ABSTRACT  This experiment was conducted to evaluate the chemical composition and standardized ileal digestibility of corn grain and to use these data to develop prediction equations for estimating total amino acids (TAAAs) and standardized ileal digestible amino acids (SIDAAs) for broiler chickens. Four types of corn grains were obtained from different origins (Brazil, Ukraine, Russia, and Iran). Eighty-day-old Ross 308 male broiler chicks were fed a standard diet until day 18, and experimental diets were fed from 19 to 24 D of age. Five dietary treatments consisted of 4 semi-purified diets containing corn from each origin as the only source of amino acid (AA) and a N-free diet for determination of basal endogenous AA losses. Assay diets contained 939 g of test corn/kg. The concentration of crude protein and gross energy ranged from 7.58 to 8.39% (coefficient of variation [CV] = 4.72%) and 4,121 to 4,621 kcal/kg (CV = 5.09%), respectively. There was significant variation among the 4 corn grains in standardized ileal digestibility (SID) for CP, Phe, Leu, Asp, Glu, Ser, Gly, Ala, and Tyr (P ≤ 0.05). The results of linear regression showed that linear prediction equations based on protein content can be used to predict the TAA and SIDAA contents (e.g., TLys = 0.041 × CP, adj $R^2 = 95.9$, standard error of prediction $[SEP] = 0.05$; SIDLys = 0.0356 × CP, adj $R^2 = 96$, SEP = 0.051). Inclusion of other proximate components of test samples into the regression equation increased the $R^2$ value and decreased the SEP value (e.g., TLys = 0.329 × crude fiber [CF] − 0.209 × Ash, adj $R^2 = 99.9$, SEP = 0.005; SIDLys = −1.1591 + 0.836 × CF − 0.055 × Ash, adj $R^2 = 99.9$, SEP = 0.001). The concentration of TAA and SIDAA was highly correlated (adj $R^2 > 89\%$) for most AA and showed that the amount of SIDAA could be predicted from its total concentration with a high degree of accuracy (e.g., SIDLys = 0.0023 + 0.861 × TLys, adj $R^2 = 99.9$, SEP = 0.0001). In conclusion, this in vitro assays and equations accurately predicted TAA and SIDAA corn grain samples for broiler chickens and can serve as a reference analysis to develop calibration equations for rapid feed quality evaluation methods such as near-infrared reflectance spectroscopy.

Key words: amino acid, broiler, corn, prediction equation, standardized ileal digestibility

INTRODUCTION

Corn grain is a commonly used ingredient in the diets of broiler chickens in many regions of the world. The concentration of crude protein (CP) in corn grain is generally lower than that in other cereal grains, and it usually varies between 70 and 126 g/kg DM (Bryden et al., 2009; Zuber and Rodehutscord, 2017). Nevertheless, as corn grain is often used in high quantities in diets for broilers, it provides significant amounts of amino acid (AA). For example, 65% of corn grain in the diets provides 0.12% of dietary lysine, which is equal to 12% of broiler requirement in a finishing period (Ross 308 nutrition specifications, 2014). Corn grain nutrient value varies in terms of composition from year to year. There are numerous factors that could impact corn grain nutrient value and digestibility such as the plant species, geographical location, agronomic conditions, postharvest processing, and storage (Collins et al., 2001;
Galon et al., 2011; Gehring et al., 2012). Da Silva et al. (2008) demonstrated that similar grains may have different chemical compositions which affect their nutritional values.

Determination of digestibility coefficients for dietary AA helps us to formulate well-balanced diets to meet bird requirement and reduce nitrogen excretion to the environment. Moreover, diets formulated based on digestible AA will have economic benefits (Baker, 1994; Baker and Han, 1994). There is a growing consensus that the standardized ileal amino acid digestibility (SIAAD) assay is a suitable method for evaluating the AA digestibility of feed ingredients (Bryden and Li, 2010). Adedokun et al. (2015) determined the prececal digestibility of AA in 3 corn samples and discovered values ranging from 83 to 93% and 90 to 93% for Lys and Met, respectively. Furthermore, Leeson and Summers (2009) suggested that the total amino acids (TAA) and standardized ileal digestible amino acids (SIDAA) of the corn grain need to be assessed 2 times and one time per year, respectively. However, high-pressure liquid chromatography and bioassays to determine the AA are costly and require a significant investment of time and labor. Therefore, rapid and accurate in vitro assays that can predict TAA and SIDAA would be useful tools for broiler nutritionists to evaluate currently available corn. Regression, one of the prediction methods for nutritive value of a feed ingredient from its chemical composition, is advantageous for its simplicity and rapid turnaround time (NRC, 1994; Ebadi et al., 2011; Sedghi et al., 2014). Thus, linear regression and artificial neural network have been used to predict the AA profiles of feed ingredients based on proximate analysis (Cravener and Roush, 2001). Information about TAA content of feedstuffs is important; however, it is more essential for a nutritionist to know the SIDAA contents of feed ingredients when formulating poultry diets. The central hypothesis was that a linear regression using CP and other proximate component values would provide more robust and accurate prediction of TAA and SIDAA of corn grains. Therefore, the main objective of this study was to develop linear regression to predict TAA and SIDAA of corn grains for broilers based on their CP content and other proximate components and also the SIDAA content from the TAA.

