Estimation of the net energy requirement for maintenance in broilers

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Objective: The net energy requirement for the maintenance (NE_m) of broilers was determined using regression models by the indirect calorimetry method (ICM) or the comparative slaughter method (CSM).

Methods: A 2×4 factorial arrangement of treatments including the evaluation method (ICM or CSM) and feed intake (25%, 50%, 75%, or 100% of ad libitum recommended) was employed in this experiment. In the ICM, 96 male Arbor Acres (AA) birds aged d 15 were used with 4 birds per replicate and 6 replicates in each treatment. In the CSM, 116 male AA birds aged d 15 were used. Among these 116 birds, 20 were selected as for initial data and 96 were assigned to 4 treatments with 6 replicate cages and 4 birds each. The linear regression between retained energy (RE) and metabolizable energy intake (MEI) or the logarithmic regression between heat production (HP) and MEI were used to calculate the metabolizable or net energy requirement for maintenance (ME_m) or NE_m, respectively.

Results: The evaluation method did not detect any differences in the metabolizable energy (ME), net energy (NE), and NE:ME of diet, and in the MEI, HP, and RE of broilers. The MEI, HP, and RE of broilers decreased (p<0.01) as the feed intake decreased. No evaluation method×feed intake interaction was observed on these parameters. The ME_m and NE_m estimated from the linear relationship were 594 and 386 kJ/kg of body weight (BW)^0.75/d in the ICM, and 618 and 404 kJ/kg of BW^0.75/d in the CSM, respectively. The ME_m and NE_m estimated by logarithmic regression were 607 and 448 kJ/kg of BW^0.75/d in the ICM, and were 619 and 462 kJ/kg of BW^0.75/d in the CSM, respectively.

Conclusion: The NE_m values obtained in this study provide references for estimating the NE values of broiler diets.

Keywords: Broiler Maintenance Energy Requirement; Comparative Slaughter Method; Feed Intake; Heat Production; Indirect Calorimetry Method

INTRODUCTION

The net energy (NE) is assumed to represent the most accurate energy value of a feed [1]. The NE is usually partitioned into NE for maintenance (NE_m) and production (NE_p). Therefore, the determination of the NE value of a feed will be influenced by NE_m evaluation. Fasting heat production (FHP), which represents the basal metabolic rate of animals, is usually used as a surrogate for NE_m [2]. However, the determined FHP value may be affected by types (breed, age, sex, etc.) of animals, the length of the fasting period [3], and previous feeding conditions with a lower FHP at lower feed intake [4]. An alternative method to estimate NE_m is to feed animals at several levels of feed intake to build the logarithmic regression between heat production (HP) and metabolizable energy intake (MEI) [5]. Then, the NE_m can be calculated by extrapolating the HP to zero MEI from the logarithmic regression [6]. Furthermore, the metabolizable energy for maintenance (ME_m) can be calculated by extrapolating the HP being equal to MEI. However, the traditional method for ME_m is to use the linear relationship between retained energy (RE) and MEI [7]. The FHP (i.e., NE_m) can also be obtained from this linear regression. Moreover, the linear regression...
was used to calculate the ME<sub>m</sub> and the logarithmic regression was used to calculate NE<sub>m</sub> in some research, respectively [8,9]. However, the comparison of using logarithmic regression and linear regression to calculate both ME<sub>m</sub> and NE<sub>m</sub> are lacking. Furthermore, evaluations of the NE<sub>m</sub> in laying hens and broiler breeder pullets have been reported [8,10,11]. Similar research is scarce in broiler chickens.

The HP is frequently determined by the comparative slaughter method (CSM) [5,8]. Compared with the CSM, the indirect calorimetry method (ICM) is easily operated without killing animals and widely applied to HP determination for pigs. However, few studies have been done with the ICM for poultry, and no data can be found on the comparison of the two methods in HP determination. The objective of this study was to estimate the NE<sub>m</sub> in broilers using the CSM and ICM. The effect of regression model selection on ME<sub>m</sub> and NE<sub>m</sub> values was also compared.

