Validation of a 3-point model for the determination of energy values using the regression method in broiler chickens

T. L. Wise, and O. Adeola [1]

Purdue University, Department of Animal Sciences, West Lafayette, IN 47907, USA

ABSTRACT Two experiments (**Exp.**) were conducted to validate a 3-point model for the regression method of determining ME, using canola meal (**CM**) and wheat as test ingredients (**TI**). Corn-soybean meal-based test diets (**TD**) contained 0, 100, 200, or 300 g/kg CM, added at the proportional expense of all energy contributing ingredients for Exp. 1, and 0, 150, 300, or 450 g/kg wheat for Exp. 2. For each Exp., 192 Cobb 500 male broiler chickens were weighed and allotted by BW to 1 of 4 treatments at d 21 post hatching in a randomized complete block design. Growth performance and metabolizability responses were evaluated for linear and quadratic effects using orthogonal contrasts, and ileal digestible energy (**IDE**), ME, and MEn of TI were determined by regressing the TI-associated energy against the dry matter intake of TI using a generalized linear model. Four data sets were used to determine ME, using all possible 3 and 4-point combinations of TD in each Exp. Increasing TI inclusion elicited linear decreases ($P < 0.01$) in the digestibility and metabolizability of DM and GE in the 2 studies. The ME of CM obtained from the 4 data sets ranged from 1,731 to 1,992 kcal/kg DM, however, excluding the highest concentration of CM produced the highest estimate of ME, whereas the other 3 sets ranged from 1,731 to 1,793 kcal/kg DM. The ME of wheat from the 4 data sets had a smaller range of 3,041 to 3,106 kcal/kg DM. Excluding the highest concentration of either TI produced higher standard errors for the estimate of ME compared to the other 3 sets (42 and 36% greater SE, respectively). Results for IDE and MEn were similar. These data indicate that there is no difference in the variation of estimates between the 3 and 4-point models, provided that the inclusion of the TI is adequate and both models represent the linearity and variability of responses.

Key words: broiler chicken, canola meal, metabolizable energy, regression method, wheat

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INTRODUCTION

It is well understood that feed is the most substantial cost when it comes to live animal production and may constitute as much as 70% of the expenses of production (Donohue and Cunningham, 2009). With this high cost, it is important to supply the necessary nutrients to animals with as little excess as possible. This can help to reduce costs as well as play a role in improving the efficiency of the animals. However, the accuracy of feed formulation is largely dependent on the available information for the ingredients being utilized. Due to this, the importance of correct ingredient evaluations cannot be understated and requires significant consideration. This is of particular consequence when considering nutrients that are highly limiting to growth, such as amino acids or energy, which also happen to contribute the largest proportion of feed costs (Pesti et al., 1986). Because of this, the evaluation of ingredients for their content and availability of these nutrients has garnered much attention.

There are a number of methods for determining the energy of feed ingredients, and one such is the regression method. This method has been used to produce ME and MEn values for a wide variety of ingredients and has consistently produced reproducible results that are similar to that of the direct and difference methods (Bolarinwa and Adeola, 2012; Zhang and Adeola, 2017; Zhang et al., 2021). The direct method tends to be most useful for cereal grains, as they can reasonably constitute a large proportion of diets, however, its application for ingredients with lower practical inclusions may fall short. The difference method can be useful in these applications as it requires smaller ingredient inclusions, however, it only utilizes a single inclusion level. The regression method

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[1]Corresponding author: ladeola@purdue.edu

utilizes multiple inclusion levels of an ingredient and therefore estimates the energy of that ingredient across a distribution of intakes, possibly producing more robust estimates compared to the other methods (Olukosi, 2021). The regression method will typically utilize 3 experimental diets that include a reference diet devoid of the test ingredient (**TI**), and 2 test diets (**TD**) that will have an intermediate and high inclusion of the TI, respectively. The multiple inclusion levels of the TI ingredient allow for the regression of the TI associated energy against TI intake, and the slope of the resulting curve represents the energy of the TI. This method captures variability of the energy of the TI over a distribution of intakes, which can help to produce a very robust estimate.

The regression method consistently proves to be a reliable way to evaluate the ME of feed ingredients, however, there are limited published data validating that the method cannot be improved by the use of more dietary inclusions. Having a greater number of experimental diets may allow the regression model to better explain the variation within the spread of dietary intakes and has the potential to reduce the standard error (**SE**) of the energy estimates obtained utilizing this method. The objective of this study was to evaluate the use of the 3-point model for the determination of ileal digestible energy (**IDE**), ME, and MEn against a 4-point model utilizing the regression method in broiler chickens.

Table 1. Ingredient and calculated composition of diets (Experiment 1).

