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Energetic values of feedstuffs for broilers determined with *in vivo* assays and prediction equations

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ABSTRACT

Two experiments were conducted to test the suitability of a set of prediction equations to predict the nitrogen-corrected apparent metabolizable energy (AMEn) of protein and energetic ingredient concentrates used by the poultry feed industry. Nine protein concentrates and nine energetic concentrates were evaluated in six replicates each via substitution for 300 and 400 g/kg of the basal diet, respectively. These values were compared to the AMEn estimated via equations that utilized data on the chemistry composition of the feedstuffs. All the equations were efficient in estimating the AMEn values of the tested feedstuffs. We concluded that the prediction equations studied can be utilized to estimate the AMEn of protein and energetic concentrate ingredients used by the poultry feed industry. The equation AMEn = 4101.33 + 5.628EE−23.297ASH−2.486aNDFom + 1.042ADFom (R^2 = 0.84; RSD = 0.4137; P-value<0.0001; n = 574) was most applicable in the prediction of energetic values of evaluated feedstuffs.

1. Introduction

The production of poultry meat throughout the world is growing significantly because of the increased world-wide demand for food. Brazil is the third largest producer in this market, contributing around 11.3 million tons of meat and exporting more than 3.6 million tons each year (USDA, 2009). However, to adequately meet the nutrient requirements of animals, it is necessary to elaborate diets that improve the nutrient utilization and the bird's performance and decrease the pollutant power of this activity.

Dietary energy level is the main factor influencing feed intake. Therefore, dietary nutrients (protein, amino acids, vitamins and minerals) should vary depending on the energy content of the diet. The model most frequently used to express the energetic values of feedstuffs for broilers is the metabolizable energy (ME) model. Nevertheless, metabolic bioassays are necessary to determine the ME of these ingredients, but these are onerous and require time. Thus, tables are commonly used to obtain the energetic values of ingredients used in diets. However, several factors can affect table values, including the

Abbreviations: ADFom, acid detergent fiber exclusive of residual ash; AOAC, Association of Official Analytical Chemists; AME, apparent metabolizable energy; AMEn, nitrogen-corrected AME; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; GE, gross energy; aNDFom, neutral detergent fiber assayed with heat stable amylase exclusive of residual ash; R^2, coefficient of determination of regression.

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Table 1
Chemistry and energetic composition of protein concentrate feedstuffs (g/kg, DM).[a,b]

Feedstuff	Composition							
	DM	GE (MJ/kg)	CP	EE	CF	aNDFom	ADFom	Ash
Soybean meal 1	876.3	18.15	471.6	13.7	75.8	164.6	97.4	66.1
Soybean meal 2	882.0	18.42	493.2	19.6	51.6	153.6	88.0	66.8
Soybean meal 3	891.4	18.17	474.3	19.5	50.2	149.5	92.0	64.7
Soybean meal 4	887.0	18.08	479.4	19.8	56.6	149.1	94.3	61.9
Semi-integral soybean meal	907.6	19.31	426.2	102.4	89.7	156.4	101.1	57.4
Full-fat extruded soybean	910.7	21.91	359.6	212.5	72.0	157.7	102.8	54.5
Texturized soybean protein	930.6	18.57	533.1	7.5	11.7	41.5	24.4	57.6
Integral micronized soy	939.2	23.06	398.5	258.5	13.8	198.7	55.6	53.5
Maize gluten meal	899.3	22.51	687.0	34.1	13.3	64.8	105.7	16.2
Average	902.7	19.80	480.3	76.4	48.3	137.3	84.6	55.4
Standard deviation	21.5	2.07	93.5	95.2	29.3	50.4	27.0	15.5
Minimum	876.3	18.08	359.6	7.5	11.7	41.5	24.4	16.2
Maximum	939.2	23.06	687	258.5	89.7	198.7	105.7	66.8

[a] Analysis made in the Animal Nutrition Laboratory of the Animal Science Department of UFLA.
[b] Dry matter (DM), gross energy (GE), crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (aNDFom), acid detergent fiber (ADFom).

chemistry composition of the feedstuffs (Zhou et al., 2010), the broiler age (Wiseman, 2006) and the methodology utilized to determine the energetic value (Losada et al., 2010).

Several researchers (Rodrigues et al., 2001; Zhao et al., 2008; Nascimento et al., 2009) have obtained prediction equations to estimate the ME using the chemistry composition of the feedstuffs; however, their results have been inconsistent or applicable only to one feedstuff group. Thus, it is necessary to combine information derived from collected data in different conditions to obtain results that are more consistent.

Recently, the use of meta-analyses to obtain prediction equations has shown promising results. A meta-analysis combines the results of several studies that address a set of related research hypotheses, increasing the statistical power of the conclusion (Fagard et al., 1996). Based on this technique, equations to predict the nitrogen-corrected metabolizable energy (AMEn) were developed by Nascimento (2007) and Nascimento et al. (2009) utilizing information gathered by numerous experiments and circumstances involving energetic and protein feedstuffs for broilers.