## MATERIALS AND METHODS

### Equipment

Four open-circuit respiration chambers of approximately 0.43 m<sup>3</sup> were used in this study based on a design similar to that of Van Milgen et al [12]. Briefly, the respiration chamber was air conditioned to maintain a constant temperature and humidity using an air conditioner and a heater. Gas was extracted continuously from the respiration chamber by a vacuum pump. Gas concentrations in each chamber were measured at 3-min intervals by an analyzer. The O<sub>2</sub> was measured with a zirconium oxide sensor (Model 65-4-20; The Advanced Micro Instruments, Huntington Beach, CA, USA), whereas CO<sub>2</sub> was measured with a nirdisperser infrared sensor (AGM 10; Sensors Europe GmbH, Erkrath, Germany) in the analyzer. The analyzer had a range of measurement of 0 to 25% for O<sub>2</sub> and 0 to 2.5% for CO<sub>2</sub>.

### Experimental procedures

The experimental procedures for animal trials were approved by the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences and performed according to the guidelines for animal experiments set by the National Institute of Animal Health. The diet was based on corn, soybean meal, and casein (Table 1) and was formulated to meet the nutrient requirements of Arbor Acres (AA) broilers. The experiment employed a 2×4 factorial arrangement of treatments using the same diet. Factors were the evaluation method (ICM or CSM) and feed intake. Four levels of feed intake were calculated as 25%, 50%, 75%, or 100% of the recommended ad libitum feed intake by the Arbor Acres Broiler Commercial Management Guide each day. In the ICM, 96 male AA broilers aged d 15 were used with 4 birds per replicate and 6 replicates in each feed intake treatment. In the CSM, 116 male AA birds aged d 15 were used. Among these 116 birds, 20 were selected for the initial data and 96 were assigned to 4 feed intake treatments with 6 replicate cages and 4 birds each.

### Table 1. Ingredients and nutrient composition of diet

| Items              | Amount |
|--------------------|--------|
| Ingredient (%)     |        |
| Corn               | 57.94  |
| Soybean meal       | 26.12  |
| Casein             | 6.96   |
| Soybean oil        | 4.60   |
| Dicalcium phosphate| 2.11   |
| Limestone          | 1.38   |
| Salt               | 0.28   |
| DL-Methionine      | 0.03   |
| Vitamin-mineral premix<sup>1)</sup> | 0.50 |
| Choline            | 0.08   |

#### Calculated nutrient composition

- **ME (MJ/kg)**: 13.20
- **Protein (%):** 22.00
- **Calcium (%):** 1.00
- **Total phosphorus (%):** 0.68
- **Available phosphorus (%):** 0.46
- **Methionine (%):** 0.46
- **Methionine+cysteine (%):** 0.76
- **Lysine (%):** 1.31

ME, metabolizable energy.

<sup>1</sup> Provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D<sub>3</sub>, 3,500 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K<sub>3</sub>, 3 mg; thiamine hydrochloride, 0.01 mg; riboflavin, 8.00 mg; pyridoxine hydrochloride, 4.5 mg; vitamin B<sub>6</sub>, 0.02 mg; nicotinic acid, 34 mg; calcium pantothenate 12 mg; folic acid, 0.5 mg; biotin, 0.2 mg; Fe, 80 mg; Cu, 8 mg; Zn, 80 mg; Mn, 80 mg; I, 0.7 mg; Se 0.3 mg.

All bird management was consistent with the recommendations of the Arbor Acres Broiler Commercial Management Guide.

### Indirect calorimetry method

The measurements were conducted in 6 periods. In each of 6 measurement periods, 16 male birds were selected at approximately equal body weights (BW) and randomly assigned into 4 respiration chambers at d 14, with 4 birds from one replicate of each feed intake treatment per respiration chamber, to acclimate to the new environments with ad libitum access to diet and water. After 8 h of fasting being enforced by the withdrawal of feed [13], birds in each of 4 respiration chambers were weighed and were fed their respective level of feed intake at d 15. The amounts of O<sub>2</sub> consumption and CO<sub>2</sub> production were determined from d 15 to 21 to calculate the HP, using the Brouwer [14] equation without correction for urinary nitrogen excretion. The respiration quotient (RQ) was determined as the volume of CO<sub>2</sub> produced, divided by the volume of O<sub>2</sub> consumed. Water was offered ad libitum at all times. Measurement was suspended for 2 h each day to replenish feed and to collect excreta. The collected excreta were pooled for each chamber over 5 d, stored in a freezer, dried, and ground to pass through a 0.5-mm screen. On d 21, birds were weighed to determine their weight gain and feed:gain.