MATERIALS AND METHODS

Data Collection and Chemical Analysis

Four types of corn grains were obtained from different origins for this study. Three of the corn grains were obtained from commercial suppliers and were imported from Brazil, Ukraine, and Russia. The other corn grain was obtained directly from the suppliers (Single Cross variety-produced in the Moghan Plain of Iran). Three samples from each of the 4 corn grains were taken and evaluated for dry matter (DM), ash, CP, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), and gross energy (GE) by the chemical laboratory of the College of Agriculture and Natural Resources University of Tehran following Association of Official Analytical Chemists International (AOAC, 2000) analytical methods. Nitrogen-free extract (NFE) was determined by mathematical calculation.

For AA analysis, samples were prepared by 6N HCl hydrolysis for 24 h at 110°C followed by neutralization with 15 mL of NaOH (9.8 N) and then cooled to room temperature. Afterward, sodium citrate buffer was added to a 100-mL volume of the mixture (AOAC, 2000). Methionine and cysteine (sulfur-containing AAs) were analyzed by performic acid oxidation at 0°C, followed by acid hydrolysis (Moore, 1963). The AAs in the hydrolyzate were determined by HPLC Agilent 1100 and 1260 (Institute of Agrifood Research and Technology, IRTA-Spain) using a reverse-phase chromatography with precolumn derivatization with orthophthalaldialdehyde with 2 replicates.

Bird Husbandry

This project was approved by the Animal Care Committee of the University of Tehran, Iran. Five dietary treatments consisted of 4 semi-puriﬁed diets containing corn from each origin as the only source of AA and one N-free diet (NFD) for determination of basal endogenous AA losses. The composition of the assay diets is presented in Table 1. Assay diets contained 939 g of test corn/kg. In the NFD, corn starch and dextrose were used as energy sources. The calculated CP levels for the diets containing Brazil, Russian, Ukraine, and Iran corn were 7.88, 7.85, 7.80, and 7.12%, respectively. All the diets were balanced in terms of calcium and phosphorus and supplemented with equal amounts of vitamin and mineral premixes (NRC, 1994). Celite (Celite 281, MilliporeSigma, Burlington, MA), a source of acid-insoluble ash (AIA), was added to all diets at 1% as an indigestible marker. The analyzed CP and AA contents of the diets are presented in Table 2. All diets were fed in mash form.

In this trial, 80 one-day-old Ross 308 male chicks were obtained from a commercial hatchery and received vaccinations for Newcastle disease (7 and 18 D) and infectious bronchitis (1 D). Chicks were weighed and randomly allotted into 20 grower battery cages so that each cage of chicks had a similar initial weight and cage weight distribution (4 replicates and 4 birds per cage; 0.18 m²/bird), and each cage was equipped with one trough-feeder and one trough-waterer. Battery cages were located in a solid-sided house with a temperature control system. Temperature was set to 33°C at placement and was decreased gradually to 24°C by the end of experimentation, with continuous fluorescent lighting. Chicks were allowed ad libitum access to a corn-soybean meal conventional diet until 18 D of age; the starter diet contained 3,000 kcal/kg metabolizable energy and 23% CP (1–10 D), and the grower diet contained 3,100 kcal/kg metabolizable energy and 21.5% CP (11–18 D). On 19 D, after an over-night fast, chicks
were given ad libitum access to the experimental diets and NFD. On day 24, all the birds were euthanized by CO₂ asphyxiation, and ileal digesta were collected from the distal two-thirds of the ileum (portion of the small intestine from Meckel’s diverticulum to approximately 1 cm anterior to the ileocecal junction) by flushing with distilled water (Kluth and Rodehutscord, 2005).

Collected ileal samples from 3 birds within a cage were pooled and stored in a freezer at 220°C for further analyses of AIA and AA. Frozen digesta samples were thawed, lyophilized, and ground using an electric coffee grinder to make finely ground samples while avoiding significant loss. AIA concentrations of diets and digesta were determined in duplicate based on the method reported by Van Keulen and Young (1977). The concentrations of AA were analyzed as described previously. The SIAAD values were obtained by multiplying TAA contents and standardized ileal digestibility coefficients.

In the present study, 17 AAs (Asp, Thr, Ser, Gly, Glu, Pro, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, and Arg) were selected to assess the relationship among TAA, SIDAA, and the corn chemical compositions.

Calculation

Apparent ileal AA digestibility (AIAAD) was calculated using the following equation (Lemme et al., 2004): AIAAD = [(AA/AIA) diet − (AA/AIA) digesta]/(AA/AIA) digesta diet. Ileal endogenous AA (IEAA) flow in broilers fed the NFD was calculated as milligrams of AA flow per kilogram of DM intake (DMI) using the following equation (Adedokun et al., 2008): IEAA, mg/kg, of DMI = ileal AA, mg/kg, × [(AIA)diet/(AIA)digesta]. Apparent IAAD coefficients were standardized using the determined IEAA flows using the following equation: SIAAD = AIAAD [(IEAA flow

