

Metabolizable energy of corn, soybean meal and wheat for laying hens

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ABSTRACT Feed formulation using apparent metabolizable energy (AME) corrected to zero nitrogen retention (AMEn) is widely used by poultry nutritionists. Most available tabulated data are from experiments using adult cockerels or growing broilers. Specific values are rarely available for laying hens. A study was conducted to evaluate AME, AMEn, and AMEs (AME adjusted to 50% nitrogen retention) of corn, soybean meal (SBM) and wheat in laying hens using the reference diet substitution and regression methods. Forty eight 42-wk-old Hy-Line Brown hens were used, 2 birds per cage with six replicates per diet. Test diets contained 30% test ingredient (as is basis) and 65.7% reference diet (as is basis) with limestone, other minerals, vitamins, and amino acids held constant across

the reference and test diets. Using the reference diet substitution method, AME values obtained for corn, SBM, and wheat were 3,791, 2,621, and 3,565 kcal/kg (DM), respectively. The corresponding AMEn values were 3,722, 2,496, and 3,479 kcal/kg (DM), and AMEs were 3,784, 2,835, and 3,562 kcal/kg (DM), respectively. Calculation of AME, AMEn, and AMEs of ingredients using regression based on the inclusion rate (DM) of dietary ingredients and reference diet gave identical values to those obtained by the reference diet substitution method. In addition, the measured AMEn values of ingredients using laying hens in this study were close to those calculated from proximate composition using the European Union prediction equation based on adult cockerels.

Key words: metabolizable energy, laying hens, corn, wheat, soybean meal

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INTRODUCTION

Little data are available on ingredient apparent metabolizable energy (AME) and AMEn assay values using commercial laying hens in peak production. Most nutritionists formulating layer feed use energy data obtained from adult cockerels or growing broilers (Janssen, 1989; Bourdillon et al., 1990a). The EU prediction equations are often used in conjunction with NIRS proximate estimates or wet chemistry proximate analysis to predict AMEn of ingredients for formulation (Janssen, 1989). While the prediction and NIRS-based methods have the advantage of being cost effective and fast, they may not accurately predict AME and AMEn values for laying hens without accurate bioassay data available. The *in vivo* methods are either based on apparent or true metabolizable energy with or without adjustments made to zero nitrogen retention (Hill and Anderson, 1958) or to a standardized nitrogen retention coefficient (Cozannet et al., 2010).

The AME assay uses either adult birds or, more frequently, growing broiler chickens with an adaptation feeding period followed by a 3 to 4-D measure-

ment period (Bourdillon et al., 1990a). In the reference diet substitution method, a portion of the reference or basal diet is replaced with test ingredient (usually 30 to 40% for grains and lower for other ingredients) (Bourdillon et al., 1990a). In these methods, the reference and test diets are generally supplemented with minerals and vitamins to ensure the diets are similar and balanced. Failure to take this into account in the calculation may cause erroneous results. Assuming the GE, AME, AMEn, AMEs (standardized AME equal to 50% of N intake; Cozannet et al., 2010; Wu et al., 2019), and NE of a complete diet consist of energy additively contributed by individual ingredients, the energy value of those ingredients can also be calculated by linear regression equations. This method has been applied in pigs by Noblet et al. (1993) for a set of 13 ingredients and also in broilers by Lopez and Leeson (2008) for calculating the AME and AMEn values of corn and SBM.

Although the AME classical total collection bioassay using young broilers has become a preferred method for formulating growing broiler feed (Farrell, 1999), the application of those values or values obtained from adult cockerels to laying hens or other bird species is debatable. Different species, breeds, and ages of poultry may have variable ability to digest and metabolize feed components (Ravindran et al., 2004; Adeola et al., 2018). For instance, the AMEn of wheat dried distillers

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Table 1. Composition of ingredients and diets (% as is).¹

