

# Determination and prediction of standardized ileal amino acid digestibility of corn distillers dried grains with soubles in broiler chickens

Huimei Wang,<sup>1</sup> Fang Yan,<sup>1</sup> Fangshen Guo, Xingpeng Liu, Xiaojun Yang, and Xin Yang<sup>2</sup>

*College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China*

**ABSTRACT** The experiment was conducted to evaluate ileal digestibility of amino acids (**AA**) in 8 corn distillers dried grains with solubles (**DDGS**) fed to broilers and to establish prediction equations for standardized ileal digestibility (**SID**) of AA for broilers based on the physicochemical properties. A total of 1,152 1-day-old male broilers were divided into 2 test stages (from day 9 to 14 and from day 23 to 28). In each stage, 576 broilers were randomly allotted to 1 of 9 diets (8 replicates, 8 birds per replicate) including a nitrogen-free diet and 8 corn DDGS test diets. Titanium dioxide (0.5%) was included in all diets as an external marker. In 8 corn DDGS samples, the contents of aflatoxin B<sub>1</sub>, deoxynivalenol, zearalenone, and zein were from 1.54 to 15.50 ppb, 0.44 to 5.12 ppm, 127.10 to 1062.46 ppb, and 3.10 to 26.89%, respectively; the content of lysine and methionine (**Met**) ranged from 0.36 to 0.67% (CV

21.51%) and from 0.16 to 0.74% (CV 58.04%), respectively. The SID of AA, except for valine and alanine, were significantly different ( $P < 0.05$ ) at day 28. A positive correlation was observed ( $P < 0.05$ ) between degree of lightness and SID of CP, Met, and total amino acid (**TAA**) at day 14. A negative correlation was observed ( $P < 0.05$ ) between mycotoxins and SID of CP, lysine, Met, and TAA at day 28. The  $R^2$  value of stepwise regression equations for predicting the SID of AA at day 14 and day 28 was best for glutamic acid ( $R^2 = 1.000$  using ether extract, crude fiber, CP, aflatoxin B<sub>1</sub>, and neutral detergent fiber) and TAA ( $R^2 = 0.904$  using ether extract), respectively. In conclusion, this experiment suggested mycotoxin can be used to predict the SID of AA in corn DDGS with reasonable accuracy, and the results of SID and prediction equations could be used to evaluate the digestibility of corn DDGS in broilers.

**Key words:** broiler, corn distillers dried grains with soluble, prediction equation, mycotoxin, standardized ileal amino acid digestibility

2020 Poultry Science 99:4990–4997

<https://doi.org/10.1016/j.psj.2020.06.041>

## INTRODUCTION

Distillers dried grains with solubles (**DDGS**) is a by-product from the ethanol industry, formed after fermenting the starch from corn. With the growth of the ethanol industry in China, the application of corn DDGS in animal industry is increasingly being accepted. Previous research in one study demonstrated that corn DDGS can be incorporated into broiler diets at levels up to 24% to maintain good performance (Shim et al., 2011). However, differences in processing

procedures and grain source may lead to large variations in the physicochemical properties of corn DDGS (Belyea et al., 2010; Meloche et al., 2014; Adedokun et al., 2015). Furthermore, nutritional composition and level of corn DDGS directly affects nutritional value and nutrient utilization efficiency. Especially, the data of amino acid (**AA**) composition and digestibility are important bases for precision application of feed-stuff because low-protein amino acid diets are trending in China. However, the data of the AA digestibility in standard of broiler breeding in China were based on the experimental data of laying hens and roosters, which ignore the unique characters of nutrient use in broiler, and restricted to the application in broiler production.

The methods of AA digestibility are relatively expensive and take much time and effort, therefore many studies were conducted to develop some useful parameters to predict AA digestibility. Some studies found

© 2020 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received March 4, 2020.

Accepted June 20, 2020.

<sup>1</sup>These authors contributed equally to this work.

<sup>2</sup>Corresponding author: [yangx0629@163.com](mailto:yangx0629@163.com)