Ingredient, g/kg	CM0[1]	CM100	CM200	CM300
Ground corn	604.60	539.50	474.41	409.31
Solvent-extracted canola meal[2]	0.00	100.00	200.00	300.00
Soybean meal, 48% CP	293.90	263.27	232.64	202.01
Soybean oil	41.00	36.73	32.45	28.18
Ground limestone	11.00	11.00	11.00	11.00
Monocalcium phosphate	11.00	11.00	11.00	11.00
Salt	4.00	4.00	4.00	4.00
L-Lysine HCl	2.00	2.00	2.00	2.00
DL-Methionine	1.50	1.50	1.50	1.50
L-Threonine	1.00	1.00	1.00	1.00
Vitamin-mineral premix[3]	5.00	5.00	5.00	5.00
TiO_2 premix[4]	25.00	25.00	25.00	25.00
Total	1,000	1,000	1,000	1,000
Calculated composition, g/kg				
MEn, kcal/kg	3,108	2,984	2,860	2,736
CP	195	213	230	248
EE[5]	65.15	61.58	58.01	54.44
Ca	6.91	7.48	8.06	8.64
P	6.08	6.77	7.46	8.16
nPP[6]	3.65	3.94	4.24	4.54

[1]CM0, CM100, CM200, and CM300 represent inclusions of 0, 100, 200, and 300 g/kg canola meal in the diets, respectively.

[2]Proximate analysis: 4,218 kcal/kg gross energy, 377.4 g/kg crude protein (N × 6.25), 14.0 g/kg crude fiber, 34.6 g/kg crude fat, 10.7 g/kg total P, 6.2 g/kg total Ca, 60.9 g/kg ash, and 877.0 g/kg dry matter.

[3]Provided the following quantities per kg of complete diet: vitamin A, 8,575 IU; vitamin D_3, 4,300 IU; vitamin E, 28.58 IU; menadione, 7.30 mg; riboflavin, 9.15 mg; D-pantothenic acid, 18.33 mg; niacin, 73.5 mg; choline chloride, 1,285 mg; vitamin B_{12}, 0.02 mg; biotin, 0.09 mg; thiamine mononitrate, 3.67; folic acid, 1.65 mg; pyridoxine hydrochloride, 5.50 mg; I, 1.85 mg; Mn, 178.5 mg; Cu, 7.40 mg; Fe, 73.5 mg; Zn, 178.5 mg.

[4]5 g of TiO_2 mixed into 20 g ground corn.

[5]Ether extract.

[6]Non-phytate phosphorus.

MATERIALS AND METHODS

All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

Birds, Experimental Design, and Diets

Two experiments (**Exp.**) were conducted utilizing Cobb 500 male broiler chickens obtained at day of hatch from a commercial hatchery, individually tagged, and housed in temperature-controlled battery cages (model SB 4T, Alternative Design Manufacturing, Siloam Springs, AR). From d 0 to 21, birds were fed a commercial starter diet formulated to meet the nutrient requirements of broiler chickens (NRC, 1994; Cobb-Vantress, 2018). On d 21 post hatching in each study, birds were individually weighed and allotted into 1 of 4 dietary treatments in a randomized complete block design with BW as the blocking factor. Each dietary treatment contained 8 replications with 6 birds per cage (192 birds/Exp). Broiler chickens had free access to feed and water for the duration of the Exp.

In Exp. 1, experimental diets consisted of 4 corn-soybean meal (**SBM**)-based diets including a reference diet (**RD**) with corn, SBM, and soybean oil as the sole energy contributing ingredients formulated to contain 19.5 g/kg CP with the addition of crystalline amino acids (Table 1). The TD were created by adding 100, 200, or 300 g/kg solvent-extracted canola meal (**CM**), respectively, at the proportional expense of all other energy contributing ingredients in the RD (Table 1). Together the 4 dietary treatments for Exp. 1 were CM0, CM100, CM200, and CM300. Progressive additions of CM resulted in a reduction of formulated MEn values of the diets from 3,108 to 2,736 kcal/kg from CM0 to CM300, respectively. In Exp. 2, there were also 4 corn-SBM-based experimental diets, including a RD identically formulated to that of Exp. 1. The TD were created for this Exp. by adding 150, 300, or 450 g/kg of wheat at the proportional expense of all energy contributing ingredients in the RD (Table 2), similarly to Exp. 1. The experimental treatments for Exp. 2 were W0, W150, W300, and W450. The progressive addition of wheat elicited a more modest reduction in the formulated MEn in the TD (3,108 to 3,056 kcal/kg). Titanium dioxide was added to all diets to serve as an indigestible marker in both Exp. Canola meal was sourced from Consumers Supply Distributors (grown in South Dakota), and wheat was provided by the Purdue University Agronomy Department (grown in West-Central Indiana) and ground by hammer mill (6.4-mm screen).

Sample Collection and Chemical Analyses

For each Exp., on d 26 post hatching, BW of all birds and feed intake (**FI**) per cage were evaluated for the experimental period and used to determine the BW gain and feed efficiency. Birds were euthanized by CO_2

Table 2. Ingredient and calculated composition of diets (Experiment 2).

