Prediction of the true digestible amino acid contents from the chemical composition of sorghum grain for poultry

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ABSTRACT Accurate knowledge of true digestible amino acid (TDAA) contents of feedstuffs is necessary to accurately formulate poultry diets for profitable production. Several experimental approaches that are highly expensive and time consuming have been used to determine available amino acids. Prediction of the nutritive value of a feed ingredient from its chemical composition via regression methodology has been attempted for many years. The artificial neural network (ANN) model is a powerful method that may describe the relationship between digestible amino acid contents and chemical composition. Therefore, multiple linear regressions (MLR) and ANN models were developed for predicting the TDAA contents of sorghum grain based on chemical composition. A precision-fed assay trial using cecectomized roosters was performed to determine the TDAA contents in 48 sorghum samples from 12 sorghum varieties differing in chemical composition. The input variables for both MLR and ANN models were CP, ash, crude fiber, ether extract, and total phenols whereas the output variable was each individual TDAA for every sample. The results of this study revealed that it is possible to satisfactorily estimate the TDAA of sorghum grain through its chemical composition. The chemical composition of sorghum grain seems to highly influence the TDAA contents when considering components such as CP, crude fiber, ether extract, ash and total phenols. It is also possible to estimate the TDAA contents through multiple regression equations with reasonable accuracy depending on composition. However, a more satisfactory prediction may be achieved via ANN for all amino acids. The R^2 values for the ANN model corresponding to testing and training parameters showed a higher accuracy of prediction than equations established by the MLR method. In addition, the current data confirmed that chemical composition, often considered in total amino acid prediction, could be also a useful predictor of true digestible values of selected amino acids for poultry.

Key words: prediction model, sorghum, true digestible amino acid

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) *Moench*] is a drought-resistant grain and thus is an important diet ingredient in semiarid regions of world. Several reports have indicated the adverse effects of some sorghum samples on growth rate and feed conversion efficiency of nonruminant animals (Duodu et al., 2003; Ebadi et al., 2005). Low protein and amino acid digestibilities of sorghum grain are the important factors affecting poultry performance and production. Because protein and amino acids are the most expensive parts of a poultry ration, accurate knowledge of true digestible amino acid (**TDAA**) contents of feedstuffs is necessary to formulate poultry diets to achieve profitable production. In vitro (enzymatic, chemical, and microbiological assays), indirect (plasma amino acid assays), and direct (digestibility and growth assays) methods have been used to determine amino acids availability for poultry (Ravindran and Bryden, 1999). Digestibility trials using live animals have become the most common technique for estimating amino acid digestibility but are expensive and time consuming. Therefore, nutritionists are highly interested in finding rapid, inexpensive, and accurate methods for assessing TDAA contents of feedstuffs. Several studies have shown that amino acid digestibility of sorghum is correlated with its chemical composition (Ebadi et al., 2005; Selle et al., 2010). Prediction of nutritive value of a feed ingredient from its chemical composition has been attempted for many years based on regression methods (NRC, 1994; Urriola

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et al., 2009). The interdependencies of the involved factors in such problems limit the use of simple regression analysis and need a more accurate and satisfactory method. The artificial neural network (**ANN**) model may accurately show the nonlinear behavior with complex relationships and it may estimate the TDAA content in feedstuffs. The ANN model has been applied successfully to the prediction and control of nonlinear systems and systems with unknown models and has been used in poultry feed evaluation (Roush and Cravener, 1997; Cravener and Roush, 1999, 2001; Ahmadi et al., 2008; Perai et al., 2010). Previously, multiple linear regression (**MLR**) and ANN models have been used to predict the total amino acid content in feed ingredients based on chemical composition (Roush and Cravener, 1997). Information about total amino acid content of feedstuffs is important; however, it is more essential for a nutritionist to know the TDAA contents for specific feed ingredients when formulating poultry diets. Differences in chemical composition may be responsible for a substantial amount of the variability in the nutritional value of sorghum grain that can affect amino acid content and digestibility. Because of a high level of antinutritional factors, amino acid digestibility is a main concern in the use of sorghum as a feed component for poultry. The relationship between sorghum chemical composition and TDAA content is not yet documented. Therefore, the main objective of this study was to develop MLR and ANN models for poultry to predict the sorghum grain TDAA content based on its chemical composition.