### Comparative slaughter method

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After 8 h of fasting being enforced by the withdrawal of feed on d 15, the birds were weighed. Twenty birds used as the initial slaughter group were euthanized by cervical dislocation, with their feathers being removed and weighed. Then, the feathers and carcasses were frozen (–20°C). The remaining 96 birds were fed using the respective experimental feed intake until d 21 when the birds were killed in the same way. The excreta from each cage were collected from d 15 to 21, pooled together, and processed as previously described. The frozen carcasses from the same replicate were first cut in small pieces, then mixed and ground with a meat grinder. Ground carcass samples were accurately weighed before and after freeze-drying to calculate the dry matter (DM) content and finely ground for further analyses. The pooled feathers were also ground for further analyses.

### Chemical analysis

The gross energy (GE) content of diet, excreta, carcass, and feather samples from each evaluation method were determined in a bomb calorimeter (C2000, IKA, Guangzhou, China) using benzoic acid as a standard. The nitrogen content of carcasses and feathers were determined with a combustion analyzer (Duma-therm, Gerhardt, Germany) using ethylenediaminetetraacetic acid as a calibration standard, with crude protein being calculated by multiplying percentage N by a correction factor (6.25). The fat content was analyzed using the classical Soxhlet petroleum-ether extraction.

### Calculations

For the ICM, the RE was calculated as the difference between the MEI and HP. For the CSM, the RE was calculated as the difference between final GE content of the total body (d 21) and initial GE content of the total body (d 15), and the HP was calculated as the difference between the MEI and RE. The MEI was calculated as follows:

$$\text{MEI (kJ)} = \text{ME} \times \text{FI},$$

where FI is the feed intake (kg of DM).

Energy retained as fat (RE\(_f\)) and protein (RE\(_p\)) were calculated as follows:

$$\text{RE}_f (\text{kJ}) = [\text{total body fat content d 21 (g)} - \text{total body fat content d 15 (g)}] \times 38.2 \text{ kJ/g},$$

$$\text{RE}_p (\text{kJ}) = [\text{total body protein content d 21 (g)} - \text{total body protein content d 15 (g)}] \times 23.6 \text{ kJ/g},$$

where the values of 38.2 and 23.6 kJ/g are energy values per gram of fat and protein, respectively, and were according to Larbier and Leclercq [15].

The ME and NE of the diet were determined using the following equations:

$$\text{ME (kJ/kg of DM)} = \frac{(\text{GEI} - \text{GEE})}{\text{FI}},$$

$$\text{NE (kJ/kg of DM)} = \frac{(\text{RE} + \text{FHP})}{\text{FI}},$$

where GEI is the gross energy intake (kJ/kg), GEE is the gross energy output of excreta (kJ/kg), and FI is the feed intake (kg of DM). The results for the MEI, RE, and HP were expressed as kJ/kg of BW\(^{0.75}\)/d.

The relationship between the RE and MEI were calculated using the following linear regression [7]:

$$\text{RE} = a + b \times \text{MEI},$$

where a is the FHP (kJ/kg of BW\(^{0.75}\)), and b is constant.

The logarithmic relationship between the HP and MEI were calculated using the following regression [5]:

$$\log(\text{HP}) = \log(a) + b \times \text{MEI},$$

### Statistical analyses

The O\(_2\) consumption, CO\(_2\) production, and RQ data measured by the ICM and RE, and RE, by the CSM were analyzed by one-way analysis of variance (ANOVA) of SPSS 19.0 (2010, SPSS Inc., Chicago, IL, USA). All other data were analyzed by two-way ANOVA as a 2×4 factorial arrangement of treatments using the general linear model procedure of SPSS to test the main effects of the evaluation method, the feed intake, and their interaction. Differences among treatment means were determined using a Duncan’s means comparison when the significance of the factor was p<0.05.

