Relationship between chemical composition and standardized ileal digestible amino acid contents of corn grain in broiler chickens

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ABSTRACT This experiment was conducted to evaluate the chemical composition and standardized ileal amino acid digestibility of corn grain and to use these data to develop prediction equations for estimating total amino acids (**TAAs**) 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 ($P \leq 0.05$). 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

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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;

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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 orthophthaldialdehyde 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 m^2/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

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

Item	Diets[1]				
	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
Dicalcium 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 premix[2]	0.6	0.6	0.6	0.6	0.60
Solka-Floc[3]	-	-	-	-	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					
AME_n, 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

[1]The corn samples were obtained from Brazil (corn-1), Russia (corn-2), Ukraine (corn-3), and Iran (corn-4), respectively.
[2]Provided the following (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3 (chole-calciferol), 2,000 IU; vitamin E (all-rac-tocopherol acetate), 20 IU; vitamin K (bisulfate menadione complex), 3 mg; riboflavin, 5 mg; pantothenic acid (d-calcium pantothenate), 10 mg; nicotinic acid, 30 mg; pyridoxine (pyridoxine·HCl), 3 mg; thiamin (thiamin mononitrate), 1 mg; vitamin B12 (cyanocobalamin), 12 µg; d-biotin, 0.15 mg; choline (choline chloride), 300 mg; folic acid, 0.5 mg; Se (Na2SeO3), 0.1 mg; I (KI), 2.0 mg; Cu (CuSO4·5H2O), 10 mg; Fe (FeSO4·7H2O), 30 mg; Mn (MnSO4·H2O), 100 mg; Zn (ZnO), 100 mg; and ethoxyquin, 110 mg.
[3]Purified cellulose (Contain Dietary Fiber).

were given *ad libitum* access to the experimental diets and NFD. On day 24, all the birds were euthanized by CO_2 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).

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).[1]

Item	Diets[2]				
	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

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

Collected ileal samples from 3 birds within a cage were pooled and stored in a freezer at −20°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) 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 g/kg of DMI)/(AA content of the diet, g/kg of DM)] × 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:

$$y_i = \beta_0 + \beta_{1\times1} + \beta_{2\times2} + \ldots + \varepsilon_i,$$

where y_i = TAA and SIDAA, β_0 = intercept of the regression equation, β_j = regression coefficient, x_j = CP and other proximate components, and ε_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):

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

where *Y* is the TAA and SIDAA determined in the chick bioassay, Ý 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, 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-

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

	Corn[2]						
Item	Corn-1	Corn-2	Corn-3	Corn-4	Mean	*P* value	SEM
CP	89.170[a]	88.390[a]	82.558[b]	88.670[a]	87.20	0.030	1.077
Essential AA							
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	94.60[a]	93.82[a]	88.62[b]	93.79[a]	92.71	0.037	0.964
Ile	91.27	89.85	85.72	91.21	89.51	0.269	1.328
Leu	92.43[a]	92.87[a]	87.65[b]	92.97[a]	91.48	0.017	0.867
Lys	86.78	87.16	86.63	87.15	86.93	0.998	1.310
Nonessential AA							
Asp	92.08[a]	92.22[a]	85.74[b]	90.94[a]	90.25	0.038	1.054
Glu	95.22[a]	95.04[a]	90.97[b]	94.90[a]	94.03	0.024	0.708
Ser	96.01[a]	95.87[a]	89.14[b]	95.41[a]	94.11	0.015	1.028
Gly	89.96[a]	86.68[a]	80.03[b]	86.54[a]	85.80	0.003	1.206
Ala	92.40[a]	93.26[a]	87.60[b]	91.89[a]	91.29	0.013	0.822
Tyr	94.39[a]	93.87[a]	87.82[b]	94.07[a]	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 ($P \leq 0.05$).

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

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

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

	Corn[2]					
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	9.83
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	72.33	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.

[1]Nutrients were analyzed in triplicate sample.