Ingredient, g/kg	W0[1]	W150	W300	W450
Ground corn	604.60	506.96	409.31	311.67
Wheat[2]	0.00	150.00	300.00	450.00
Soybean meal, 48% CP	293.90	247.95	202.01	156.06
Soybean oil	41.00	34.59	28.18	21.77
Ground limestone	11.00	11.00	11.00	11.00
Monocalcium phosphate	11.00	11.00	11.00	11.00
Salt	4.00	4.00	4.00	4.00
L-Lysine HCl	2.00	2.00	2.00	2.00
DL-Methionine	1.50	1.50	1.50	1.50
L-Threonine	1.00	1.00	1.00	1.00
Vitamin-mineral premix[3]	5.00	5.00	5.00	5.00
TiO_2 premix[4]	25.00	25.00	25.00	25.00
Total	1,000	1,000	1,000	1,000
Calculated composition, g/kg				
MEn, kcal/kg	3,108	3,091	3,074	3,056
CP	195	183	170	157
EE[5]	65.15	57.38	49.61	41.84
Ca	6.91	6.83	6.75	6.66
P	6.08	6.50	6.93	7.35
nPP[6]	3.65	3.48	3.31	3.14

[1]W0, W150, W300, and W450 represent inclusions of 0, 150, 300, and 450 g/kg wheat in the diets, respectively.

[2]Proximate analysis: 3,809 kcal/kg gross energy, 101.5 g/kg crude protein (N × 6.25), 22.3 g/kg crude fiber, 16.5 g/kg crude fat, 3.5 g/kg total P, 0.3 g/kg total Ca, 15.6 g/kg ash, and 868.0 g/kg dry matter.

[3]Provided the following quantities per kg of complete diet: vitamin A, 8,575 IU; vitamin D_3, 4,300 IU; vitamin E, 28.58 IU; menadione, 7.30 mg; riboflavin, 9.15 mg; D-pantothenic acid, 18.33 mg; niacin, 73.5 mg;choline chloride, 1,285 mg; vitamin B_{12}, 0.02 mg; biotin, 0.09 mg; thiamine mononitrate, 3.67; folic acid, 1.65 mg; pyridoxine hydrochloride, 5.50 mg; I, 1.85 mg; Mn, 178.5 mg; Cu, 7.40 mg; Fe, 73.5 mg; Zn, 178.5 mg.

[4]5 g of TiO_2 mixed into 20 g ground corn.

[5]Ether extract.

[6]Non-phytate phosphorus.

asphyxiation, dissected, and digesta were collected from the distal two-thirds of the ileum (i.e., from Meckel's diverticulum to approximately 20 mm proximal to the ileocecal junction). The ileal digesta were flushed with distilled water into plastic containers, pooled by cage and stored at −20°C until further analyses. Excreta were collected during the last 3 d of the experimental period, then both excreta and digesta were dried in a forced-air drying oven at 55°C until constant weight. Diets, excreta, and ileal digesta samples were ground (< 0.05 mm) by centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany). Experimental diets, excreta, and ileal digesta samples were analyzed for dry matter (**DM**) by drying at 105°C for 24 h in a forced-air drying oven (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006), gross energy (**GE**) by an isoperibol bomb calorimeter (Parr 6200; Parr Instrument Co., Moline, IL), N by a combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and Ti by a dry ashing digestion adapted from Short et al. (1996). The concentration of CP was calculated by multiplying 6.25 with the concentration of analyzed N in samples.

Calculations and Statistical Analysis

Calculations of GE digestibility were based on an index method suggested by Kong and Adeola (2014). The apparent ileal digestibility (**AID**) of GE and DM in the TI were calculated by the following equation:

$$AID,\ \% = \left[1 - \left(\frac{Mi}{Mo}\right) \times \left(\frac{No}{Ni}\right)\right] \times 100$$

where Mi and Mo represent the concentration of marker, that is, TiO_2 (g/kg DM) in experimental diets (input) and ileal digesta samples (output), respectively; Ni and No represent the concentration of nutrient (g/kg DM) in experimental diets (input) and ileal digesta (output) samples, respectively. The apparent total tract utilization (**ATTU**) of GE, DM, and N are calculated using the equation for AID values by replacing ileal digesta with excreta. The IDE and ME (kcal/kg DM) as well as retainable N (g/kg DM) in experimental diets are calculated as follows:

$$IDE\ (kcal/kgDM) = GEi \times (AID/100)$$

$$ME\ (kcal/kgDM) = GEi\ \times\ (ATTU/100)$$

$$retainable\ N\ (g/kgDM) = Ni\ \times\ (ATTU/100)$$

where GEi and Ni are the concentration of GE (kcal/kg DM) and N (g/kg DM) in experimental diets, respectively. The ME in experimental diets is corrected to zero N retention using a factor of 8.22 kcal/g (Hill and Anderson, 1958) to calculate the MEn (kcal/kg DM) in experimental diets as follows:

$$MEn\ (kcal/kgDM) = ME\ - (8.22\ \times retainable\ N)$$

Data were analyzed by ANOVA using GLM procedure of SAS (SAS Inst. Inc., Cary, NC.). Model included diets and block as independent variables. Linear and quadratic effects of graded concentration of TI were determined by orthogonal contrasts. Regression of the test ingredient-associated IDE, ME, or MEn intake in kilocalories against kilograms of test ingredient DM intake per cage of birds were conducted using multiple linear regression for each experiment using the following SAS statements: Proc GLM; class TI; Model Y = TI × DMintake/solution; the solution option was used to generate intercepts and slopes. Y is test ingredient-associated IDE, ME, or MEn intake in kilocalories, TI is test ingredient and DMintake is test ingredient intake in kilograms of DM, which was used as a regressor (Bolarinwa and Adeola, 2012). A total of 4 data sets were used to determine the IDE, ME and MEn of the TI. Set 1 contained all four diets while each subsequent set excluded one of the TD (Set 1 = RD, TD1, TD2, TD3; Set 2 = RD, TD1, TD2; Set 3 = RD, TD1, TD3; Set 4 = RD, TD2, TD3). This made set 1 a 4-point regression while all others represented different 3-point regressions. The experimental unit was the cage, and statistical significance was declared at $P \leq 0.05$ with a statistical trend at $0.05 < P \leq 0.10$.

RESULTS

Experiment 1

Increasing dietary CM concentrations did not produce any significant effect on the growth performance of broiler

Table 3. Growth performance of broilers fed diets with increasing concentrations of canola meal in Exp. 1 or wheat in Exp. 2.[1]

Diet	Initial BW, g	Final BW, g	BW gain, g	FI[2], g	G:F[3], g:kg
Canola meal (Exp. 1)					
CM0[4]	731	1,089	358	514	699
CM100	731	1,061	329	503	656
CM200	731	1,099	368	504	731
CM300	731	1,084	354	487	728
SEM	0.3	11.7	11.7	9.3	20.0
P-values					
Linear	0.335	0.642	0.624	0.069	0.084
Quadratic	0.485	0.551	0.538	0.706	0.337
Wheat (Exp. 2)					
W0[5]	730	1,061	331	509	649
W150	730	1,048	318	520	611
W300	729	1,033	303	508	597
W450	730	1,006	277	498	556
SEM	0.3	11.0	10.9	13.4	8.4
P-values					
Linear	0.477	0.002	0.002	0.447	< 0.001
Quadratic	0.628	0.543	0.533	0.432	0.871

[1]Values represent least squares means from 8 replicate cages.
[2]Feed intake.
[3]Gain:Feed ratio.
[4]CM0, CM100, CM200, and CM300 represent inclusions of 0, 100, 200, and 300 g/kg canola meal in the diets, respectively.
[5]W0, W150, W300, and W450 represent inclusions of 0, 150, 300, and 450 g/kg wheat in the diets, respectively.

chickens, however, there was a linear trend ($P = 0.07$) of decreasing feed intake with higher CM inclusions (Table 3). This produced a linear trend of an increase ($P = 0.08$) in the feed efficiency of birds as CM increased in the diet. Linear decreases ($P < 0.01$) in the AID of DM and GE were observed as dietary CM concentrations were increased, and this resulted in the subsequent decrease ($P < 0.01$) in the IDE of the TD from 3,760 to 3,090 kcal/kg DM (Table 4). Table 4 also displays that the metabolizability of DM, GE, and nitrogen were linearly decreased ($P < 0.01$) by increasing CM concentration. Linear decreases ($P < 0.01$) in the ME and MEn of the TD were observed as 3,635 to 3,013 kcal/kg DM and 3,462 to 2,840 kcal/kg DM, respectively.

The 4 data sets used to determine the energies of CM all displayed significant fits with a linear model. The IDE estimates produced were 1,560, 1,587, 1,613, and 1,624 kcal/kg DM for sets 1 to 4, respectively (Table 5). Estimates of the ME of CM ranged from 1,731 to 1,992 kcal/kg DM, however, when Set 2 (set without highest inclusion of TI) was excluded, the values ranged from only 1,731 to 1,793 kcal/kg DM (Table 5). The estimate produced by Set 2 was 13 % higher than the mean estimate from the other 3 sets. The estimates of MEn follow a similar pattern where the estimate from Set 2 was 11 % higher than the mean of the other sets (1,751 vs. 1,575 kcal/kg DM). For all energy metrics, Set 2 produced a greater SE of the estimate relative to the means of the other 3 sets (IDE, 254 vs. 188 kcal/kg DM; ME, 126 vs. 89 kcal/kg DM; MEn, 116 vs. 87 kcal/kg DM).

