

# METABOLISM AND NUTRITION

## Net energy prediction and energy efficiency of feed for broiler chickens

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**ABSTRACT** Global consumption of chicken meat has increased at a faster rate than any other animal protein source, and thus refinements in energy formulation techniques for feed have continued to gain importance. Formulation of animal feed based on net energy (NE) has been implemented in ruminants and pigs but not in poultry. A closed-circuit respiratory calorimetry system was employed on 25- to 28-day-old broilers fed 19 diets formulated with varying nutrient composition to produce equations to predict NE and apparent metabolizable energy (AME) efficiency of feed for broiler chickens. Performance, energy and N balance, respiratory quotient, and energy utilization were measured in the

birds. Linear regression analysis was performed to generate prediction equations for dietary energy content and AME efficiency. The NE content was positively related to AME and ether extract, but negatively to crude protein. The study generated equations that can accurately predict NE, and NE/AME using AME value and chemical composition of feeds. The NE prediction equations were further validated on a separate set of diets with high correlation ( $r = 0.99$ ) and accuracy. The outcomes are an important step for the broiler industry to adapt to an NE system in place of AME systems for the formulation of broiler chicken feeds following robust validation experiments.

**Key words:** net energy, metabolizable energy, energy efficiency, heat increment, broiler chickens

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## INTRODUCTION

Energy is a major cost component of broiler feed. In recent years, the energy cost of ingredients has become increasingly variable as a result of increased demand and biofuel production. It is now correlated to energy yielding non-agricultural commodities (Yu et al., 2006; Harri et al., 2009). As such, refinements in energy formulation techniques have gained importance. Currently, most broiler feeds are formulated on the basis of apparent or, to an extent, true metabolizable energy, corrected to zero nitrogen retention (**AMEn**, **TMen**). As growing broilers are indeed retaining nitrogen, it has been proposed that correction to 50% nitrogen retention as standard AME (AMEs) may be more representative of the metabolic situation (Cozannet et al., 2010). Formulation of broiler feed on a net energy (NE) basis may further enhance efficiency and profitability as it accounts for the energy lost as heat or heat increment

(HI) evolved during metabolism of nutrients. Resultant formulations may therefore more accurately meet the energy requirement of the bird and thereby reduce over- or underformulation of energy required for production and maintenance. The NE/AME ratio (or efficiency of AME for NE) has been quantified in poultry (Fraps and Carlyle 1942; de Groot 1974; Swick et al., 2013). In a recent study, the efficiency of AMEn utilization for NE in growing chicks has been determined to be 78, 85, and 68% for carbohydrates, fat, and protein, respectively (Carre et al., 2014); the corresponding HI values were then 22, 15, and 32% of AME, respectively. Such results reinforce the justification for proposing an NE system for poultry feeds.

The NE of a feed is equal to its AME minus HI. It can also be described as the energy used for maintenance (i.e., fasting heat production [**FHP**]) plus the energy retained in BW gain or as eggs in poultry (Noblet et al., 2010a). Heat increment is the heat produced by a fed animal in excess of that associated with its FHP, FHP being measured according to different methods or simply estimated from the literature (Noblet et al., 2015; Liu et al., 2017a). Energy retention can be measured over time directly in carcasses of birds fed a known

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amount of AME (Carre et al., 2014). Energy retention can also be determined using indirect calorimetry by measuring gaseous exchange and calculating heat production (**HP**) by the Brouwer equation (Brouwer 1965; McLean 1972), where energy retention is then equal to the difference between AME intake and HP. Net energy of diets thus calculated can be used to generate NE prediction equations with regression models to estimate NE of both diets and ingredients (Fraps 1946; Pirgozliev and Rose 1999). Overall, NE calculation methods rely on values of FHP (Noblet et al., 2015). In addition, when determining HP and HI in fed birds, environmental temperature, age and genotype of birds, housing conditions, amount of feed consumed and feed texture (mash vs. pellets) must be accurately controlled or standardized as these factors influence HP and HI (Zhou and Yamamoto 1997; Koh and Macleod 1999).

Prediction equations estimating HI and NE are based on chemical composition of the diets and/or digestibility of various feed components similar to the way it has been done in pigs (Noblet et al., 1994). In spite of differences in efficiencies of AME utilization for NE of nutrients, Carre et al. (2014) proposed more sophisticated equations involving digestible CP to AMEn ratio in a linear or quadratic relationships to predict NE in chickens. In other studies, the variation of AME efficiency for NE was not found to be related to feed nutrient composition, e.g., fat, protein, and fiber (Noblet et al., 2010b). This lack of nutrient effect on energy efficiency may have been due to genetics, environmental conditions, accuracy of HP measurement, number of observations, and limited effect of the diets used in the studies. The accuracy of the measured values may also be questionable as the diets used in the study were nutritionally imbalanced (Liu et al., 2017b). Literature data can sometimes be conflicting and thus a simple prediction equation for the estimation of NE values of poultry feed ingredients has been desirable through more complete design of the experiment and accurate measurement of gaseous exchange.

The present study was undertaken to generate a prediction equation for NE in fast growing modern broilers fed balanced diets varying in chemical composition. The resulting NE equations were examined for prediction accuracy using a different set of nutritionally balanced or imbalanced diets.

## MATERIALS AND METHODS

### Birds

The study was approved by the Animal Ethics Committee of the University of New England and designed to follow the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC 2013).

Prior to each calorimetry run (15), 40 male Ross 308 day-old chicks were purchased from a commercial

hatchery (Baiada Poultry, Tamworth, NSW, Australia). Chick brooding and rearing conditions followed Ross 308 management guidelines (Aviagen 2014). Birds were reared in floor pens until 22 d for acclimatization and measurements (Swick et al., 2013). On day 22, 2 birds were moved into each of 16 calorimeter chambers with lids open and air pumps running in a climate controlled room for acclimatization. Test diets were fed during this period. Feed and water were provided ad libitum. Energy balance and respiratory measurements were taken from day 25 to 28.

### Experiments and Diets

The starter diet (day 0 to 10) was based on maize, wheat, sorghum, soybean meal, canola meal, meat and bone meal, and canola oil and was pelleted and crumbled. Calculated nutrient content (DM basis) was as follows: 14.1 MJ/kg AMEn, 252 g/kg CP, 14.3 g/kg digestible lysine, 10.6 g/kg digestible methionine + cysteine, 11.8 g/kg Ca, and 5.6 g/kg non-phytate phosphorus (**NPP**). A common standard grower diet was fed to birds until day 18 and based on wheat, sorghum, canola meal, meat and bone meal, and canola oil in pellet form. Calculated nutrient content (DM basis) was 14.8 MJ/kg AMEn, 247 g/kg CP, 124 g/kg digestible lysine, 9.4 g/kg digestible methionine + cysteine and 9.0 g/kg Ca, and 4.5 g/kg NPP.

Experiment 1 was performed with a single diet, 4 replicates per run with 6 runs conducted for a total of 24 replicates. The first experiment was used to determine NE variation between chambers. Experiment 2 was performed with 16 diets per run and 8 replicate runs and was used to measure the NE content of diets formulated for different nutrient composition. Experiment 3 was performed with 2 diets, 8 replicates each in a single run with 16 chambers. This final experiment was designed to have high and low NE/AMEn diets so that the variation in NE effected by different CP and ether extract (**EE**) contents of feed could be assessed. Table 1 shows the summarized information of ingredients and nutrient composition of diets used in the 3 experiments. A wide range of pre-analyzed ingredients was chosen to formulate diets varying in the levels of CP, starch, fiber components, lipid, and estimated AMEn. Although the levels of nutrients varied, diets were formulated to meet or exceed the Ross 308 nutrient specifications (Aviagen 2014) for digestible amino acids, essential fatty acids, AME, vitamins and minerals, except 4 diets with imbalanced nutrients used in the validation of prediction equations as described later. Total amino acid content of each ingredient was measured (AOAC 2016), and the Evonik AminoDat 4.0 standard ileal amino acid digestibility coefficients (**SIAAD**) were applied to calculate SIAAD levels of diets.