The objective of this work was to evaluate the proposed equations by Nascimento (2007) and Nascimento et al. (2009) and to estimate the AMEn values of the protein and energetic concentrate feedstuffs used by the poultry feed industry.

2. Materials and methods

2.1. Ingredients

A total of nine protein concentrate ingredients and nine energetic concentrate ingredients were simultaneously obtained from a commercial establishment, considering the availability in Brazil and the chemical variation among the feedstuffs. The protein concentrate ingredients were four samples of commercial trademark soybean meal and one sample each of semi-integral soybean meal, full-fat extruded soybean, texturized soybean protein, integral micronized soy and maize gluten meal. The energetic concentrate ingredients were as follows: samples of two different maize hybrids, two samples of sorghum, and one sample each of broken rice, integral rice meal, pre-gelatinized maize, wheat meal and broken maize.

Feedstuffs samples were analyzed immediately upon collection and the chemical composition is shown in Tables 1 and 2.

2.2. Experimental procedures

The AME values of the feedstuffs were determined *in vivo* using the substitution method. The experiments were approved by the Ethics Committee of the Federal University of Lavras.

Two bioassays were conducted, one with protein concentrate ingredients and another with energetic concentrate ingredients. Experimental diets were manufactured by substituting the protein concentrate ingredients studied for 300 g/kg of a basal diet or the energetic concentrate ingredients for 400 g/kg of the same diet. In both bioassays, each one of the nine dietary treatments was offered to six cages of five male chicks (Cobb 500) that were maintained in metabolic cages from days 15 to 25 post-hatch. Broilers were kept in an environmentally controlled room at a temperature of 24 °C under constant 24-h incandescent lighting, with free access to feed and water.

The basal diet was a maize and soybean meal containing 200 g/kg of crude protein. The estimative of energetic value and digestibility of nutrients of basal diet was 12.52 MJ/kg of ME, 11.3 g/kg of digestible lysine, 82.0 g/kg of digestible methionine plus cysteine, 88.0 g/kg of calcium and 44.0 g/kg of available phosphorus, according to Rostagno et al. (2005).

Total excreta output and feed intake were determined from 23 to 25 days post-hatching (Rodrigues et al., 2005). Daily excreta collections were then pooled within a cage and weighed. Representative excreta samples were retained and frozen.

Table 2
Chemistry and energetic composition of energetic concentrate feedstuffs (g/kg, DM).[a,b]

Feedstuff	Composition							
	DM	GE (MJ/kg)	CP	EE	CF	aNDFom	ADFom	Ash
Maize 1	875.3	17.70	95.4	41.1	17.3	149.1	58.0	12.1
Maize 2	862.2	19.53	103.7	44.0	13.5	129.4	71.9	14.0
Sorghum 1	867.2	17.62	113.5	33.2	27.7	156.5	64.2	17.7
Sorghum 2	878.3	17.50	101.8	33.4	28.0	141.0	61.1	18.2
Broken rice	859.2	18.62	97.0	9.7	3.8	35.3	50.7	10.7
Integral rice meal	885.3	19.50	141.2	197.4	88.9	213.5	161.2	104.3
Pre-gelatinized maize	879.1	17.81	91.5	20.0	21.1	117.5	12.8	11.7
Wheat meal	865.7	19.27	185.2	49.0	94.6	469.7	130.0	59.4
Broken maize	856.8	17.89	94.1	48.8	30.3	129.5	60.5	15.6
Average	869.9	18.38	113.7	53.0	36.1	171.3	74.5	29.3
Standard deviation	9.9	0.85	30.8	55.7	32.6	121.1	44.3	31.9
Minimum	856.8	17.50	91.5	9.7	3.8	35.3	12.8	10.7
Maximum	885.3	19.53	185.2	197.4	94.6	469.7	161.2	104.3

[a] Analysis made in the Animal Nutrition Laboratory of the Animal Science Department of UFLA.
[b] Dry matter (DM), gross energy (GE), crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (aNDFom), acid detergent fiber (ADFom).

The total droppings were dried in a forced air oven (65 °C) to a constant weight. After drying, the excreta samples were ground (in a hammer mill, 1.0 mm screen) and stored at 4 °C prior to chemical analysis.

2.3. Chemical analyses

All analyses were carried out in duplicate. Ingredients were analyzed for dry matter (DM) by oven-drying the sample (method 934.01), ash by muffle furnace incineration (method 942.05), crude protein (CP) by the Kjeldahl method (method 954.01), ether extract (EE) without acids hydrolysis (method 920.39), acid detergent fiber (ADFom) (index no. 973.18) and crude fiber (CF) (method 962.09) according to the AOAC (1995). aNDFom content was analyzed following the method of Van Soest et al. (1991), with samples treated with α-amylase before aNDFom extraction. Gross energy (GE) was determined in a bomb calorimeter (model 1261, Parr Instrument Company, Moline, IL, USA).

