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

Huimei Wang, Fang Yan, Fangshen Guo, Xingpeng Liu, Xiaojun Yang, and Xin Yang

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 B1, 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 R² value of stepwise regression equations for predicting the SID of AA at day 14 and day 28 was best for glutamic acid (R² = 1.000 using ether extract, crude fiber, CP, aflatoxin B1, and neutral detergent fiber) and TAA (R² = 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

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
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: NWAFAC 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°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...
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 cages (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°C until required for chemical analysis.

**Calculations**

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

$$\text{AID} \% = 1 - \left[ \frac{\text{AA}_1 \times \text{TDC}_d}{\text{AA}_d \times \text{TDC}_i} \right] \times 100\%$$

in which $\text{AA}_1$ 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 (IAA$_{end}$, 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:

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

in which $\text{AA}_1$ 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 IAA$_{end}$ of each AA, the SID of each AA was calculated using the following equation:

$$\text{SID} = \text{AID} + \left( \frac{\text{IAA}_{end}}{\text{AA}_d} \right) \times 100\%$$
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 DDGS 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%, 18.34 to 19.11, 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.

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 ($P < 0.05$)
The best equation for predicting the SID was the best for Glu ($R^2 = 0.529$, 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 ($R^2 = 0.543–0.835$, $P < 0.05$).

### DISCUSSION

In the present study, there were considerable differences in the chemical composition of 8 corn DDGS.
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-Amezcu et al., 2007). The EE (9.11–12.64%) in the present study was higher than the values observed by Acedokun 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.

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

### 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).

| Prediction equations | R²   | P-value |
|----------------------|------|---------|
| Day 14               |      |         |
| 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 - 3.751 L* | 0.970 | 0.002   |
| SID Met = 153.396 - 2.526 CP | 0.531 | 0.035   |
| SID Phe = -105.68 + 2.352 L* | 0.713 | 0.008   |
| SID Cys = 81.118 - 1.517AFB1 | 0.747 | 0.006   |
| SID Glu = 70.106 + 6.043 EE - 3.922CF - 1.785 | 1.000 | <0.001  |
| CP + 0.666AFB1 + 0.444NDF |      |         |
| SID Ser = 155.795 - 2.997CF | 0.614 | 0.021   |
| SID Tyr = -39.018 + 1.828 L* - 2.943Ash | 0.899 | 0.003   |
| Day 28               |      |         |
| SID CP = -0.934 + 9.439 L* | 0.756 | 0.005   |
| SID TAA = 49.687 + 2.846 EE | 0.906 | <0.001  |
| SID Arg = 88.742 - 0.011ZEN | 0.736 | 0.006   |
| SID His = 81.495 - 2.78SDON | 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 | 0.001   |
| SID Gln = 91.915 - 0.014ZEN | 0.858 | 0.001   |
| SID Ser = 87.676 - 3.471DON | 0.835 | 0.002   |
| SID Tyr = 78.444 + 0.489ZEN | 0.808 | 0.002   |

### 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).

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.
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 date 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 R² 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 R² 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, CP, 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.

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