**Table 1.** Ingredients and nutrient composition of diets (g/kg DM basis unless noted).

| Experiment  | 1     | 2     | 3     |       |       |       |
|---|-------|-------|-------|-------|-------|-------|
|   |       | Mean  | Min   | Max   | 3-1   | 3-2   |
| 2-1 to 2-16 (n = 16)                                  |       |       |       |       |       |       |
| Diet  | 1-1   |       |       |       |       |       |
| <i>Ingredients</i>                                    |       |       |       |       |       |       |
| Wheat   | 648   | 256.5 | 0     | 700.8 | 252.5 | 200.0 |
| Corn  | 0     | 206.4 | 0     | 612.9 | 285.2 | 171.8 |
| Sorghum   | 0     | 79.9  | 0     | 599.4 | 0     | 150.0 |
| Peas  | 0     | 49.6  | 0     | 543.0 | 0     | 80.0  |
| Soybean meal  | 193   | 209.8 | 0     | 389.5 | 367.2 | 178.2 |
| Meat meal   | 53.4  | 11.8  | 0     | 41.1  | 0     | 27.5  |
| Wheat pollard   | 0     | 23.4  | 0     | 150.0 | 0     | 0     |
| Oat groats  | 0     | 17.5  | 0     | 100.0 | 0     | 100.0 |
| Canola meal solvent                                   | 52.4  | 59.1  | 0     | 200.0 | 50.1  | 30.0  |
| Canola meal expeller                                  | 0     | 6.3   | 0     | 100.0 | 0     | 0     |
| Canola oil  | 38.0  | 25.9  | 0     | 64.2  | 0.1   | 36.8  |
| Cottonseed oil  | 0     | 0.4   | 0     | 4.9   | 0     | 0     |
| Oat hulls   | 0     | 5.0   | 0     | 7.0   | 0     | 0     |
| Rice hulls  | 0     | 0     | 0     | 0     | 20    | 0     |
| Wheat starch  | 0     | 6.3   | 0     | 100.0 | 0     | 0     |
| Canola seed   | 0     | 8.8   | 0     | 140.0 | 0     | 0     |
| Wheat gluten  | 0     | 6.3   | 0     | 70.0  | 0     | 0     |
| Dicalcium phosphate                                   | 0     | 4.71  | 0     | 8.86  | 6.52  | 2.66  |
| Limestone   | 4.50  | 9.34  | 5.70  | 11.98 | 10.38 | 8.15  |
| L-lysine HCl, 78.4%                                   | 2.27  | 2.22  | 0     | 3.17  | 0     | 3.10  |
| D,L-methionine, 99%                                   | 2.19  | 2.59  | 0.69  | 4.81  | 0     | 3.25  |
| L-threonine, 99%                                      | 1.31  | 1.52  | 0     | 4.87  | 0     | 1.84  |
| Salt  | 1.67  | 2.11  | 1.63  | 2.80  | 2.42  | 1.88  |
| Sodium bicarbonate                                    | 1.50  | 2.09  | 2.00  | 3.50  | 2.00  | 2.00  |
| Vitamin premix <sup>1</sup>                           | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  |
| Trace mineral premix <sup>2</sup>                     | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  |
| Phytase 5000FTU/g                                     | 0     | 0.10  | 0.10  | 0.10  | 0.10  | 0.10  |
| Xylanase 40,000 BXU/g <sup>3</sup>                    | 0     | 0.06  | 0     | 0.50  | 0.05  | 0.05  |
| Choline 70%   | 0.37  | 0.77  | 0.08  | 1.45  | 0.35  | 0.85  |
| <i>Nutrients assayed</i>                              |       |       |       |       |       |       |
| GE, MJ/kg   | 19.05 | 19.30 | 18.52 | 19.93 | 18.52 | 19.60 |
| AMEn, MJ/kg   | 12.59 | 13.25 | 11.15 | 15.46 | 12.21 | 13.50 |
| Arginine  | 16.1  | 13.6  | 11.8  | 1.8   | 13.65 | 13.14 |
| Lysine  | 14.3  | 13.5  | 12.2  | 15.7  | 13.76 | 13.36 |
| Methionine  | 5.81  | 6.0   | 5.1   | 6.9   | 6.23  | 6.30  |
| Cysteine  | 4.0   | 3.7   | 2.5   | 4.7   | 3.49  | 3.48  |
| Tryptophan  | 3.13  | 3.0   | 2.3   | 4.1   | 2.40  | 2.39  |
| Isoleucine  | 10.51 | 9.1   | 8.1   | 11.5  | 9.28  | 8.47  |
| Threonine   | 9.95  | 9.9   | 8.8   | 14.0  | 9.17  | 9.34  |
| Valine  | 12.3  | 10.5  | 9.0   | 13.0  | 11.03 | 9.67  |
| CP  | 246   | 232   | 185   | 299   | 276   | 221   |
| EE  | 50    | 55    | 27    | 89    | 16    | 66    |
| Crude fiber   | 33    | 33    | 24    | 50    | 38    | 25    |
| Ash   | 55    | 52    | 47    | 62    | 63    | 47    |
| NSP total   | 94    | 98    | 82    | 135   | 115   | 82    |
| NSP soluble   | 12    | 10    | 6     | 12    | 12    | 11    |
| NSP insoluble   | 81    | 81    | 75    | 124   | 103   | 71    |
| NDF   | 119   | 110   | 71    | 182   | 109   | 108   |
| ADF   | 47    | 57    | 38    | 88    | 63    | 46    |
| Starch  | 367   | 413   | 304   | 472   | 370   | 460   |
| Sugars  | 41    | 44    | 33    | 64    | 48    | 33    |
| <i>Nutrients calculated, as fed basis<sup>4</sup></i> |       |       |       |       |       |       |
| AMEn MJ/kg  | 12.66 | 12.95 | 11.3  | 14.02 | 11.72 | 13.6  |
| dig Arginine  | 11.8  | 12.4  | 10.2  | 16.7  | 15.4  | 11.4  |
| dig Lysine  | 11.0  | 11.1  | 9.8   | 12.8  | 11.8  | 11.0  |
| dig Methionine + cystine                              | 8.0   | 8.4   | 7.5   | 8.9   | 8.4   | 8.4   |
| dig Tryptophan  | 2.6   | 2.4   | 1.8   | 3.3   | 3.0   | 2.0   |
| dig Isoleucine  | 7.4   | 7.9   | 6.7   | 10.2  | 9.7   | 7.5   |
| dig Threonine   | 7.3   | 7.5   | 6.5   | 11.0  | 7.3   | 7.3   |
| dig Valine  | 8.8   | 9.2   | 7.9   | 11.8  | 11.1  | 8.4   |
| Calcium   | 7.2   | 8.0   | 8.0   | 8.0   | 8.0   | 8.0   |
| Phosphorus, available                                 | 3.6   | 4.0   | 4.0   | 4.0   | 4.0   | 4.0   |

Abbreviations: ADF, acid detergent fiber; AMEn, apparent metabolizable energy corrected with N retention; CP, crude protein; dig, digestible; DM, dry matter; EE, ether extract or fat; GE, gross energy; HCl, Hydrochloric acid; NDF, neutral detergent fiber; NSP, non-starch polysaccharides;

<sup>1</sup>Vitamin premix supplied the following per kg of diet: A 13,000 IU; D3 5,000 IU; E 80 IU; K 3.2 mg; thiamin 3.2 mg; riboflavin 8.6; niacin 60 mg; pantothenic acid 17; pyridoxine 5.4 mg; biotin 0.30 mg; folic acid 2.20 mg; cyanocobalamin 0.017 mg.

<sup>2</sup>Trace mineral premix supplied the following per kg of diet: Cu 16 mg; I 1.25 mg; Fe 20 mg; Mn 120 mg; Se 0.30 mg; Zn 110 mg.

<sup>3</sup>All diets containing wheat or wheat by-products were supplemented with xylanases except 1-1.

<sup>4</sup>g/kg, unless indicated; digestibility coefficients from AminoDat 4.0 applied to raw materials; dig = digestible.

For the validation of prediction equations, 16 additional diets were used with 12 reported previously (Swick et al., 2013). Among those, 4 diets were nutritionally imbalanced: high AMEn (14.1 MJ/kg) and low CP (17.9%); high EE (10.9%), high CP (25.2%), and low AMEn (12.2 MJ/kg); doubled Na level (0.35%); extremely high lysine (3.84% L-lysine HCl).

### **Analysis of Diets and Excreta**

Feed intake (**FI**) was measured, and total excreta was collected daily in each calorimetry chamber. Excreta was collected daily, mixed, and weighed after 3 d collections. All the variables were adjusted to a total of 72 h for calculation. Feed and excreta were thoroughly homogenized with subsamples taken for analysis. Feed samples were analyzed per as-is basis and results were expressed on DM basis. 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 was freeze dried to a constant weight for gross energy (**GE**) and N analysis. Wet excreta DM was calculated by correction for the loss of moisture during both freeze- and oven-drying. Gross energy was analyzed using an adiabatic bomb calorimeter (IKA® Werke, C7000, GMBH and CO., Staufen, Germany). Feed samples were analyzed for CP, EE, crude fiber (**CF**), ash, neutral detergent fiber (**NDF**) and acid detergent fiber (**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).

### **Respiratory Chambers and Measurements of O<sub>2</sub> Consumption, CO<sub>2</sub> Expiration, and Performance**

Closed-circuit calorimetry chambers were similar in design as those described by Farrell (1972) with modifications as described by Swick et al. (2013). Briefly, the chamber bases were constructed of stainless steel and were 100 cm long × 76 cm high × 70 cm wide and each were equipped with a wire-mesh cage (89 cm long × 60 cm high × 60.5 cm wide) suspended above a removable, stainless steel excreta collection tray. Water was used to seal the chamber by sitting a transparent polycarbonate chamber lid into the trough sited at the top rim of the chamber base. The pressure was controlled using a barometric sensor connected to an electronic switch that activated a solenoid valve to control oxygen release. Temperature and humidity in each chamber were monitored constantly using temperature and humidity sensors with electronic display and memory capabilities. A 28 L/min diaphragm air pump first circulated chamber air through a rubber cork-capped plastic bottle containing 2.0 L of a 320 g/kg

KOH solution and a bubbler assembly to absorb CO<sub>2</sub> exhaled by the birds, after which the air was passed through a trap containing 3 kg of dried silica gel to absorb humidity, before being returned to the chamber. Humidity was maintained at less than 70% for the entire run and CO<sub>2</sub> concentrations were maintained at less than 10 mL/L. Medical-grade O<sub>2</sub> was provided by equipping each chamber with a 490-L cylinder fitted with a regulator and a reducing valve to replenish the volume of O<sub>2</sub> consumed in the chamber by the birds.

