Arginine	85.39^{d}	86.38^{c,d}	88.97^{b}	91.36^{a}	86.84^{c,d}	88.06^{b,c}	0.40	<0.001	87.83	2.43
Isoleucine	88.62^{c}	88.96^{c}	89.78^{c}	92.15^{a}	91.34^{a,b}	90.11^{b,c}	0.28	<0.001	90.16	1.51
Leucine	88.82^{c}	89.08^{c}	90.24^{b,c}	92.11^{a}	90.20^{b,c}	90.72^{b}	0.25	<0.001	90.20	1.32
Valine	84.92^{c}	85.19^{b,c}	85.89^{b,c}	89.69^{a}	87.25^{b}	87.27^{b}	0.37	<0.001	86.70	2.04
Histidine	84.58^{d}	85.16^{c,d}	86.43^{b,c,d}	90.37^{a}	87.04^{b,c}	87.53^{b}	0.42	<0.001	86.85	2.36
Phenylalanine	89.49	91.99	90.63	94.65	92.75	91.90	0.59	0.181	91.90	1.93
Mean	85.93^{c}	86.00^{c}	87.09^{b,c}	89.87^{a}	87.27^{b,c}	87.93^{b}	0.32	0.001	87.35	1.67
Dispensable amino acids										
Cysteine	88.99	86.01	88.68	89.61	89.07	88.58	0.37	0.071	88.49	1.43
Glycine	81.98^{b}	82.48^{b}	83.42^{b}	87.20^{a}	84.22^{b}	83.72^{b}	0.41	0.001	83.84	2.20
Serine	87.20^{c}	87.61^{b,c}	86.70^{c}	91.40^{a}	89.44^{a,b}	89.41^{a,b}	0.38	<0.001	88.63	2.00
Proline	91.88^{c}	93.81^{b}	93.83^{b}	95.86^{a}	94.05^{b}	94.15^{b}	0.27	<0.001	93.93	1.35
Alanine	81.70^{c}	82.83^{b,c}	82.96^{b,c}	86.52^{a}	84.38^{a,b}	84.52^{a,b}	0.40	0.003	83.82	2.02
Aspartic acid	80.64^{c}	81.82^{b,c}	81.04^{c}	86.23^{a}	83.70^{b}	83.59^{b}	0.45	<0.001	82.84	2.53
Glutamic acid	95.14^{c}	95.15^{c}	95.97^{b}	96.83^{a}	95.57^{b,c}	95.45^{b,c}	0.12	<0.001	95.69	0.67
Mean	86.79^{b}	87.10^{b}	87.51^{b}	90.52^{a}	88.63^{b}	88.49^{b}	0.31	0.001	88.17	1.55

[a-d]Within an age group, means in the same row with different superscripts are different at $P < 0.05$.
[1]Data are means of 6 pens of broilers with 10 broilers per pen, CV = coefficient of variation.

97.99 mg/kg of DM feed intake, respectively) and the highest flow in Glu (689.90 mg/kg of DM feed intake). The ileal endogenous AA were obviously different for all AA, and these basal endogenous AA losses in the present study were similar to those reported by Toghyani et al. (2015), who also fed an NFD to broiler chicks. In general, the endogenous 0loss of dispensable AA was higher than that of indispensable AA.

Standardized Ileal Digestible AA of Wheats for Broilers

The determination of endogenous AA loss enables us to establish the SID AA values of feed ingredients by correcting the basic endogenous AA loss, and AA digestibility increased with age after standardization (Adedokun et al., 2008). Table 5 shows the SID AA values. Of the 11 indispensable AA, the average SID AA values for Met and Phe were the highest (91.64 and 91.90%), while those for Lys, Thr, and Trp were the lowest (82.28, 81.61, and 81.86%, respectively). Of the 7 dispensable AA, the average SID AA value was highest for Glu (95.7%) and lowest for Asp (82.8%). Overall, the average SID AA values (81.61 for Thr to 91.90% for Phe) for the indispensable AA in wheat samples in the present study were lower than those previously reported by Bandegan et al. (2011) for 6 wheat samples (ranged from 83.7 for Lys to 93.8% for Phe) fed to broilers but were higher than those reported by Osho et al., (2019) for 1 wheat sample (ranged from 70.4 to 87.8%) fed to broilers. There are several reasons that could explain the differences in SID AA estimates between these studies; however, the CP content of the feed ingredients was likely a major factor. The wheat sample in the study of Osho et al., (2019) had low CP value which was 11.19%, but those studied by Bandegan et al. (2011) were high (range, 14.8–18.4%); the values of the wheat samples in the present study were of intermediate level (range, 10.48–15.41%). Because AID AA increases correspondingly with dietary AA intake (Adedokun et al., 2015) and because endogenous AA remains relatively stable, SID AA maintains a rising trend. Consistent with this conclusion, the W-4 SID AA value (CP was 15.41%) was greater ($P < 0.05$) than those of other wheats, except Lys, Thr, Phe, and Cys ($P > 0.05$); however, the W-1 SID AA value (CP was 10.48%) was the lowest among the 6 wheat samples ($P < 0.05$). As SID AA estimates may be affected by analytical conditions, we

Table 6. Apparent retention of gross energy (GE), AME, and AMEn contents (on DM basis) of wheats from different origins fed to broiler chicks[1].