[2]The 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

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

	Corn[2]											
	Corn-1		Corn-2		Corn-3		Corn-4		Mean		CV%	
Component	Total	SID	Total	SID	Total	SID	Total	SID	Total	SID	Total	SID
Essential AA												
His	0.172	0.162	0.171	0.156	0.170	0.153	0.146	0.135	0.165	0.152	7.69	7.90
Thr	0.288	0.244	0.290	0.249	0.288	0.230	0.245	0.214	0.278	0.235	7.79	6.68
Arg	0.360	0.331	0.344	0.308	0.317	0.273	0.276	0.244	0.324	0.289	11.3	13.2
Val	0.287	0.265	0.273	0.238	0.264	0.218	0.235	0.210	0.265	0.233	8.22	10.5
Met	0.181	0.170	0.160	0.138	0.171	0.148	0.158	0.142	0.167	0.149	6.34	9.50
Phe	0.251	0.237	0.260	0.244	0.263	0.233	0.233	0.219	0.252	0.233	5.33	4.64
Ile	0.239	0.218	0.236	0.212	0.230	0.197	0.214	0.195	0.230	0.206	4.90	5.51
Leu	0.624	0.577	0.676	0.628	0.697	0.611	0.610	0.567	0.652	0.596	6.40	4.83
Lys	0.437	0.379	0.274	0.239	0.312	0.270	0.316	0.275	0.355	0.291	21.2	20.9
Nonessential AA												
Asp	0.559	0.514	0.537	0.495	0.541	0.464	0.471	0.428	0.527	0.475	7.29	7.90
Glu	1.120	1.067	1.164	1.106	1.199	1.091	1.014	0.963	1.124	1.057	7.13	6.12
Ser	0.275	0.264	0.296	0.284	0.302	0.270	0.255	0.244	0.282	0.265	7.61	6.34
Gly	0.277	0.249	0.268	0.232	0.258	0.206	0.217	0.188	0.255	0.219	10.2	12.3
Ala	0.439	0.406	0.459	0.428	0.465	0.408	0.383	0.352	0.436	0.398	8.62	8.20
Tyr	0.207	0.195	0.222	0.208	0.223	0.196	0.187	0.175	0.209	0.194	8.07	6.96
Cys	0.167	0.140	0.175	0.145	0.158	0.129	0.152	0.126	0.163	0.135	6.20	6.48
Pro	0.481	0.421	0.509	0.440	0.481	0.410	0.465	0.408	0.484	0.420	3.83	3.54

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

[1]Amino acids were analyzed in duplicate sample.

[2]The 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]