Oxygen consumption was measured by subtracting the weight of the O<sub>2</sub> cylinder at the end of each run from the weight of the cylinder at the beginning of each run. The density of O<sub>2</sub> (1.331 g/L) at normal temperature and pressure (defined as 20°C and 101.325 kN/m<sup>2</sup>) was used for the conversion of weight (g) to volume (L). Subsamples of KOH from each chamber were taken after the solution from each KOH bottle was made up to 2.0 L. The recovery of CO<sub>2</sub> was estimated according to the method described by Annison and White (1961) based on a BaCl<sub>2</sub> precipitation technique. One milliliter of KOH solution was accurately pipetted into a dried and pre-weighed 15 mL centrifuge tube in duplicate. Subsequently, 1.5 mL of NH<sub>4</sub>Cl was added to each tube. The solution was gently swirled and mixed thoroughly. After the addition of 5 mL of BaCl<sub>2</sub> to the tubes, the mixture was centrifuged for 15 min at 2,800 *g*. The supernatant from each tube was carefully decanted and the carbonate pellet was then resuspended in 5 mL of distilled water, followed by centrifugation for 30 min at 2,800 *g*. The supernatant was subsequently decanted and the tubes were dried overnight at 105°C. Finally, tubes were cooled in a desiccator and accurately weighed to record the BaCO<sub>3</sub> recovered from the 1.00 mL aliquot of KOH solution. The CO<sub>2</sub> exhaled by the birds was then calculated by multiplying the weight of BaCO<sub>3</sub> (extrapolated yield from 2 L KOH) by 0.2229 (the molecular weight of CO<sub>2</sub> divided by that of BaCO<sub>3</sub>). The density of CO<sub>2</sub> (1.842 g/L) at normal temperature and pressure (defined as 20°C and 101.325 kN/m<sup>2</sup>) was used for the conversion of weight (g) to volume (L).

### **AME, Total HP, and NE**

AME was determined by the total collection method previously described by Bourdillon et al. (1990) with modifications. Values were corrected to zero nitrogen retention (AMEn) using 8.22 kcal/g of N (or 34.4 kJ/g) as the correction factor where AMEn was used (Hill and Anderson 1958). Total HP was calculated for 3 d by measuring O<sub>2</sub> consumption and CO<sub>2</sub> production in sealed chambers as described. Each day during the collection period, the system was suspended for about 2 h for replenishing feed, water, KOH, and silicone gel and for excreta collection. Heat production values were obtained by applying chamber CO<sub>2</sub> and O<sub>2</sub> data to the modified Brouwer equation (Brouwer 1965; McLean

1972) by removing methane and N in expired gas from the equation as:

$$\text{Total heat (kcal)} = 3.866 \times \text{oxygen consumed (L)} \\ + 1.200 \times \text{CO}_2 \text{ exhaled (L)}$$

The respiratory quotient (**RQ**) of the birds was calculated as the ratio of CO<sub>2</sub> volume exhaled to O<sub>2</sub> volume consumed by birds. Heat increment was calculated by subtracting FHP from total HP. The 450 kJ/BW<sup>0.70</sup> FHP estimate of Noblet et al. (2015) was used in the calculations; it corresponds to the asymptotic HP (at zero activity) over a 24 h fasting. NE was calculated as AME intake minus HI divided by feed consumed.

## Statistical Analyses

The performance and nitrogen balance data were expressed per bird per day, with FI and FCR reported on a DM basis. Energy balance data were expressed per bird per kg BW<sup>0.70</sup> per day due to the linear correlation between FHP and BW<sup>0.70</sup> of growing broiler chickens (Noblet et al., 2015). Energy values diets were expressed per kg DM, and energy utilization data were expressed as percent. All data analyses were performed using SAS software, version 9 (SAS Institute Inc., Cary, NC).

PROC GLM was used to determine the effects of: (a) replicate runs in experiment 1; (b) replicate runs and diets in experiment 2; and (c) diets in experiment 3. The model was:

$$Y_{ij} = \mu + a_i + b_j + \epsilon_{ij}$$

where Y<sub>ij</sub> is the response expected independent variables,  $\mu$  is the mean,  $a_i$  is the effect of diet,  $b_j$  is the effect of replicate run, and  $\epsilon_{ij}$  is the random error.  $a_i$  and  $b_j$  were excluded in experiment 1 and 3, respectively, owing to the lack of such effects. Tukey's range test of 2 separate means was performed to determine the differences between diets in experiment 2. Differences were considered significant at  $P < 0.05$ .

PROC CORR procedure was performed to determine the correlations between the nutrients of diets used for regression analysis to produce the NE prediction equation. The diets were formulated to minimize the correlations between the energy-yielding nutrients so that the effect of the nutrients could be established in the regression analysis. PROC CORR procedure was also used to determine the correlation level between the predicted and measured NE values of the diets with balanced and imbalanced nutrients in the validation process.

PROC REG was used for assessing the energy contribution of nutrient compositions to the energy content

of diet by multiple linear regression with or without intercept ( $\beta_0$ ). The regression model was:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n + \epsilon$$

where  $x_1, x_2, \dots, x_n$  are the amount of chemical components;  $\beta_1, \beta_2, \dots, \beta_n$  are the partial coefficients of respective chemical components; and  $\epsilon$  is the corresponding random error.

PROC REG was performed to predict energy efficiency and energy contents of diets by their chemical components using stepwise linear regression with an intercept where the significance threshold for inclusion and exclusion of the variables was set to  $P < 0.10$ . The regression model was:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n + \epsilon$$

where  $x_1, x_2, \dots, x_n$  are the amount of chemical components;  $\beta_1, \beta_2, \dots, \beta_n$  are the partial coefficients of respective chemical components; and  $\epsilon$  is the corresponding random error.

## RESULTS

### Performance, Dietary Characteristics and Energy Balance

The performance data showed that growth of birds in all the 3 trials was above the Ross performance objectives of Ross 2014 (Aviagen 2014). In experiment 1, variability (as RSD) for the measured parameters, HP and AME intake, was low indicating the uniformity of the test system. In experiments 2 and 3, the results showed that diet significantly affected bird performance. Interestingly, AME intakes were not affected, but BW, FI, FCR, daily NE intake, AME/BW gain, and NE/BW gain were affected by diet in experiments 2 and/or 3 ( $P < 0.05$ ) (Table 2). Further, nitrogen (g/bird/d) and energy balances (kJ/kg BW<sup>0.70</sup>/d) in birds were affected by diet ( $P < 0.001$ , or 0.01), but not HI and HP. Respiratory quotients averaged 1.029 within the range of 0.975 to 1.090. As expected, diets with higher EE levels resulted in lower RQ relative to those with lower EE levels (data not shown).

As indicated in Table 3, the measured chemical components (DM basis) of the diets were in agreement with those calculated during formulation. The AMEn means of each diet ranged from 11.66 to 15.02 MJ/kg, CP from 18.5 to 29.9%, EE from 1.6 to 11.1%, starch from 30.4 to 47.2%, and CF from 2.4 to 5.0% (DM basis). The efficiencies of energy utilization of the diets were measured in each experiment (Table 4). The efficiency of GE for AME was 73% with the lowest mean being 65% and highest mean being 80%. The energy difference from

**Table 2.** Effect of diet composition on performance, energy balance, and energy efficiency of diets in broilers.

| Item   | Experiment 1 |       | Experiment 2 |       |                                  |                         | Experiment 3 |       |                         |
|--|--------------|-------|--------------|-------|----------------------------------|-------------------------|--------------|-------|-------------------------|
|  | Mean         | RSD   | Mean         | RSD   | Replicate run ( <i>P</i> -value) | Diet ( <i>P</i> -value) | Mean         | RSD   | Diet ( <i>P</i> -value) |
| <b>Growth performance</b>                        |              |       |              |       |                                  |                         |              |       |                         |
| BW, g  | 1,523        | 32    | 1,666        | 103   | <0.001                           | <0.01                   | 1,782        | 50    | NS                      |
| FI, g DM/d                                       | 144          | 11    | 153          | 11    | <0.01                            | <0.001                  | 150          | 10    | <0.05                   |
| BW gain, g/d                                     | 104          | 9     | 112          | 10    | <0.001                           | NS                      | 114          | 14    | NS                      |
| FCR (g/g DM)                                     | 1.388        | 0.095 | 1.366        | 0.088 | <0.05                            | <0.001                  | 1.322        | 0.074 | NS                      |
| AME intake (kJ/d)                                | 1,935        | 128   | 2,131        | 144   | NS                               | NS                      | 2,087        | 142   | NS                      |
| NE intake (kJ/d)                                 | 1,423        | 109   | 1,592        | 116   | <0.05                            | <0.01                   | 1,528        | 109   | NS                      |
| AME/BW gain, kJ/g                                | 18.66        | 1.28  | 19.09        | 1.17  | <0.001                           | <0.001                  | 18.46        | 1.05  | <0.01                   |
| NE/BW gain, kJ/g                                 | 13.73        | 1.04  | 14.25        | 0.88  | <0.01                            | <0.001                  | 13.53        | 0.88  | <0.01                   |
| <b>Nitrogen balance, g/d</b>                     |              |       |              |       |                                  |                         |              |       |                         |
| Intake   | 6.00         | 0.47  | 5.69         | 0.41  | <0.01                            | <0.001                  | 5.91         | 0.39  | <0.001                  |
| Retained   | 3.63         | 0.26  | 3.50         | 0.23  | <0.01                            | <0.001                  | 3.38         | 0.23  | <0.01                   |
| <b>Energy balance, kJ/kg BW<sup>0.70</sup>/d</b> |              |       |              |       |                                  |                         |              |       |                         |
| AME intake                                       | 1,443        | 83    | 1,496        | 120   | <0.01                            | <0.001                  | 1,392        | 73    | NS                      |
| HP   | 832          | 30    | 826          | 23    | <0.001                           | NS                      | 823          | 34    | <0.05                   |
| RE   |              |       |              |       |                                  |                         |              |       |                         |
| Total  | 611          | 72    | 665          | 80    | <0.001                           | <0.001                  | 570          | 60    | NS                      |
| As protein                                       | 403          | 26    | 365          | 28    | <0.001                           | <0.001                  | 336          | 18    | <0.001                  |
| As fat   | 208          | 56    | 300          | 58    | <0.01                            | <0.001                  | 234          | 54    | <0.01                   |
| HI   | 382          | 30    | 381          | 53    | NS                               | NS                      | 373          | 34    | <0.05                   |
| NE intake  | 1,061        | 72    | 1,118        | 97    | <0.01                            | <0.001                  | 1,020        | 60    | NS                      |
| <b>Respiratory quotient</b>                      | 1.023        | 0.023 | 1.029        | 0.023 | <0.001                           | <0.001                  | 1.028        | 0.029 | NS                      |