Item	W-1	W-2	W-3	W-4	W-5	W-6	SEM	*P*-value	Average	CV
GE, %	66.53^{b}	71.26^{a}	68.11^{b}	73.42^{a}	68.89^{b}	68.53^{b}	2.86	<0.001	69.46	3.56
AME, MJ/kg	11.05^{b}	11.96^{a}	11.38^{b}	12.15^{a}	11.22^{b}	11.24^{b}	0.50	<0.001	11.50	3.88
AMEn, MJ/kg	10.66^{c}	11.52^{a,b}	11.07^{b,c}	11.83^{a}	10.85^{c}	10.89^{c}	0.59	0.001	11.14	4.02

[a-c]Within an age group, means in the same row with different superscripts are different at $P < 0.05$.
Abbreviations: AME, apparent metabolizable energy; AMEn, nitrogen-corrected apparent metabolizable energy.
[1]Data are means of 6 pens of broilers with 10 broilers per pen, CV = coefficient of variation.

Table 7. Effects of dietary supplementation of xylanase on standardized ileal amino acid digestibility in broilers[1].

Item (%)	W-6	W-6 + XYL	*P*-value
CP	86.15 ± 2.37	87.93 ± 1.65	0.161
Indispensable amino acids			
Methionine	92.28 ± 0.75	93.53 ± 0.55	0.008
Methionine + cysteine	90.10 ± 1.29	91.01 ± 0.81	0.173
Lysine	82.71 ± 2.63	87.39 ± 0.68	0.002
Threonine	82.51 ± 4.60	84.40 ± 2.10	0.382
Tryptophan	84.04 ± 1.84	86.97 ± 1.00	0.007
Arginine	88.06 ± 1.18	90.99 ± 0.58	0.001
Isoleucine	90.11 ± 1.03	91.99 ± 0.70	0.004
Leucine	90.72 ± 0.93	92.37 ± 0.28	0.006
Valine	87.27 ± 1.00	89.35 ± 1.12	0.007
Histidine	87.53 ± 0.88	88.88 ± 1.42	0.075
Phenylalanine	91.90 ± 2.66	93.11 ± 3.30	0.500
Dispensable amino acids			
Cysteine	88.58 ± 1.96	89.21 ± 1.33	0.529
Glycine	83.72 ± 1.39	85.74 ± 1.37	0.029
Serine	89.41 ± 0.90	91.16 ± 1.28	0.021
Proline	94.15 ± 0.87	95.25 ± 0.68	0.034
Alanine	84.52 ± 1.07	87.45 ± 1.23	0.001
Aspartic acid	83.59 ± 1.23	86.48 ± 1.39	0.003
Glutamic acid	95.45 ± 0.52	96.73 ± 0.40	0.001

[1]Data are means of 6 pens of broilers with 10 broilers per pen.

measured and recorded the reproducibility of ileal digestibility analysis in wheat as previously reported (Ravindran et al., 2017).

Metabolizable Energy Content of Wheats for Broilers

As shown in Table 6, the average apparent GE retention for all wheats was 69.46%, and average AME and AMEn contents were 11.50 and 11.14 MJ/kg, respectively. These values were lower than the 13.30 and 12.19 MJ/kg reported by Moss et al. (2017) on 27-day-old broilers fed wheat; this difference was due to a higher fat content (2.04%) than those of the wheats used in this study (1.64%). Compared with other wheats, W-4 had a greater apparent GE retention ($P < 0.001$), which could be attributed to its higher CP and AA digestibility. The AME and AMEn contents were greater ($P < 0.001$) for W-4 than other wheats, which was due to the greater content and apparent retention of GE. Therefore, wheat AME and AMEn values for broilers differed based on different origins and ranged from 11.05 to 12.15 MJ/kg and from 10.66 to 11.83 MJ/kg, respectively, in the present study.