MATERIALS AND METHODS

Data Collection

Twelve varieties of sorghum grain were grown under similar environmental conditions. Four samples from each of the 12 varieties were taken and evaluated for CP, ash, crude fiber (**CF**), and ether extract (**EE**) following AOAC International (2000) analytical methods (990.03, 920.39, 978.10, and 942.05, respectively). Total phenolics were assayed by the Folin-Denis method (952.03; AOAC International, 2000).

Amino acid concentrations in sorghum grain samples were analyzed by ion exchange chromatography following hydrolysis in 6 *N* HCl for 24 h at 110°C in sealed tubes, with at least 2 replicates per sample. Derivation with ninhydrin was accomplished (Andrews and Baldar, 1985) and the quantity of each amino acid was determined using the Bechman Biochrom 20 Amino Acid Analyzer at the University of Manitoba (Winnipeg, Canada). Methionine and Cys were determined on samples that had been oxidized in performic acid before acid hydrolysis (Moore, 1963).

This project was approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran. Single comb White Leghorn roosters were cecectomized according to Parson's method (Parsons, 1985). After a recovery period and 24 h of feed deprivation, roosters were randomly given a 30-g sorghum sample via crop intubation (6 roosters/sorghum sample). Six additional roosters were fed 30 g of glucose to measure endogenous amino acids excreted (Green et al., 1987; McNab and Blair, 1988). The excreta were collected over a 48-h period and stored in a freezer. After all excreta were freeze dried, the concentrations of amino acids were analyzed as described previously. True amino acid digestibility coefficient was calculated by the Sibbald (1986) method. The TDAA values were obtained by multiplying total amino acid contents and true amino acid digestibility coefficients. In the present study, 16 amino acids (Asp, Thr, Ser, Glu, Pro, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, and Arg) were selected to assess the relationship between these amino acid digestibilities and the sorghum chemical compositions.

Model Development

Two methods of MLR and ANN models were used to predict TDAA contents in 48 sorghum grain samples. The input variables for both MLR and ANN models were CP, CF, EE, ash, and total phenols. Each individual TDAA content was the output variable. To avoid any bias, the 48 data lines were randomly split into training and testing sets with 34 and 14 data lines, respectively. An algorithm of feed-forward multilayer perceptron with 5 inputs, 1 output (with a linear activation function), and 6 hidden neurons (with a hyperbolic tangent activation function) was considered to construct the ANN model. A training algorithm of Quasi-Newton was used to train the network (Lou and Nakai, 2001; Ahmadi and Golian, 2010). The ANN models were made using Statistica Neural Networks software (version 8.0; StatSoft, 2009). Evaluation of model performance was based on the accuracy of the prediction on the testing data.

Data from the training set (34 data lines) were also fitted for linear regression using SAS PROC REG (SAS Institute, 2003). Quantitative examination of the predictive ability of both MLR and ANN models was determined using R^2, MS error, and bias for each amino acid (Roush et al., 2006).

RESULTS AND DISCUSSION

Average, minimum, maximum, and SD values (n = 48) of chemical composition and TDAA content for all 48 sorghum samples are shown in Table 1. The results indicate a great variability in both the sorghum chemical composition and TDAA content obtained in sorghum grain. The difference between maximum and minimum values for TDAA content of sorghum samples suggests that the formulation of diets based on average nutritive value may not be the most accurate method.

