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Broiler Diets Formulated Based on Digestible Amino Acid Values as Determined by *in vivo* and Prediction Methods

Research Article

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ABSTRACT

The aim of the present study was to assess whether near infrared reflectance spectroscopy (NIRS) and regression equations are the practical and accurate approach of nutritional assessment of common feedstuffs. Therefore two experiments were conducted to study the effect of amino acid determination methods on broiler performance. In experiment I, two hundred thirty four male Ross broiler chicks were fed a commercial starter diet from d 1 to 16 of age followed by the test diets from d 17 to 21 to determine the digestible amino acids content of wheat, corn and soybean meal. The 3 test diets consisted of corn, wheat or soybean meal was used as the sole sources of protein. On d 21, birds were killed and ileal digesta were collected for determining standardized ileal amino acid digestibility (SID). The *in vivo* values of SID were compared with values predicted by NIRS and regression methods. Experiment II was conducted to compare the effect of feed formulation based on SID determined by *in vivo* with regression and NIRS prediction methods on broiler performance. The results showed that the SID contents of wheat and corn predicted by NIRS and regression method were close to *in vivo* values. Values predicted by NIRS and regression for soybean meal varied when compared with those of *in vivo* assay. Although, these differences were not appeared when comparing performance of broiler fed diets formulated based on SID values estimated through *in vivo* trial, NIRS and regression methods. This study reveals that feed formulation based on digestible amino acids estimated by NIRS and regression methods are reliable for feed formulation in commercial poultry production.

KEY WORDS near infrared reflectance spectroscopy, performance, regression, standardized ileal.

INTRODUCTION

Poultry diets have generally been formulated based on a crude protein or total amino acid (AA). More recently, it has been suggested that diets formulation based on digestible AA can be more advantageous and decrease feed cost and nitrogen excretion into environment and also improve poultry performance (Applegate *et al.* 2008; Rostagno *et al.* 1995). Amino acids of feed ingredients vary considerably not only among different feedstuff but also the same ingredients of different batches which may be due to several factors such as genotype, crushing and anti nutritional factors (De Coca Sinova *et al.* 2008; Sedghi *et al.* 2012). So determination of digestible AA content of feed ingredient is necessary to accurately formulate poultry diets. Different approaches have been developed to determine AA digestibility of feed ingredient like animal assay (Sibbald, 1987; Rostagno *et al.* 1995; Dari *et al.* 2005) and prediction methods (Ebadi *et al.* 2011; Sedghi *et al.* 2012). A question often posed by commercial nutritionists is that what digestible AA determination method is more appropriate in feed formulation for poultry production. It should be appreciated if nutritionists can analyze poultry feed materials for digestible values via *in vivo* assay; however, determination of digestible AA prior to feed formulation via animal assay is limited due to time and cost.

Prediction methods, such as regression or near infrared reflectance spectroscopy (NIRS), are advantageous for their simplicity and rapidity turnaround time (Harrison *et al.* 1991; NRC, 1994; Fontaine *et al.* 2001; Fontaine *et al.* 2002; Ebadi *et al.* 2011; Liu *et al.* 2012). 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 *et al.* 2009). Cravener and Roush, (2001) had been used liner regression and artificial neural network to predict the AA profiles of feed ingredients, given proximate analysis.

Also Ebadi *et al.* 2011 and Sedghi *et al.* (2012) successfully applied regression method to predict amino acid digestibility of sorghum. One of the fastest and easiest methods uses regression equations based on moisture and crude protein (CP).

Generally CP is used because amino acid content is closely and positively correlated with protein level. The relationship between CP and amino acid content works quite well for prediction of unknown samples in most ingredients. It is suggested that in practical feed formulation, the amino acid content can be estimated based on the dry matter and crude protein content by regression equations (Amino Dat, 2010). The Amino NIR is another service which has been used for the fast determination of amino acids in raw materials. These methods are generally reproducible and require no animal, which is favored by many institutions and nutritionists.

The published results show that the NIRS method can be used for the determination of amino acids in raw materials, with acceptable results (Harrison *et al.* 1991; Fontaine *et al.* 2001; Fontaine *et al.* 2002; Liu *et al.* 2012). Koch (2002) compared the Amino NIRS results with wet chemical analysis for wheat, soybean and meat meals for lysine and methionine.