Experiment 2

Increases in the dietary concentration of wheat resulted in linear reductions ($P < 0.01$) in BW gain and feed efficiency of 331 to 277 g and 649 to 556 g:kg, respectively (Table 3). Linear reductions ($P < 0.05$) were observed in the AID of DM and GE as well as a reduction ($P < 0.01$) in the IDE of the TD from 3,413 to 3,136 kcal/kg DM as the inclusion of wheat increased (Table 4). Wheat also linearly reduced ($P < 0.01$) the metabolizability of DM and GE as well as produced a decrease ($P < 0.01$) in the utilization of nitrogen. These reductions resulted in linear decreases ($P < 0.01$) in the ME (3,580−3,312 kcal/kg DM) and MEn (3,444−3,208 kcal/kg DM) of the test diets (Table 4). Wheat resulted in more modest reductions in the ME (7.5 %) and MEn (6.9 %) of the TD compared the effects of CM, which were 17 and 20% for ME and MEn, respectively.

All data sets used for the determination of IDE, ME, and MEn of wheat displayed significant fits with a linear model. The estimates obtained from the 4 data sets for the IDE of wheat ranged from 2,911 to 3,245 kcal/kg DM, however, Set 2 yielded the highest estimate of 3,245 kcal/kg DM while the other 3 sets had a mean of 2,957 kcal/kg DM (Table 6). The SE of the IDE estimate from Set 2 was elevated compared to the other 3 sets (14%), however, it was not to the same magnitude as was observed for CM (35%). The estimates for ME and MEn varied less than IDE with ranges of 3,041 to 3,106 kcal/kg DM and 2,935 to 3,035 kcal/kg DM, respectively. Although the estimates of ME and MEn from Set 2 did not vary from the other 3, the SE of those estimates were larger relative to the mean of the other 3 sets: 131 vs. 96 kcal/kg DM and 124 vs. 91 kcal/kg DM, respectively. These derivations in the SE for the estimates of ME and MEn of wheat were similar to what was observed for CM in Exp. 1, with average increases of 25 and 27% for CM and wheat, respectively.

DISCUSSION

In Exp. 1, the diets with high inclusions of CM had very high CP concentrations, and this excess CP was

Table 4. Digestibility and metabolizability of parameters and for diets with increasing concentrations of canola meal or wheat.[1]

	Digestibility			Metabolizability				
Item:	Dry matter, %	Gross energy, %	IDE[2], kcal/kg DM	Dry matter, %	Gross energy, %	Nitrogen, %	ME, kcal/kg DM	MEn, kcal/kg DM
Canola meal (Exp. 1)								
CM0[3]	79.0	79.9	3,760	74.8	77.8	71.2	3,635	3,462
CM100	75.2	76.3	3,603	68.5	72.2	64.5	3,410	3,243
CM200	67.9	69.6	3,308	65.0	69.0	63.6	3,278	3,093
CM300	63.7	65.4	3,090	59.3	63.8	56.9	3,013	2,840
SEM	0.96	0.83	37.7	0.44	0.40	0.99	18.9	18.6
P-values								
Linear	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic	0.865	0.712	0.418	0.497	0.588	0.989	0.298	0.386
Wheat (Exp. 2)								
W0[4]	69.5	72.0	3,413	71.6	75.7	61.3	3,580	3,444
W150	69.1	71.3	3,345	70.4	74.1	61.8	3,478	3,345
W300	68.2	70.6	3,297	69.2	73.5	59.3	3,407	3,282
W450	66.2	68.2	3,136	68.9	72.0	54.5	3,312	3,208
SEM	1.11	1.08	50.3	0.67	0.54	1.17	25.7	24.3
P-values								
Linear	0.040	0.019	0.001	0.003	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic	0.511	0.444	0.362	0.507	0.951	0.026	0.910	0.612

[1]Values represent least squares means from 8 replicate cages.
[2]Ileal digestible energy.
[3]CM0, CM100, CM200, and CM300 represent inclusions of 0, 100, 200, and 300 g/kg canola meal in the diets, respectively.
[4]W0, W150, W300, and W450 represent inclusions of 0, 150, 300, and 450 g/kg wheat in the diets, respectively.

likely metabolized for energy, a process that is less efficient than the metabolism of starches and fats, which may help to explain the linear reductions of DM and GE metabolizability as CM increased in the diets (D'Mello, 2003). Furthermore, the observed reduction in the nitrogen metabolizability can be largely explained by the digestibility of nearly all dispensable and indispensable amino acids being lower for CM compared with SBM (Kim et al., 2012), and the CP digestibility was reduced by progressive additions of these less available amino acids at the expense of those from SBM. In Exp. 2, progressive additions of dietary wheat produced linear decreases in the metabolizability of DM, GE, and nitrogen. The reduction in the metabolizability of GE was relatively modest (75.7−72.0%) and can be primarily attributed to the lower ME of wheat compared with corn (Rostagno, 2017), and the reductions in nitrogen and DM metabolizability can be contributed to that, as well as the reduction in dietary CP.