### Table 1. Ingredient composition of diets fed to broilers from 19 to 24 D of age (% as-fed basis).

| Item                | Corn-1 | Corn-2 | Corn-3 | Corn-4 | N-Free |
|---------------------|--------|--------|--------|--------|--------|
| Ingredient          |        |        |        |        |        |
| Corn                | 93.9   | 93.9   | 93.9   | 93.9   | -      |
| Corn-starch         | -      | -      | -      | -      | 45.65  |
| Dextrose            | -      | -      | -      | -      | 43.00  |
| Soybean oil         | 1.00   | 1.00   | 1.00   | 1.00   | 1.00   |
| Dicelium phosphate  | 1.85   | 1.85   | 1.85   | 1.85   | 2.50   |
| Limestone           | 1.2    | 1.2    | 1.2    | 1.2    | 0.85   |
| Salt                | 0.36   | 0.36   | 0.36   | 0.36   | 0.40   |
| Vitamin-mineral premix² | 0.6   | 0.6    | 0.6    | 0.6    | 0.60   |
| Solka-Floc³         | -      | -      | -      | -      | 5.00   |
| Celite              | 1.00   | 1.00   | 1.00   | 1.00   | 1.00   |
| Total               | 1,000  | 1,000  | 1,000  | 1,000  | 1,000  |

Calculated energy and nutrient

| AMEn, kcal/kg | 3,258 | 3,258 | 3,258 | 3,258 | 3,191 |
| Protein, %    | 7.88  | 7.85  | 7.80  | 7.12  | -     |
| Ca, %         | 0.87  | 0.87  | 0.87  | 0.87  | 0.87  |
| Available P, %| 0.44  | 0.44  | 0.44  | 0.44  | 0.44  |
| Sodium, %     | 0.18  | 0.18  | 0.18  | 0.18  | 0.18  |

Table 2. Analyzed amino acids (AA) and CP composition of the semi-purified diets fed to broilers from 19 to 24 D of age (% as-fed basis).

| Item                | Corn-1 | Corn-2 | Corn-3 | Corn-4 | N-Free |
|---------------------|--------|--------|--------|--------|--------|
| CP                  | 7.52   | 7.65   | 7.26   | 6.66   | 0.27   |
| Essential AA        |        |        |        |        |        |
| His                 | 0.182  | 0.158  | 0.147  | 0.161  | 0.003  |
| Thr                 | 0.247  | 0.305  | 0.266  | 0.254  | 0.003  |
| Arg                 | 0.418  | 0.359  | 0.321  | 0.290  | 0.005  |
| Val                 | 0.283  | 0.270  | 0.248  | 0.244  | 0.005  |
| Met                 | 0.168  | 0.164  | 0.149  | 0.145  | 0.0001 |
| Phe                 | 0.284  | 0.295  | 0.247  | 0.238  | 0.005  |
| Ile                 | 0.191  | 0.252  | 0.208  | 0.200  | 0.003  |
| Leu                 | 0.637  | 0.733  | 0.625  | 0.628  | 0.009  |
| Lys                 | 0.197  | 0.259  | 0.294  | 0.232  | 0.005  |
| Nonessential AA     |        |        |        |        |        |
| Asp                 | 0.533  | 0.595  | 0.503  | 0.455  | 0.006  |
| Glu                 | 1.171  | 1.257  | 1.086  | 1.056  | 0.014  |
| Ser                 | 0.315  | 0.299  | 0.281  | 0.280  | 0.005  |
| Gly                 | 0.310  | 0.267  | 0.263  | 0.243  | 0.005  |
| Ala                 | 0.440  | 0.475  | 0.423  | 0.411  | 0.006  |
| Tyr                 | 0.236  | 0.236  | 0.208  | 0.197  | 0.001  |
| Cys                 | 0.123  | 0.130  | 0.145  | 0.136  | 0.0001 |
| Pro                 | 0.485  | 0.554  | 0.499  | 0.492  | 0.001  |

Table 2. Analyzed amino acids (AA) and CP composition of the semi-purified diets fed to broilers from 19 to 24 D of age (% as-fed basis).

1Values reported from the analysis conducted at the Chemical Laboratory, Institute for Food and Agricultural Research and Technology (IRTA), Catalonia, Spain. Samples were analyzed in duplicate.

2The corn samples were obtained from Brazil (corn-1), Russia (corn-2), Ukraine (corn-3), and Iran (corn-4), respectively.

3Purified cellulose (Contain Dietary Fiber).
$g/kg$ of DMI)/($AA$ content of the diet, $g/kg$ of DM) $\times 100$.

**Statistical Analyses**

Data were analyzed using a randomized complete block design (SAS, 2003). Pen location was the blocking factor. GLM procedure and lsmeans method were used to compare mean SIAAD coefficients.

Simple and multiple linear regressions were used to predict TAA and SIDAA contents in corn grain samples using SPSS version 19 (IBM Corp., NY) with the following model (Statistic, 2011). The input variables were proximate components (moisture, CP, GE, EE, CF, NDF, ADF, ash, and NFE) and TAA in the SIDAA equations. Each individual TAA and SIDAA contents were the output variable:

$$y_i = \beta_0 \beta_{1x1} + \beta_{2x2} + \ldots + \epsilon_i,$$

where $y_i$ = TAA and SIDAA, $\beta_0$ = intercept of the regression equation, $\beta_j$ = regression coefficient, $x_i$ = CP and other proximate components, and $\epsilon_i$ = random error of the regression model. The coefficient of determination ($R^2$), adjusted $R^2$, $P$-value regression, $P$-value coefficients, and standard error of prediction (SEP) were used to define the equation with the best fit. Statistical significance was considered at $P \leq 0.05$.