They demonstrated that Amino NIRS provides accurate and reliable estimation of AA contents in raw materials with different origins. More recently, Owens *et al.* (2009) showed similar results with wheat samples.

They suggested that the NIRS is a useful, rapid and accurate tool for determination of wheat nutritive values and could be extremely beneficial to be used in poultry nutrition. Although the applicability of NIRS for feed quality was described in many published studies, however the applicability of these methods for digestible AA estimation before feed formulation is unknown for some nutritionists.

Therefore the objective of this study was to compare standardized ileal digestible AA values which obtained by chick assay, NIRS and regression methods. In addition the performance of broilers was compared when feed formulation was based on SID values obtained by chick assay, NIRS and regression methods.

MATERIALS AND METHODS

Digestibility trial

An experiment was conducted to determine the standardized ileal AA digestibility (SID) of corn, wheat and soybean meal. Two hundred thirty four day-old male broiler chicks with 41 ± 2 g body weight (Ross 308) were placed in 18 units of battery brooders. A corn-soybean meal broiler diet, adequate in all nutrients was fed from 1 to 16 d of age. Chicks were randomly assigned to 3 dietary treatments with 6 replicate cage containing 13 chicks each. Three experimental diets were formulated to contain corn, wheat or soybean meal as the sole source of protein. Chromic oxide was added at 0.3% of the diets as an indigestible marker. Diets were prepared in mash form and were fed *ad libitum* to broiler chicks from 17 to 21 d of age. Composition of the assay diets are shown in Table 1. Ileal digesta samples were collected after euthanizing the birds at 21 d of age. The digesta samples from the ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileo-cecal junction) were collected by gently squeezing the contents of the ileum into sample bags.

Table 1 Composition of the diets used in the amino acid digestibility assay

Ingredient (%)	Corn	Wheat	Soybean meal
Tested ingredient	93.28	93.28	48.00
Sucrose	0.00	0.00	45.63
Vegetable oil	2.00	2.00	2.00
Limestone	1.47	1.47	1.22
DCP	1.95	1.95	1.85
Salt	0.50	0.50	0.50
Vitamin mix[1]	0.25	0.25	0.25
Mineral mix[2]	0.25	0.25	0.25
Cr_2O_3[3]	0.30	0.30	0.30
Calculated composition			
TMEn (kcal/kg)	3300	3100	2827
CP	7.5	12.5	23

[1] Vitamin mix provided the following (per kg of diet): vitamin A: 4500 IU; vitamin D: 2250 IU; vitamin E: 50 IU; vitamin B3: 0.02 mg; Thiamin HCl: 15 mg; Riboflavin 15 mg; Nicotinic acid: 50 mg; Folic acid: 6 mg; Pyridoxine: 6 mg; Biotin: 0.6 mg; Choline Cl: 2500 mg; Di-calcium pantothenate: 20 mg; Menadione sodium bisulfite: 1.5 mg; Butylatedhydroxytoluene: 100 mg and Glucose to make 12 g.

[2] Mineral premix provides per kilogram of diet: Mn ($MnSO_4 \cdot H_2O$): 65 mg; Zn (ZnO): 55 mg; Fe ($FeSO_4 \cdot 7H_2O$): 50 mg; Cu ($CuSO_4 \cdot 5H_2O$): 8 mg; I [$Ca(IO_3)_2 \cdot H_2O$]: 1.8 mg; Se: 0.30 mg; Co (Co_2O_3): 0.20 mg and Mo: 0.16 mg.

[3] Cr_2O_3: chromic oxide.

DCP: di-calcium phosphate; TMEn: true metabolizable energy corrected for nitrogen and CP: crude protein.

Digesta from birds within a cage were pooled into one bag and frozen (-20 ˚C). Immediately after collection the feed and digesta samples were analyzed for AAs content by

Evonic industries (Hanau-Wolfgang, Germany) using ion-exchange chromatography with post-column derivatization with ninhydrin (Llames and Fontaine 1994).

Sulfur AA (Met and Cys) were first oxidized with performic acid and then neutralized with sodium metabisulfite (Commission Directive, 1998).

AA concentrations were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Chromium concentrations were determined by atomic absorption spectroscopy (Williams *et al*. 1962).