Abbreviations: AME, apparent metabolizable energy; BW, body weight; DM, dry matter; FCR, feed conversion ratio; FI, feed intake; GE, gross energy; HI, heat increment; HP, heat production; NE, net energy; RE, energy retention; RSD, residual standard deviation.

**Table 3.** Main measured characteristics of the diets used in the NE prediction equations.

| Item                          | Mean  | N   | Measurement range <sup>1</sup> |       | Mean range <sup>2</sup> |       |
|-------------------------------|-------|-----|--------------------------------|-------|-------------------------|-------|
|                               |       |     | Min                            | Max   | Min                     | Max   |
| Diets composition, % DM basis |       |     |                                |       |                         |       |
| Ash                           | 5.26  | 19  | 4.66                           | 6.26  | —                       | —     |
| CP                            | 23.5  | 19  | 18.5                           | 29.9  | —                       | —     |
| EE                            | 5.32  | 19  | 1.61                           | 8.90  | —                       | —     |
| Starch                        | 41.1  | 19  | 30.4                           | 47.2  | —                       | —     |
| CF                            | 3.30  | 19  | 2.36                           | 5.00  | —                       | —     |
| NDF                           | 11.08 | 19  | 7.05                           | 18.22 | —                       | —     |
| ADF                           | 5.67  | 19  | 3.79                           | 8.75  | —                       | —     |
| NSP                           | 9.80  | 19  | 8.15                           | 13.51 | —                       | —     |
| Sugars                        | 4.34  | 19  | 3.31                           | 6.42  | —                       | —     |
| Energy values, MJ/kg DM       |       |     |                                |       |                         |       |
| GE                            | 19.26 | 19  | 18.52                          | 19.93 | —                       | —     |
| AME                           | 13.99 | 135 | 11.84                          | 16.34 | 12.36                   | 15.87 |
| AMEn                          | 13.20 | 135 | 11.15                          | 15.46 | 11.66                   | 15.02 |
| AMEs                          | 13.85 | 135 | 11.77                          | 16.12 | 12.29                   | 15.68 |
| NE                            | 10.43 | 135 | 8.55                           | 12.87 | 9.10                    | 12.00 |
| Energy utilization, %         |       |     |                                |       |                         |       |
| AME/GE                        | 72.6  | 135 | 62.8                           | 82.0  | 65.5                    | 79.6  |
| AMEn/GE                       | 68.5  | 135 | 59.1                           | 77.6  | 61.8                    | 75.3  |
| AMEs/GE                       | 71.8  | 135 | 62.4                           | 80.9  | 65.1                    | 78.6  |
| NE/AME                        | 74.5  | 135 | 62.8                           | 82.0  | 71.4                    | 76.2  |
| NE/AMEn                       | 78.9  | 135 | 71.2                           | 83.2  | 76.0                    | 80.1  |
| NE/AMEs                       | 75.2  | 135 | 67.1                           | 80.4  | 71.5                    | 77.3  |

Abbreviations: ADF, acid detergent fiber; AME, apparent metabolizable energy; AMEn, AME corrected with N retention; AMEs, AME corrected with 50%; CF, crude fiber; CP, crude protein; DM, dry matter; EE, ether extract or fat; GE, gross energy; MJ, megajoules; NDF, neutral detergent fiber; NE, net energy; NSP, non-starch polysaccharides.

<sup>1</sup>Mean and range of individual data (n = 135).

<sup>2</sup>Range of means per diet (n = 19).

GE to AME is represented as fecal organic dry matter and urinary energy losses of mainly uric acid. The efficiency of AME for NE ranged from 71 to 76% with a mean of 74%. The energy difference between AME and NE was through HI as a result of digestion, metabolism,

and activity. Across tested diets, the efficiencies of GE conversion to AMEn were lower than GE to AME and similarly, AMEn to NE were higher than AME to NE due to the correction to zero nitrogen retention. The efficiencies of conversion of GE to AMEs were close to

**Table 4.** Correlations between nutrient parameters of 19 diets used for the prediction of net energy values.

| Nutrients   |         | Ash    | CP     | EE     | Starch | CF     | NDF    | ADF    | Free sugars | NSP    | GE     | AME    |
|-------------|---------|--------|--------|--------|--------|--------|--------|--------|-------------|--------|--------|--------|
| CP          | r       | 0.492  |        |        |        |        |        |        |             |        |        |        |
|             | P-value | <0.05  |        |        |        |        |        |        |             |        |        |        |
| EE          | r       | -0.229 | -0.348 |        |        |        |        |        |             |        |        |        |
|             | P-value | NS     | NS     |        |        |        |        |        |             |        |        |        |
| Starch      | r       | -0.730 | -0.755 | 0.073  |        |        |        |        |             |        |        |        |
|             | P-value | <0.001 | <0.001 | NS     |        |        |        |        |             |        |        |        |
| CF          | r       | 0.088  | 0.136  | -0.285 | -0.350 |        |        |        |             |        |        |        |
|             | P-value | NS     | NS     | NS     | NS     |        |        |        |             |        |        |        |
| NDF         | r       | 0.088  | 0.436  | -0.482 | -0.495 | 0.630  |        |        |             |        |        |        |
|             | P-value | NS     | NS     | <0.05  | <0.05  | <0.01  |        |        |             |        |        |        |
| ADF         | r       | 0.028  | 0.320  | -0.521 | -0.313 | 0.736  | 0.668  |        |             |        |        |        |
|             | P-value | NS     | NS     | <0.05  | NS     | <0.001 | <0.01  |        |             |        |        |        |
| Free sugars | r       | 0.705  | 0.329  | -0.155 | -0.611 | 0.207  | 0.357  | 0.093  |             |        |        |        |
|             | P-value | <0.001 | NS     | NS     | <0.01  | NS     | NS     | NS     |             |        |        |        |
| NSP         | r       | 0.451  | 0.423  | -0.496 | -0.613 | 0.677  | 0.840  | 0.538  | 0.635       |        |        |        |
|             | P-value | NS     | NS     | <0.05  | <0.01  | <0.01  | <0.001 | <0.05  | <0.01       |        |        |        |
| GE          | r       | -0.249 | -0.062 | 0.922  | -0.073 | -0.270 | -0.320 | -0.362 | -0.170      | -0.420 |        |        |
|             | P-value | NS     | NS     | <0.001 | NS     | NS     | NS     | NS     | NS          | NS     |        |        |
| AME         | r       | -0.237 | -0.460 | 0.826  | 0.373  | -0.559 | -0.810 | -0.736 | -0.246      | -0.717 | 0.710  |        |
|             | P-value | NS     | <0.05  | <0.001 | NS     | <0.05  | <0.001 | <0.001 | NS          | <0.001 | <0.001 |        |
| NE          | r       | -0.297 | -0.552 | 0.844  | 0.418  | -0.496 | -0.786 | -0.699 | -0.255      | -0.703 | 0.701  | 0.987  |
|             | P-value | NS     | <0.05  | <0.001 | NS     | <0.05  | <0.001 | <0.001 | NS          | <0.001 | <0.001 | <0.001 |

Abbreviations: ADF, acid detergent fiber; CF, crude fiber; CP, crude protein; EE, ether extract or fat; GE, gross energy; AME, apparent metabolizable energy; NDF, neutral detergent fiber; NE, net energy; NS, not significant; NSP, non-starch polysaccharides.

those of GE to AME, and the efficiencies of conversion of AMEs to NE were close to those of AME to NE.