**Table 1.** Analyzed physicochemical properties of corn distillers dried grains with solubles sources (%).

Item	Corn distillers dried grains with solubles sources								Mean	CV
	1	2	3	4	5	6	7	8		
GE (MJ/kg)	18.73	18.43	18.46	20.94	19.21	22.71	20.92	21.17	20.07	7.87
CP	32.66	33.30	32.57	32.08	32.03	31.96	28.61	28.25	31.43	6.06
Ether extract	9.33	9.20	9.11	12.32	9.36	12.64	11.29	12.91	10.77	15.71
Crude fiber	7.76	6.81	6.94	9.18	7.03	9.81	9.15	7.22	7.99	15.07
Crude ash	3.06	3.02	3.21	2.85	3.42	0.99	3.76	2.34	2.83	30.03
Neutral detergent fiber	32.80	29.94	31.23	35.31	31.14	40.14	35.80	31.97	33.54	10.01
Acid detergent fiber	10.07	8.69	9.06	12.03	9.09	12.28	11.80	9.79	10.35	14.16
Aflatoxin B1 (ppb)	14.99	15.50	15.40	2.69	12.31	2.13	2.02	1.54	8.32	80.96
Deoxynivalenol (ppm)	4.63	2.74	3.71	1.65	5.12	0.40	1.69	2.20	2.77	58.29
Zearalenone (ppb)	967.83	683.46	791.45	200.67	1,062.46	127.10	181.81	166.00	522.60	75.55
L*	64.96	64.77	63.38	69.35	67.08	65.18	69.73	72.32	67.09	4.60
a*	7.09	6.35	6.56	8.32	7.15	7.42	7.53	7.53	7.24	8.49
b*	31.30	30.43	29.97	32.59	28.78	23.59	25.86	28.07	28.82	10.24
Bulk weight (g/L)	398.53	378.89	380.17	407.86	377.44	383.73	392.61	380.66	387.49	2.85
Zein (%)	3.10	3.24	3.09	23.13	9.44	26.89	19.82	13.88	13.88	73.40
Total amino acid	13.69	13.23	12.74	21.03	15.18	25.59	16.59	18.38	17.05	26.11
Indispensable amino acid										
Arginine	0.74	0.64	0.63	0.94	0.61	1.02	1.25	0.83	0.83	27.10
Histidine	0.51	0.48	0.45	0.56	0.45	0.64	0.37	0.47	0.49	16.64
Isoleucine	0.59	0.52	0.51	0.92	0.67	1.10	2.50	0.80	0.95	69.29
Leucine	1.73	1.55	1.46	3.03	2.02	3.96	0.73	2.62	2.14	47.84
Lysine	0.52	0.49	0.44	0.67	0.42	0.67	0.36	0.54	0.52	21.51
Methionine	0.21	0.20	0.16	0.46	0.23	0.57	0.74	0.31	0.36	58.04
Phenylalanine	0.64	0.59	0.55	1.16	0.74	1.45	0.47	1.06	0.83	42.34
Threonine	0.74	0.65	0.60	0.95	0.70	1.09	0.76	0.87	0.79	20.88
Valine	1.03	0.95	0.86	1.29	1.06	1.50	0.19	1.26	1.02	38.49
Dispensable amino acid										
Alanine	0.67	1.49	1.41	1.95	1.83	2.42	1.45	1.72	1.62	31.23
Aspartic acid	1.06	0.91	0.84	1.33	1.07	1.79	1.30	1.38	1.21	25.28
Cystine	0.18	0.16	0.17	0.23	0.20	0.25	1.02	0.18	0.30	98.41
Glutamic acid	2.92	2.64	2.77	4.45	2.98	5.47	2.95	3.58	3.47	28.72
Glycine	0.82	0.74	0.69	0.88	0.79	0.94	0.67	0.81	0.79	11.60
Serine	0.90	0.79	0.82	1.33	0.89	1.60	0.89	1.18	1.05	27.61
Tyrosine	0.44	0.43	0.38	0.89	0.53	1.12	0.93	0.78	0.69	40.53

Abbreviations: a\*, degree of redness; b\*, degree of yellowness; L\*, degree of lightness.

that the color scores may provide a rapid method for identifying corn DDGS sources with good or poor AA digestibility (Ergul et al., 2003; Fastinger et al., 2006). In addition, equation for ME of corn DDGS based on nutrient composition or the color score has been developed for use in broilers (Meloche et al., 2014). Zhang et al. (2020) established prediction equation of AA digestibility of rapeseed meals for Pekin ducks based on chemical composition. However, other features such as mycotoxin such as aflatoxin B1 (AFB1), deoxynivalenol (DON), and zearalenone (ZEN) too may ultimately limit corn DDGS use in poultry diets because they can lead to the loss of the resistance and the performance of all the birds. The content of mycotoxin in corn DDGS may show some regularity owing to the influence of growing environment, climate, process technology, and other factors, hence, they may be a kind of new and very effective potential predictors. No analogous equation especially based on mycotoxins as predictive factors has been reported for AA digestibility. Therefore, the objective of this study to determine the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AA of corn DDGS in broilers and to establish regression prediction equations based on physicochemical properties.

## MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee of Northwest A&F University (Permit Number: NWAAC 1008).

### Corn DDGS Samples

Eight samples of corn DDGS used in the current experiment were collected from 4 provinces of Heilongjiang, Jilin, Inner Mongolia, and Henan in China. Before chemical analysis and being fed to broilers, all corn DDGS samples were ground to pass through a sieve with 40 mesh size and stored in  $-20^{\circ}\text{C}$  for further analysis. The physicochemical properties of the collected corn DDGS are shown in Table 1.

### Experimental Procedures

A total of 1,152 1-day-old male broilers (Arbor Acres) were obtained from a commercial hatchery and received a standard starter diet before feeding the experimental diets. Broilers were provided ad libitum access to water and feed throughout the experiment. The experimental diets included 1 nitrogen-free diet and 8 test diets (Table 2). In the test diets, corn DDGS was the only

**Table 2.** Ingredient composition of the experimental diets (g/kg, as-fed basis).

Ingredients	DDGS diets	N-free diet
Corn DDGS	63.63	-
Corn starch	-	20.07
Glucose	27.37	64.00
Carboxymethylcellulose	-	5.00
Soybean oil	5.00	5.00
Multivitamin premix <sup>1</sup>	0.02	0.02
Mineral premix <sup>2</sup>	0.20	0.20
CaHPO <sub>4</sub>	1.70	-
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	-	1.90
NaCl	0.30	-
NaHCO <sub>3</sub>	-	0.75
KCl	-	0.29
MgO	-	0.20
Choline chloride(50%)	0.25	0.50
Limestone	1.00	1.30
K <sub>2</sub> CO <sub>3</sub>	-	0.26
TiO <sub>2</sub>	0.50	0.50
Antioxidant	0.03	0.01
Total	100.00	100.00

Abbreviation: DDGS, distillers dried grains with solubles sources.

<sup>1</sup>The premix provided the following per kg of diets: (1–21 D) VA, 11,200 IU; VD3, 3,360 IU; VE, 20 mg; VK3, 4.0 mg; VB1, 2.2 mg; VB2, 7.28 mg; VB6, 4.8 mg; (21–42 D) VA, 8,400 IU; VD3, 2,520 IU; VE, 15.75 mg; VK3 3.0 mg; VB1, 1.64 mg; VB2, 5.46 mg; VB6, 3.6 mg.