### RESULTS

#### Broiler growth performance

The growth performance of broilers is presented in Table 2. There was no evaluation method and feed intake interaction for growth performance. Feed intake had significant effects on final BW, BW gain, and feed:gain (p<0.01). Feed:gain increased (p<0.01) as the feed intake decreased, except that of the treatment of 25% of ad libitum feed intake. Birds fed 25% of ad libitum feed intake had negative BW gain and feed:gain.

#### Energy value of the diet and energy balance of broilers

Table 3 shows the data on the dietary energy values and energy balance of broilers. The evaluation method did not detect any differences in the ME, NE, and NE:ME of diet, and in the MEI, HP, and RE of broilers. The ME of the diet in the treatment of 25% of ad libitum feed intake was higher (p<0.01) than that in birds fed 100% of ad libitum feed intake. The dietary NE and NE:ME in the treatment of 100% of ad libitum feed intake were lower (p<0.01) than these values in the other three treatments.
The MEI, HP, and RE of broilers decreased (p<0.01) as the feed intake decreased. No evaluation method×feed intake interaction was observed on these parameters.

As presented in Table 4, the \( O_2 \) consumption×feed intake interaction decreased. The RQ decreased (p<0.01) from 0.97 to 0.73 with the level of feed intake reducing from 100% to 25%. Similarly, energy retained as fat and protein measured by the CSM decreased (p<0.01) as the feed intake decreased.

**Energy requirement for maintenance**

The linear regression equations between the RE and MEI, and the logarithmic regression equations between the HP and MEI are shown in Figure 1 to 4. The values of \( ME_{\text{rfr}} \), \( NE_{\text{rfr}} \), and \( K_m \) are shown in Table 5. From the linear regression equations (Equations 1 and 3), the \( ME_{\text{rfr}} \), which was calculated by extrapolating the MEI to zero energy retention, was 594 kJ/kg of BW\(^{0.75}\)/d in the ICM and 618 kJ/kg of BW\(^{0.75}\)/d in the CSM, and the \( NE_{\text{rfr}} \) calculated as the ratio between \( NE_{\text{r}} \) and \( ME_{\text{rfr}} \) was 65.0% in the ICM and 65.4% in the CSM. From the logarithmic regression equations (Equations 2 and 4), the calculated \( ME_{\text{rfr}} \) was 607 kJ/kg of BW\(^{0.75}\)/d in the ICM and 619 kJ/kg of BW\(^{0.75}\)/d in the CSM, and the \( NE_{\text{rfr}} \) calculated by extrapolating the HP to zero MEI was 448 kJ/kg of BW\(^{0.75}\)/d.
in the ICM and 462 kJ/kg of BW^{0.75}/d in the CSM. The K_{m} was 73.8% in the ICM and 75.0% in the CSM.

**DISCUSSION**

**Broiler growth performance**

The birds fed lower feed intakes had poorer growth performance [16]. However, the growth performance in the same feed intake was not affected by the evaluation method, which indicated that in the respiration chambers could provide a similar growth environment as that in the CSM for broilers. Birds fed 25% of ad libitum feed intake had negative BW gain and feed:gain. It has been suggested that when the MEI is below the maintenance requirement, the energy used by broilers will not only be supplied by their diet, but also by their body reserves [6].

**Table 5.** Regression of the RE and logarithm of the HP as a function of the MEI, values of ME_{m}, NE_{m}, and K_{m} of broilers

| Method | Equation number | Regression equations | ME_{m} (kJ/kg of BW^{0.75}/d) | NE_{m} (kJ/kg of BW^{0.75}/d) | K_{m} (%) |
|--------|-----------------|----------------------|--------------------------------|-------------------------------|----------|
| ICM    | 1               | RE = -386 + 0.65 × MEI | 594                            | 386                           | 65.0     |
| ICM    | 2               | log (HP) = 6.11 + (4.83 × 10^{-4}) × MEI | 607                            | 448                           | 73.8     |
| CSM    | 3               | RE = -404 + 0.63 × MEI | 618                            | 404                           | 65.4     |
| CSM    | 4               | log (HP) = 6.14 + (4.65 × 10^{-4}) × MEI | 619                            | 462                           | 75.0     |