			Statistical parameter[2]					
Amino acids	Basis	Prediction equations	R^2	Adjusted R^2	P-value regression	P-value coefficients		SEP
TMet	CP	Y = 0.0205 × CP	99.8	99.7	0.0001	CP	0.0001	0.007
	CF, NFE	Y = − 0.631 + 0.149 × CF + 0.007 × NFE	99.9	99.9	0.005	CF	0.004	0.014
						NFE	0.006	
TCys	CP	Y = 0.020 × CP	99.9	99.8	0.0001	CP	0.0001	0.005
	NFE, ADF	Y = − 0.760 + 0.014 × NFE − 0.026 × ADF	99.9	99.9	0.014	NFE	0.011	0.000
						ADF	0.009	
	CP, CF, EE	Y = 0.017 × CP + 0.047 × EE − 0.102 × CF	99.8	99.7	0.006	CF	0.091	0.002
						EE	0.081	
						CP	0.080	
TMet + Cys	CP	Y = 0.0245 + 0.0375 × CP	84.0	76.0	0.084	CP	0.084	0.005
	CP	Y = 0.0405 × CP	99.9	99.9	0.0001	CP	0.0001	0.005
	NDF, Ash	Y = −0.0175 + 0.038 × NDF + 0.024 × Ash	99.9	99.9	0.009	NDF	0.007	0.001
						Ash	0.022	
TLys	CP	Y = 0.041 × CP	96.9	95.9	0.002	CP	0.002	0.050
	Ash	Y = 0.692 − 0.283 × Ash	98.3	97.5	0.008	Ash	0.008	0.008
	CF, Ash	Y = 0.329 × CF − 0.209 × Ash	99.9	99.9	0.0001	CF	0.001	0.005
						Ash	0.005	
TThr	CP	Y = −0.1826 + 0.056 × CP	99.0	98.4	0.005	CP	0.005	0.004
	CP, moisture	Y = 0.0415 × CP − 0.005 × Moisture	99.9	99.9	0.0001	CP	0.0001	0.005
						Moisture	0.013	
TIle	CP	Y = 0.0009 + 0.028 × CP	94.0	91.0	0.003	CP	0.003	0.002
	CP, EE	Y = −0.065 + 0.0258 × CP + 0.019 × EE	99.9	99.8	0.024	CP	0.018	0.000
						EE	0.062	
TLeu	CP	Y = 0.0798 × CP	99.8	99.7	0.0001	CP	0.0001	0.029
	CP, NDF	Y = 0.183 × CP − 0.101 × NDF	99.9	99.9	0.0001	CP	0.017	0.008
						NDF	0.051	
THis	CP	Y = −0.100 + 0.0325 × CP	99.9	99.9	0.0001	CP	0.0001	0.000
	CP, NFE	Y = 0.0346 × CP − 0.0016 × NFE	99.9	99.8	0.0001	CP	0.005	0.001
						NFE	0.026	
TVal	CP	Y = −0.172 + 0.0535 × CP	88.1	82.2	0.061	CP	0.061	0.006
	CP	Y = 0.0324 × CP	99.9	99.8	0.0001	CP	0.0001	0.009
	CP. GE	Y = −0.2277 + 0.0359 × CP + 0.00004617 × GE	99.9	99.7	0.020	CP	0.027	0.000
						GE	0.037	
TArg	CP	Y = −0.389 + 0.0875 × CP	84.2	76.3	0.082	CP	0.082	0.012
	CP	Y = 0.0398 × CP	99.6	99.5	0.0001	CP	0.0001	0.020
	EE, Moisture	Y = 0.140 × EE − 0.0266 × Moisture	99.9	99.9	0.0001	EE	0.004	0.004
						Moisture	0.015	
TPhe	CP	Y = 0.0308 × CP	99.9	99.9	0.0001	CP	0.0001	0.005
	CP, NDF	Y = 0.050 × CP − 0.0188 × NDF	99.9	98.6	0.0001	CP	0.006	0.001
						NDF	0.039	

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).

accuracy (adj $R^2 \geq 76$) with linear regression based on protein content and other proximate components. 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 (% protein) + b_2 (% moisture) + b_3 (% fat) + b_4 (% fiber) + b_5 (% 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 × CP], (R^2 = 97%, SEP = 0.051); [−1.1591 + 0.836 × CF − 0.055 × ash], (R^2 = 99.9, SEP = 0.001); and [0.0023 + 0.861 × TLys], (R^2 = 99.9%, 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).[1]