<sup>2</sup>The premix provided the following per kg of diets: Cu, 8.4 mg; Fe, 54 mg; Zn, 49.5 mg; Mn, 70.5 mg.

source of CP and AA. The nitrogen-free diet was used to measure the basal endogenous losses of AA. The analyzed composition of the test diets is shown in Table 3. Titanium dioxide was used as an exogenous indicator in each diet; vitamins and minerals were supplemented to meet or exceed the estimated nutrient requirements for broilers as recommended by the NRC (1994). Each diet was fed for 5 D before ileal content collection on days 14 and 28. The broilers in 8 replicate cages ( $n = 8$  broilers per cage) were euthanized, and the ileal contents were collected on day 14. The remaining broilers were fed the standard starter diet until day 23, when 576 broilers were randomized to 8 replicate cage ( $n = 8$  broilers per cage) for each treatment diet. Broilers were euthanized, and the ileal content were removed on day 28. All euthanasia was by carbon dioxide asphyxiation, and all ileal digesta from the lower half of the ileum were collected by gently flushing with distilled water into aluminum containers. The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction. The ileal digesta from all broilers with a cage were pooled and frozen immediately after collection and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at  $-4^{\circ}\text{C}$  until required for chemical analysis.

## Measurements

All corn DDGS and diets used in this experiment were analyzed for CP (method 984.13; AOAC, 2006), ether extract (EE; Thiex et al., 2003), crude fiber (CF; method 978.10; AOAC, 2006), and crude ash (method 942.05; AOAC, 2006). Neutral detergent fiber (NDF) and acid

detergent fiber (ADF) were determined using filter bags and fiber analyzer equipment (Fiber Analyzer; Ankom Technology, Macedon, NY) following a modification of the procedures described by Van Soest et al. (1991). The GE was analyzed using a bomb calorimeter (Parr 6300 Calorimeter; Moline, IL) as per ISO 9831 (1998).

Bulk weight of corn DDGS samples were analyzed following the method prescribed by the USDA (1999). The degree of lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) were measured using a colorimeter (Shengguang WSC-S, Shanghai, China). The zein content of all corn DDGS was determined as per a modified method described by Larson and Hoffman (2008). The contents of AFB1, DON, and ZEN were determined by HPLC (Wu et al., 2016).

Samples of corn DDGS, diets, and ileal digesta were analyzed for their AA content. For AA (lysine [Lys]; methionine [Met]; arginine [Arg]; isoleucine [Ile]; leucine [Leu]; phenylalanine [Phe]; histidine [His]; threonine [Thr]; valine [Val]; aspartic acid [Asp]; serine [Ser]; glutamic acid [Glu]; glycine [Gly]; alanine [Ala]; cysteine [Cys]; tyrosine [Tyr]) analysis, first, samples of corn DDGS, diets, and ileal digesta were hydrolyzed with 6 N HCl for 24 h at  $110^{\circ}\text{C}$  (method 982.30 E; AOAC, 2006), filtered, and subsequently content of AA were analyzed by an automatic AA analyzer (Membra A-300, Germany). Tryptophan was not determined. The titanium dioxide content in diets and ileal digesta was measured as described by Short et al. (1996).

## Calculations

The AID for each AA was calculated using the following equation:

$$\text{AID (\%)} = 1 - [\text{AA}_i \times \text{TDC}_d / (\text{AA}_d \times \text{TDC}_i)] \times 100\%$$

in which  $\text{AA}_i$  and  $\text{TDC}_i$  were the concentrations of AA and titanium dioxide in the ileal digesta (g/kg DM), respectively, whereas  $\text{AA}_d$  and  $\text{TDC}_d$  were the concentrations of AA and titanium dioxide in the test diets (g/kg DM), respectively.

The basal endogenous loss of each AA ( $\text{IAA}_{\text{end}}$ , g/kg DM) at the distal ileum was determined based on the outflow obtained when broilers were fed a nitrogen-free diet using the following equation:

$$\text{IAA}_{\text{end}} = \text{AA}_i \times (\text{TDC}_d / \text{TDC}_i)$$

in which  $\text{AA}_i$  and  $\text{TDC}_i$  were the concentrations of AA and titanium dioxide in the ileal digesta collected after feeding the nitrogen free diet (g/kg DM), respectively, whereas  $\text{TDC}_d$  was the titanium dioxide concentration in the nitrogen-free diet (g/kg DM).

By correcting the AID of each AA that was calculated for each sample for the  $\text{IAA}_{\text{end}}$  of each AA, the SID of each AA was calculated using the following equation:

$$\text{SID} = \text{AID} + (\text{IAA}_{\text{end}} / \text{AA}_d) \times 100\%$$

**Table 3.** Analyzed composition of the experimental diets (%).

Item	Corn distillers dried grains with solubles sources diet							
	1	2	3	4	5	6	7	8
CP	18.48	18.82	18.17	19.11	18.34	22.16	20.02	18.57
Total amino acid	9.39	8.22	8.71	13.45	10.88	18.68	13.78	13.80
Indispensable amino acid								
Arginine	0.45	0.39	0.42	0.61	0.39	0.72	0.59	0.66
Histidine	0.32	0.30	0.31	0.37	0.29	0.48	0.37	0.38
Isoleucine	0.38	0.33	0.34	0.56	0.47	0.80	0.56	0.57
Leucine	1.09	0.94	0.98	1.92	1.37	2.82	2.00	1.97
Lysine	0.32	0.29	0.32	0.43	0.29	0.48	0.43	0.44
Methionine	0.15	0.16	0.16	0.28	0.19	0.41	0.29	0.33
Phenylalanine	0.42	0.37	0.38	0.76	0.57	1.05	0.77	0.76
Threonine	0.47	0.43	0.44	0.57	0.59	0.81	0.60	0.59
Valine	0.67	0.56	0.64	0.81	0.84	1.11	0.82	0.85
Dispensable amino acid								
Alanine	1.05	0.91	0.97	1.30	1.26	1.76	1.30	1.28
Aspartic acid	0.69	0.59	0.62	0.92	0.96	1.32	0.98	0.95
Cystine	0.12	0.09	0.11	0.16	0.14	0.18	0.15	0.14
Glutamic acid	1.87	1.61	1.71	2.76	2.01	3.94	2.87	2.83
Glycine	0.51	0.46	0.50	0.60	0.51	0.73	0.57	0.58
Serine	0.57	0.51	0.52	0.82	0.64	1.25	0.87	0.86
Tyrosine	0.31	0.28	0.29	0.60	0.37	0.82	0.61	0.59

## Statistical Analysis

The data were analyzed using one-way ANOVA procedures of SPSS (ver. 21.0 for Windows; SPSS Inc. Chicago, IL). The cage and corn sample were the experimental units for analyzing the data. Means were compared using the Tukey's studentized range test. The relationship between physical characteristic, chemical composition, AID, and SID of AA were analyzed using bivariate correlation analysis by SPSS procedure. The linear regression equations for predicting the SID value of AA of the corn DDGS from the physical characteristic and chemical composition were calculated with the forward stepwise regression procedure. In all analysis, the differences were considered significant if  $P < 0.05$ .