RE, retained energy; HP, heat production; MEI, metabolizable energy intake; ME_{m}, metabolizable requirement for maintenance; NE_{m}, net energy requirement for maintenance; K_{m}, the ratio between NE_{m} and ME_{m}; ICM, indirect calorimetry method; CSM, comparative slaughter method.
Energy value of the diet and energy balance of broilers
The ME content of diet was not affected by feed intake when the level of feed intake was more than 50% of ad libitum, which is in agreement with the observation reported by Hill and Anderson [17]. The increased dietary ME content at the lowest feed intake level might be ascribed to the change in the metabolic and endogenous energy losses of broilers. The determined feed NE increased as the feed intake decreased. The dietary NE value is usually calculated as the sum of the FHP (i.e., NE<sub>0.75</sub>) and RE [18]. Therefore, the increased NE value in a lower feed intake may be supported by previous observations that feed intake affects the FHP [4]. The dietary NE:ME in 100% of the ad libitum feed intake group was 67.7%, which was lower than the mean NE:ME value (76.4%) observed by Carré and Juin [19] and 70.5% observed by Yang et al [20]. This could be associated with the differences in the diet composition and types (breed, age, sex, etc.) of poultry. The NE:ME of diet increased as the feed intake decreased, which means that the proportion of ME transformed into heat increment decreased as the feed intake decreased. This may be another reason for the higher NE value in the lower feed intake group.

According to De Lange et al [21], the MEI is partitioned into the thermal effect of feeding (HP<sub>t</sub>), activity HP (HP<sub>a</sub>), plateau fasting HP (FHP<sub>p</sub>), and RE. Therefore, the reduction of the HP<sub>t</sub> and RE that accompanies a reduction in the MEI may result in the reduction of the HP. Energy retained as protein was positive and as fat was negative in 25% of the ad libitum feed intake group measured by the CSM, which means that broilers can deposit protein by expending body lipid at a lower MEI [22,23]. On the other hand, the RQ for broilers receiving 25% of ad libitum feed intake measured by the ICM dropped to 0.73, which also indicated that broilers utilized body fat deposits to maintain energy metabolism [24].

Energy requirement for maintenance
The traditional method for estimating the ME<sub>n</sub> requirement is to use the linear relationship between the RE and MEI by extrapolating to the MEI at zero energy retention (i.e., the intercept on the X-axis) [7,9]. Furthermore, when the MEI is equal to zero, the intercept on the Y-axis of this equation represents the FHP (i.e., NE<sub>0.75</sub>) [7]. According to this method, the estimated ME<sub>n</sub> values were 594 kJ/kg of BW<sup>0.75</sup>/d in the ICM and 618 kJ/kg of BW<sup>0.75</sup>/d in the CSM. The ME<sub>n</sub> values determined herein are similar to the value (602 kJ/kg of BW<sup>0.75</sup>/d) determined from broiler breeder pullets (4 wks of age) by Sakomura et al [8] at 22°C, and were in the ranges of values estimated by Nieto et al [25] for male broiler chickens (519 to 628 kJ/kg of BW<sup>0.75</sup>/d). The NE<sub>n</sub> data for broilers calculated from linear regression are limited. The NE<sub>n</sub> values of 386 kJ/kg of BW<sup>0.75</sup>/d in the ICM and 404 kJ/kg of BW<sup>0.75</sup>/d in the CSM were in agreement with the values of 395 and 387 kJ/kg of BW<sup>0.75</sup>/d for two breeds of laying hens measured by the same method [7].