Covariate analysis indicated no differences between experiments for performance or energy partitioning of diets. As such, the data from all 3 experiments were combined for the regression of chemical components. The combination of diets from experiments 1, 2, and 3 was used to perform regression as an objective of the study. Maximum nutrient levels varied between diets to provide enough variation to allow for significant regression analysis and to allow predictions to be used in practical situations. Minimum and maximum nutrient levels are shown in Table 1. The correlation analysis between chemical components of 19 diets indicated minimal correlations due to unavoidable relationships as shown in Table 4. As expected, CF was positively correlated to ADF ( $R = 0.736$ ;  $P < 0.001$ ), NDF ( $R = 0.630$ ;  $P < 0.01$ ), and NSP ( $R = 0.667$ ;  $P < 0.01$ ). Interestingly, EE was not correlated with CP or starch but starch was negatively correlated with CP ( $R = -0.755$ ;  $P < 0.001$ ). Therefore, regression equations to predict energy using chemical components could be generated.

### **Prediction of GE, AME, NE and Energy Efficiency by Dietary Nutrients**

Linear regression was performed to compute the contributions of each energy yielding chemical component to the energy values of diets (Table 5). As shown, the coefficients of CP for dietary energy were reduced from 0.239 for GE ( $SE = 0.003$ ) to 0.129 for AME ( $SE = 0.017$ ) and further to 0.064 for NE ( $SE = 0.029$ ), which were 46 and 50% reduction of CP contributions from GE to AME and from AME to NE respectively. Starch

also showed slightly reduced coefficients with those for AME being 94% of the coefficients for GE, and further reduction from AME to NE (79%). In contrast, the energy coefficient of EE for AME (0.429) was higher than that for GE (0.393) being 109%. The coefficient of EE for NE remained high at 0.364 being 85% of AME. Similar effects of energy yielding components of diets on AMEn and AMEs are shown in Table 5. As expected, CP coefficients for AMEn were lower than those for AME and AMEs due to correction for zero N retention.

The development of the prediction equations for dietary energy content and the efficiency values were achieved by stepwise regression against the chemical components and AME values of experimental diets (Tables 6 and 7). The results showed that EE positively affected AME/GE, while CP and CF content negatively affected AME/GE. In contrast to the significant effect of multiple variables on AME/GE, only CP and EE showed significant negative and positive effects, respectively, on NE/AME. When AMEn or AMEs was used instead of AME, the effects of nutrients were rather similar.

The effects of EE, starch, and fiber content (CF or NDF and ADF) were significant in the prediction of AME. For NE prediction, AME positively affected NE as expected (Table 7). Whereas CP was negatively related, EE was positively related to NE estimated by the equation.

### **Validation of Prediction Equation for NE and Ingredient NE Prediction**

Prediction equations for NE were validated with a series of 16 additional diets. Twelve of these were

**Table 5.** Contributions of diet energy-yielding nutrients (% DM basis) to gross energy, AME, AMEn, AMEs, and NE (MJ/kg DM basis) in broilers.<sup>1</sup>

| Equation no | Energy | Equation |       |        |             |       |       | RSD   |
|-------------|--------|----------|-------|--------|-------------|-------|-------|-------|
|             |        | CP       | EE    | Starch | Free sugars | Res1  | Res2  |       |
| 1           | GE     | 0.239    | 0.393 | 0.176  | 0.166       | 0.175 |       | 0.109 |
| 2           |        | 0.239    | 0.393 | 0.176  |             |       | 0.174 | 0.105 |
| 3           | AME    | 0.129    | 0.429 | 0.166  | 0.340       | 0.027 |       | 0.482 |
| 4           |        | 0.137    | 0.446 | 0.163  |             |       | 0.069 | 0.496 |
| 5           | AMEs   | 0.140    | 0.411 | 0.161  | 0.289       | 0.025 |       | 0.462 |
| 6           |        | 0.148    | 0.427 | 0.159  |             |       | 0.064 | 0.475 |
| 7           | AMEn   | 0.112    | 0.411 | 0.161  | 0.289       | 0.025 |       | 0.462 |
| 8           |        | 0.120    | 0.427 | 0.159  |             |       | 0.064 | 0.475 |
| 9           | NE     | 0.064    | 0.364 | 0.131  | 0.251       | 0.026 |       | 0.367 |
| 10          |        | 0.071    | 0.378 | 0.128  |             |       | 0.060 | 0.380 |

Abbreviations: AME, apparent metabolizable energy; AMEn, AME corrected with N retention; AMEs, AME corrected with 50% N retention; CP, crude protein; DM, dry matter; EE, ether extract or fat; GE, gross energy; MJ, mega joules; NE, net energy; Res, residue; Res1, the organic matter in the diets except CP, EE, starch and free sugars; Res2, Res1 and free sugars; RSD, residual standard deviation.

<sup>1</sup>The analysis was performed using linear regression without intercept with means of the measurements on 19 diets.

**Table 6.** Prediction of efficiencies of GE for AME and AME for NE in broilers (AME/GE, AMEn/GE, AMEs/GE, NE/AME, NE/AMEn; NE/AMEs; %) from diet composition (% DM basis).<sup>1</sup>

| Equation no | Equation <sup>2</sup> |      |       |      |       | RSD  |
|-------------|-----------------------|------|-------|------|-------|------|
|             | Intercept             | CP   | EE    | CF   |       |      |
| 11          | AME/GE                | 88.6 | -0.52 | 0.70 | -2.32 | 2.91 |
| 12          | AMEn/GE               | 85.4 | -0.57 | 0.69 | -2.20 | 2.80 |
| 13          | AMEs/GE               | 85.8 | -0.43 | 0.65 | -2.20 | 2.79 |
| 14          | NE/AME                | 79.2 | -0.26 | 0.26 |       | 1.50 |
| 15          | NE/AMEn               | 81.9 | -0.18 | 0.25 |       | 1.59 |
| 16          | NE/AMEs               | 81.7 | -0.35 | 0.32 |       | 1.51 |

Abbreviations: AME, apparent metabolizable energy; AMEn, AME corrected for zero N retention; AMEs, AME adjusted corrected for 50% N retention; CF, crude fiber; CP, crude protein; EE, ether extract or fat; GE, gross energy; NE, net energy; RSD, residual standard deviation.

<sup>1</sup>From 135 measurements on 19 diets.

**Table 7.** Prediction of AME, AMEn, AMEs, and NE content (MJ/kg DM basis) of broilers diets from chemical composition (% DM basis).<sup>1,2</sup>

| Equation no | Energy (MJ/kg DM) | Equation  |       |       |       |        |       |        |        |      |     | RSD  |
|-------------|-------------------|-----------|-------|-------|-------|--------|-------|--------|--------|------|-----|------|
|             |                   | Intercept | AME   | AMEn  | AMEs  | CP     | EE    | Starch | CF     | NDF  | ADF |      |
| 17          | AME               | 11.57     | —     | —     | —     | 0.325  | 0.046 | -0.364 |        |      |     | 0.55 |
| 18          |                   | 14.34     | —     | —     | —     | 0.236  | 0.034 | -0.146 | -0.119 | 0.48 |     |      |
| 19          | AMEn              | 10.37     | —     | —     | —     | 0.319  | 0.054 | -0.326 |        |      |     | 0.53 |
| 20          |                   | 13.05     | —     | —     | —     | 0.235  | 0.025 | -0.139 | -0.106 | 0.46 |     |      |
| 21          | AMEs              | 11.75     | —     | —     | —     | 0.308  | 0.040 | -0.352 |        |      |     | 0.53 |
| 22          |                   | 14.37     | —     | —     | —     | 0.224  | 0.012 | -0.142 | -0.108 | 0.46 |     |      |
| 23          | NE                |           | 0.781 |       |       | -0.028 | 0.029 |        |        |      |     | 0.21 |
| 24          |                   |           |       | 0.808 |       | -0.017 | 0.031 |        |        |      |     | 0.21 |
| 25          |                   |           |       |       | 0.808 | -0.039 | 0.031 |        |        |      |     | 0.21 |

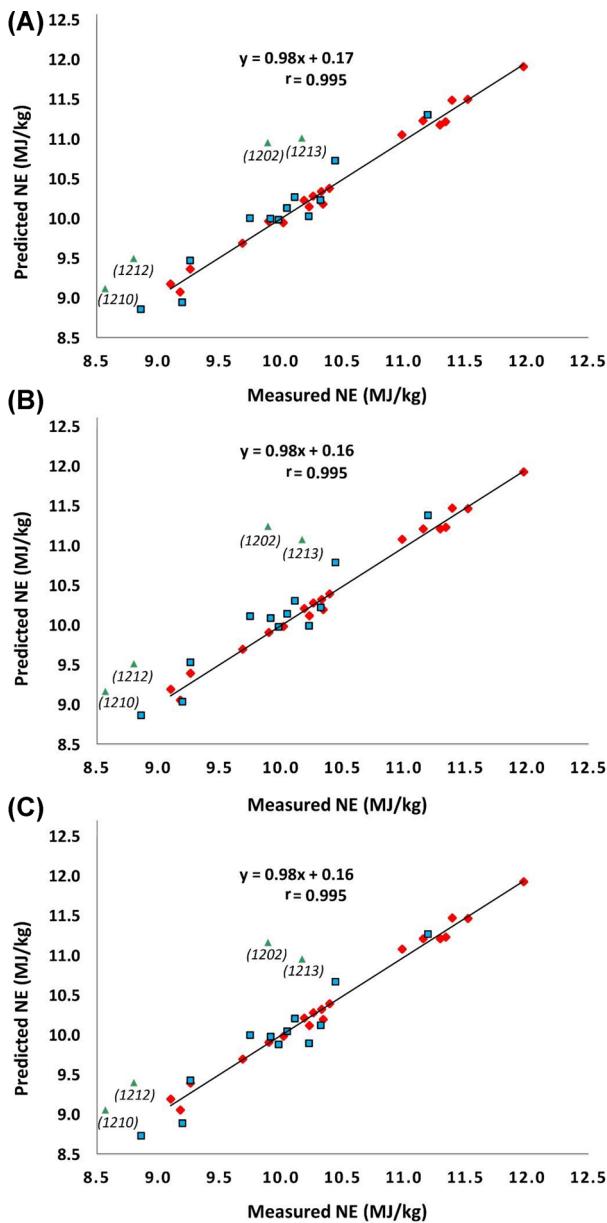
ADF, acid detergent fiber; AME, apparent metabolizable energy; AMEn, AME corrected for N retention; AMEs, AME corrected for 50% N retention; CF, crude fiber; CP, crude protein; DM, dry matter; EE, ether extract or fat; GE, gross energy; MJ, mega joules; NDF, neutral detergent fiber; NE, net energy; RSD, residual standard deviation.