	Ingredients			Reference diet	Test diets		
	Corn	Soybean meal	Wheat		Corn	Soybean meal	Wheat
Nutrients							
Dry Matter	88.0	89.9	89.6	89.8	89.5	90.1	90.0
GE, kcal/kg ²	3920	4189	3918	3641	3601	3634	3602
Crude protein	8.6	47.5	11.2	17.6	14.2	25.7	14.8
Crude fiber	1.7	3.3	2.3	1.8	1.7	2.2	1.9
Ether extract	2.7	1.7	1.4	3.6	3.3	2.9	3.0
Ash	1.5	6.5	1.5	14.3	13.8	15.2	13.7
ADF	3.4	5.1	2.6	3.4	3.2	3.7	3.0
NDF	8.1	8.4	9.8	7.0	7.0	7.1	7.6
Starch	48.9	0.1	63.9	29.6	34.1	19.4	38.6
NSP total	5.7	12	8.1	6.6	6.1	7.9	6.8
NSP soluble	0.3	0.8	1.3	0.4	0.4	0.5	0.3
NSP insoluble	5.4	11.2	6.8	6.2	5.7	7.4	4.1
Calcium	0.03	0.26	0.05	4.1	3.7	5.1	2.7
Avail Phosphorus	0.08	0.23	0.12	0.4	0.4	0.4	0.4
Lysine	0.30	2.90	0.40	0.93	0.73	1.43	0.74
Methionine	0.16	0.60	0.16	0.52	0.48	0.60	0.48
Threonine	0.31	1.90	0.33	0.75	0.62	1.08	0.63
Arginine	0.39	3.40	0.51	1.04	0.80	1.62	0.84
Valine	0.41	2.20	0.50	0.82	0.66	1.21	0.69
Isoleucine	0.32	2.10	0.40	0.68	0.54	1.01	0.57

¹Measured values.

²Abbreviations: GE = gross energy, ADF = acid detergent fibre; NDF = neutral detergent fibre; NSP = non-starch polysaccharide.

grains with solubles (**DDGS**) showed the highest value in adult cockerels followed by adult layers and growing broilers. The ability of laying hens to digest energy as AMEn was 97 and 93% for diets and DDGS, respectively, compared to adult cockerels (Cozannet et al., 2010). Differences in length of intestine and passage rate likely affect the energy utilization of various classes of birds. A longer intestine and slower passage rate may decrease microbial fermentation and reduce energy utilization in broilers compared to Leghorn layers (Shires et al., 1987). Other work found layers utilize more AMEn from corn, SBM, and wheat bran compared to broiler strains (Pishnamazi et al., 2005). Thus, the potential of the broiler to utilize dietary energy is less than layers (Lopez and Leeson, 2005). The objective of this study was to measure the metabolizable energy of 3 major ingredients (corn, wheat, and soybean meal) by the reference diet substitution and regression methods in laying hens.

MATERIALS AND METHODS

Birds and Diets

The study was approved by the Animal Ethics Committee of the University of New England (UNE) and designed to follow the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013). Forty eight Hy-Line Brown hens, 42 wk of age, laying at 93% hen day production (HDP) were housed 2 birds per cage. The sloping floor cages were 55 cm by 50 cm, by 50 cm average height and were in an open-sided shed at the University of New England, Australia with 16 h light per day in spring

season; the average temperatures were between 22 and 24°C. Cages were fitted with individual feeders, nipple drinkers, and 75 × 70 cm dropping collection trays.

Flint corn, Argentina SBM, and hard red wheat were sourced from the local market. Nutrient composition (%) of the three ingredients is given in Table 1. The ingredients and nutrient composition of reference and test diets (fed as mash) are shown in Table 2. The reference diet was based on corn, SBM, canola oil, and added amino acids (ref diet) and the three experimental diets contained 30% of each test ingredient (as is) and a constant level (65.7%) of ref diet (as is). Supplementary vitamins, minerals (including calcium), and other non-energy ingredients were adjusted to have equivalent inclusion rates across all diets. Each of the 4 diets was fed to six replicate cages for a 7-D adaptation period followed by a 3-D experimental period with feed intake measured and total excreta collected and measured. Birds had ad libitum access to water and experimental diets. Feed spillage was measured from the under cage collection tray and deducted from feed intake. Feathers and down were removed from the collected excreta.

Measurements and Analysis

Feed intake, egg production, and egg weight were measured for each cage. The total excreta voided daily were pooled from each cage and weighed. Multiple subsamples were collected and homogenized from the total of each cage at the end of the collection period. A 30 g representative sample of excreta was weighed and freeze-dried to a constant weight for gross energy and N analysis. Samples of feed and freeze-dried excreta were

Table 2. Ingredients composition (%) of the reference and test diets (as is).