The estimates of ME and MEn obtained from the current studies are largely in agreement with previous work evaluating CM and wheat. The average ME and MEn obtained from Exp. 1 were 1,824 and 1,619 kcal/kg DM, respectively for CM. Adewole et al. (2017) reported that the AMEn of CM ranged from 1,691 to 2,041 kcal/kg DM based on the evaluation of CM obtained from 6 Canadian canola seed processing plants, while Woyengo et al. (2010) reported average AMEn values of 1,801 and 2,694 kcal/kg DM for solvent extracted and expeller extracted canola meals, respectively. The ME results from Exp. 1 fall within these reported ranges, however, some authors report the ME or AMEn to range from 2,000 to 2,486 kcal/kg DM (NRC, 1994; Zhang and Adeola, 2017; Veluri and Olukosi, 2020). One explanation for these values being greater than what was observed in the current study is the fat content of the CM evaluated. The CM used by Zhang and Adeola (2017) was reported to have a fat content of 10.5%, while the CM used in the current study was analyzed to contain 3.46% crude fat, and subsequently the AMEn values observed were different. Adewole et al. (2017) reported on CM that ranged from 1.6 to 4.9% crude fat, and produced estimates close to those of the current study. This highlights the importance of fat content on the energy of CM. There is a great deal of variation in the ME and MEn reported for CM, and this is likely due to the different processing techniques used in the manufacture of CM that can result in variable concentrations of residual canola oil and other nutrients (Woyengo et al., 2010). Solvent-extracted CM, as used in this experiment, have approximately 3 to 4% residual oil, and are the result of a multi-step process typically performed in the crushing plant prior to crushing. Expeller-extracted CM can be even more variable in nutrient content and may range from 8 to 20% residual oil, as this process is often performed in smaller facilities and with variations in the conditions and equipment used (Spragg and Mailer, 2007). This can result in large deviations in ME of CM from the various processing techniques and may be partially

Table 5. Ileal digestible energy, metabolizable energy, and nitrogen corrected metabolizable energy of canola meal (Experiment 1).

Item:	Slope[1], kcal/kg DM	SE, kcal/kg DM	Intercept[2], kcal	SE, kcal
Set 1 (4 points)				
IDE[3]	1,560	184	14.57	14.86
ME	1,793	91	1.39	7.43
MEn	1,594	86	1.53	7.06
Set 2 (CM300[4] omitted)				
IDE	1,587	254	13.28	14.47
ME	1,992	126	-3.81	7.29
MEn	1,751	116	-2.56	6.71
Set 3 (CM200[5] omitted)				
IDE	1,613	174	14.18	13.64
ME	1,731	88	-1.05	7.02
MEn	1,545	89	-0.33	7.05
Set 4 (CM100[6] omitted)				
IDE	1,624	205	2.83	18.46
ME	1,780	88	4.20	7.87
MEn	1,587	85	3.14	7.60

[1]Estimate of the slope of the regression of the test ingredient-associated energy and the test ingredient DM intake.
[2]Estimate of the Y-intercept of the regression between test ingredient-associated energy and the test ingredient DM intake.
[3]Ileal digestible energy.
[4]300 g/kg inclusion of canola meal.
[5]200 g/kg inclusion of canola meal.
[6]100 g/kg inclusion of canola meal.

responsible for the wide range of reported energies for this ingredient.

It should be noted that due to the excessive protein present in these diets (particularly in the CM 200 and CM300 diets) the ME values may be underestimated due to the evaluation being done under excess protein resulting in exorbitant catabolism of protein, which may result in the under partitioning of dietary energy assigned to CM. This could have also been exacerbated by the diets with the highest inclusions of CM containing suboptimal concentrations of dietary energy overall. As discussed below, greater emphasis should be placed on the N-corrected values for CM obtained in the current study.

The results for ME and MEn for wheat obtained from Exp. 2 were largely in agreement with previously published works that report ME and AMEn ranging from 2,600 to 3,411 kcal/kg DM (NRC, 1994; Gutierez del Alamo et al., 2008; Karunaratne et al., 2018; Lu et al., 2020). Bolarinwa and Adeola (2012) reported an IDE of 3,413 kcal/kg DM for wheat which is much greater than the average value obtained from the current experiment of 3,029 kcal/kg DM. This difference may be due to a difference in wheat cultivar used in the evaluation or the region in which the wheat was produced as this can result in variations of ME and other nutrient contents (Kim et al 2003; Gutierez del Alamo, 2008). It is also possible that the values obtained in this Exp. were lower because of the imbalance of energy and protein caused by the progressive additions of wheat lowering the CP of the diets. This is the inverse of the excessive protein catabolism in the CM study but may still help to lend explanation to these slightly lower than average ME and MEn values. However, the findings for ME and MEn