<sup>1</sup>From 135 measurements on 19 diets.

<sup>2</sup>Composition is expressed as % of DM.

balanced and had sufficient required nutrients, and 4 of these were imbalanced with poor performance or low RQ indicating low metabolic rate. The NE values of these diets were measured to compare with their predicted NE values using CP and EE as well as AME (Figure 1a), AMEn (Figure 1 b), and AMEs (Figure 1c) values in the prediction equations. The predicted and measured NE values of 12 balanced diets were close,

whereas the predicted and measured NE values of 4 imbalanced diets were divergent. Among these 4 imbalanced diets, diet 1202 had high AME (14.06 MJ/kg) and low CP (17.9%) compared to Ross 308 nutrition specifications (Aviagen) with AME of 12.97 MJ/kg and CP of 21.5%. This led to very little growth of the birds (average daily BW gain of 16 g/d) and very poor FCR (5.32). Diet 1212 had Na levels above requirement



**Figure 1.** Validation of the NE prediction equations by the comparisons between predicted and measured NE values of the 19 diets used for stepwise regression to produce the prediction equations (♦), 12 independent diets with balanced nutrients (□), and 4 independent diets with imbalanced nutrients (▲) where numbers denoting individual diets. NE of independent diets were predicted using regression equations nos 23, 24, 25 and based on AME (a), AMEn (b), and AMEs (c) together with CP and EE. The measured values were the means obtained by the measurements in the birds fed the corresponding diets with at least 4 replicate measurements in the closed-circuit calorimetry chambers.

(0.35% vs. 0.16% of Ross 308 nutrition specification) that resulted in wet excreta and lower AME intake ( $1,259 \text{ kJ/d/BW}^{0.70}$ ), although the daily BW gain (92 g/d) and FCR (1.34) appeared to be normal. Diet 1213 was formulated with excess L-lysine HCl (3.84% vs. Ross 308 nutrition specification of 1.29%) and consequently produced wet excreta, low average daily BW gain (56 g/d) and high FCR (1.95). Diet 1210 had relatively high EE content (10.9%), high CP (25.2% vs. Ross 308 nutrition specification of 21.5%)

but low AME (12.18 MJ/kg vs. Ross 308 nutrition specification of 12.97 MJ/kg) leading to low RQ (0.93). However, apparent performance deficiency was not observed in the birds fed this diet with an FI of 151 g/d, BW gain of 107 g/d, and FCR of 1.43.

## DISCUSSION

As has been indicated earlier, the performance data showed that growth of birds in all the 3 experiments was as expected. The GE utilization for AME (AME/GE) averaged 73% with a range of 66–80% for the 19 diets used. The range of GE efficiency for AME observed in this study is in agreement with those observed in other studies (Hughes et al., 2001; Carré et al., 2013; Liu et al., 2016). The AME/GE was found to be positively dependent on EE and starch, and negatively dependent on CP and CF, which is consistent with high digestibilities of EE and starch and poor digestibility of the dietary fiber fraction in broiler chickens; the negative effect of CP is directly dependent on the urinary excretion of absorbed protein in excess of requirements. Nitrogen retention in birds in the current study was also within the expected range (3.1 to 4.0 g, mean 3.5 g) per bird per day. The N retention coefficients were 51 to 71% (mean 62%) and were similar to previously reported values (Ao et al., 2009; Cozannet et al., 2010). Gross energy contributions from CP, EE, and carbohydrates were consistent with the direct combustion analyses performed using pure products (Kienzle et al., 2001) and the prediction equation reported previously (Tran and Sauvant 2004), indicating accurate laboratory analyses of dietary GE and proximate analysis. The in vitro and in vivo data collected in this study provide a foundation for the accurate prediction of NE and utilization of AME for NE (NE/AME).

The linear regression prediction of feed AME was achieved using CP, EE, and starch content of the feed. The AME values of these energy yielding components agree with those reported previously (Naber and Touchburn 1969; Sibbald and Kramer 1978; Wiseman et al., 1986; NRC 1994), further indicating the accuracy of laboratory analyses on GE, CP, EE, and starch of feed and excreta as described above. The extra caloric value of EE demonstrated as the energy efficiency of GE for AME was approximately 109%, being consistent with previous reports in the literature (Mateos and Sell 1980; Pesti and Smith 1984; Murugesan et al., 2013; Cao and Adeola 2015; Poorghassemi et al., 2015). This has been attributed to improved digestion of other nutrients by additional EE (Mateos and Sell 1980). While starch GE was utilized as AME to a great extent (94%), the protein AME was only 54% of GE (12.9 MJ/kg/23.9 MJ/kg), likely due to digestive and metabolic losses in the case of excessive CP supply. As has been reported elsewhere and observed in the current study, the CP (or N) retention in a balanced diet for meat chickens is >60% (Ao, et al., 2009). This

suggests that utilization of protein (or AA) for protein accretion or as energy for maintenance or activities requires relatively more energy compared to EE or starch. The lower AME value for CP of 12.9 MJ/kg obtained in the current study is likely the reflection of what truly occurs *in vivo* and does not agree with higher CP AME values of 16.1 MJ/kg inferred from previously determined AME prediction equations (Vohra 1966; Leeson and Summers, 2001).

The NE/AME ratio represents the efficiency of AME utilization for NE. In the current study, the measured ratio in the experimental diets varied between 0.71 and 0.76 and showed the ratio to vary with changes in dietary levels of CP and EE. This is comparable to reported ratios between 0.73 and 0.80 (Carre, et al., 2014) and between 0.74 and 0.76 (Liu, et al., 2017b). High protein and low EE levels in a diet leads to low utilization of AME for NE, meaning high energy loss as HI. This conclusion in broiler chickens is in agreement with the observation in growing pigs (Noblet, et al., 1994) with overall lower ME/NE in chickens than pig: CP (49.6% vs 58.2%), EE (84.8% vs 89.8%) and starch (78.9% vs 82.3%). Carre, et al. (2014) examined digestible nutrient effects on NE/AME and found consistent AME for NE values as those in the current study: starch, 0.781 (digestible) vs. 0.785 (crude), and fat, 0.849 (digestible) vs. 0.848 (crude). However, the NE/AME values for CP differed between the 2 studies: 0.680 (digestible) vs. 0.518 (crude). This suggests that similar efficiencies between digestible and crude fat or starch may be due to their relatively high digestibility in broilers at the age of the assay (Abdollahi, et al., 2013), whereas NE/AME efficiency of CP is lower than that of digestible protein. This is likely due to differences in metabolism and heat production between undigested and digested amino acids (Yang, et al., 2008).

The current study shows that NE for broilers can be predicted from AME, EE, and CP. Both AME and EE increase the value of NE while CP reduces NE. It has been reported by others (Carre, et al., 2014) that in addition to AMEn, CP was positively related to NE. Interestingly, higher AME efficiency for NE of digestible EE than other nutrients was reported in the same study (Carre, et al., 2014), but the effect of EE in the NE/AME prediction was not observed. Emmans (1994), however, suggested that CP was negatively related to NE (effective energy) as inferred from the study of Hartel (1977). Neither study detected effects of dietary EE on NE for chickens. No dietary EE nor CP effects were detected on NE/AME in the initial report of Noblet et al. (2010b), but later results showed significant EE effects (Noblet, personal communication). In growing pigs, EE also showed positive and CP showed negative effects on NE in addition to starch and fiber (Noblet et al., 1994). This agrees with the findings from the current study for EE and CP. Such agreement observed in growing pigs (Noblet et al., 1994) and broilers (this study) suggests the importance of dietary EE and CP as contributory factors to HI of feeding. It

is plausible that fiber may not predict NE in broilers as its digestion is limited (Carré et al., 1990) and contribution as an energy source is also limited as shown in this study (2.7 MJ/kg AME and 2.6 MJ/kg NE in contrast to GE 17.5 MJ/kg). In addition, the current prediction equation was developed using diets that contained enzymes (fiber degrading enzymes and phytase). This meant that any anti-nutritive effects of soluble fiber on nutrient digestion and gut health, and perhaps on NE, were minimized. Dietary starch however was found to be correlated to CP ( $R = -0.755$ ) and less difference between its contribution to AME and NE (16.6 vs. 13.1 MJ/kg), compared to CP (12.9 and 6.4 MJ/kg), may lead to its removal from the equation during the stepwise regression process. In addition, variation in starch levels across diets may not have been large enough to show significant effects. Nevertheless, when the accuracy of the prediction equation was validated in a different set of diets and performance measurements, there was a high degree of accuracy between predicted and measured NE values of diets balanced in nutrients. However, NE values of nutritionally imbalanced diets could not be accurately predicted, presumably due to anomalous metabolic activities in the birds (D'Mello 1994). This is evidenced by limited FI and growth and high (poor) FCR in some of those diets. It can be concluded that the prediction equations can accurately predict NE or NE/AME values of diets with balanced nutrients and the ingredients used in feed formulation. Further work may to include more variables such as individual fiber, NSPs, or starch components (amylopectin vs. amylose as resistant or rapidly digestible) to explore possible roles of these variables in their contributions to NE utilization efficiency. Furthermore, digestible nutrient contents including AAs may be beneficial for the fine-tuning of NE efficiency in broiler chickens.