Item	Reference diet	Test diets			Ingredient DM (%)
		Corn	SBM	Wheat	
Ingredient, %					
Corn	60.5	39.8	39.8	39.8	89.4
Soybean meal	25.8	16.9	16.9	16.9	90.2
Corn	0.0	30.0	0.0	0.0	89.4
Soybean meal	0.0	0.0	30.0	0.0	90.2
Wheat	0.0	0.0	0.0	30.0	90.5
Canola oil g	1.3	0.8	0.8	0.8	100.0
Limestone	9.7	9.7	9.6	9.6	99.5
Dicalcium phosphate	1.6	1.6	1.6	1.6	98.0
Salt	0.3	0.3	0.3	0.3	99.6
Na bicarbonate	0.2	0.2	0.2	0.2	100.0
Vitamin, mineral premix ¹	0.2	0.2	0.2	0.2	99.0
Choline Cl 60%	0.08	0.08	0.08	0.08	75.0
L-lysine HCl	0.08	0.08	0.08	0.08	99.5
D,L-methionine	0.27	0.27	0.27	0.27	99.5
L-threonine	0.09	0.09	0.09	0.09	99.5
CP (DM)	19.7	15.9	28.4	16.9	
Energy yielding ingredients (%) ²	86.9	57.4	57.3	57.2	
a (% DM) ³	100.0	66.0	65.9	65.8	
b (% DM) ⁴	0.0	29.5	29.7	29.8	

¹Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D, 3500 IU; vitamin E, 40 mg; vitamin K, 2 mg; nicotinic acid B3, 50 mg; pantothenic acid B5, 11 mg; folic acid, 1.5 mg; riboflavin B2, 6 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; pyridoxine B6, 5 mg; thiamine B1, 2 mg; Cu, 8 mg; Co, 0.3; Mo, 1 mg; I, 1 mg; Se, 0.3 mg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg; Endox (antioxidant), 25 mg.

²The total amount of energy-yielding ingredients including grains, SBM, canola oil, and amino acids in the reference and test diets (%).

³The contributions of energy-yielding ingredients from the reference diet in the test diet.

⁴Substitution level of each ingredient in the test diet (DM basis).

finely ground to ensure homogeneity. Approximately 2 g of diet and 3 g of freeze-dried excreta samples were dried in crucibles in a drying oven at 105°C for 16 h to determine DM. Excreta DM was calculated by correction for the loss of moisture during both freeze- and oven-drying. The gross energy content of feed and excreta was determined in triplicate 0.5 g samples using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH, and Co., Staufen, Germany) with benzoic acid as the calibration standard. Ingredient samples were analyzed for GE, CP, EE, crude fibre (**CF**), ash, neutral detergent fibre (**NDF**) and acid detergent fibre (**ADF**) (AOAC, 2016), starch (Megazyme Total Starch Kit, Megazyme International Ireland Ltd., Bray, Ireland), free sugars (mono- and disaccharides) (Annison et al., 1996), total non-starch polysaccharides (**NSP**), soluble NSP and insoluble NSP (Englyst and Hudson, 1987; Theander and Westerlund, 1993). Nitrogen was measured using a LECO model FP-2000 N analyzer in triplicate on 0.15 g samples of ingredients, diets, and excreta. The LECO analysis was calibrated by pure reference EDTA. Feed intake (g, DM), hen day egg production (%), egg weight (g), egg mass (g), and feed conversion ratio (FCR) were measured daily and cumulated over a period of 3 D.

Calculations

Egg mass was calculated as the product of average egg weight and hen day production. FCR was calculated as the ratio of feed intake to egg mass. Nitrogen intake

and nitrogen retained in the body were measured for all the birds fed reference and test diets as g/b/d.

Dietary AME (kcal/kg of DM) values were calculated according to the following equations:

$$\begin{aligned} \text{AME (kcal/kg DM of diet)} \\ = [(F_i \times \text{GE}_f) - (E \times \text{GE}_e)] / F_i \end{aligned}$$

$$\begin{aligned} \text{AMEn (kcal/kg DM of diet)} \\ = [\text{AME} - [8.22 \times (N_i - N_e)]] / F_i \end{aligned}$$

$$\begin{aligned} \text{AMEs (kcal/kg DM of diet)} \\ = \text{AMEn} + 8.22 \times N_i\% \times 10 \times 50\% \end{aligned}$$

Where GE_f is the gross energy of feed (kcal/g DM) and GE_e is the gross energy of excreta (kcal/g DM); F_i = feed intake (g DM/d/hen); E = excreta output (g DM/day/hen) and 8.22 kcal/g of N as nitrogen correction factor for each gram of N retained in the body and in eggs (Hill and Anderson, 1958); N_i is nitrogen intake from the diet (g/day) and N_e is the nitrogen output from the excreta (g/day). AMEs is the AME corrected for a retention of N equal to 50% of nitrogen intake; this ratio corresponds to what occurs under practical conditions in laying hens (Cozannet et al., 2010; Barzegar et al., unpublished results).