The SEP was calculated using the following equation (Yegani et al., 2013):

$$SEP = \sqrt{\frac{\sum (Y - \bar{Y})^2}{N}}$$

where $Y$ is the TAA and SIDAA determined in the chick bioassay, $\bar{Y}$ is the predicted TAA and SIDAA values based on the *in vitro* data, and $N$ is the number of corn grain samples tested.

**RESULTS AND DISCUSSION**

The analyzed CP contents in the Brazil, Russian, Ukraine, and Iran corn and NFD were 7.52, 7.65, 7.26, and 6.66, and 0.27%, respectively, which were close to formulated values (Table 2). The AA concentrations in the diets were in general agreement with reported values by Kong and Adeola (2013).

Overall, the level of SIAAD was high for most AA in the experimental diets and varied little among diets (Table 3). Ukraine corn grain had significantly lower SID of CP (82.56%) and most of the AAs (Phe, Leu, Asp, Glu, Ser, Gly, Ala, and Tyr) than the other corn samples ($P \leq 0.05$). However, corn samples from Brazil, Russia, and Iran had similar SID of CP (89.17–88.39%) and AA. Cys showed the lowest mean digestibility (82.6%), whereas Ser showed the highest mean digestibility (94.11%). The SID of Trp values were omitted because of levels being too low to be detected in the ileal assays. The results indicate that SID of CP and AA can be different depending on the origin. Therefore, the formulation of diets based on average nutritive and digestibility values may not be the most accurate method. The SID of CP and AA from Brazil, Russia, and Iran in the present study was within the range of values previously reported by Adedokun et al. (2008), Kim et al. (2011), and Kong and Adeola (2013) and was relatively higher than reported values by Iyayi and Adeola (2014) in 26-day-old broilers and by Szczurek (2009) in 30-day-old broilers.

Table 3. Coefficient of standardized ileal digestibility (%) of CP and amino acid (AA) of the diet in broilers of 24 D of age.1

| Item | Corn 1 | Corn 2 | Corn 3 | Corn 4 | Mean | P value | SEM |
|------|--------|--------|--------|--------|------|---------|-----|
| CP   | 89.17* | 88.390* | 82.558 | 88.670a | 87.20 | 0.030   | 1.077 |
| His  | 94.29   | 91.58   | 89.64   | 92.78   | 92.07 | 0.143   | 0.737 |
| Thr  | 84.84   | 86.11   | 80.04   | 87.40   | 84.60 | 0.438   | 1.546 |
| Arg  | 92.12   | 89.61   | 86.27   | 88.55   | 89.14 | 0.366   | 1.088 |
| Val  | 92.33   | 87.25   | 82.62   | 89.18   | 87.85 | 0.099   | 1.603 |
| Met  | 93.97   | 86.26   | 86.54   | 89.74   | 89.13 | 0.425   | 1.794 |
| Phe  | 94.60a  | 93.82a  | 88.62a  | 93.79a  | 92.71 | 0.037   | 0.964 |
| Ile  | 91.27   | 89.85   | 85.72   | 91.21   | 89.51 | 0.269   | 1.328 |
| Leu  | 92.43a  | 92.87a  | 87.63a  | 92.97a  | 91.48 | 0.017   | 0.867 |
| Lys  | 86.78   | 87.16   | 86.63   | 87.15   | 86.93 | 0.998   | 1.310 |
| Asp  | 92.08a  | 92.22a  | 85.74b  | 90.94a  | 90.25 | 0.038   | 1.054 |
| Glu  | 95.22a  | 95.04a  | 90.97b  | 94.90a  | 94.03 | 0.024   | 0.708 |
| Ser  | 96.01*  | 95.87a  | 89.14*  | 95.41*  | 94.11 | 0.015   | 1.028 |
| Gly  | 89.96*  | 86.68*  | 80.03*  | 86.34*  | 85.80 | 0.003   | 1.206 |
| Ala  | 92.40a  | 93.20a  | 87.60a  | 91.89a  | 91.29 | 0.013   | 0.822 |
| Tyr  | 94.39a  | 93.87a  | 87.82a  | 94.07a  | 92.54 | 0.011   | 1.005 |
| Cys  | 83.47   | 82.53   | 81.62   | 82.79   | 82.60 | 0.991   | 1.878 |
| Pro  | 87.59   | 86.46   | 85.21   | 87.75   | 86.75 | 0.893   | 0.649 |

Means within a row, not sharing a common superscript lowercase letter, are significantly different ($P \leq 0.05$).

1There were 4 cages of 4 chicks each per treatment.

2The corn samples were obtained from Brazil (corn-1), Russia (corn-2), Ukraine (corn-3), and Iran (corn-4), respectively.
old broilers. However, it was relatively lower than that reported by Adedokun et al. (2015) in 21-day-old broilers. Zuber and Rodehutscord (2017) reported that AA digestibility of corn grain varied among corn samples. The differences in these studies could be due to variety, growing conditions, the presence of antinutritional factors (such as nonstarch polysaccharides of tannins), age of birds, or the method used in the estimation (Choct and Hughes, 1999; Ravindran et al., 1999).