The AMEn (Hill and Anderson 1958) has been considered the accepted norm for describing the energy value of commercial poultry feed and ingredients. Correction for N retention is based on data obtained in non-growing adult roosters to allow application of data to growing chickens. However, the accuracy of using AME values corrected to zero nitrogen retention has been controversial (Vohra 1972; Lopez and Leeson 2007, 2008). Studies to compare AME values across different genetics, genders, ages, and environments are warranted to investigate how these factors affect the energy values used in formulation. This may be a moving target with continuous improvement of broiler chicken genetics, production management, and dietary energy sources. As growing chickens retain more than half of the nitrogen consumed, the concept of AMEs has been proposed, where N retention is assumed to be 50% N retention (Cozannet et al., 2010). Equations for prediction of NE using AME, AMEn, and AMEs have been generated in the current study. These are provided in the tables to accommodate the needs of those who may wish to explore the use of AME, AMEn, or

**Table 8.** Calculated NE and NE/AMEn (DM basis) of broiler feed ingredients.<sup>1</sup>

| Nutrients                | Corn  | Wheat soft | Sorghum | Soybean meal | Canola meal | Canola, full fat | Meat and bone meal | Soy oil | Wheat bran |
|--------------------------|-------|------------|---------|--------------|-------------|------------------|--------------------|---------|------------|
| AMEn, MJ/kg <sup>2</sup> | 15.16 | 13.94      | 15.61   | 10.50        | 6.65        | 18.98            | 11.63              | 38.08   | 7.69       |
| Protein % <sup>2</sup>   | 9.4   | 12.1       | 10.9    | 49.4         | 38.0        | 20.7             | 53.3               | 0.0     | 17.0       |
| Fat, % <sup>2</sup>      | 4.3   | 1.7        | 3.4     | 1.9          | 2.6         | 45.6             | 12.3               | 98.6    | 3.9        |
| NE <sup>3</sup> , MJ/kg  | 12.22 | 11.11      | 12.53   | 7.71         | 4.81        | 16.40            | 8.87               | 33.83   | 6.05       |
| NE/AMEn                  | 0.806 | 0.797      | 0.803   | 0.734        | 0.723       | 0.864            | 0.763              | 0.888   | 0.786      |

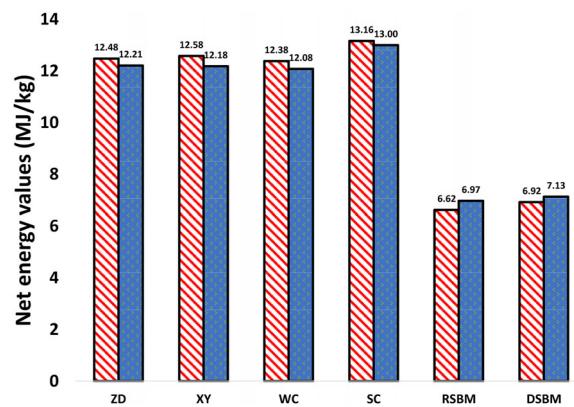
<sup>1</sup>Based on the AMEn and nutrient values from INRA table (Sauvant et al., 2004).

<sup>2</sup>DM calculated according to the DM provided in the referenced tables.

<sup>3</sup>NE calculated according to equation 24 (Table 7): NE = 0.808 × AMEn (MJ/kg) - 0.017 × CP (%) + 0.031 × EE (%)

AMEn values following robust field validation of NE system.

While the NE and NE/AME prediction equations are validated with various diets, the ultimate goal for creating such equations is to predict NE and NE/AME values of feed ingredients possibly for feed formulation. Therefore, it is essential that the equations accurately estimate the NE and NE/AME values in ingredients. According to the prediction equation produced in the current study, we were able to compute the NE values and AME efficiencies for NE of grains, oil, and protein sources commonly used in broiler feed formulation as shown in Table 8. Grains had NE values in a range of 11.1 to 12.5 MJ/kg with an order of sorghum > corn > wheat. The NE values of protein sources were in a range of 4.8 to 8.9 MJ/kg with an order of meat meal > soybean meal > solvent-extracted canola meal. The NE value of soy oil was 33.8 MJ/kg. The NE/AME ratio was up to 0.89 for oil, 0.72 for canola meal, and 0.80 for grains. Based on the prediction, oil sources had the highest, grains had intermediate, and protein sources had the lowest NE efficiency. This is consistent with previous observations in poultry (de Groot 1974; Carre et al., 2014; Liu et al., 2017b) and pigs (Noblet et al., 1994). Further, we applied equation 23 in Table 7 to calculate the NE values of corn and soybean meal (shown in Figure 2) reported recently in Liu et al. (2017b). The resultant NE values (in MJ/kg: 12.21, 12.18, 12.08, 13.00, 6.97, and 7.13) are close to the measured NE values (in MJ/kg: 12.48, 12.58, 12.38, 13.16, 6.62, and 6.92), in 2 normal, 1 waxy, and 1 sweet corn and regular and dehulled soybean meal samples, respectively. It is interesting to notice that calculated NE values are all slightly lower for corn samples but higher for soybean meal samples than the measured NE values. We speculate that this may be due to energy utilization differences between growing (present study) and mature chickens used by Liu et al. (2017b), or the replacement of basal diets by the ingredients in the latter study, which may have led to an imbalance in the nutrient profile of the diets resulting in inaccurate ingredient NE measurements. Nevertheless, the consistency between the ingredient NE values predicted using the equation of this study and reported in the literature suggests that the prediction equations proposed in the current study can be applied to feed formulation once the NE system has been validated in the field and implemented.



**Figure 2.** Comparisons between NE values of corns (ZD, XY, WC, SC) and soybean meals (RSBM, DSBM) measured (▨) in Liu et al. (2017b) and predicted using regression equations NE = 0.781 × AME - 0.028 × CP + 0.029 × EE (equation 23 in Table 7) produced in the current study (■). The energy unit of kcal/kg in Liu, et al. (2017b) was converted to MJ/kg by dividing 239. Abbreviations (Liu et al., 2017b): ZD, Zheng Dan 958; XY, Xian Yu 335; WC, waxy corn; SC, sweet corn; RSBM, regular soybean meal; and DSBM, dehulled soybean meal.

In conclusion, the relationship between AME, NE, AME for NE, and GE for AME and dietary chemical components including EE, protein, and fiber were determined using balanced diets fed to broiler chickens. Particular emphasis was placed on practical relevance of the experimental design and respective equations to predict energy components from dietary chemical components in ingredients were then generated and validated in closed-circuit calorimetry chambers. Based on the prediction equations, a database of NE values can be easily established according to existing or analyzed ingredient AME, EE, CP, and CF values. In addition, computed NE and NE/AMEn values appear to be appropriate with balanced diets according to the consensus from scattered values estimated or measured by other studies (Fraps and Carlyle 1942; Carre et al., 2014; Liu et al., 2016). Further validation studies are warranted to assess potential economic benefits of using NE in place of AMEn in commercial feed formulation. These studies will be needed to assess whether the NE system is beneficial in regard to the utilization efficiency of the feed, feed costs for production, broiler growth, uniformity, and carcass quality.