## RESULTS

### Physicochemical Properties of Corn DGGS

The analyzed composition of the corn DGGS samples is shown in Table 1. There was considerable variation in the chemical compositions of the 8 corn DDGS, especially the content of mycotoxin. A higher CV was found for AFB1, DON, and ZEN, which were 80.96, 58.29, and 75.55%, respectively. The content of AFB1, DON, and ZEN ranged from 1.54 to 15.50 ppb, 0.44 to 5.12 ppm, and 127.10 to 1062.46 ppb, respectively. The content of GE, CP, EE, CF, crude ash, NDF, and ADF were from 18.43 to 22.71%, 28.25 to 32.66%, 9.11 to 12.91%, 6.81 to 9.81%, 0.99 to 3.76%, 29.94 to 40.14%, and 8.69 to 12.28%, respectively, and the CV of these compositions with the exception of GE and CF were greater than 10%.

The average bulk weight of corn DDGS samples was 387.49 g/L ranging from 377.44 to 407.86 g/L. The CV of bulk weight was only 2.85%, and yet, the CV of zein

was 73.40%. The content of zein of corn DDGS samples was from 3.09 to 26.89%. For the color measurements, the average  $L^*$ ,  $a^*$ , and  $b^*$  of 8 corn DDGS samples were 67.09, 7.24, and 28.82, and the CV of these color characteristics were 4.60, 8.49, and 10.24%, respectively.

For indispensable AA values, the content of Lys, Met, Arg, Ile, Leu, Thr, Val, Phe, and His were from 0.36 to 0.67%, 0.21 to 0.74%, 0.61 to 1.25%, 0.51 to 2.50%, 1.46 to 3.96%, 0.60 to 1.09%, 0.19 to 1.29%, 0.47 to 1.45%, and 0.37 to 0.64%, respectively. For dispensable AA values, the concentration of Asp, Ser, Glu, Gly, Ala, Cys, and Tyr were from 0.84 to 1.79%, 0.82 to 1.60%, 2.64 to 5.47%, 0.67 to 0.94%, 0.67 to 2.42%, 0.16 to 1.02%, and 0.38 to 1.12%, respectively. The highest CV for Ile and Cys were 69.29 and 98.41%, respectively.

### Digestibility of CP and AA

The SID of the 8 corn DDGS in broilers is shown in Table 4. At day 14, the SID of CP and AA with the exception of His and Gly were different ( $P < 0.01$ ) among the 8 corn DDGS sources tested. The SID of CP, Met, Lys, and Thr ranged from 43.75 to 62.97%, 63.11 to 89.37%, 27.23 to 75.97%, and 45.28 to 77.00% with averages of 53.64, 74.36, 49.56, and 53.47%, respectively. At day 28, the SID of CP and AA with the exception of Phe and Gly was different ( $P < 0.01$ ) among the 8 corn DDGS sources. The mean SID of CP was 67.44% and varied from 55.44 to 73.85%. The SID of Met, Lys, and Thr ranged from 82.55 to 94.93%, 53.85 to 78.67%, and 57.60 to 86.79% with averages of 89.23, 70.67, and 68.96%, respectively.

### Correlation Analysis

Table 5 shows the correlation between physicochemical properties and the SID of CP and AA of corn DDGS. On day 14, a position correlation was observed ( $P < 0.05$ )

**Table 4.** Coefficients of standardized ileal digestibility of CP and amino acids in 8 corn distillers dried grains with solubles sources fed to broilers chickens (%).