Among all the ingredients, the moisture, GE, and NDF differed with a coefficient of variation (CV) ranging from 5 to 10%, and the concentration of ADF and ash showed the greatest difference with a CV higher than 10% (Table 4). On DM basis, the concentration of CP and GE ranged from 7.58 to 8.39% and 4,121 to 4,621 kcal/kg, respectively. Mean contents of CP (8.15%), DM (88.93%), EE (4.42%), and CF (1.82%) in this experiment were in good agreement with those reported by Feedstuffs (2014) and NRC (1994). The results of TAA and SIDAA (Table 5) showed that most AA values were variable (CV > 6%) except Phe, Ile, and Pro (CV < 6%). Mean contents of TAA and SIDAA values were within the range reported by Bryden et al. (2009). The present study also revealed considerable variation in total and SID of Lys (CV = 21.2 and 20.9) of corn grain in broilers and confirmed the variation in total Lys and AA digestibility observed by Zuber and Rodehutscord (2017).

The linear regression equations for prediction of TAA contents of corn grain samples from protein content and other proximate components are shown in Table 6. Corn grain TAA levels were predicted with high

| Component | Corn-1 | Corn-2 | Corn-3 | Corn-4 | Mean | CV% |
|-----------|--------|--------|--------|--------|------|-----|
| DM        | 89.20  | 89.31  | 89.85  | 87.35  | 88.93| 1.22|
| Moisture  | 10.8   | 10.69  | 10.15  | 12.65  | 11.07| 0.93|
| GE, kcal/kg | 4,621 | 4,332  | 4,198  | 4,121  | 4,318| 5.09|
| CP        | 8.39   | 8.35   | 8.30   | 7.58   | 8.15 | 4.72|
| EE        | 4.57   | 4.50   | 4.23   | 4.37   | 4.42 | 3.34|
| CF        | 1.90   | 1.77   | 1.80   | 1.80   | 1.82 | 3.17|
| NDF       | 9.00   | 8.30   | 8.23   | 7.77   | 8.33 | 6.11|
| ADF       | 3.90   | 3.47   | 4.60   | 3.87   | 3.96 | 11.89|
| Total Ash | 0.91   | 1.48   | 1.37   | 1.28   | 1.26 | 19.58|
| NFE       | 73.44  | 73.22  | 74.14  | 72.83  | 73.28| 1.02|

Abbreviations: ADF, acid detergent fiber; CF, crude fiber; CV, coefficient of variation; EE, ether extract; GE, gross energy; NDF, neutral detergent fiber; NFE, nitrogen-free extract.

1Nutrients were analyzed in triplicate sample.

2The corn samples were obtained from Brazil (corn-1), Russia (corn-2), Ukraine (corn-3), and Iran (corn-4), respectively.

| Component | Corn-1 | Corn-2 | Corn-3 | Corn-4 | Mean | CV% |
|-----------|--------|--------|--------|--------|------|-----|
| DM        | 89.20  | 89.31  | 89.85  | 87.35  | 88.93| 1.22|
| Moisture  | 10.8   | 10.69  | 10.15  | 12.65  | 11.07| 0.93|
| GE, kcal/kg | 4,621 | 4,332  | 4,198  | 4,121  | 4,318| 5.09|
| CP        | 8.39   | 8.35   | 8.30   | 7.58   | 8.15 | 4.72|
| EE        | 4.57   | 4.50   | 4.23   | 4.37   | 4.42 | 3.34|
| CF        | 1.90   | 1.77   | 1.80   | 1.80   | 1.82 | 3.17|
| NDF       | 9.00   | 8.30   | 8.23   | 7.77   | 8.33 | 6.11|
| ADF       | 3.90   | 3.47   | 4.60   | 3.87   | 3.96 | 11.89|
| Total Ash | 0.91   | 1.48   | 1.37   | 1.28   | 1.26 | 19.58|
| NFE       | 73.44  | 73.22  | 74.14  | 72.83  | 73.28| 1.02|

Table 4. Determined chemical composition of the corn tested (%, DM basis).1

Table 5. Determined total amino acids (TAAs) and standardized ileal digestible amino acid (SIDAA) of the corn tested (%, DM basis).1

| Component | Corn-1 | Corn-2 | Corn-3 | Corn-4 | Mean | CV% |
|-----------|--------|--------|--------|--------|------|-----|
| DM        | 89.20  | 89.31  | 89.85  | 87.35  | 88.93| 1.22|
| Moisture  | 10.8   | 10.69  | 10.15  | 12.65  | 11.07| 0.93|
| GE, kcal/kg | 4,621 | 4,332  | 4,198  | 4,121  | 4,318| 5.09|
| CP        | 8.39   | 8.35   | 8.30   | 7.58   | 8.15 | 4.72|
| EE        | 4.57   | 4.50   | 4.23   | 4.37   | 4.42 | 3.34|
| CF        | 1.90   | 1.77   | 1.80   | 1.80   | 1.82 | 3.17|
| NDF       | 9.00   | 8.30   | 8.23   | 7.77   | 8.33 | 6.11|
| ADF       | 3.90   | 3.47   | 4.60   | 3.87   | 3.96 | 11.89|
| Total Ash | 0.91   | 1.48   | 1.37   | 1.28   | 1.26 | 19.58|
| NFE       | 73.44  | 73.22  | 74.14  | 72.83  | 73.28| 1.02|

Abbreviations: CV, coefficient of variation; SID, standardized ileal digestibility.