The excreta were analyzed for DM, CP and GE.

2.4. AMEn determination

The AME values of the diets were calculated using the following formula with appropriate corrections made for differences in dry matter (DM) content:

$$\text{AME} = \frac{(\text{feed intake} \times \text{GE}_{\text{diet}}) - (\text{excreta output} \times \text{GE}_{\text{excreta}})}{\text{feed intake}}$$

Nitrogen-corrected AME (AMEn) was calculated by correction to zero nitrogen retention according to Hill and Anderson (1958). The AMEn of each feedstuff, determined by *in vivo* bioassay, was calculated using the equation proposed by Matterson et al. (1965):

$$\text{AMEn of feedstuff} = \text{AMEn bd} + \frac{\text{AMEn td} - \text{AMEn bd}}{\text{inclusion level of test ingredient on basal diet (g/kg)}/1000}$$

where AMEn td is the AMEn of the tested diet and AMEn bd is the AMEn of the basal diet.

In parallel, the AMEn of the feedstuffs (kcal/kg DM) was determined by prediction equation based on the chemical compositions (g/kg DM) and converted to MJ/kg by multiplying by a factor of 0.004187. Equations were proposed by Nascimento (2007) and Nascimento et al. (2009), utilizing the meta-analysis principle:

(i) *Specific equation for protein concentrate feedstuffs* (Nascimento, 2007):
AMEn = 2707.71 + 5.863EE − 1.606aNDFom.
(R^2 = 0.81; RSD = 0.4847; P-value<0.0001; n = 199).

(ii) *Specific equations for energetic concentrate feedstuffs* (Nascimento, 2007):
AMEn = 4371.18 − 2.648CP + 3.065EE − 12.693ASH − 5.226CF − 2.514aNDFom + 2.440ADFom.
(R^2 = 0.81; RSD = 0.4689; P-value<0.0001; n = 375).
AMEn = 4205.23 + 3.058EE − 13.035ASH − 5.829CF − 2.831aNDFom + 1.671ADFom.
(R^2 = 0.81; RSD = 0.4771; P-value<0.0001; n = 375).

(iii) *General equations for energetic and protein concentrate feedstuffs* (Nascimento et al., 2009):

Table 3
Nitrogen-corrected apparent metabolizable energy (MJ/kg DM) of protein concentrate feedstuffs obtained by metabolism assays ($n = 6$) with broilers or by prediction equations.

Feedstuff	Observed average value	Standard deviation	Lower limit	Higher limit	Calculated by (i)	Calculated by (ii)	Calculated by (iii)
Soybean meal 1	9.74	0.56	9.29	10.18	10.57	9.76	9.71
Soybean meal 2	9.86	0.69	9.30	10.42	10.78	9.90	9.88
Soybean meal 3	10.03	0.47	9.66	10.40	10.81	10.17	10.12
Soybean meal 4	10.37	0.39	10.06	10.69	10.82	10.46	10.39
Texturized soybean protein	11.76	0.51	11.35	12.17	11.24	11.40	11.51
Semi-integral soybean meal	13.23	0.66	12.69	13.76	12.80	12.80	12.71
Integral micronized soy	15.79	0.66	15.27	16.32	16.35	16.22	16.40
Full-fat extruded soybean	15.82	0.46	15.46	16.19	15.49	15.67	15.60
Maize gluten meal	16.47	0.91	15.74	17.20	11.74	16.18	15.83
Average	12.56		–	–	12.29	12.51	12.46
Estimative-standard error between observed and calculated values	–		–	–	1.79	0.28	0.39

(i) Specific equation for protein feedstuffs: AMEn = 2707.71 + 5.863EE − 1.606aNDFom (Nascimento, 2007) (values in g/kg of DM).
(ii) General equation for energetic and protein feedstuffs 1: AMEn = 4101.33 + 5.628EE − 23.297ASH − 2.486aNDFom + 1.042ADFom (Nascimento et al., 2009) (values in g/kg of DM).
(iii) General equation for energetic and protein feedstuffs 2: AMEn = 4095.41 + 5.684EE − 22.526ASH − 2.224aNDFom (Nascimento et al., 2009) (values in g/kg of DM).

AMEn = 4101.33 + 5.628EE − 23.297ASH − 2.486aNDFom + 1.042ADFom.
($R^2 = 0.84$; RSD = 0.4137; P-value<0.0001; $n = 574$).
AMEn = 4095.41 + 5.684EE − 22.526ASH − 2.224aNDFom.
($R^2 = 0.83$; RSD = 0.4171; P-value<0.0001; $n = 574$).