Item	Corn distillers dried grains with solubles sources number								SEM	P-value	Mean	CV
	1	2	3	4	5	6	7	8				
Day 14												
CP	47.53	43.99	57.36	59.31	53.84	43.75	60.34	62.97	1.825	<0.001	53.64	14.20
Total amino acid	60.85	57.84	54.98	60.22	63.22	66.67	66.87	82.64	1.743	0.002	64.41	13.21
Indispensable amino acid												
Arginine	76.84	69.76	70.02	68.69	65.31	61.19	66.54	87.66	2.115	0.003	70.76	11.55
Histidine	65.46	64.56	68.56	65.87	62.59	63.00	62.48	78.71	1.419	0.019	66.43	8.09
Isoleucine	64.43	67.40	65.02	64.41	69.58	64.62	63.49	83.29	1.623	0.001	67.96	9.67
Leucine	70.08	73.57	71.42	77.86	74.07	78.05	77.02	88.87	1.248	<0.001	77.08	7.58
Lysine	53.10	49.90	53.12	42.74	45.27	30.90	47.46	75.97	3.080	0.003	49.56	25.74
Methionine	71.49	69.58	74.47	69.54	70.54	73.35	73.72	89.37	1.514	0.001	74.63	8.69
Phenylalanine	51.42	56.30	57.01	66.12	65.16	68.06	68.65	81.05	1.802	<0.001	65.52	14.14
Threonine	43.48	45.03	89.70	41.06	57.39	47.51	47.67	65.64	2.398	<0.001	53.47	30.48
Valine	65.20	66.08	66.79	59.82	70.72	62.15	62.21	79.15	1.448	0.005	66.47	9.20
Dispensable amino acid												
Alanine	77.03	79.01	79.05	75.86	79.96	74.27	74.84	86.85	0.997	0.008	78.18	5.13
Aspartic acid	48.31	50.53	87.07	43.73	62.68	48.26	48.87	67.76	2.279	<0.001	55.83	26.12
Cystine	55.38	50.76	65.62	79.47	65.22	71.60	73.59	86.30	2.359	<0.001	69.93	16.91
Glutamic acid	65.66	66.10	66.97	72.72	66.99	74.42	72.55	87.70	1.487	<0.001	72.65	10.11
Glycine	57.36	56.51	62.92	55.72	59.24	50.29	52.11	71.89	1.750	0.016	57.85	11.69
Serine	55.98	58.52	58.67	60.56	57.71	60.19	61.95	79.05	1.902	0.001	62.27	11.72
Tyrosine	68.99	71.52	70.28	76.89	72.01	76.40	77.22	88.94	1.359	<0.001	76.11	8.37
Day 28												
Crude protein	69.15	58.57	55.44	73.85	70.33	70.10	69.57	72.53	1.196	<0.001	67.44	9.91
Total amino acid	75.29	78.17	73.57	86.83	76.80	85.45	81.64	84.92	1.184	0.005	79.82	6.34
Indispensable amino acid												
Arginine	77.68	86.46	78.01	89.04	76.18	85.73	84.60	86.96	0.919	<0.001	82.83	6.02
Histidine	68.22	79.62	70.05	80.96	64.52	76.84	70.54	79.48	1.143	<0.001	73.43	8.44
Isoleucine	73.90	86.17	75.10	85.87	79.41	84.08	78.63	82.51	0.991	0.001	80.56	5.84
Leucine	83.05	88.65	84.80	92.02	88.01	89.98	87.76	89.45	0.696	0.007	87.83	3.26
Lysine	67.31	77.72	68.21	78.67	53.85	71.70	70.31	78.13	1.554	<0.001	70.67	11.60
Methionine	82.55	94.93	83.29	94.07	84.86	93.26	91.65	92.95	1.164	0.003	89.23	5.83
Phenylalanine	82.81	87.69	81.59	90.64	86.33	88.04	86.43	88.00	0.781	0.023	86.29	3.42
Threonine	57.60	67.27	86.79	70.93	62.05	72.14	65.11	69.13	1.511	<0.001	68.96	12.58
Valine	76.98	85.15	82.34	85.91	82.48	86.86	80.06	82.04	0.959	0.162	82.58	3.93
Dispensable amino acid												
Alanine	88.75	89.38	85.56	91.80	84.77	92.50	86.11	88.57	0.915	0.288	88.32	3.20
Aspartic acid	64.92	73.64	85.91	70.34	69.97	73.62	66.22	72.62	1.191	<0.001	72.27	8.90
Glutamic acid	77.19	85.65	78.70	92.00	78.76	88.80	87.42	90.04	0.995	<0.001	84.41	6.86
Glycine	64.12	78.17	70.06	76.74	67.15	68.47	65.05	75.99	1.327	0.025	70.63	7.82
Serine	71.31	79.22	70.01	83.35	71.94	85.55	80.06	83.13	1.100	<0.001	77.53	7.91
Tyrosine	75.16	83.41	80.60	91.54	83.76	89.92	89.26	88.22	0.960	<0.001	84.89	6.53

between the L\* value and the SID of CP, total AA (TAA), and Met. There was a negative correlation between the CP content of corn DDGS and the SID of Met and TAA ( $P < 0.05$ ). In addition, on day 28, the content of DON had a negative relationship with the SID of Met, Lys and TAA ( $P < 0.05$ ), and the content of ZEN also had a negative relationship with the SID of Met, Lys and TAA ( $P < 0.05$ ). A positive correlation was observed ( $P < 0.05$ ) between the a\* value and SID of CP and TAA.

### Prediction Equations for CP and AA

Stepwise regression equations for predicting SID of CP, TAA, Met, Leu, Glu, Ser, and Tyr in broilers on days 14 and 28 based on the physicochemical properties of corn DDGS are presented in Table 6. On day 14, the  $R^2$  value of stepwise regression equations for predicting the SID was the best for Glu ( $R^2 = 1.000$  using EE, CF, CP, AFB1, and NDF), then followed by Leu ( $R^2 = 0.970$ , using L\*, crude ash, and a\*) and least significant for CP

( $R^2 = 0.529$ , using L\*) with intermediate values for SID of TAA, Met, Phe, Cys, Ser, and Tyr ( $R^2 = 0.551$ – $0.899$ ,  $P < 0.05$ ). The best-fit equation for SID of CP was  $SID\ CP = -66.752 + 1.794\ L^*$  ( $R^2 = 0.529$ ,  $P < 0.05$ ). The best fit equation for SID of Met was  $SID\ Met = 153.396 - 2.526\ CP$  ( $R^2 = 0.551$ ,  $P < 0.05$ ). On day 28, the  $R^2$  value of stepwise regression equations for predicting the SID was the best for TAA ( $R^2 = 0.904$ , using EE), then followed by Glu ( $R^2 = 0.858$ , using ZEN), and least significant for His ( $R^2 = 0.528$ , using DON) with intermediate values for SID of CP, Met, Arg, Leu, Ala, Ser, and Tyr ( $R^2 = 0.543$ – $0.835$ ,  $P < 0.05$ ). The best fit equation for SID of CP was  $SID\ CP = -0.934 + 9.439\ L^*$  ( $R^2 = 0.756$ ,  $P < 0.01$ ). The best fit equation for SID of Met was  $SID\ Met = 97.004 - 2.641\ DON$  ( $R^2 = 0.672$ ,  $P < 0.05$ )

### DISCUSSION

In the present study, there were considerable differences in the chemical composition of 8 corn DDGS.