Calculation of test ingredient AME, AMEn, or AMEs according to the reference diet substitution method is

as follows:

$$\begin{aligned} \text{AME}_{\text{ingr}} (\text{kcal/kg}) \\ = (\text{AME}_{\text{test}} - \text{AME}_{\text{ref}} \times a\%) / b\% \end{aligned}$$

Where AME_{ingr} is the AME value of the test ingredient, AME_{test} is the mean of measured AME value of test diet less the AME contributed by the supplemental L-lysine HCl, D, L-methionine and L-threonine; AME_{ref} is the mean of measured AME value of the reference diet less the AME contributed by the supplemented D, L-methionine, L-lysine HCl and L-threonine; $a\%$ is the inclusion level of energy-yielding ingredients from the reference diet in the test diet and $b\%$ is the substitution level of the ingredient in the test diet. All the energy-yielding ingredients (including AA's), minerals and vitamins inclusion rates in reference and test diets were converted to DM basis in order to express the a and b values on a DM basis (Table 2). The AME values contributed by supplemental amino acids (D, L-methionine, L-lysine HCl and L-threonine) added in reference and test diet was considered as GE of these AAs (from INRA feeding tables; Sauvante et al., 2004) as it has been assumed the digestibility of supplemented AAs is close to 100% (Karakas et al., 2001).

The AMEn contribution of supplemented AAs was calculated from AME by:

$$\begin{aligned} \text{AMEn}_{\text{AA}} (\text{kcal/kg}) \\ = \text{AME}_{\text{AA}} - 8.22 \times N\% \times 10 \end{aligned}$$

AMEn_{AA} is the AMEn of the supplemental AA; $N\%$ is the percentage of N in the AA which is converted to g/kg by multiplying 10.

The AMEs contribution of supplemented AAs was calculated from AMEn by:

$$\begin{aligned} \text{AMEs}_{\text{AA}} (\text{kcal/kg}) \\ = \text{AMEn}_{\text{AA}} + 8.22 \times N\% \times 10 \times 50\% \end{aligned}$$

AMEs_{AA} is the AMEs of the supplemental AA; 50% is the % of N intake that is retained.

Statistical Analysis

Energy values of diets and other data referring to diets were distributed normally and thus subjected to a one-way ANOVA analysis with a randomized design of treatments using the PROC GLM of SAS 9.2 (SAS, 2010) and least squares means option at $P < 0.05$. The four diets (one ref diet and 3 test diets) contained variable levels of the reference diet (100, 66.0, 65.9 and 65.8%, DM) and of each test ingredient (corn, soybean meal and wheat at 0, 29.5, 29.7, and 29.8%, DM, respectively, see Table 2) using six replicates of each diet. It can then be assumed that measured GE, AME, AMEs, or AMEn of diets consisted of

the additive contributions of the corresponding GE, AME, AMEs, or AMEn contents of the reference diet and test ingredients multiplied by their inclusion rates (DM/DM). The coefficients of the regression correspond to the energy values (kcal/kg DM) of the reference diet and the 3 test ingredients.

RESULTS AND DISCUSSION

Feed intake and performance measured in the three days of bioassay measurement in the laying hens were not affected ($P > 0.05$) by feeding diets containing different energy values and protein contents (Table 3). Feed intake was similar to breeder performance recommendations (Hy-Line (2016)).

The N retention values were 1.28, 1.14, 1.25, and 1.36 (g/b/d) ($P > 0.05$) for those birds fed the reference diet and the test diets including additional corn, wheat, and SBM, respectively. Therefore, feeding diets with various CP content had no effect on N retention (g/b/d) ($P > 0.05$); the lowest CP and amino acids (0.74% total lysine, for instance) levels in the diets of this study were then sufficient to meet the protein requirements of laying hens and to maintain their production.