Near infrared reflectance spectroscopy and regression method

The NIRS, a long established method for crude nutrient analysis has been extended to total AA content. The functioning of NIRS and the main principles has been discussed by a number of authors (Harrison *et al*. 1991). The advantages of this method are speed, high accuracy and low analytical costs (Harrison *et al*. 1991). The NIRS generally calibrated for total AA prediction. The predicted AAs have been used for digestible AAs determination using constant digestibility coefficient. We used amino NIRS to predict AA content of wheat, corn and soybean samples. Amino NIRS is a service for the fast analysis of AAs in feed raw materials that is offered by Evonic. The SID content of tested feed ingredient obtained from NIRS AA data × constant SID coefficient.

The digestibility coefficient of soybean meal was corrected based on heat damage value (Koch, 2002). In the regression method, ileal digestible AAs were predicted based on DM and CP contents of each ingredient using Amino Dat 4.0.

Performance trial

Ninety day-old male chickens with 41 ± 2 g body weight were obtained from a commercial hatchery, weighed on arrival and randomly assigned to 18 cages of 5 birds each. The nutrient compositions of the three tested diets were formulated based on Ross 308 recommendations (Aviagen, 2007). Digestible AAs of raw material which were calculated based on *in vivo* assay NIRS and regression methods were used to formulate diets. The ingredient composition of the three diets and their calculated chemical compositions are given in Table 2. Feed intake and weight gain were recorded at the end of the experiment (42 day of age) and feed conversion ratio (FCR) was calculated after correction for mortality.

Data analysis

Cage means were the experimental unit. Data were subjected to the analysis of variance as a completely randomize design of treatments using the general linear models procedure of SAS (SAS, 2004). The model used, was:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where:
μ: common mean.
A: effect of treatment.
e_{ij}: random error.

Tukey's test was applied to compare the treatment means when the treatment effect was significant at $P < 0.05$.

RESULTS AND DISCUSSION

The standardized ileal digestible AA content of wheat, corn and soybean meal determined by *in vivo* assay NIRS and regression methods are shown in Table 3. Digestible AA values which predicted by NIRS and regression methods were similar to *in vivo* assay values for wheat and corn samples; Whereas digestible AA predicted by these two latter methods for soybean meal sample varied when compared with *in vivo* values, especially for phenylalanine (phe), arginine (Arg), valine (Val), isoleucine (Ile) and Leucine (Leu).

Also the comparison between predicted methods (NIRS and regression) and *in vivo* assay showed that these two methods generally over estimated the SID values of wheat, corn and soybean meal.

Broiler performance

The efficiency of the broiler chickens performance fed with diets formulated based on different digestible AAs determination methods (*in vivo* and prediction) is shown in Table 4. The results indicated that determination of digestible AA via different methods did not have a significant effect on body weight gain, feed intake and feed conversion ratio. Body weight gain in birds fed diets formulated base on data obtained by regression method numerically tended to be lower than NIRS and chick assay data. These results thus suggest that NIRS and regression method are acceptable tools which can be used for the rapid prediction of the digestible AA values of tested feedstuffs. The ileal digestibility of AA determination of feedstuffs is too costly and time-consuming. Recently researchers tried to find simple and rapid precise methods for feed quality control that can be replaced with *in vivo* assay (Fontain *et al*. 2002; Sedghi *et al*. 2011; Liu *et al*. 2012; Soleimani Roudi *et al*. 2012). The NIRS is a method that can be used for digestible AA determination of feedstuffs. So we compared standardized ileal digestible AA values which obtained by chick assay, NIRS and regression methods.

Table 2 Standardized digestible amino acid content of wheat, corn and soybean meal obtained by regression, NIRS and amino acid digestibility assay

Ingredient	Regression method			NIRS			Amino acid digestibility assay		
	Wheat	Soy	Corn	Wheat	Soy	Corn	Wheat	Soy	Corn
Methionine	0.179	0.542	0.158	0.179	0.543	0.157	0.178	0.526	0.154
Cystine	0.259	0.526	0.164	0.259	0.525	0.163	0.253	0.487	0.162
Met + Cys	0.441	1.069	0.320	0.442	1.069	0.321	0.431	1.020	0.317
Lysine	0.298	2.336	0.234	0.298	2.337	0.233	0.287	2.293	0.208
Threonine	0.310	1.451	0.266	0.318	1.451	0.266	0.296	1.412	0.260
Arginine	0.519	2.851	0.360	0.520	2.852	0.360	0.516	2.770	0.333
Isoleucine	0.415	1.711	0.288	0.415	1.711	0.287	0.395	1.631	0.275
Leucine	0.787	2.897	1.015	0.787	2.897	1.015	0.775	2.781	1.000
Valine	0.493	1.802	0.374	0.493	1.802	0.373	0.470	1.730	0.354
Histidine	0.275	1.061	0.232	0.275	1.060	0.232	0.275	1.015	0.221
Phenylalanine	0.553	1.905	0.407	0.553	1.906	0.407	0.546	1.824	0.381

NIRS: near infrared reflectance spectroscopy.