			Statistical parameter[2]					
Amino acids	Basis	Prediction equations	R^2	Adjusted R^2	P-value regression	P-value coefficients		SEP
SID Met	CP	Y = 0.0183 × CP	99.4	99.2	0.0001	CP	0.0001	0.011
	CF, NFE	Y = −0.5327 + 0.0033 × NFE + 0.241 × CF	99.9	99.8	0.010	NFE	0.037	0.002
						CF	0.007	
SID Cys	CP	Y = 0.0165 × CP	99.8	99.8	0.0001	CP	0.0001	0.005
	CP, ADF	Y = 0.0432 + 0.0177 × CP − 0.0133 × ADF	99.9	99.9	0.002	CP	0.002	0.000
						ADF	0.002	
SID Met + Cys	CP	Y = 0.035 × CP	99.8	99.8	0.0001	CP	0.0001	0.012
	NDF	Y = −0.021 + 0.0367 × NDF	96.6	95.0	0.017	NDF	0.017	0.003
SID Lys	CP	Y = 0.0356 × CP	97.0	96.0	0.002	CP	0.002	0.051
	CF, Ash	Y = −1.1591 + 0.836 × CF − 0.055 × Ash	99.9	99.9	0.001	CF	0.005	0.001
						Ash	0.017	
SID Thr	CP	Y = 0.0287 × CP	99.8	99.7	0.0001	CP	0.0001	0.006
	CP, ADF	Y = −0.0123 + 0.0375 × CP − 0.015 × ADF	99.9	99.9	0.0001	CP	0.0001	0.000
						ADF	0.0001	
SID Ile	CP	Y = 0.0252 × CP	99.9	99.8	0.0001	CP	0.0001	0.024
	EE, moisture	Y = 0.059 × EE − 0.005 × Moisture	99.9	99.9	0.0001	EE	0.002	0.024
						Moisture	0.043	
SID Leu	CP	Y = 0.073 × CP	99.9	99.8	0.0001	CP	0.0001	0.063
	CP, CF	Y = 0.827 + 0.0625 × CP − 0.4078 × CF	99.9	99.8	0.024	CP	0.019	0.058
						CF	0.020	
SID His	CP	Y = −0.0877 + 0.0293 × CP	94.5	91.8	0.028	CP	0.028	0.013
	CP, GE	Y = −0.1077 + 0.0223 × CP + 0.0000166 × GE	99.9	99.9	0.014	CP	0.015	0.019
						GE	0.037	
SID Val	CP	Y = 0.0285 × CP	99.5	99.4	0.0001	CP	0.0001	0.034
	NDF, ADF	Y = − 0.0841 + 0.0453 × NDF − 0.0153 × ADF	99.8	99.5	0.043	NDF	0.029	0.033
						ADF	0.052	
SID Arg	CP	Y = 0.0355 × CP	99.3	99.1	0.0001	CP	0.0001	0.040
	GE, CF	Y = 0.000243 × GE − 0.4185 × CF	99.9	99.9	0.0001	GE	0.011	0.036
						CF	0.012	
SID Phe	CP	Y = 0.0285 × CP	99.9	99.9	0.0001	CP	0.0001	0.020
	NDF, Ash	Y = 0.0227 × NDF + 0.035 × Ash	99.9	99.9	0.0001	NDF	0.002	0.019
						Ash	0.031	
	CF, NDF	Y = 0.374 − 0.232 × CF + 0.0336 × NDF	99.5	98.4	0.057	CF	0.042	0.020
						NDF	0.036	

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).[1]

		Statistical parameter[2]				
Amino acids	Prediction equations	R^2	Adjusted R^2	P-value regression	P-value coefficient	SEP
SID Met	Y = 0.8936 × TMet	99.9	99.8	0.0001	0.0001	0.005
SID Cys	Y = −0.0083 + 0.879 × TCys	97.7	96.6	0.011	0.011	0.001
SID Met + Cys	Y = 0.863 × TMetCys	99.9	99.9	0.0001	0.0001	0.007
SID Lys	Y = 0.0023 + 0.861 × TLys	99.9	99.9	0.0001	0.0001	0.000
SID Thr	Y = 0.842 × TThr	99.9	99.9	0.0001	0.0001	0.007
SID Ile	Y = 0.894 × TIle	99.9	99.9	0.0001	0.0001	0.024
SID Leu	Y = 0.913 × TLeu	99.9	99.9	0.0001	0.0001	0.056
SID His	Y = −0.0039 + 0.895 × THis	93.2	89.8	0.035	0.035	0.021
SID Val	Y = 0.880 × TVal	99.8	99.8	0.0001	0.0001	0.031
SID Arg	Y = −0.0444 + 1.028 × TArg	97.2	95.8	0.014	0.014	0.035
SID Phe	Y = 0.926 × TPhe	99.9	99.9	0.0001	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 ($R^2 = 0.96$ 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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