The AME, AMEs, and AMEn values of reference diet, test diets and test ingredients measured by different methods are given in Tables 3 and 4. All the AMEs values were greater than the AMEn (kcal/kg DM), either for diets or ingredients. The difference was not consistent, with the highest difference of 7% for soybean meal diet followed by 4 and 3% for wheat and corn diets. Similarly, this was the highest for soybean meal (high-protein ingredient) compared to cereals (12 vs. 2%). The highest difference between AMEs and AMEn was observed for the soybean meal diet (185 kcal/kg DM) in this experiment. That difference was 140 kcal/kg DM for different categories of birds fed different diets (Cozannet et al., 2010). AME values were also higher than AMEn (kcal/kg, DM) for both diets and ingredients. The highest difference observed as 4% for soybean meal diet compared to 3% for both of corn and wheat diets. The AME of ingredients were 5 and 2% higher than correspondent AMEn values for soybean meal and cereals. Lopez and Leeson (2008) reported that difference of AME and AMEn by 7–12% and 3–5% for soybean meal and corn in broilers. The AMEs and AME values of low-protein diets and ingredients (cereals) were close to each other. However, the AMEs was higher than the AME for soybean meal diet (+3%) and soybean meal ingredient (+8%) because of an excessive N catabolism due to a N supply higher than the requirement in the soybean meal diet.

To conclude, the AMEn values are not representative of production conditions, in particular for the high-protein ingredients. In addition, AME values as obtained from the difference method should be interpreted with caution as it is affected by the CP content of the test diet. AMEs would then be the most representative of productive conditions.

Table 3. Effect of diet composition on laying hens performance, N balance and diets energy values.

Items	Reference diet	Test diets			RSD	P value
		Corn	SBM	wheat		
Laying performance						
FI, g	90.7	95.2	97.0	96.5	5.2	0.19
HDP, %	55.6	61.1	83.3	56.7	24.1	0.20
Egg weight, g	59.9	59.7	61.1	60.4	5.3	0.97
Egg mass, g	33.2	37.0	51.3	34.6	15.0	0.17
FCR	1.47	1.99	1.03	1.57	0.97	0.42
N balance (g/b/d)						
Intake	2.86 ^b	2.41 ^c	4.41 ^a	2.60 ^c	0.16	< 0.001
Excreta	1.58 ^b	1.27 ^c	3.05 ^a	1.35 ^{b,c}	0.15	< 0.001
Retained ¹	1.28	1.14	1.36	1.25	0.15	0.14
Energy values, kcal/kg DM ²						
AME	3195 ^a	3228 ^a	2885 ^b	3166 ^a	45	< 0.001
AMEs	3211 ^a	3236 ^a	2959 ^b	3176 ^a	39	< 0.001
AMEn	3083 ^{a,b}	3134 ^a	2774 ^c	3067 ^b	39	< 0.001

Abbreviations: FI, feed intake (g DM/bird/d); DM, dry matter; HDP, average hen day production (%); Egg weight, average egg weight (g); Egg mass as average egg weight × average HDP (g of egg/bird/d); FCR, feed conversion ratio (g of feed DM/g of egg); AME, apparent metabolizable energy; AMEs, AME corrected with nitrogen retention equal to 50% of nitrogen intake; AMEn, AME corrected with zero N retention.

¹Total N retained calculated as N intake—N in excreta (g/b/d).

²By reference diet substitution method. Data are means of 6 replicates of 2 hens per dietary treatment during 3 experimental days. ($P < 0.05$) by one-way ANOVA.

^{a-c}Means within rows with different superscripts are different ($P < 0.05$).

Table 4. GE, AME, AMEs, and AMEn (kcal/kg DM) and metabolizability (%) values of corn, wheat and soybean meal in laying hens; comparison with the literature values.

Method	GE ¹	AME	AMEs	AMEn	AME/GE	AMEs/GE	AMEn/GE	EU table ² AMEn	INRA table ³ AMEn
Bioassay method ⁴									
Reference diet	3971	3195	3211	3083					
Test ingredients:									
Corn	4455	3791	3784	3722	85.1	84.9	83.6	3725	3662
SBM	4659	2621	2835	2496	56.3	60.8	53.6	2579	2614
wheat	4373	3565	3562	3479	81.5	81.5	79.6	3494	3429
Regression equation ⁵									
Reference diet	3971	3195	3211	3083					
Test ingredients:									
Corn	4437	3791	3784	3722	85.4	85.3	83.9		
SBM	4539	2621	2835	2496	57.7	62.5	55.0		
wheat	4418	3565	3562	3479	80.7	80.6	78.7		
RSD	–	45	39	39					

Abbreviations: GE, gross energy; AME, apparent metabolizable energy; AMEs, AME corrected with 50% nitrogen intake; AMEn, AME corrected with zero N retention.