Table 6. Ileal digestible energy, metabolizable energy, and nitrogen corrected metabolizable energy of wheat (Experiment 2)

Item:	Slope[1], kcal/kg DM	SE, kcal/kg DM	Intercept[2], kcal	SE, kcal
Set 1(4 points)				
IDE[3]	2,980	147	7.54	18.18
ME	3,096	95	3.35	12.02
MEn	3,018	90	3.00	11.25
Set 2 (W450[4] omitted)				
IDE	3,245	175	-4.77	15.17
ME	3,041	131	5.76	11.77
MEn	2,935	124	6.70	11.03
Set 3 (W300[5] omitted)				
IDE	2,911	154	6.56	18.52
ME	3,106	85	1.60	10.55
MEn	3,035	81	1.78	9.93
Set 4 (W150[6] omitted)				
IDE	2,980	159	6.48	21.86
ME	3,072	108	9.88	15.14
MEn	2,996	102	8.70	14.36

[1]Estimate of the slope of the regression of the test ingredient-associated energy and the test ingredient DM intake.
[2]Estimate of the Y-intercept of the regression between test ingredient-associated energy and the test ingredient DM intake.
[3]Ileal digestible energy.
[4]300 g/kg inclusion of wheat.
[5]200 g/kg inclusion of wheat.
[6]100 g/kg inclusion of wheat.

from the current studies fall comfortably in the range of reported data for wheat as well as for CM.

The diets in each of the current studies had significantly imbalanced amino acid and N contents, and as discussed briefly above, this can potentially affect estimated ME values. Correcting ME to zero N retention (MEn) is one way to account for these differences, however, because of the large deviations in N metabolizability it may be prudent to consider standardized ME (**MEs**) values for this study (Cozannet et al., 2010; Noblet et al., 2022). The MEs corrects for a 50% N retention, rather than zero N retention like that of MEn, and may better correct for modern production conditions. It has been argued that this metric more closely corresponds to the practical N retention of broilers being fed complete diets, which may well be true for the current study (Cozannet et al., 2010). Standardized ME has been proposed for use when considering feed ingredients across different poultry species (for example broilers vs. turkeys) and production types (for example broilers vs. layers) and may prove explanatory for the current study, due to the amino acid and N imbalance of the diets (Noblet et al., 2022).

The MEs values for each study were calculated using Set 3 (without the second highest inclusion; CM0, CM100, and CM300 in Exp.1 or W0, W150, and W450 in Exp.2) for the purpose of discussion. The MEs estimates for CM and wheat were 1,480 ± 117.0 and 3,060 ± 89.1 kcal/kg DM, respectively. For CM, the ME, MEs, and MEn values were 1,731, 1,480, and 1,545 kcal/kg DM, respectively with the MEs value lower than the ME and MEn estimates, while for wheat the ME, MEs, and MEn values were 3,106, 3,060, and 3,035 kcal/kg DM, with the MEs estimate intermediate to that of ME and MEn. There were differences in grams of N retention between the 2 studies with birds in the wheat study retaining approximately 65% of the N retained by birds in the CM study resulting in greater correction of ME for N retention in the CM study. Thus, ME correction for N retention resulted in 98 or 97% of ME for MEs or MEn, respectively in the wheat study, but 85 or 89% of ME for MEs or MEn, respectively in the CM study. In Exp. 2, the N retention of the 4 diets continuously decreased as both the N content of the diet and N metabolizability decreased with progressive additions of wheat, and the correction for 50% N retention did not cause large changes in these corrections. This was not the case for Exp. 1, where the N metabolizability was decreased in parallel with an increase in the N content of the diets, meaning the N retention of the broilers was relatively consistent as CM increased in the diet. However, when the correction was made for 50% N retention, the calculated N retention for each diet increased with the inclusion of CM. This caused a more disproportionate correction from ME to MEs in the higher substituted diets and subsequently reduced the slope (estimate) of the regression for MEs. Furthermore, if the MEs value is calculated by a difference method it averages 1,594 kcal/kg DM across all 3 substitution levels, which would more closely resemble the results observed for wheat. Because of these factors, it is difficult to determine if the use of MEs values is appropriate in the context of this study, or if it provides any additional utility beyond what is provided by correction for zero N retention. Perhaps the value of MEs estimates lies more with experimental diets that are better balanced for amino acids and N.