2.5. Statistical analysis

The AMEn values predicted with each equation were compared with those determined *in vivo* bioassay. The validation procedure was realized by fitting a simple linear regression model ($Y = a + bX$) of observed (dependent variable) to predicted values (independent variable) using simultaneous hypotheses tested by F test, in accordance with the method of Mayer et al. (1994):

$$H_0 : \beta_0 = 0 \tag{1}$$

$$H_0 : \beta_1 = 1 \tag{2}$$

Predicted and observed values were considered similar when both null hypotheses were not rejected. If this was not the case, the simplified equation was adjusted, abolishing the parameter intercept (reduced model: $Y = bX$) (Neter et al., 1985; Roseler et al., 1997). In conditions of acceptance of nullity hypothesis to slope ($H_0 : \beta_1 = 1$), bias between observed and predicted values was measured by the following equation (Paulino et al., 2005):

$$B = (\beta - 1) \times 100$$

where B = estimated bias (%) and β = estimated angular coefficient of the adjusted equation without consideration of the intercept parameter (reduced model).

The estimative-standard error, which measures the variability around the regression line, was calculated considering the set of predicted values from those observed (Neter et al., 1985):

$$s_{\text{est}} = \sqrt{\frac{\sum (Y - Y')^2}{N - 2}}$$

where S_{est} = standard error of estimative; Y = predicted values; Y' = observed values; $N-2$ = degrees of freedom of the residue obtained in the regression variation analysis.

The statistical analyses were performed using the Sas (2004). For all statistical procedures, $\alpha = 0.05$ was adopted.

3. Results

The AMEn values of the protein and energetic concentrate feedstuffs determined by *in vivo* bioassay, and those calculated with prediction equations, and their respective standard errors are shown in Tables 3 and 4, respectively.

Table 4
Nitrogen-corrected apparent metabolizable energy (MJ/kg DM) of energetic feedstuffs obtained by metabolism assays ($n = 6$) with broilers or by prediction equations.

Feedstuff	Observed average value	Standard deviation	Lower limit	Higher limit	Calculated by (i)	Calculated by (ii)	Calculated by (iii)	Calculated by (iv)
Wheat meal	8.13	0.29	7.89	8.36	8.03	8.03	8.21	8.34
Integral rice meal	11.23	0.45	10.87	11.59	11.18	10.87	10.13	10.02
Sorghum 1	14.78	0.51	14.37	15.18	14.93	14.98	14.88	14.81
Sorghum 2	15.06	0.26	14.85	15.27	15.16	15.11	14.98	14.91
Pre-gelatinized maize	15.17	0.64	14.66	15.68	15.35	15.41	15.33	15.43
Broken maize	15.39	0.50	14.99	15.79	15.65	15.53	15.72	15.63
Maize 1	15.49	0.38	15.19	15.79	15.77	15.69	15.66	15.60
Maize 2	15.96	0.43	15.62	16.31	16.05	16.05	15.81	15.67
Broken rice	16.17	0.45	15.81	16.53	16.84	16.99	16.21	16.04
Average	14.15	–	–	–	14.33	14.29	14.10	14.05
Estimative-standard error between observed and calculated values	–	–	–	–	0.29	0.35	0.42	0.47

(i) Specific equation for energetic feedstuffs 1: AMEn = 4371.18 − 2.648CP + 3.065EE − 12.693ASH − 5.226CF − 2.514aNDFom + 2.440ADFom (Nascimento, 2007) (values in g/kg of DM).
(ii) Specific equation for energetic feedstuffs 2: AMEn = 4205.23 + 3.058EE − 13.035ASH − 5.829CF − 2.831aNDFom + 1.671ADFom (Nascimento, 2007) (values in g/kg of DM).
(iii) General equation for energetic and protein feedstuffs 1: AMEn = 4101.33 + 5.628EE − 23.297ASH − 2.486aNDFom + 1.042ADFom (Nascimento et al., 2009) (values in g/kg of DM).
(iv) General equation for energetic and protein feedstuffs 2: AMEn = 4095.41 + 5.684EE − 22.526ASH − 2.224aNDFom (Nascimento et al., 2009) (values in g/kg of DM).

Table 5
Parameter estimates, probability values (F test) for the null hypothesis and regression coefficient (R^2) between observed and predicted values for AMEn of protein feedstuffs ($n = 9$).

Prediction equation	Intercept		Slope		R^2
	Estimate	P value[a]	Estimate	P value[b]	
(i)	0.11225	0.9774	1.0133	0.9665	0.5542
(ii)	−0.01354	0.9780	1.0057	0.8809	0.9894
(iii)	−0.01150	0.9860	1.0093	0.8572	0.9808

(i) Specific equation for protein feedstuffs: AMEn = 2707.71 + 5.863EE − 1.606aNDFom (Nascimento, 2007) (values in g/kg of DM).
(ii) General equation for energetic and protein feedstuffs 1: AMEn = 4101.33 + 5.628EE − 23.297ASH − 2.486aNDFom + 1.042ADFom (Nascimento et al., 2009) (values in g/kg of DM).
(iii) General equation for energetic and protein feedstuffs 2: AMEn = 4095.41 + 5.684EE − 22.526ASH − 2.224aNDFom (Nascimento et al., 2009) (values in g/kg of DM).
[a] $H_0:\beta_0 = 0$; $H_a:\beta_0 \neq 0$.
[b] $H_0:\beta_1 = 1$; $H_a:\beta_1 \neq 1$.