**Table 5.** Correlation coefficients between physicochemical properties and coefficients of standardized ileal digestibility of CP and some amino acids of corn distillers dried grains with solubles sources (n = 8).

	Day 14 SID					28 D SID				
	CP	Lys	Met	Thr	TAA	CP	Lys	Met	Thr	TAA
GE	0.150	0.067	0.504	-0.265	0.641	0.664	0.376	0.400	0.023	0.885**
CP	-0.702	-0.593	-0.749*	-0.178	-0.813*	-0.483	-0.222	-0.184	0.107	-0.465
Ether extract	0.346	0.302	0.655	-0.182	0.770*	0.685	0.560	0.520	0.063	0.951**
Crude fiber	-0.031	-0.250	0.097	-0.549	0.220	0.549	0.331	0.362	-0.054	0.685
Crude ash	0.402	0.074	-0.335	0.079	-0.407	-0.237	-0.352	-0.392	-0.188	-0.548
Neutral detergent fiber	-0.137	-0.296	0.143	-0.406	0.270	0.492	0.254	0.319	0.026	0.644
Acid detergent fiber	0.097	-0.152	0.186	-0.510	0.302	0.616	0.377	0.379	-0.051	0.737*
Aflatoxin B1	-0.443	-0.253	-0.588	0.233	-0.720*	-0.718*	-0.445	-0.429	0.008	-0.916**
Deoxynivalenol	-0.030	0.051	-0.297	0.295	-0.497	-0.261	-0.709*	-0.809*	-0.268	-0.829*
Zearalenone	-0.318	-0.180	-0.502	0.202	-0.678	-0.433	-0.709*	-0.749*	-0.205	-0.894**
L*	0.727*	0.698	0.732*	-0.095	0.758*	0.714*	0.322	0.181	-0.261	0.669
a*	0.486	0.207	0.385	-0.394	0.420	0.869**	0.279	0.141	-0.178	0.790*
b*	0.126	0.141	-0.277	-0.030	-0.417	-0.173	0.062	-0.112	-0.045	-0.317
Bulk weight	0.186	0.063	0.019	-0.546	-0.016	0.486	0.379	0.209	-0.246	0.349
Zein	0.300	0.067	0.441	-0.322	0.588	0.702	0.373	0.389	0.011	0.917**

\* 0.01 < P ≤ 0.05; \*\* 0.001 < P ≤ 0.01.

Abbreviations: a\*, degree of redness; b\*, degree of yellowness; Lys, lysine; L\*, degree of lightness; Met, methionine; SID, standard ileal digestibility; Thr, threonine; TAA, total amino acid.

Many factors can contribute to the large variability in chemical composition of corn DDGS, which include raw material (Spiehs et al., 2002), drying step of the process (Kingsly et al., 2010), and proportion of solubles added back to the distillers dried grains (Martinez-Amezcuca et al., 2007). The EE (9.11–12.64%) in the present study was higher than the values observed by Adedokun et al. (2015) (mean 9.09%) and Jie et al. (2013) (mean 8.81%). This may be due to the high

proportion of distillers dried soluble in the DDGS. The CP content (mean 31.43%) was much higher than the values reported by Fastinger et al. (2006) and Batal and Dale (2006). The researchers found that the content of CP in corn DDGS was from 27.0 to 29.3% and 23.0 to 30.0%, respectively. The high proportions of CP in present experiment corn DDGS samples were likely owing to the more advanced technology used for fermentation of corn. Meanwhile, we also found there is a variation in each AA (CV from 11.60 to 98.41%) among the 8 corn DDGS samples, which may be caused by the aforementioned reasons. Compared with the AA model recommended by the NRC (1994), the proportion of various AA in corn DDGS was unbalanced. This may be because some free AA are susceptible to heat treating, resulting in a decrease in their content.

**Table 6.** Prediction equations of standardized ileal digestibility of CP and some amino acids based on the physicochemical properties of corn distillers dried grains with solubles sources of broilers chickens (n = 8).

Prediction equations	R <sup>2</sup>	P-value
<b>Day 14</b>		
SID CP = -66.752 + 1.794 L*	0.529	0.041
SID TAA = 180.981 - 3.717CP	0.694	0.010
SID Leu = -31.155 + 2.164 L* - 3.707Ash - 3.751a*	0.970	0.002
SID Met = 153.396 - 2.526 CP	0.551	0.035
SID Phe = -105.68 + 2.532 L*	0.713	0.008
SID Cys = 81.118 - 1.517AFB1	0.747	0.006
SID Glu = 70.106 + 6.043 EE - 3.492CF - 1.785 CP + 0.668AFB1 + 0.444NDF	1.000	<0.001
SID Ser = 155.795 - 2.997CP	0.614	0.021
SID Tyr = -39.018 + 1.828 L* - 2.943Ash	0.899	0.003
<b>Day 28</b>		
SID CP = -0.934 + 9.439 L*	0.756	0.005
SID TAA = 49.687 + 2.846 EE	0.904	<0.001
SID Arg = 88.742 - 0.011ZEN	0.736	0.006
SID His = 81.495 - 2.788DON	0.528	0.041
SID Leu = 85.09 + 0.207ZEN	0.543	0.037
SID Met = 97.004 - 2.641DON	0.672	0.013
SID Ala = 44.136 - 2.694Ash + 0.134BW	0.833	0.011
SID Glu = 91.915 - 0.014ZEN	0.858	0.001
SID Ser = 87.676 - 3.471DON	0.835	0.002
SID Tyr = 78.444 + 0.489Zein	0.808	0.002

Abbreviations: Ala, alanine; AFB1, aflatoxin B1; Arg, arginine; Ash, crude ash; Asp, aspartic acid; a\*, degree of redness; BW, bulk weight; b\*, degree of yellowness; CF, crude fiber; Cys, cysteine; DON, deoxynivalenol; EE, ether extract; Glu, glutamic acid; His, histidine; Leu, leucine; Lys, lysine; L\*, degree of lightness; NDF, neutral detergent fiber; Phe, phenylalanine; Ser, serine; SID, standardized ileal digestibility; Thr, threonine; Tyr, tyrosine; ZEN, zearalenone.