The MLR and ANN models prediction efficiency, as R^2, MS error, and bias obtained for each amino acid and chosen ANN models architecture, is shown

Table 1. Maximum, minimum, average, and SD for chemical composition and true digestible amino acids content obtained by 48 sorghum grain samples[1]

	Chemical composition					True digestible amino acid content														
Item	Total phenol	CP	EE	CF	Ash	Asp	Thr	Ser	Glu	Pro	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg
Maximum	0.617	13.825	3.883	9.797	2.942	1.031	0.444	0.610	3.197	1.248	1.487	0.219	0.761	0.261	0.597	1.861	0.525	0.717	0.253	0.247	0.396
Minimum	0.039	10.898	2.489	2.117	1.457	0.361	0.143	0.206	1.020	0.178	0.489	0.087	0.283	0.054	0.223	0.326	0.093	0.158	0.064	0.098	0.119
Average	0.319	12.985	3.222	4.029	1.855	0.689	0.276	0.405	2.043	0.671	0.969	0.146	0.506	0.149	0.379	1.118	0.304	0.432	0.159	0.169	0.249
SD	0.219	1.294	0.409	2.498	0.460	0.244	0.101	0.150	0.808	0.348	0.346	0.051	0.174	0.058	0.132	0.521	0.158	0.198	0.064	0.052	0.103

[1]Chemical composition and true digestible amino acids content are expressed based on percentage of DM in the sorghum grain. CF = crude fiber; EE = ether extract.

Table 2. Statistic values derived from linear regression and artificial neural network models to estimate the true digestible amino acid content of sorghum grain based on ash, ether extract, crude fiber, CP, and total phenols[1]

	Amino acid															
Model and statistic[2]	Asp	Thr	Ser	Glu	Pro	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg
MLR training																
R^2	0.68	0.81	0.80	0.85	0.85	0.81	0.87	0.80	0.71	0.81	0.77	0.77	0.84	0.75	0.40	0.67
MS error	0.017	0.002	0.004	0.088	0.018	0.019	0.0	0.005	0.001	0.003	0.053	0.005	0.006	0.001	0.001	0.003
Bias	0.000	0.000	0.000	0.000	0.000	0.001	−0.001	0.000	0.000	0.000	0.000	0.000	−0.002	0.000	0.000	0.000
MLR testing																
R^2	0.93	0.97	0.95	0.96	0.94	0.94	0.92	0.87	0.72	0.86	0.91	0.95	0.95	0.78	0.51	0.84
MS error	0.115	0.020	0.034	0.784	0.163	0.269	0.004	0.083	0.023	0.075	0.562	0.031	0.046	0.012	0.026	0.031
Bias	−0.050	−0.032	−0.028	−0.128	−0.074	−0.034	−0.006	−0.001	−0.004	0.015	−0.044	0.002	−0.028	0.000	0.002	−0.002
ANN training																
R^2	0.99	0.97	0.97	0.99	0.98	0.98	0.93	0.98	0.91	0.97	0.99	0.99	0.99	0.91	0.85	0.95
MS error	0.001	0.000	0.001	0.006	0.002	0.002	0.000	0.001	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000
Bias	0.000	0.000	0.000	0.000	0.002	0.003	0.000	−0.001	0.000	0.000	0.000	0.000	0.000	0.000	−0.002	−0.001
ANN testing																
R^2	0.99	0.96	0.99	0.99	0.97	0.99	0.96	0.99	0.95	0.99	0.99	0.99	0.99	0.94	0.92	0.98
MS error	0.001	0.001	0.001	0.006	0.003	0.002	0.000	0.001	0.000	0.001	0.003	0.000	0.001	0.000	0.001	0.000
Bias	−0.020	−0.026	−0.024	−0.028	0.001	−0.023	−0.006	−0.009	−0.011	−0.009	−0.010	−0.004	−0.012	−0.004	−0.005	−0.010

[1]Type of network = 3 layers perception; training algorithm = Quasi-Newton; no. of hidden neurons = 6; type of activation function in hidden neurons = hyperbolic tangent.