Estimates for the parameters of the regression equation, values of estimated parameters, the probability of the null hypothesis and regression coefficient (R^2) between the observed and predicted values for AMEn of protein and energetic feedstuffs are shown in Tables 5 and 6, respectively.

For the protein concentrate feedstuffs, the statistical analysis of the intercept and the slope of the straight line was consistent (P>0.05) with the null hypothesis ($H_0:\beta_0 = 0$ and $H_0:\beta_1 = 1$), indicating that the observed values for AMEn are equivalent to those predicted by the equations, although a smaller standard error was obtained with the general equations (Table 3).

For the energetic concentrate feedstuffs, the intercept and the slope of the straight line obtained with the general equation for energetic and protein feedstuffs 1 and 2 (Eqs. (iii) and (iv), Table 6) were consistent with the null hypothesis ($H_0:\beta_0 = 0$; $H_0:\beta_1 = 1$). Meanwhile, for specific equations (Eqs. (i) and (ii), Table 6), it was verified that the slope of the straight line and its respective probability values were sufficient to reject the null hypothesis ($H_0:\beta_0 = 0$). When a new regression equation was created, in which the parameter relative at the intercept was abolished (reduced model), the slope did not differ (P>0.05) between the two equations, showing that there are only bias in these equations, but that these can be used to estimated the AMEn of the feedstuffs. In this case, the bias were 1.38% and 1.22% for the specific equations (1) and (2), respectively.

4. Discussion

The evaluated feedstuffs (Tables 1 and 2) showed chemical compositions values different from those reported in the literature (National Research Council, 1994; Lesson and Summers, 1997; Generoso et al., 2008; Batal and Dale, 2009; Mello

Table 6
Parameter estimates, probability values (F test) for the null hypothesis regression coefficient (R^2) between observed and predicted values for AMEn (MJ/kg) of energetic feedstuffs ($n = 9$).

Prediction equation	Complete regression model				R^2	Simple model	
	Intercept		Slope			Slope	
	Estimate	P value[a]	Estimate	P value[b]		Estimate	P value[b]
(i)	0.63838	0.0729	0.9432	0.0290	0.9961	0.9862	0.0729
(ii)	0.94577	0.0640	0.9239	0.0369	0.9919	0.9878	0.0640
(iii)	1.05609	0.1690	0.9286	0.1805	0.9790	–	–
(iv)	0.92863	0.2930	0.9414	0.3388	0.9713	–	–

(i) Specific equation for energetic feedstuffs 1: AMEn = 4371.18 – 2.648CP + 3.065EE – 12.693ASH – 5.226CF – 2.514aNDFom + 2.440ADFom (Nascimento, 2007) (values in g/kg of DM).
(ii) Specific equation for energetic feedstuffs 2: AMEn = 4205.23 + 3.058EE – 13.035ASH – 5.829CF – 2.831aNDFom + 1.671ADFom (Nascimento, 2007) (values in g/kg of DM).
(iii) General equation for energetic and protein feedstuffs 1: AMEn = 4101.33 + 5.628EE – 23.297ASH – 2.486aNDFom + 1.042ADFom (Nascimento et al., 2009) (values in g/kg of DM).
(iv) General equation for energetic and protein feedstuffs 2: AMEn = 4095.41 + 5.684EE – 22.526ASH – 2.224aNDFom (Nascimento et al., 2009) (values in g/kg of DM).
[a] H_0: $\beta_0 = 0$; H_a: $\beta_0 \neq 0$.
[b] H_0: $\beta_1 = 1$; H_a: $\beta_1 \neq 1$.

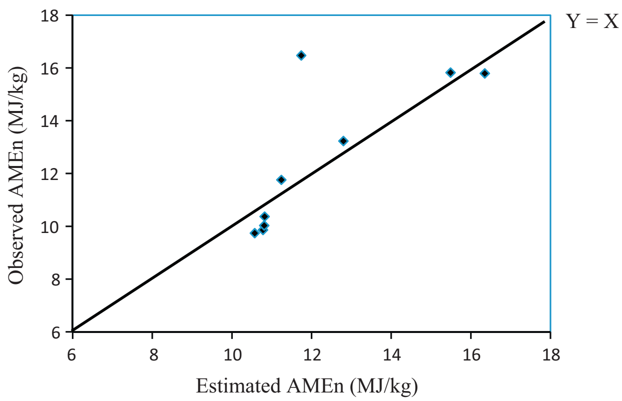

Fig. 1. Relationship between observed AMEn values with protein concentrate feedstuffs and values predicted by the specific equation for protein feedstuffs (AMEn = 2707.71 + 5.863EE – 1.606aNDFom).

et al., 2009). Several factors can affect the chemical composition of feedstuffs, including soil fertility, cultivation conditions, climate, genetics, storage, grain processing and method of analysis as well.