Mycotoxins, a very adverse factor for feed utilization, are found everywhere in nature, harvested cereal crops and livestock diets worldwide, which are harmful for health of birds and result in financial damages in poultry production (Awad et al., 2013; Pitt et al. 2016). The mycotoxin content of feed might be changed with elements such as corn-growing areas, variable climate, and especially processing technology. Furthermore, the content of 3 mycotoxins examined in the study is an inspection-required item stipulated in the feeding stuffs regulations of China. So, it might serve as a potential predictor of SID to reflect the quality of corn DDGS for broiler chickens. Feed hygiene standards in China stipulated the critical content of AFB1, DON, and ZEN as 50 ppb, 5 ppm, and 1,500 ppb, respectively. In the study, the content of mycotoxin in 8 corn DDGS was low relatively but had a wide range (AFB1, 1.54 ppb to 15.50 ppb, CV 80.96%, mean 8.32 ppb; DON, 0.44 ppm to 5.12 ppm, CV 58.29%, mean 2.77 ppm; ZEN, 127.10 ppb to 1062.46 ppb, CV 75.55%, mean 522.60 ppb). Compared with samples 1, 2, 3, and 5, samples 4, 6, 7, and 8 have moderately low levels of mycotoxin. Taken together, the results suggest

at least 2 main kinds of processing technology for corn DDGS might exist in China.

In our study, there were significant differences in the digestibility of CP and most AA. These results agreed with studies with pigs and layers (Stein et al., 2006; Liu, 2010), which have indicated that there were differences in AID and SID of AA when pigs and layers were fed corn DDGS from different sources. In general, processing technology (Stein et al., 2005) and associated heat damage for some DDGS samples (Stein and Shurson, 2009) were responsible for the variability of the SID of AA values. Similar to previous studies, our data also showed that there were differences in the digestibility of CP and most AA between at days 14 and 28, indicating that age factor have an important effect on the digestibility of CP and AA (Huang et al., 2005). Therefore, this factor should be taken into account in the formulation design.

As the important limiting AA in broilers, Met has a relatively high SID (74.63% on day 14 and 89.23% on day 28) than the SID of other AA in corn DDGS in the present study, which makes corn DDGS become a good source of Met for broilers. However, the Lys had a relatively low SID (49.56% at day 14 and 70.67% at day 28) in the corn DDGS samples, which is in agreement with the results reported by Fastinger et al. (2006) and Xue et al. (2012). The reason of that may be the excessive heating during the processing of corn DDGS, which may cause Maillard reaction between Lys and carbohydrate moieties to form Amadori compounds (Stein et al., 2005), and the Lys that is bound in these Amadori compounds is called blocked and is biologically unavailable (Tanghe et al., 2015).

To the best of our knowledge, there has not been any study on the evaluation the SID of AA based on the mycotoxin content. In the present study, on day 28, we found that the content of some mycotoxin, such as DON and ZEN, and the SID of some AA showed a negative correlation. The  $R^2$  value of stepwise regression equations for predicting SID of Met, His and Ser based on DON were 0.672, 0.528 and 0.835, respectively, and the  $R^2$  value of stepwise regression equations for predicting SID of Arg and Glu based on ZEN was 0.736 and 0.858, respectively. The stepwise regression equations indicate that DON or ZEN could be a suitable prediction of SID of some AA in corn DDGS, which may be owing to that pepsin activity levels of broilers increased gradually along with the increase of age (Zhang et al., 2005), whereas AA digestibility gradually increased to a certain degree, the content of nutrients is no longer the main factors affecting AA digestibility and antinutrients such as mycotoxin hindered the absorption and utilization of nutrients. In addition, we found there was a significantly positive correlation between  $L^*$  values and the SID of CP or some AA. The result was in line with that of previous findings. Fastinger and Mahan (2006) found that the SID of AA in grower-finisher pigs was reduced with decreasing the  $L^*$  values in corn DDGS; Batal and Dale (2006) reported there was a positive correlation between the SID of some AA for poultry and  $L^*$  values.

Therefore, the  $L^*$  values could be a reasonable predictor of SID for CP and some AA. We also found that other factors, such as CP, EE, CF, and Ash, were also essential for estimating the SID of CP and some AA.

## CONCLUSIONS

In summary, the results of this study indicate that physicochemical properties and the SID of CP and most AA in corn DDGS showed a huge difference. There was a relation between SID of CP and AA and the physicochemical properties of corn DDGS. Therefore, we could develop the prediction equation for SID of CP and AA based on the chemical composition, color score, and mycotoxin content of corn DDGS samples.

## ACKNOWLEDGMENTS

This work was supported by the Yangtze River Scholar and Innovation Research Team Development Program (IRT0945), and Program for Shaanxi Science and Technology of China under Grant (2019ZDXM3-02 and 2017ZDXM-NY-087).