¹GE values for bioassay method were measured by bomb calorimeter in the laboratory and GE values for regressions were calculated by regression of diet GE against inclusion levels of ingredients and reference diet in test diets.

²According to Janssen (1989).

³Based on the AMEn values of INRA table for adult cockerels (Sauvant et al., 2004).

⁴By reference diet substitution method applied on mean value of each diet.

⁵Estimated by regressing the determined GE, AME, AMEn, and AMEs values (kcal/kg DM) of diets on the inclusion rates of reference diet and corn, SBM and wheat in the diets (n = 24; no intercept).

Estimation of AME values of ingredients by regressing their inclusion rate in the reference and test diets against the measured AME value of the diets is a statistical approach to validate the AME, AMEs, and AMEn values measured using the reference diet substitution method. In our conditions, values obtained for ingredients AME, AMEs, and AMEn according to the reference diet substitution method were the same as those obtained by diet-ingredient-inclusion-regression (Table 4). Using multiple linear regression to extrapolate the AME and AMEn of ingredients by their inclusion rate in diets has been examined by others (Applegate, 2005) with conclusions similar to ours.

The ingredient AMEn (kcal/kg DM) values for laying hens derived from the reference diet substitution method were compared to those predicted by applying measured proximate values in equations from the EU table of energy values for poultry feedstuffs (Janssen, 1989) and INRA tables (Sauvant et al., 2004). A summary is given in Table 4. In all methods, the highest values of AMEn were obtained for corn followed by wheat and SBM, respectively. However, the corn and wheat AMEn values obtained in our trial differed by less than 15 kcal/kg from those calculated by the EU prediction equation and 84 kcal/kg lower for SBM.

The mineral supplement inclusion rate in the diet affects energy utilization in animals. Providing limestone as a source of calcium for egg production leads to endogenous energy loss and decreases the AMEn content of ingredients in laying hens compared to roosters (Cozannet et al., 2010) and in pigs (Noblet and Van Milgen, 2013). Provision of limestone in layer diets in the current study might explain the differences between AMEn values of corn, SBM, and wheat grains compared to diets formulated for adult cockerels in EU tables (Table 4). The ability of laying hens to metabolize energy has been reported to be lower than cockerels and more than broilers. Slinger et al. (1964) showed higher metabolizable energy values in layer chickens compared to broiler chickens. Bourdillon et al. (1990b) reported higher AMEn values for different diets in adult cockerels compared to growing broilers. Although laying hens showed intermediate AMEn values for different diets and one grain compared to highest and lowest correspondent values by cockerels and broiler (Cozannet et al., 2010), the AME values of feedstuffs using adult cockerels can be used for laying hens (Farrell, 1999). The data achieved in the current study confirm these observations.

Utilization of GE for different AME values are shown in Table 4. The AME/GE was 85, 82, and 56% for corn, wheat, and soybean meal, respectively. The lower metabolizability of energy of soybean meal is related to its high-CP content and subsequent excessive N oxidation and excretion and, more importantly, to the presence of poorly digested NSP (Dale, 2000). Utilization of GE for AME, AMEs and AMEn were almost similar for cereals (83%, on average) while values for soybean meal were 56, 61, and 54% of GE.

The regression method is an alternative calculating method to extrapolate the AME, AMEs, AMEn values of ingredients based on their inclusion rates in dietary treatments in substitution method experiment. The results in the current study showed agreement between the reference diet substitution and regression methods to estimate ingredients ME content because the same starting values and inclusion rates were used. The regression method has the advantage over the diet substitution method that a series of practical diets can be formulated with varying levels of ingredients that remain balanced in nutrient content with no deficiencies or excesses or with conventional maximum inclusion rates. However, attention should be paid to get inclusion rates of ingredients that are not significantly correlated (Noblet et al., 1993). Overall, this regression approach is able to provide more representative energy values and applicable in practical diets.

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