Given that the null hypothesis (H_0:$\beta_0 = 0$ and H_0:$\beta_1 = 1$) was accepted for the prediction equations (Table 5), we concluded that the regression analyses simply indicated a similarity between the observed and estimated values but did not indicate which prediction equation best reconciles the estimated values with the observed values. In other words, regression analysis only verifies that the relationship between two whole values is existent and without bias, assuming the pre-established form of $Y = 0 + 1X$ or simply $Y = X$ (Figs. 1–3). In the present work, failing to reject the null hypothesis for all of the regression equations implies that the tested prediction equations for the protein concentrate feedstuffs adequately estimated the AMEn values of the feedstuffs without bias, indicating their applicability in predicting the AMEn values of protein feedstuffs for broilers.

By numeric values, the equation AMEn = 4101.33 + 5.628EE – 23.297ASH – 2.486aNDFom + 1.042ADFom (Eq. (ii), Table 3), came closest to the observed values, with a lower standard error, indicating good applicability to the prediction of the AMEn of protein concentrate feedstuffs. The higher regression coefficient (R^2) obtained confirms its applicability (Table 5). This result explains the lower average difference between the observed and predicted values, which was 0.40% against 2.15% to the specific equation (Eq. (i), Table 3) and 0.80% to the general equation (2) (Eq. (iii), Table 3).

The other general equation (Eq. (iii), Table 3) has also shown good applicability. The lower coefficient of determination obtained with the specific equation (Eq. (i), Table 3) does not invalidate its use for estimating the AMEn of protein feedstuffs utilized for broilers.

Generally, the values estimated by the prediction equations for maize gluten meal were less accurate than the observed values. This trend is likely explained by the higher percentage of CP in this ingredient and by the absence of this variable in the prediction equations for protein concentrate feedstuffs. For this ingredient, the general equation (1) (Eq. (ii), Table 3) was the one that produced values most similar to the observed values, possibly because of the inclusion a larger number of variables influencing the energy content of feedstuffs (EE, aNDFom, ADFom and Ash). Based on animal nutrition studies,

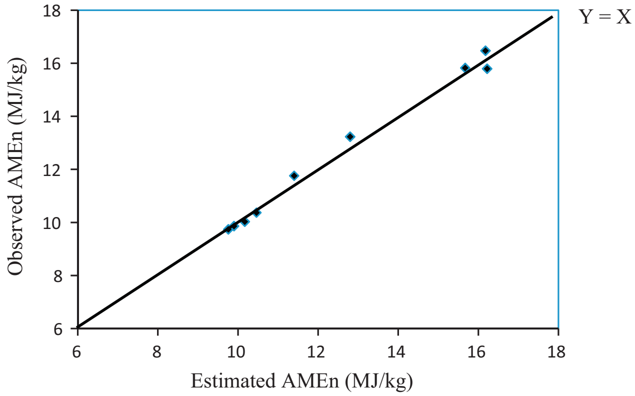

Fig. 2. Relationship between observed AMEn values with protein concentrate feedstuffs and values predicted by the general equation for concentrate feedstuffs 1 (AMEn = 4101.33 + 5.628EE – 23.297ASH – 2.486aNDFom + 1.042ADFom).

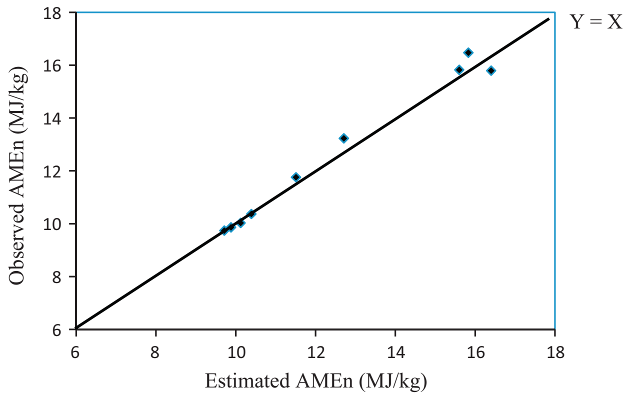

Fig. 3. Relationship between observed AMEn values with protein concentrate feedstuffs and values predicted by the general equation for concentrate feedstuffs 2 (AMEn = 4095.41 + 5.684EE – 22.526ASH – 2.224aNDFom).

1.0 g of protein has an average heat combustion of 23.67 J. Thus, the equations studied may have overestimated the energetic value of foods with higher CP content. Additionally, the integral micronized soy also presented overestimated values. The biggest differences were observed when the specific equation (Eq. (i), Table 3) and general equation (2) (Eq. (iii), Table 3) were used. This was probably due to the high percentage of EE of this ingredient and the absence of ADFom in these two equations.