1Amino acids were analyzed in duplicate sample.

2The corn samples were obtained from Brazil (corn-1), Russia (corn-2), Ukraine (corn-3), and Iran (corn-4), respectively.
Table 6. Regression equations for prediction of total amino acids (TAAs) composition from protein content and proximate components of corn grain (DM basis).1

| Amino acids | Basis | Prediction equations | $R^2$ | Adjusted $R^2$ | $P$-value regression | $P$-value coefficients | SEP |
|-------------|-------|----------------------|-------|---------------|----------------------|-------------------------|------|
| TMet        | CP    | $Y = 0.0205 \times CP$ | 99.8  | 99.7          | 0.0001               | CP, 0.0001              | 0.007 |
|             | CF, NFE | $Y = -0.631 + 0.149 \times CP + 0.007 \times NFE$ | 99.9  | 99.9          | 0.005                | CF, 0.004               | 0.014 |
| TMet + Cys  | CP    | $Y = 0.020 \times CP$ | 99.9  | 99.8          | 0.0001               | CP, 0.0001              | 0.005 |
|             | NFE, ADF | $Y = -0.760 + 0.014 \times NFE - 0.026 \times ADF$ | 99.9  | 99.9          | 0.014                | NFE, 0.011              | 0.005 |
|             | CP, CF, EE | $Y = 0.017 \times CP + 0.047 \times EE - 0.102 \times CF$ | 99.8  | 99.7          | 0.006                | EE, 0.009               | 0.002 |
| Tlys        | CP    | $Y = 0.041 \times CP$ | 96.9  | 95.9          | 0.002                | CP, 0.002               | 0.050 |
|             | Ash, Ash | $Y = 0.692 - 0.283 \times Ash$ | 98.3  | 97.5          | 0.008                | Ash, 0.008              | 0.008 |
|             | CP, Ash | $Y = 0.329 \times CF - 0.209 \times Ash$ | 99.9  | 99.9          | 0.004                | CP, 0.001               | 0.005 |
| TThr        | CP    | $Y = -0.1826 + 0.056 \times CP$ | 99.0  | 98.4          | 0.005                | CP, 0.005               | 0.004 |
|             | CP, moisture | $Y = 0.0415 \times CP - 0.005 \times Moisture$ | 99.9  | 99.9          | 0.001                | CP, 0.001               | 0.005 |
| TLeu        | CP    | $Y = 0.009 + 0.028 \times CP$ | 94.0  | 91.0          | 0.003                | CP, 0.003               | 0.002 |
|             | CP, EE | $Y = -0.065 + 0.0258 \times CP + 0.019 \times EE$ | 99.9  | 99.8          | 0.024                | CP, 0.018               | 0.000 |
| TLeu        | CP    | $Y = 0.0798 \times CP$ | 99.8  | 99.7          | 0.001                | CP, 0.001               | 0.029 |
|             | CP, NDF | $Y = 0.183 \times CP - 0.101 \times NDF$ | 99.9  | 99.9          | 0.001                | CP, 0.017               | 0.008 |
| THis        | CP    | $Y = -0.100 + 0.0325 \times CP$ | 99.9  | 99.9          | 0.001                | CP, 0.001               | 0.000 |
|             | CP, NFE | $Y = 0.0346 \times CP - 0.0016 \times NFE$ | 99.9  | 99.8          | 0.001                | CP, 0.005               | 0.001 |
| TVal        | CP    | $Y = -0.172 + 0.0535 \times CP$ | 88.1  | 82.2          | 0.061                | CP, 0.061               | 0.006 |
|             | CP, GE | $Y = -0.2277 + 0.0359 \times CP + 0.00004617 \times GE$ | 99.9  | 99.7          | 0.020                | CP, 0.027               | 0.000 |
| TArg        | CP    | $Y = -0.389 + 0.0875 \times CP$ | 84.2  | 76.3          | 0.082                | CP, 0.082               | 0.012 |
|             | CP    | $Y = 0.0918 \times CP$ | 99.6  | 99.5          | 0.0001               | CP, 0.001               | 0.020 |
|             | EE, Moisture | $Y = 0.140 \times EE - 0.0266 \times Moisture$ | 99.9  | 99.9          | 0.0001               | EE, 0.004               | 0.004 |
| TPhe        | CP    | $Y = 0.0308 \times CP$ | 99.9  | 99.9          | 0.0001               | CP, 0.001               | 0.005 |
|             | CP, NDF | $Y = 0.050 \times CP - 0.0188 \times NDF$ | 99.9  | 98.6          | 0.0001               | CP, 0.006               | 0.001 |

Abbreviations: ADF, acid detergent fiber; CF, crude fiber; EE, ether extract; GE, gross energy; NDF, neutral detergent fiber; NFE, nitrogen-free extract; SEP, standard error of prediction.

1 Analyzed with SPSS statistical software and stepwise and inter procedures.

2 $R^2$ is the coefficient of determination, adjusted $R^2$ adjusted for the number of predictors in the model. $P$-value < 0.05 is statistically significant (Yegani et al., 2013).