Effect of SID AA and AMEn With Xylanase Addition in Wheat Diets

Wheat NSP is of relatively high content and is an antinutritional factor that prevents the effective utilization of nutrients in wheat to some extent. It is interesting that most of the recent publications on exogenous enzymes in the diet of monogastric animals have focused on the effects on AA digestibility (Cowieson and Roos, 2014) and metabolizable energy (Rouhollah et al., 2018). The results in the present experiment (Table 7 and Figure 1) showed that a beneficial effect of exogenous xylanase addition to a wheat-based broiler diet is to increase SID AA and AME. These responses are comparable with previous observations (Liu and Kim, 2017; Peiman et al., 2018). Exogenous xylanase in diets could increase SID CP (86.15 vs. 87.93%, $P = 0.161$); it was not significant. The addition of exogenous xylanase resulted in an increase ($P < 0.05$) in the SID AA values of Met (+1.25%), Trp (+2.93%), Arg (+2.93%), Ile (+1.88%), Leu (+1.65%), Val (+2.08%), Gly (+2.02%), Ser (+1.75%), Pro (+1.10%), Ala (+2.93%), Asp (+2.89%), and Glu (+1.28%). The xylanase used in this study is extremely effective in hydrolyzing xylans and arabinoxylans into xylooligosaccharides in a variety of wheats. In the present study, the addition of xylanase increased the SID AA values by more than 1.96% (mean of all AA). This is consistent with previous reports that exogenous xylanase is capable of significantly increasing the apparent ileal digestibility of several important AA (Kiarie et al., 2014; Liu and Kim, 2017). In addition, exogenous xylanase supplementation increased the ileal digestibility of starch, especially in diets with low protein and digestible AA content. Exogenous xylanase addition can also improve the solubility of the wheat starch and protein matrix, and increase

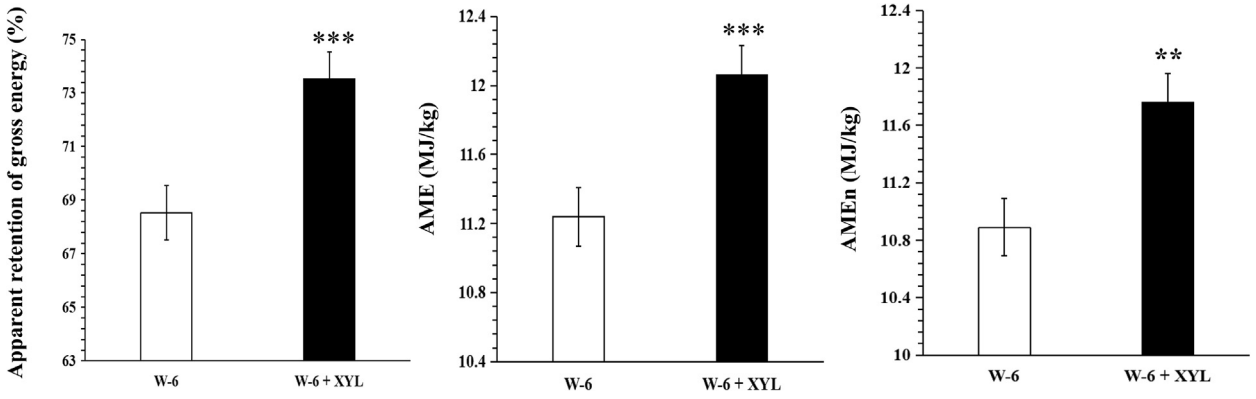

Figure 1. Effects of dietary supplementation of xylanase on apparent retention of gross energy (GE), apparent metabolizable energy (AME), and nitrogen-corrected apparent metabolizable energy (AMEn) contents (on DM basis) of W-6 wheat in broilers. (n = 6 chicks/group). Data represent the mean ± SEM. $^{**}P < 0.01$,$^{***}P < 0.001$.

intestinal soluble starch. The supply of AA to the intestine may stimulate the kinetics of starch digestion and indirectly improve AA digestibility (Liu and Selle, 2015). In the present study, dietary xylanase addition increased AME values from 11.24 to 12.06 MJ/kg (+0.82 MJ/kg) and AMEn values from 10.89 to 11.76 MJ/kg (+0.87 MJ/kg), which is in close agreement with other reported responses (Kalmendal and Tauson, 2012; Kiarie et al., 2014. Amerah et al., 2015). Clearly, the AME and AMEn levels increased by exogenous xylanase observed in the present study suggested an enzymatic benefit owing to the more efficient energy gain through higher feed nutrient digestibility. By adding exogenous xylanase to wheat-based broiler diets, the SID AA contents increased more than expected, meaning nutrients that generate a lot of energy, or total nutrient allocation, work together. The effect of dietary protein and AA content on starch digestibility of grain in broilers is a valuable research direction (Cowieson et al., 2019).

In conclusion, the results showed that the variability exists in SID AA and AMEn for broilers because the different origins of wheat, in general, followed the same trend as that of digestible CP. Therefore, it is of great importance to determine or obtain the SID AA and AMEn values before inclusion in the feed, and the CP content of wheats provide a general and simple prediction of these values. These data provide baseline for the variation that could be expected for wheat originating from these 6 major wheat-producing areas in China. Furthermore, xylanase added to a wheat-based poultry diet effectively improved SID AA and AME values and should be considered when formulating broilers' feed.

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