Dolz and De Blas (1992) and Rodrigues et al. (2002) observed that the adjustment of models with two independent variables can be applied in the estimation of the energetic values of the feedstuffs. This was also true in the present work to verify that all tested equations accepted the null hypothesis (Table 5). Nevertheless, equations with more than two variables were better adjusted between the observed and estimated values.

According to Nascimento (2007), the values of aNDFom were important to the prediction equation. When the author removed this variable from the databank, the generated equation showed an R^2 reduction from 81 to 71%. Wan et al. (2009), evaluating the use of prediction equations to determine the energetic values of wheat and its sub-products for ducks, verified that the equation composed only of this variable explained 94% of the variation in energetic values for these feedstuffs. Nevertheless, Carre et al. (1984) mentioned that the aNDFom do not include all indigestible carbohydrates in broilers, citing as an example the pectic substances in the cellular wall. According to these authors, others variables must be included in the prediction equations. The aNDFom variable, for example, is also important because it accounts for the feedstuff fraction containing cellulose and lignin, which have a much-reduced digestibility in birds.

In addition to aNDFom, the EE also can be considered an important variable responsible for the energetic variability of the feedstuffs. Nunes et al. (2001), in elaborating prediction equations for the energetic values of wheat and its sub-products for broilers, observed that the EE had a positive correlation with ME values. This result can be linked to the high energy content (GE: 38.1 J/g) of the EE compared to the other contents of the feedstuffs.

Moreover, Rodrigues et al. (2002) reported that the ash is also important in the energetic estimation of the feedstuffs because it represents, in the inverse form, the organic fraction of these. This observation could explain why the general equations for concentrate feedstuffs have been more applicable in the prediction of the AMEn values for protein ingredients.

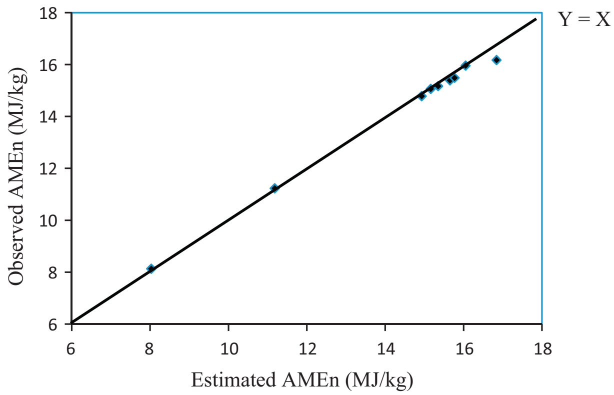

Fig. 4. Relationship between observed AMEn values with energetic concentrate feedstuffs and values predicted by the specific equation for energetic feedstuffs 1 (AMEn = 4371.18 – 2.648CP + 3.065EE – 12.693ASH – 5.226CF – 2.514aNDFom + 2.440ADFom).

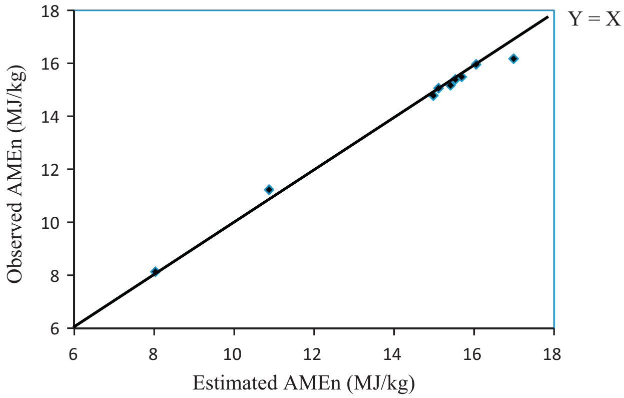

Fig. 5. Relationship between observed AMEn values with energetic concentrate feedstuffs and values predicted by the specific equation for energetic feedstuffs 2 (AMEn = 4205.23 + 3.058EE – 13.035ASH – 5.829CF – 2.831aNDFom + 1.671ADFom).

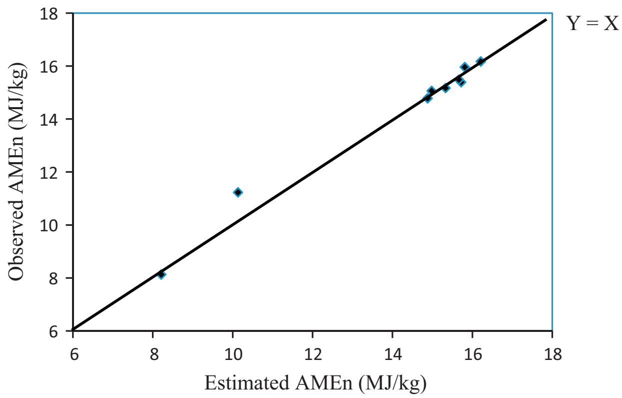

Fig. 6. Relationship between observed AMEn values with energetic concentrate feedstuffs and values predicted by the general equation for concentrate feedstuffs 1 (AMEn = 4101.33 + 5.628EE – 23.297ASH – 2.486aNDFom + 1.042ADFom).