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## REFERENCES

- Abdollahi, M., V. Ravindran, and B. Svhuis. 2013. Influence of grain type and feed form on performance, apparent metabolisable energy and ileal digestibility of nitrogen, starch, fat, calcium and phosphorus in broiler starters. *Anim. Feed Sci. Technol.* 186:193–203.
- Annison, G., R. Hughes, and M. Choct. 1996. Effects of enzyme supplementation on the nutritive value of dehulled lupins. *Br. Poult. Sci.* 37:157–172.
- Annison, E., and R. White. 1961. Glucose utilization in sheep. *Biochem. J.* 80:162–169.
- Ao, T., A. Cantor, A. Pescatore, M. Ford, J. Pierce, and K. Dawson. 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poult. Sci.* 88:111–117.
- AOAC. 2016. Official Methods of Analysis of AOAC International. 20th ed.
- Aviagen, 2014. Ross 308 Broiler Performance Objectives Aviagen, [http://en.aviagen.com/assets/Tech.Center/Ross\\_Broiler/Ross-308-Broiler-PO-2014-EN.pdf](http://en.aviagen.com/assets/Tech.Center/Ross_Broiler/Ross-308-Broiler-PO-2014-EN.pdf). Accessed 18 September, 2018.
- Bourdillon, A., B. Carré, L. Conan, M. Francesch, M. Fuentes, G. Huyghebaert, W. Janssen, B. Leclercq, M. Lessire, and J. McNab. 1990. European reference method of in vivo determination of metabolisable energy in poultry: reproducibility, effect of age, comparison with predicted values. *Br. Poult. Sci.* 31:567–576.
- Brouwer, E. 1965. Report of sub-committee on constants and factors. Pages 441–443 in Blaxter, K.L., Ed. Energy Metabolism, Academic Press, London, UK.
- Cao, M., and O. Adeola. 2016. Energy value of poultry byproduct meal and animal-vegetable oil blend for broiler chickens by the regression method. *Poult. Sci.* 95:268–275.
- Carré, B., L. Derouet, and B. Leclercq. 1990. The digestibility of cell-wall polysaccharides from wheat (bran or whole grain), soybean meal, and white lupin meal in cockerels, muscovy ducks, and rats. *Poult. Sci.* 69:623–633.
- Carré, B., M. Lessire, and H. Juin. 2013. Prediction of metabolisable energy value of broiler diets and water excretion from dietary chemical analyses. *Animal* 7:1246–1258.
- Carre, B., M. Lessire, and H. Juin. 2014. Prediction of the net energy value of broiler diets. *Animal* 8:1395–1401.
- Cozannet, P., M. Lessire, C. Gady, J. Metayer, Y. Primot, F. Skiba, and J. Noblet. 2010. Energy value of wheat dried distillers grains with solubles in roosters, broilers, layers, and turkeys. *Poult. Sci.* 89:2230–2241.
- D'Mello, J. 1994. Amino Acid Imbalances, Antagonisms And Toxicities, CAB International, Wallingford, Oxon, UK.
- de Groote, G. 1974. A comparison of a new net energy system with the metabolisable energy system in broiler diet formulation, performance and profitability. *Br. Poult. Sci.* 15:75–95.
- Emmans, G. 1994. Effective energy: a concept of energy utilization applied across species. *Br. J. Nutr.* 71:801–821.
- Englyst, H., and G. Hudson. 1987. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. *Food Chem.* 24:63–76.
- Farrell, D. 1972. An indirect closed circuit respiration chamber suitable for fowl. *Poult. Sci.* 51:683–688.
- Fraps, G. S. 1946. Composition and productive energy of poultry feeds and rations. Bull. No. 678. Texas Agricultural Experiment Station, College Station.
- Fraps, G. S., and E. C. Carlyle. 1942. Productive energy of some feeds and foods as measured by gains of energy by growing chicks. *Bull. Texas Agric. Exp. Stat.* 625:1–51.
- Harri, A., L. Nalley, and D. Hudson. 2009. The relationship between oil, exchange rates, and commodity prices. *J. Agric. Appl. Econ.* 41:501–510.
- Hartel, H. 1977. Beziehungen zwischen der N korrigierten umsetzbaren Energie und den Nahrstoffgehalten des Futters beim Huhn. *Arch. Geflugelkd.* 41:152–181.
- Hill, F., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 64:587–603.
- Hughes, R., M. Choct, and R. Van Barneveld. 2001. Factors influencing the energy values of Australian cereal grains fed to broilers. *Proc. Aust. Poult. Sci. Symp.* 13:30–38.
- Kienzle, E., I. Schrag, R. Butterwick, and B. Opitz. 2001. Calculation of gross energy in pet foods: new data on heat combustion and fibre analysis in a selection of foods for dogs and cats. *J. Anim. Physiol. Anim. Nutr.* 85:148–157.
- Koh, K., and M. G. Macleod. 1999. Effects of ambient temperature on heat increment of feeding and energy retention in growing broilers maintained at different food intakes. *Br. Poult. Sci.* 40:511–516.
- Leeson, S., and J. Summers. 2001. Scott's Nutrition of the Chicken, in Scott's nutrition of the chickenUniversity Books, Guelph, Ontario, Canada.
- Liu, W., C. H. Lin, Z. K. Wu, G. H. Liu, H. J. Yan, H. M. Yang, and H. Y. Cai. 2017a. Estimation of the net energy requirement for maintenance in broilers. *Asian-Australas J Anim Sci.* 30:849–856.
- Liu, W., G. Liu, R. Liao, Y. Chang, X. Huang, Y. Wu, H. Yang, H. Yan, and H. Cai. 2017b. Apparent metabolizable and net energy values of corn and soybean meal for broiler breeding cocks. *Poult. Sci.* 96:135–143.
- Liu, S., C. Sydenham, and P. Selle. 2016. Feed access to, and inclusions of fishmeal and corn starch in, sorghum-based broiler diets influence growth performance and nutrient utilisation as assessed by the Box-Behnken response surface design. *Anim. Feed Sci. Technol.* 220:46–56.
- Lopez, G., and S. Leeson. 2007. Relevance of nitrogen correction for assessment of metabolizable energy with broilers to forty-nine days of age. *Poult. Sci.* 86:1696–1704.
- Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. *Poult. Sci.* 87:298–306.
- Mateos, G. G., and J. L. Sell. 1980. Influence of carbohydrate and supplemental fat source on the metabolizable energy of the diet. *Poult. Sci.* 59:2129–2135.
- McLean, J. 1972. On the calculation of heat production from open-circuit calorimetric measurements. *Br. J. Nutr.* 27:597–600.
- Murugesan, G. R., B. J. Kerr, and M. E. Persia. 2013. Evaluation of energy values of various oil sources when fed to broiler chicks. *Anim. Ind. Rep.* 659:55.
- Naber, E. C., and S. P. Touchburn. 1969. Effect of hydration, gelatinization and ball milling of starch on growth and energy utilization by the chick. *Poult. Sci.* 48:1583–1589.
- NHMRC. 2013. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, Australian Government Public Service.
- Noblet, J., S. Dubois, E. Labussiere, B. Carre, and J. Van Milgen. 2010a. Metabolic utilization of energy in monogastric animals and its implementation in net energy systems. Energy and Protein Metabolism and Nutrition, 3rd EAAP International Symposium on Energy and Protein Metabolism and Nutrition, Parma, Italy, 6–10 September, 2010:573–582.
- Noblet, J., S. Dubois, J. Lasnier, M. Warpechowski, P. Dimon, B. Carré, J. Van Milgen, and E. Labussière. 2015. Fasting heat production and metabolic BW in group-housed broilers. *Animal* 9:1138–1144.
- Noblet, J., H. Fortune, X. Shi, and S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. *J. Anim. Sci.* 72:344–354.
- Noblet, J., J. Van Milgen, and S. Dubois. 2010b. Utilisation of metabolisable energy of feeds in pigs and poultry: interest of net energy systems. *Proc. Aust. Poult. Sci. Symp.* 21:26–35.

- NRC. 1994. Nutrient Requirements of Poultry. Natl. Acad. Press Washington, DC.
- Pesti, G., and C. Smith. 1984. The response of growing broiler chickens to dietary contents of protein, energy and added fat. *Br. Poult. Sci.* 25:127–138.
- Pirgozliev, V., and S. Rose. 1999. Net energy systems for poultry feeds: a quantitative review. *Worlds Poult. Sci. J.* 55:23–36.
- Poorghasemi, M., A. Seidavi, A. Qotbi, J. Chambers, V. Laudadio, and V. Tufarelli. 2015. Effect of dietary fat source on humoral immunity response of broiler chickens. *Eur. Poult. Sci.* 79:1–8.
- Sauvant, D., J. M. Perez, and G. Tran. 2004. Tables of Composition and Nutritional Value of Feed Materials. Wageningen Academic Publishers, The Netherlands & INRA, Paris, France.
- Sibbald, I., and J. Kramer. 1978. The effect of the basal diet on the true metabolizable energy value of fat. *Poult. Sci.* 57:685–691.
- Swick, R. A., S. B. Wu, J. Zuo, N. Rodgers, M. R. Barekatain, and M. Choct. 2013. Implications and development of a net energy system for broilers. *Anim. Prod. Sci.* 53:1231–1237.
- Theander, O., and E. Westerlund. 1993. Determination of individual components of dietary fiber. Pages 77–98 in *Dietary Fiber in Human Nutrition*, G. A. Spiller, ed. CRC Press, Boca Raton, FL.
- Tran, G., and D. Sauvant. 2004. Chemical data and nutritional value. Pages 17–24 in *Tables of Composition and Nutritional Value of Feed Materials*. D. Sauvant, J. M. Perez, and G. Tran, eds. Wageningen Academic Publishers The Netherlands & INRA, Paris, France.
- Vohra, P. 1966. Energy concepts for poultry nutrition. *Worlds Poult. Sci. J.* 22:6–24.
- Vohra, P. 1972. Evaluation of metabolizable energy for poultry. *Worlds Poult. Sci. J.* 28:204–214.
- Wiseman, J., D. Cole, F. Perry, B. Vernon, and B. Cooke. 1986. Apparent metabolisable energy values of fats for broiler chicks. *Br. Poult. Sci.* 27:561–576.
- Yang, Y., P. Iji, A. Kocher, E. Thomson, L. Mikkelsen, and M. Choct. 2008. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. *Br. Poult. Sci.* 49:186–194.
- Yu, T. H., D. A. Bessler, and S. Fuller. 2006. Cointegration and causality analysis of world vegetable oil and crude oil prices. Proc. The American Agricultural Economics Association Annual Meeting, Long Beach, California.
- Zhou, W. T., and S. Yamamoto. 1997. Effects of environmental temperature and heat production due to food intake on abdominal temperature, shank skin temperature and respiration rate of broilers. *Br. Poult. Sci.* 38:107–114.