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

## REFERENCES

- Adedokun, S. A., P. Jaynes, R. L. Payne, and T. J. Applegate. 2015. Standardized ileal amino acid digestibility of corn, corn distillers' dried grains with solubles wheat middlings, and bakery by-products in broilers and laying hens. *Poult. Sci.* 94:2480–2487.
- AOAC. 2006. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- Awad, W., K. Ghareeb, J. Böhm, and J. Zentek. 2013. The toxicological impacts of the *Fusarium* mycotoxin, deoxynivalenol, in poultry flocks with special reference to immunotoxicity. *Toxins* 5:912–925.
- Batal, A. B., and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. *J. Appl. Poult. Res.* 15:89–93.
- Belyea, R. L., K. D. Rausch, T. E. Clevengerc, V. Singh, D. B. Johnston, and M. E. Tumbleson. 2010. Sources of variation in composition of DDGS. *J. Anim. Feed. Sci.* 159:122–130.
- Ergul, T., C. M. Amezcua, C. Parsons, B. Walters, J. Brannon, and S. Noll. 2003. Amino acid digestibility in corn distillers dried grains with solubles. *Poult. Sci.* 82(Suppl.1):70.
- Fastinger, N. D., J. D. Latshaw, and D. C. Mahan. 2006. Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. *Poult. Sci.* 85:1212–1216.
- Fastinger, N. D., and D. C. Mahan. 2006. Determination of the ileal amino acid and energy digestibilities of corn distillers dried grains with solubles using grower-finisher pigs. *J. Anim. Sci.* 84:1722–1728.
- Huang, K. H., V. Ravindran, X. Li, and W. L. Bryden. 2005. Influence of age on the apparent ileal amino acid digestibility of feed ingredients for broiler chickens. *Br. Poult. Sci.* 46:236–245.
- ISO. 1998. Animal Feeding Stuffs, Animal Products, and Faeces or Urine-Determination of Gross Calorific Value: Bomb Calorimeter Method, Standard 9831. International Standards Organization, Geneva, Switzerland.
- Jie, Y. Z., J. Zhang, Y. Zhao, L. H. Ma, Q. G. and C. Ji. 2013. The relationship between the metabolizable energy content, chemical composition and color score in different sources of corn DDGS. *J. Anim. Sci. Biotechnol.* 4:38.
- Kingsly, A. R. P., K. E. Ileleji, C. L. Clementson, A. Garcia, D. E. Maier, R. L. Strohshine, and S. Radcliff. 2010. The effect of process variables

- during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS)—Plant scale experiments. *Bioresour. Technol.* 101:193–199.
- Larson, J., and P. C. Hoffman. 2008. Technical note: a method to quantify prolamin proteins in corn that are negatively related to starch digestibility in ruminants. *J. Dairy Sci.* 91:4834–4839.
- Liu, Y. 2010. Evaluation of Nutritional Value of Domestic Corn DDGS on Laying Hens. Diss. Northwest A&F University, Shaanxi (in Chinese).
- Meloche, K. J., B. J. Kerr, N. Billor, G. C. Shurson, and W. A. Dozier. 2014. Validation of prediction equations for apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. *Poult. Sci.* 93:1428–1439.
- Martinez-Amezcuca, C., C. M. Parsons, V. Singh, R. Srinivasan, and G. S. Murthy. 2007. Nutritional characteristics of corn distillers dried grains with solubles as affected by the amounts of grains versus solubles and different processing techniques. *Poult. Sci.* 86:2624–2630.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Pitt, J. I., C. P. Wild, R. A. Baan, W. C. A. Gelderblom, J. D. Miller, R. T. Riley, and F. Wu. 2016. Improving Public Health through Mycotoxin Control. IARC Scientific Publication, Geneva, Switzerland, p. 158.
- Shim, M. Y., G. M. Pesti, R. I. Bakalli, P. B. Tillman, and R. L. Payne. 2011. Evaluation of corn distillers dried grains with solubles as an alternative ingredient for broilers. *Poult. Sci.* 90:369–376.
- Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.* 59:215–221.
- Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J. Anim. Sci.* 80:2639–2645.
- Stein, H. H., A. A. Pahm, and C. Pedersen. 2005. Methods to determine amino acid digestibility in corn by-products. Pages 35–49 in *Proc. 66th Minnesota Nutr. Conf.* University of Minnesota, St. Paul, MN.
- Stein, H. H., M. L. Gibson, C. Pedersen, and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. *J. Anim. Sci.* 84:853–860.
- Stein, H. H., and G. C. Shurson. 2009. Board-invited review: the use and application of distillers dried grains with solubles in swine diets. *J. Anim. Sci.* 87:1292–1303.
- Tanghe, S., J. De Boever, B. Ampe, D. De Brabander, S. De Campeneere, and S. Millet. 2015. Nutrient composition, digestibility and energy value of distillers dried grains with solubles and condensed distillers solubles fed to growing pigs and evaluation of prediction methods. *Anim. Feed Sci. Technol.* 210:263–275.
- Thiex, N. J., S. Anderson, and B. Gildemeister. 2003. Crude fat, dietary ether extraction, in feed, cereal grain, and forage (Randall/Soxtec/Submersion method): collaborative study. *J. AOAC. Int.* 86:888–898.
- USDA. 1999. *Practical Procedures for Grain Handlers: Inspecting Grain*. United States Department of Agriculture-Grain Inspection, Packers, and Stockyards Administration, Washington, D. C. Accessed Feb. 2005. <http://151.121.3.117/pubs/primer.pdf>.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Wu, L., J. J. Li, Y. H. Li, T. J. Li, Q. H. He, Y. L. Tang, H. N. Liu, Y. T. Su, Y. L. Yin, and P. Liao. 2016. Aflatoxin b1, zearalenone and deoxynivalenol in feed ingredients and complete feed from different province in China. *J. Anim. Sci. Biotechnol.* 7:63.
- Xue, P. C., B. Dong, J. J. Zang, Z. P. Zhu, and L. M. Gong. 2012. Energy and standardized ileal amino acid digestibilities of Chinese distillers dried grains, produced from different regions and grains fed to growing pigs\*. *Asian-aust. J. Anim. Sci.* 25:104–113.
- Zhang, K. X., K. Y. Zhang, T. J. Applegate, S. P. Bai, X. M. Ding, J. P. Wang, H. W. Peng, Y. Xuan, Z. W. Su, and Q. F. Zeng. 2020. Evaluation of the standardized ileal digestibility of amino acids of rapeseed meals varying in protein solubility for Pekin ducks. *Poult. Sci.* 99:1001–1009.
- Zhang, T. Y., J. Wang, and Y. Q. Li. 2005. Developments of digestive parameters in broilers with age 0~49. *China. Anim. Husb.Vet. Med.* 32:6–10 (in Chinese).