Considering the results for the energetic concentrate feedstuffs, the acceptance of both null hypotheses (H_0:β_0 = 0; H_0:β_1 = 1) (Table 6) indicates that the estimated values given by the general equations were similar to those obtained by the metabolic bioassays, demonstrating good applicability of these equations.

Conversely, rejection of the null hypothesis for the specific equations (Eqs. (i) and (ii), Table 6), which led to calculate the bias, indicates that the estimated values overestimated those observed in the *in vivo* bioassay. The means calculated for the observed and estimated values confirmed this hypothesis.

Observing the arrangements of the points along the ideal axis (Y = X) (Figs. 4–7), it is notable that the specific equations for energetic concentrate feedstuffs were more clustered around the central axis, despite the rejection of the null hypothesis

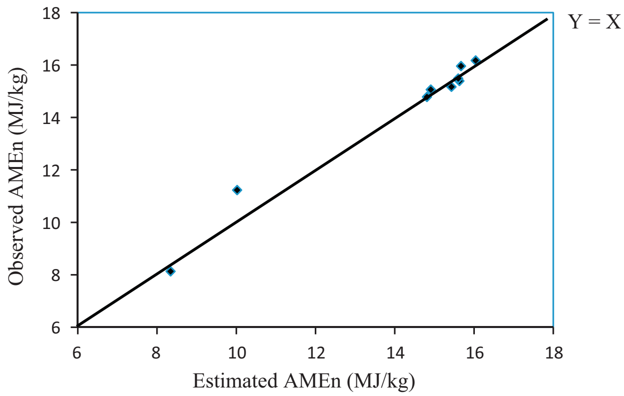

Fig. 7. Relationship between observed AMEn values with energetic concentrate feedstuffs and values predicted by the general equation for concentrate feedstuffs 2 (AMEn = 4095.41 + 5.684EE − 22.526ASH − 2.224aNDFom).

for these equations by the regression model. However, because the null hypothesis was accepted for the reduced model, we suggest that this equation can be used to estimate the AMEn values of energetic feedstuffs.

The standard error of estimates from the four equations utilized for energetic feedstuffs (Table 4) verifies that the specific equations for energetic feedstuffs are best adjusted at the observed values, particularly for equation (1). The results obtained with these equations were different from those seen for broken rice with regard to the values obtained by the metabolic bioassays. This may have occurred because this feedstuff had a lower content of CP compared to others and because this variable may subtract from the total value of the AMEn in specific equations, which does not occur with the general equations. All the others feedstuffs showed similar deviations in their mean differences.

Between the general equations, a lower average difference with regard to observed AMEn values was obtained with general equation (1) (Eq. (iii), Table 4), supporting good applicability in the estimates for the AMEn values of energetic concentrate feedstuffs, such as those observed for the protein ingredients. Considering the estimated value for integral rice meal, these values were better estimated by the general equations, unlike other ingredients. This result might be due to a higher value of ash in this ingredient, which is associated with a higher negative coefficient of this variable in the equation. According to Giacometti et al. (2003), this feedstuff is considered an unconventional ingredient for broilers because of restrictions and limitations on its use that cause a higher variability in the energetic values.

Rodrigues et al. (2001) reported that the equations composed of two and four variables explain most of the variation in AMEn values obtained for maize and its sub-products as well as millet, but that a larger number of variables increases the estimative precision of these equations. In the present study, considering the general equations for concentrate feedstuffs, which accepted the null hypotheses (did not show bias), it was observed that equations with four variables showed lower standard errors (Table 4), confirming the hypothesis of these authors. Nevertheless, a lower number of chemical composition variables facilitates the use of equations, expediting laboratory analyses and facilitating the quick calculation of energetic values for diet formulations. Thus, general equations with fewer numbers of variables may be utilized.

Thus, we have shown that these prediction equations are important for increasing the accuracy of diet formulation, allowing producers to correct energetic values in accordance with variations in the chemical composition of feedstuffs.

5. Conclusions

All the equations proposed by Nascimento (2007) adequately estimated the AMEn of energetic and protein concentrate feedstuffs, and equations with more variables showed lower standard errors in their estimates. It was shown that the equation AMEn = 4101.33 + 5.628EE − 23.297ASH − 2.486aNDFom + 1.042ADFom (R^2 = 0.84; RSD = 0.4137) was the most applicable to the prediction of energetic values of protein and energetic concentrate ingredients used in the poultry feed industry.

Acknowledgments

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