Table 3 Experimental diets formulated based on digestible values obtained by digestibility assay (SID), NIRS and regression methods

Ingredient	Starter			Grower			Finisher		
	SID	NIRS	Reg	SID	NIRS	Reg	SID	NIRS	Reg
Corn	50.16	50.40	50.38	55.84	55.86	55.85	55.79	55.93	55.92
SBM	30.96	30.94	30.94	26.61	26.61	26.61	21.52	21.51	21.51
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	15.00	15.00	15.00
DCP	2.57	2.57	2.57	2.26	2.26	2.26	2.09	2.08	2.08
Oyster shell	1.10	1.10	1.10	1.03	1.03	1.03	0.89	0.89	0.89
Soy oil	1.00	1.00	1.00	1.93	1.94	1.94	2.41	2.43	2.43
Vit[1]-Min[2] mix	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
L-lysine	0.55	0.51	0.51	0.41	0.41	0.41	0.41	0.38	0.38
DL-methionine	0.42	0.40	0.42	0.33	0.33	0.35	0.30	0.28	0.30
L-threonine	0.24	0.22	0.22	0.17	0.17	0.17	0.16	0.14	0.15
L-arginine	0.23	0.19	0.19	0.13	0.13	0.13	0.16	0.13	0.13
L-valine	0.19	0.16	0.16	0.10	0.10	0.10	0.11	0.08	0.08
L-isoleucine	0.17	0.13	0.13	0.12	0.09	0.09	0.11	0.08	0.08
$NaHCO_3$	0.33	0.31	0.31	0.26	0.26	0.26	0.27	0.25	0.25
NaCl	0.15	0.16	0.16	0.19	0.19	0.19	0.19	0.20	0.20
Sand	1.34	1.30	1.30	0.00	0.00	0.00	0.00	0.00	0.00
Calculated analysis									
ME kcal/kg	3025	3025	3025	3150	3150	3150	3200	3200	3200
CP %	20.22	20.04	20.05	18.26	18.24	18.25	16.81	16.66	16.66
Methionine	0.68	0.67	0.69	0.58	0.58	0.60	0.53	0.52	0.53
Cystine	0.68	0.67	0.69	0.59	0.59	0.61	0.53	0.53	0.54
Met + Cys	0.94	0.94	0.94	0.84	0.84	0.84	0.76	0.76	0.76
Lysine	1.27	1.27	1.27	1.10	1.10	1.10	0.97	0.97	0.97
Threonine	0.83	0.83	0.83	0.73	0.73	0.73	0.65	0.65	0.65
Tryptophan	0.20	0.20	0.20	0.18	0.18	0.18	0.16	0.16	0.16
Arginine	1.31	1.31	1.31	1.14	1.14	1.14	1.02	1.02	1.02
Isoleucine	0.85	0.85	0.85	0.78	0.75	0.75	0.67	0.67	0.67
Valine	0.95	0.95	0.95	0.84	0.84	0.84	0.75	0.75	0.75
Leucine	1.44	1.48	1.48	1.41	1.41	1.41	1.27	1.31	1.31
Histidine	0.45	0.47	0.47	0.44	0.44	0.44	0.38	0.40	0.40
Phenylalanine	0.81	0.85	0.85	0.79	0.79	0.79	0.69	0.72	0.72

[1] Vitamin mix provided the following (per kg of diet): vitamin A: 4500 IU; vitamin D: 2250 IU; vitamin E: 50 IU; vitamin B_3: 0.02 mg; Thiamin HCl: 15 mg; Riboflavin:15 mg; Nicotinic acid: 50 mg; Folic acid: 6 mg; Pyridoxine: 6 mg; Biotin: 0.6 mg; Choline Cl: 2500 mg; Di-calcium pantothenate: 20 mg; Menadione sodium bisulfite: 1.5 mg; butylatedhydroxytoluene: 100 mg and Glucose to make 12 g.