The design of the current study utilized 4 inclusion levels of each TI. This allowed for the use of 4 separate data sets with which to regress the TI associated energy against the TI intake and estimate the IDE, ME, and MEn of the TI. In Exp. 1, IDE estimates produced from all sets only had a range of 64 kcal/kg, while ME and MEn had much larger ranges. This difference was exacerbated by Set 2, as its estimates of ME and MEn were approximately 200 kcal/kg DM greater than the average of the estimates for the other 3 sets, though this result was not observed for IDE. This could be due to the influence of excessive excess protein in the CM200 and CM300 diets effecting the relative ME of the TI (Kim et al., 2022), however, it could also be an artifact of the current study. The increase in estimate is not necessarily indicative of an inaccurate estimate, however, the increase in the estimate was accompanied by a marked increase in the SE of the estimates for IDE, ME, as well as MEn for Set 2, relative to the other sets. The SE of the estimates was increased by an average of 40% for all parameters. Set 2 excluded the highest inclusion level of the TI, while it was included in all other sets. This gave Set 2 a smaller total range of inclusions compared to the other 3 sets, and this may lend explanation to the increase in the variability of the estimates. In all evaluations of ingredient energy, the concentration of the TI in the TD is important to capturing the natural variation of an ingredient. Olukosi (2021) evaluated SBM with both 3- and 4-point models using the regression method and found no significant effect of the number of inclusions on the AME estimate obtained, though this author did not report the individual SE of the regressions. However, it was observed that inclusions of SBM less than 300 g/kg using the direct method produced more variable results compared to larger inclusions. This could lend further explanation to why excluding the CM300 diet created greater variability in the estimate of ME.

This relationship was continued for Exp. 2, where there was much higher variation in the energy estimates for Set 2, relative to the means of the other sets. However, for the IDE, ME, and MEn of wheat, excluding the highest concentration of the TI did not yield numerically greater estimates relative to the other 3 sets, as was the case for CM. This difference is difficult to explain, however, it could be related to the inherent differences in the energy digestibility of protein concentrates compared with cereal grains. Additionally, it could be related to the overall higher inclusion levels that were used for wheat in Exp. 2 compared to the lower concentrations used for CM. Nevertheless, the increase in the SE of the estimates for Set 2 was still present and followed the same trend as in in Exp. 1. The reasoning for this increase is likely the same as explained for CM, where the smaller total range of intakes was responsible for the increase in variability, due to the lessoned ability to explain variation in the model. This further reinforces the notion that the concentration of test ingredient used in the evaluation of ingredient energy is paramount and has the potential to affect the obtained results (Olukosi, 2021). Furthermore, it appears that this phenomenon may be present in a variety of ingredient categories as CM and wheat represent two very different feed classes but showed very similar responses to ingredient concentration. There were some differences in the response of IDE between the 2 test ingredients. For CM, the omission of the highest concentration of the TI resulted in elevations in the estimate for ME and MEn, however, this response was not observed for IDE. For wheat, the inverse was observed, where ME and MEn were not elevated by the omission, but IDE was increased in similar magnitude to the ME in the first Exp. This may be a result of different N metabolism states caused by the 2 sets of ingredients or may have been due to the relative inclusion level of wheat being higher than that of CM, especially when the highest concentration was omitted. It is difficult to interpret these results as there is still a deficit of understanding in the exact nature of the relationship between IDE and ME.

The data from the current study largely support the use of a 3-inclusion level model for the determination of the energy values of feed ingredients. There were issues with increased estimates and variability, however, these only arose when the largest concentrations of the test ingredients were omitted. This is similar to observation by Larbier and Leclercq (1992), who found that the standard errors of the linear regressions being used for the difference method became greater when smaller TI inclusions were utilized relative to larger ones. For CM, the estimates for Sets 1, 3, and 4 differed only 62 kcal/kg for ME and the SE of the estimates for both experiments varied less than 11% for these three sets. There were only issues present when the largest concentration was omitted. This is advantageous for executing the regression method, as it means 3 diets can be used, so long as the TI is included in high enough concentration. This is supported by the findings of Olukosi (2021), who reported that there were no differences between 3- and 4-point models covering the same total range of intakes. A possible advantage of the regression method is the use of an intermediate intake of the test ingredient, which in turn allows for the evaluation of energy across a range of intakes and at practical inclusion levels. This can be especially important for oil seeds and protein concentrates, as their energy values can be incorrectly estimated with higher than practical inclusions, such as in using the direct method. Therefore, in theory, the regression method has the potential to produce estimates of energy with low associated standard errors, relative to other methods. Additionally, utilizing 3 diets rather than 4 will be valuable as it will require less birds and time, and therefore help lower costs.

Based on the data generated in these studies, the regression method was shown to be an effective method with which to estimate the IDE, ME, and MEn of CM and wheat, as current estimates are in agreement with previous work utilizing other methods. There is also evidence that the use of a 3-point model with the regression method is appropriate and not significantly improved by the addition of a subsequent inclusion level. This requires, however, that the concentration of the TI used is high enough to properly capture the variation of reasonable intakes to avoid elevated errors for the estimates.

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DISCLOSURES

The authors declare no conflicts of interest.

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