The inclusion of other proximate components of the test samples (e.g., CF, EE, NDF, NFE and so on) into the regression equation increased the adj $R^2$ (adj $R^2 > 97$) and decreased the SEP, indicating that the predictive ability was improved as compared to the protein content equations. Cravener and Roush (2001) used a linear regression model to predict the AA content in feed ingredients based on proximate analysis and reported the AA contents of feedstuffs were related to the sample proximate analysis. NRC (1994) presented an equation for estimating the TAA content of feedstuffs related to changes in protein content ($Y = a + bx$) and an equation for estimating AA content from other proximate components ($Y = a + b_1 (\% \text{protein}) + b_2 (\% \text{moisture}) + b_3 (\% \text{fat}) + b_4 (\% \text{fiber}) + b_5 (\% \text{ash})$), where the $b_n$ represents the regression coefficients. In the equations based on other proximate components for corn grain reported in NRC (1994), most of the proximate components for prediction of AA except threonine are not included, and also the accuracy of the regression equations for estimating the amount of TAA in ingredients seems to be variable and low. The prediction equations for the SID contents of the AAs from protein content, other proximate components, and TAA presented a high degree of accuracy (adj $R^2$ of above 91%), as indicated by significant $P$-value regression and coefficients (Tables 7 and 8). The SID of Lys was predicted using the following equations: %SID Lys = $0.0356 \times CP$, $(R^2 = 97\%, \text{SEP} = 0.051)$; $\{ -1.1591 + 0.836 \times CF - 0.055 \times ash \}$, $(R^2 = 99.9\%, \text{SEP} = 0.001)$; and $0.0023 + 0.861 \times \text{Tlys}$, $(R^2 = 99.9\%, \text{SEP} = 0.000)$. Zuber and
Table 7. Regression equations for prediction of standardized ileal digestible amino acid (SIDAA) composition from protein content and proximate components of corn grain (DM basis).  

| Amino acids | Basis | Prediction equations | $R^2$ | Adjusted $R^2$ | P-value regression | P-value coefficients | SEP |
|-------------|-------|---------------------|------|-------------|------------------|-----------------|-----|
| SID Met     | CP    | $Y = 0.0183 \times CP$ | 99.4 | 99.2 | 0.0001 | CP 0.0001 | 0.011 |
|             | CF, NFE | $Y = -0.327 + 0.0033 \times NFE + 0.241 \times CF$ | 99.9 | 99.8 | 0.010 | NFE 0.037 | 0.002 |
| SID Cys     | CP    | $Y = 0.0165 \times CP$ | 99.8 | 99.8 | 0.0001 | CP 0.0001 | 0.005 |
|             | CP, ADF | $Y = 0.0432 + 0.0177 \times CP - 0.0133 \times ADF$ | 99.9 | 99.9 | 0.002 | CP 0.002 | 0.000 |
| SID Met + Cys | CP | $Y = 0.035 \times CP$ | 99.8 | 99.8 | 0.0001 | CP 0.0001 | 0.012 |
|             | NDF  | $Y = -0.021 + 0.0367 \times NDF$ | 96.6 | 95.0 | 0.017 | NDF 0.017 | 0.003 |
| SID Lys     | CP    | $Y = 0.0356 \times CP$ | 97.0 | 96.0 | 0.002 | CP 0.002 | 0.051 |
|             | CF, Ash | $Y = -1.1591 + 0.836 \times CF - 0.055 \times Ash$ | 99.9 | 99.9 | 0.001 | CF 0.005 | 0.001 |
| SID Thr     | CP    | $Y = 0.0287 \times CP$ | 99.8 | 99.7 | 0.0001 | CP 0.0001 | 0.006 |
|             | CP, ADF | $Y = -0.0123 + 0.0375 \times CP - 0.015 \times ADF$ | 99.9 | 99.9 | 0.001 | CP 0.0001 | 0.000 |
| SID Ile     | CP    | $Y = 0.0252 \times CP$ | 99.9 | 99.8 | 0.0001 | CP 0.0001 | 0.024 |
|             | EE, moisture | $Y = 0.059 \times EE - 0.005 \times Moisture$ | 99.9 | 99.9 | 0.0001 | EE 0.002 | 0.024 |
| SID Leu     | CP    | $Y = 0.073 \times CP$ | 99.9 | 99.8 | 0.0001 | CP 0.0001 | 0.063 |
|             | CP, CF | $Y = 0.827 + 0.0625 \times CP - 0.4078 \times CF$ | 99.9 | 99.8 | 0.024 | CP 0.019 | 0.058 |
| SID His     | CP    | $Y = -0.0877 + 0.0293 \times CP$ | 94.5 | 91.8 | 0.028 | CP 0.028 | 0.013 |
|             | CP, GE | $Y = -0.1077 + 0.0223 \times CP + 0.000106 \times GE$ | 99.9 | 99.9 | 0.014 | CP 0.015 | 0.019 |
| SID Val     | CP    | $Y = 0.0285 \times CP$ | 99.8 | 99.5 | 0.0001 | CP 0.0001 | 0.034 |
|             | NDF, ADF | $Y = -0.0841 + 0.0453 \times NDF - 0.0153 \times ADF$ | 99.8 | 99.5 | 0.043 | NDF 0.029 | 0.033 |
| SID Arg     | CP    | $Y = 0.0355 \times CP$ | 99.3 | 99.1 | 0.0001 | CP 0.0001 | 0.040 |
|             | GE, CF | $Y = 0.000243 \times GE - 0.4185 \times CF$ | 99.9 | 99.9 | 0.0001 | GE 0.011 | 0.036 |
| SID Phe     | CP    | $Y = 0.0285 \times CP$ | 99.9 | 99.9 | 0.0001 | CP 0.0001 | 0.020 |
|             | NDF, Ash | $Y = 0.0227 \times NDF + 0.035 \times Ash$ | 99.9 | 99.9 | 0.0001 | Ash 0.031 | 0.020 |
|             | CF, NDF | $Y = 0.374 - 0.232 \times CF + 0.0336 \times NDF$ | 99.5 | 98.4 | 0.057 | CF 0.042 | 0.020 |