[2]MLR = multiple linear regressions; ANN = artificial neural network.

in Table 2. The R^2 value corresponding to the ANN model showed a relatively higher prediction accuracy compared with the equation obtained by the regression method. The best R^2 value (Table 2) obtained by ANN was 0.99 for Asp, Ser, Glu, Ala, Val, Ile, Leu, Tyr, and Phe. In terms of MS error, for the training data sets, the calculated values using the ANN model for all the amino acids in this study showed lower residuals distribution than those obtained by the regression model. The calculated model error measurement obtained by the ANN method indicated that the testing set for all amino acids yielded similar MS error values and higher R^2 values when compared with the training set. Quantified values of bias for ANN models that show any under- or overestimation for the training and testing values for all amino acids were minor in both training and testing data sets. The prediction of TDAA for the training data set using the MLR method was not as accurate as that using the ANN model. However, relatively good agreement between TDAA and chemical composition in sorghum sample was observed in the ANN testing data set. The regression R^2 showed the relatively less accurate estimation for all amino acids. For the testing data sets, the R^2 values obtained with the ANN method ranged from 0.92 to 0.99 and, for 10 amino acids, was more than 0.98. The ANN model prediction for digestible Lys from proximate analysis was lower than that for other amino acids. Urriola et al. (2009) also found lower Lys values compared with other amino acids for the prediction of digestible Lys from total concentration of Lys in corn-based distillers dried grains with solubles. Roush and Cravener (1997) suggested that when using the ANN model it must be customized to each individual amino acid to improve predictive performance. In this study, default architectures were held constant for all individual ANN models during training for each amino acid. Therefore, it is possible to improve the accuracy of the individual ANN by changing the defaults and training parameters of each network.

The linear regression equations calculated on the training data set are shown in Table 3. For most amino acids, the best relationships between TDAA and chemical composition in sorghum grain appear to be described by a linear equation. However, a minor exception to this close relationship between TDAA and chemical composition for MLR was observed for Lys, Met, Arg, and His (Table 2). This inconsistency may derive from specific features of some sorghum samples. It appears that all our selected input variables (CP, CF, EE, ash, and total phenols) in all amino acid models had a strong effect on output prediction. The MLR and ANN models have been used to predict the amino acid content in feed ingredients based on proximate analysis (Roush and Cravener, 1997) because the amino acid contents of feedstuffs are related to the sample proximate analysis. Further work indicated that when linear regression and ANN were used to predict the amino acid profile of feed ingredients (Cravener and Roush, 1999), the ANN method was more accurate than linear regression to describe the complex relationship between nutrients and feed ingredients. Cravener and Roush (2001) successfully applied ANN models to predict amino acids profile for various feed ingredients. The main factors affecting the sorghum protein and amino acid digestibility may be categorized as exogenous and endogenous. Exogenous factors are attributable to interaction of sorghum proteins with nonprotein components such as polyphenols, nonstarch polysaccharides, and phytate contents. Endogenous factors involve changes within the sorghum proteins themselves that do not involve interactions of the proteins with nonprotein components. All these factors have been shown to influence sorghum protein digestibility (Duodu et al., 2003). Phenolic compounds in sorghum grain may be divided into 3 major parts: phenolic acids, flavonoids, and tannins (Hahn et al., 1984). Polyphenols and tannins negatively affect the nutritional value of sorghum, reducing protein and amino acid digestibility in poultry. Tannins are able to form complexes with proteins and

Table 3. Multiple linear regression equations for prediction of true digestible amino acid contents of sorghum grain samples[1]