[2] Mineral premix provides per kilogram of diet: Mn ($MnSO_4.H_2O$): 65 mg; Zn (ZnO): 55 mg; Fe ($FeSO_4.7H_2O$): 50 mg; Cu ($CuSO_4.5H_2O$): 8 mg; I [Ca $(IO_3)_2.H_2O$]: 1.8 mg; Se: 0.30 mg; Co (Co_2O_3): 0.20 mg and Mo: 0.16 mg.

SID: standardized ileal amino acid digestibility; NIRS: near infrared reflectance spectroscopy; Reg: regression analysis; SBM: soybean meal (44% CP); DCP: di-calcium phosphate; ME: metabolizable energy and CP: crude protein.

The result showed that both NIRS and regression methods over predict the digestible amino acid content of feedstuffs. The sound theoretical definition for feed quality can be observe in animal performance; so the performance of broilers was compared when feed formulation was based on SID values obtained by chick assay, NIRS and regression methods. As shown in table 4 the performance of broiler chicken fed diet formulated with digestible AA obtained by

Table 4 Performance of male broilers (1-42 days) fed diets based on SID, NIRS and regression methods

Item	BWG (g)	FI (g)	FCR
Formulated based on *in vivo* SID	2430[a]	4296[a]	1.768[a]
Formulated based on NIRS	2411[a]	4278[a]	1.774[a]
Formulated based on regression method	2406[a]	4302[a]	1.788[a]
P-value	0.082	0.278	0.107

BWG: body weight gain; FI: feed intake and FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

NIRS method was close with chick assay method. However the groups fed with diets formulated with predicted by regression method data, numerically showed lower body weight gain (P=0.082).

Similar to our results, Liu *et al.* (2012) reported the performance of male broilers fed different soybean meal based on book values and the NIRS specifications (metabolizable energy and digestible AAs). Their finding showed that when diets formulated based on the NIRS nutrient values, broilers received more balanced ration, thus will have a good performance than those fed diets formulated based on book values. The application of NIRS to control feed quality has also been reported in several studies; Van Kempen and Simmins (1997) reported that the NIRS technology is a precise and economically sounds good for quality control feed formulation. Van Kempen and Bodin (1998) compared the NIRS ability to estimate the digestibility of AA in feedstuffs with the nitrogen-based regression method. They showed that NIRS was more accurate than nitrogen-based regression technique for evaluation of AAs in feedstuffs. Furthermore, Fontaine *et al.* (2002) plotted the individual data for some AA content of corn and wheat samples to validate the AAs estimated NIRS with the reference analysis (wet chemical analysis results). They concluded that the developed NIRS calibrations enable a fast and accurate prediction of essential AAs in wheat and corn. They also compared the NIRS method for AA prediction with regression method and found that, the NIRS can predict AAs contents especially for lysine and methionine in those cereals much better than crude protein regression method. Bodin and Aubret (2005) concluded that the NIRS calibrations can be used to predict metabolizable energy or digestible AAs of feed ingredients. They also demonstrated that the NIRS can be easily used to evaluate feedstuffs and allow the feed producers to reduce costs and consequently improve poultry performance. Similar to our result, all mentioned studies indicated the advantages of diet formulating based on digestible AAs prediction with NIRS. While unlike other studies we showed that the regression method may also be used to estimate the digestible AAs in feedstuffs. Due to variation in quality and some anti-nutritional factors in feedstuffs measurements of digestible AAs, using regression methods may vary between different samples. In the other word, the closeness of regression values with this obtained from *in vivo* may be due to that selected samples which accidentally closed to the mean; and regression method may predict digestible AAs with lower accuracy for samples with higher deviation from the mean of digestible AA values. Therefore the accuracy of regression method is different in various studies. Use of NIRS for digestibility measurement of AAs may solve this problem partly.

CONCLUSION

The objective of this paper was to compare the effect of feed formulation based on digestible AA values obtained by SID chick assay, NIRS and regression equation on poultry performance. The findings indicated that the values obtained NIRS can be used for feed formulation based on digestible AA value. Also the use of regression analysis to find the digestible AA contents may be used in commercial broilers production.

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