The logarithmic relationship between the HP and MEI is usually used to calculate the NE<sub>n</sub> as being the HP at zero MEI [5,26]. Similarly, the ME<sub>n</sub> can also be calculated by extrapolating the HP being equal to the MEI. In the current study, the estimated ME<sub>n</sub> values obtained by logarithmic regression were 607 kJ/kg of BW<sup>0.75</sup>/d in the ICM and 619 kJ/kg of BW<sup>0.75</sup>/d in the CSM, which were nearly equal to the respective value calculated by linear regression. The NE<sub>n</sub> data for broilers calculated form logarithmic regression are also lacking. The NE<sub>n</sub> value obtained from logarithmic regression was 448 kJ/kg of BW<sup>0.75</sup>/d in the ICM and 462 kJ/kg of BW<sup>0.75</sup>/d in the CSM, which were greater than the NE<sub>n</sub> values calculated by linear regression in this study. The NE<sub>n</sub> values of 497.48, 457.31, and 387.02 kJ/kg BW<sup>0.75</sup>/d for broiler breeder pullets (4 wks of age) at 15°C, 22°C, and 30°C, and 418.57, 334.09, and 289.32 kJ/kg BW<sup>0.75</sup>/d for laying hens (2 wks of age) at 12°C, 22°C, and 31°C were determined with logarithmic regression between the HP and MEI by Sakomura et al [8,11]. Moreover, the NE<sub>n</sub> can also be estimated by direct measurements of the FHP in fasting animals [27]. O’Neill and Jackson [10] founded that the FHP varied between 404 and 464 kJ/kg BW<sup>0.75</sup>/d for hens and between 223 and 349 kJ/kg BW<sup>0.75</sup>/d for the cockerels. Furthermore, Noblet et al [2] suggested that the present FHP values measured in modern lines of broilers should be expressed as per kg of BW<sup>0.70</sup>, and the FHP values in 0.5 to 3.0 kg broilers ranged between 410 and 460 kJ/kg BW<sup>0.70</sup>/d. These results suggest that the estimates of NE<sub>n</sub> are affected by types (breed, age, sex, etc.) of animals, the experimental environment, and measurement methods. Within one animal species, the constant FHP can be obtained by being expressed as per unit of metabolic BW after the exponent of metabolic BW being calculated for an animal over a large BW. Noblet et al [2] indicated that the FHP was linearly related to the BW<sup>0.70</sup>. In our previous study, the exponent of metabolic BW was 0.74 for AA broilers weighing 0.94 to 2.75 kg, and the FHP per kg of BW<sup>0.74</sup> were constant for broilers in this BW range [28]. Therefore, the respective NE<sub>n</sub> values of different types (breed, sex, etc.) of animals should be determined in standardized condition to calculate the NE content of a feed ingredient. The NE value of a poultry diet should express the energy cost of production (growth, egg, etc.) and NE<sub>n</sub>. The K<sub>n</sub> values of 73.8% from the ICM and 75.0% from the CSM calculated by logarithmic regression were higher than the values of 65.0% from the ICM and 65.4% from the CSM obtained by linear regression, which was caused by the lower NE<sub>n</sub> values determined by linear regression. The K<sub>n</sub> values determined in the present experiment from logarithmic regression are similar to those estimated by Sakomura et al [8,9] for broiler breeder pullets (75%, 76%, and 72% at 15°C, 22°C, and 30°C, respectively) and for broiler chickens (76%, 80%, and 76% at 13°C, 23°C, and 32°C, respectively), in which the NE<sub>n</sub> values were calculated by logarithmic regression between the HP and MEI and the ME<sub>n</sub> values were calculated by the linear relationship between the RE and MEI, respectively. Balnave [29] indicated
that the variability in the efficiency for maintenance ranged between 66% and 78%. This variability in efficiencies of energy utilization for maintenance could be related with the composition of the diets [25].

In conclusion, the $ME_m$ and $NE_m$ estimated from the linear relationship between the RE and MEI were 594 and 386 kJ/kg of BW$^{0.75}$/d in the ICM, and those in the CSM were 618 and 404 kJ/kg of BW$^{0.75}$/d. The $ME_m$ and $NE_m$ estimated by logarithmic regression between the HP and MEI were 607 and 448 kJ/kg of BW$^{0.75}$/d. The $ME_m$ and $NE_m$ estimated by logarithmic regression between the HP and MEI were 607 and 448 kJ/kg of BW$^{0.75}$/d. These results provide references for the determination of NE values of broiler feed ingredients.

**CONFLICT OF INTEREST**

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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