Amino acid	Equation[1]
Lys	$Y = 0.6507 - 0.1887 \times$ total phenols $- 0.0260 \times$ CP $- 0.0480 \times$ EE $- 0.0254 \times$ CF $+ 0.0930 \times$ ash
Met	$Y = 0.3885 - 0.2454 \times$ total phenols $- 0.0109 \times$ CP $- 0.0336 \times$ EE $- 0.0158 \times$ CF $+ 0.0830 \times$ ash
Cys	$Y = 0.3672 - 0.2376 \times$ total phenols $- 0.0109 \times$ CP $- 0.0109 \times$ EE $- 0.0141 \times$ CF $+ 0.0485 \times$ ash
Thr	$Y = 0.2946 - 0.3405 \times$ total phenols $- 0.0053 \times$ CP $- 0.0049 \times$ EE $- 0.0239 \times$ CF $+ 0.0765 \times$ ash
Ile	$Y = 0.1091 - 0.4137 \times$ total phenols $+ 0.0355 \times$ CP $- 0.0807 \times$ EE $- 0.0187 \times$ CF $+ 0.1467 \times$ ash
Leu	$Y = 1.1330 - 1.8199 \times$ total phenols $+ 0.0417 \times$ CP $- 0.1350 \times$ EE $- 0.0987 \times$ CF $+ 0.4680 \times$ ash
Val	$Y = 0.4011 - 0.5478 \times$ total phenols $- 0.0265 \times$ CP $- 0.0916 \times$ EE $- 0.0473 \times$ CF $+ 0.2271 \times$ ash
Tyr	$Y = 0.7936 - 0.5868 \times$ total phenols $- 0.0204 \times$ CP $- 0.0548 \times$ EE $- 0.0573 \times$ CF $+ 0.1986 \times$ ash
Phe	$Y = 0.7067 - 0.6973 \times$ total phenols $+ 0.0045 \times$ CP $- 0.0723 \times$ EE $- 0.0582 \times$ CF $+ 0.1970 \times$ ash
His	$Y = 0.1274 - 0.2617 \times$ total phenols $- 0.0162 \times$ CP $- 0.0644 \times$ EE $- 0.0033 \times$ CF $+ 0.0675 \times$ ash
Arg	$Y = 0.4551 - 0.3602 \times$ total phenols $+ 0.0022 \times$ CP $- 0.0692 \times$ EE $- 0.0273 \times$ CF $+ 0.1155 \times$ ash
Asp	$Y = 1.2128 - 0.8795 \times$ total phenols $- 0.0068 \times$ CP $- 0.1147 \times$ EE $- 0.0666 \times$ CF $+ 0.2685 \times$ ash
Ser	$Y = 0.3850 - 0.5211 \times$ total phenols $+ 0.0162 \times$ CP $- 0.0267 \times$ EE $- 0.0242 \times$ CF $+ 0.0903 \times$ ash
Glu	$Y = 3.3884 - 3.0571 \times$ total phenols $- 0.0268 \times$ CP $- 0.01881 \times$ EE $- 0.2314 \times$ CF $+ 0.8378 \times$ ash
Pro	$Y = 1.5805 - 1.3430 \times$ total phenols $- 0.0061 \times$ CP $- 0.1836 \times$ EE $- 0.1122 \times$ CF $+ 0.3582 \times$ ash
Ala	$Y = 0.4184 - 0.9793 \times$ total phenols $+ 0.0605 \times$ CP $- 0.0556 \times$ EE $- 0.0733 \times$ CF $+ 0.3030 \times$ ash

[1]Chemical composition is expressed based on percentage of DM in sorghum grain. Y = the true digestible amino acid content based on percentage of DM in sorghum grain; CF = crude fiber; EE = ether extract.

decrease availability for enzymatic digestion (Selle et al., 2010). However, some sorghum varieties are tannin free; it seems that low protein digestibility still exists for low tannin or tannin-free cultivars. It may be attributable to cell wall components, which are the other factors that affect amino acid digestibility by reducing the accessibility to enzymes or the formation of indigestible complexes. Bach Knudsen and Munck (1985) reported that in sorghum a large amount of protein is associated with total dietary fiber or, more specifically, cell wall components. Although many other factors may be responsible for the changes in digestible amino acid of sorghum for poultry, our results indicate that the TDAA content was well correlated with the CP, CF, EE, ash, and total phenols contents of the sorghum grain and may be predicted with MLR and ANN models.

In conclusion, the chemical composition and some antinutritional factors such as polyphenols are probably the main factors affecting TDAA values of sorghum grain for poultry. The ANN and MLR models appear to be promising tools for describing the relationship between the sorghum grain chemical composition and TDAA content. The ANN model may be able to enhance our ability to accurately predict digestible amino acid contents of feedstuffs in order to achieve optimal balance in poultry diets.

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