Abbreviations: ADF, acid detergent fiber; CF, crude fiber; EE, ether extract; GE, gross energy; NDF, neutral detergent fiber; NFE, nitrogen-free extract; SEP, standard error of prediction; SID, standardized ileal digestibility.

1 Analyzed with SPSS statistical software and stepwise and inter procedures.

2 $R^2$ is the coefficient of determination, adjusted $R^2$ adjusted for the number of predictors in the model, $P$-value $< 0.05$ is statistically significant (Yegani et al., 2013).

Rodehutscord (2017) reported that the digestibility of most AA was positively correlated with the CP concentration of the corn samples. Ebadi et al. (2011) found the best relationships between true digestible AA and chemical composition (CP, CF, EE, ash, and total phenols) in sorghum grain for most AAs. These studies show that the chemical composition of feed ingredient is related to the digestibility of AAs.

Table 8. Regression equations for prediction of SIDAA from TAA of corn grain (DM Basis).  

| Amino acids | Prediction equations | $R^2$ | Adjusted $R^2$ | P-value regression | P-value coefficient | SEP |
|-------------|---------------------|------|-------------|------------------|-----------------|-----|
| SID Met     | $Y = 0.8936 \times TMet$ | 99.9 | 99.8 | 0.0001 | CP 0.0001 | 0.005 |
| SID Cys     | $Y = -0.0039 + 0.879 \times TCys$ | 97.7 | 96.6 | 0.011 | CP 0.011 | 0.001 |
| SID Met + Cys | $Y = 0.863 \times TMetCys$ | 99.9 | 99.9 | 0.0001 | CP 0.0001 | 0.007 |
| SID Lys     | $Y = 0.0025 + 0.861 \times TLys$ | 99.9 | 99.9 | 0.0001 | CP 0.0001 | 0.000 |
| SID Thr     | $Y = 0.842 \times TTTh$ | 99.9 | 99.9 | 0.0001 | CP 0.0001 | 0.007 |
| SID Ile     | $Y = 0.894 \times Tlle$ | 99.9 | 99.9 | 0.0001 | CP 0.0001 | 0.024 |
| SID Leu     | $Y = 0.913 \times Tlle$ | 99.9 | 99.9 | 0.0001 | CP 0.0001 | 0.056 |
| SID His     | $Y = -0.0359 + 0.855 \times THis$ | 93.2 | 93.8 | 0.035 | CP 0.035 | 0.021 |
| SID Val     | $Y = 0.820 \times TVal$ | 99.8 | 99.8 | 0.0001 | CP 0.0001 | 0.031 |
| SID Arg     | $Y = -0.0444 + 1.028 \times TArg$ | 97.2 | 95.8 | 0.014 | CP 0.014 | 0.035 |
| SID Phe     | $Y = 0.926 \times TPhe$ | 99.9 | 99.9 | 0.0001 | CP 0.0001 | 0.018 |

Abbreviations: SEP, standard error of prediction; SID, standardized ileal digestibility; SIDAA, standardized ileal digestible amino acids; TAA, total amino acids.

1 Analyzed with SPSS statistical software and stepwise and inter procedures.

2 $R^2$ is the coefficient of determination, adjusted $R^2$ adjusted for the number of predictors in the model, $P$-value $< 0.05$ is statistically significant (Yegani et al., 2013).
The prediction equations of digestible AA from TAA concentration in distillers dried corn with soluble in growing pigs were reported (Urriola et al., 2009). They found a low correlation between the concentration and digestibility of AA, His (0.71), Lys (0.66), Met (0.75), and Thr (0.39), suggesting that it is desirable to develop in vitro procedures to predict digestible AA concentration. This observation differs from that reported by Van Kempen et al. (2002) for SBM in growing swine, where the amount of digestible CP and AA could be predicted from its total concentration (R² = 0.96 for Lys, 0.98 for Met, 0.90 for Thr, and 0.89 for Trp). The Lys concentration per unit CP could be an acceptable predictor of Lys SID in wheat distillers dried grains with soluble (Cozannet et al., 2010).

In conclusion, predictions based on chemical characteristics were more accurate in terms of reflecting the bioassay results. Inclusion of proximate components increased the accuracy of prediction of TAA and SIDAA for broiler chickens, but additional time and costs were associated with this approach. The goodness of fit of the regression equations may have been improved with a wider range in corn grain samples, and further research is warranted to develop robust TAA and SIDAA prediction equations for corn grains currently used by the poultry industry. As a result, the equations developed in the present study can serve as a reference to develop calibration equations for the prediction of concentration of TAA and SIDAA of corn grains for broiler chickens using near-infrared reflectance spectroscopy.

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