# Postprint: Optimal Dietary Levels of Metabolizable Energy, Crude Protein, Methionine, and Lysine for 1- to 21-Day-Old Arbor Acres × Ross Broiler Crossbreeds

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## Abstract

This study was conducted to investigate the optimal dietary levels of metabolizable energy (ME), crude protein (CP), methionine (Met) and lysine (Lys) for 1- to 21-day-old Arbor Acres (AA) × Roman meat-type crossbred chickens. An L9 (3^4) orthogonal experimental design was employed, with dietary ME levels of 11.70, 12.12 and 12.54 MJ/kg; CP levels of 19%, 20% and 21%; Met levels of 0.45%, 0.50% and 0.55%; and Lys levels of 1.00%, 1.10% and 1.20%. A total of 864 one-day-old AA × Roman meat-type crossbred chickens were selected and randomly assigned to 9 groups, with 6 replicates per group and 16 birds per replicate, for a 21-day experimental period. The feeding effects of diets with different nutrient levels were assessed through feeding trials, slaughter experiments and other methods. The results showed that: 1) Dietary CP, Met and Lys levels significantly affected the average daily gain (ADG) of experimental chickens ($P<0.05$), the 21% CP level group was significantly higher than the 20% CP level group ($P<0.05$), the 0.50% Met level group was significantly higher than the 0.55% Met level group ($P<0.05$), and the 1.20% Lys level group was significantly higher than the 1.10% Lys level group ($P<0.05$). 2) Dietary ME level significantly affected the live body energy content of 21-day-old experimental chickens ($P<0.05$), the 12.12 MJ/kg ME level group was significantly higher than the 12.54 MJ/kg ME level group ($P<0.05$); dietary Met level significantly affected the live body crude fat content of experimental chickens ($P<0.05$), the 0.50% and 0.55% Met level groups were significantly higher than the 0.45% Met level group ($P<0.05$). 3) Dietary ME and CP levels significantly affected the live body energy deposition rate of 21-day-old experimental chickens ($P<0.05$), the 11.70 MJ/kg ME level group was significantly higher than the 12.54 MJ/kg ME level group ($P<0.05$), the

21% CP level group was significantly higher than the 20% and 19% CP level groups (P<0.05); the 1.10% Lys level group had a significantly higher live body lysine deposition rate than the 1.20% Lys level group (P<0.05). 4) Dietary Lys level significantly affected serum glucose, uric acid and urea nitrogen levels of experimental chickens (P<0.05), the 1.00% Lys level group had the highest serum glucose level, which was significantly higher than the 1.20% Lys level group (P<0.05), the 1.10% Lys level group had the highest serum uric acid level, which was significantly higher than the 1.20% Lys level group (P<0.05), and the 1.20% Lys level group had the highest serum urea nitrogen level, which was significantly higher than the 1.10% Lys level group (P<0.05). 5) Regression analysis yielded the following formulas for calculating optimal dietary levels of ME, CP, Met and Lys for 1- to 21-day-old AA × Roman meat-type crossbred chickens: ME=45.33W0.75+183.84 W, CP=19.77W0.75+626.47 W, Met=1.44W0.75+10.31 W, Lys=3.01W0.75+21.28 W (W0.75 is metabolic body weight, W is average daily gain), and based on these formulas, the optimal dietary levels of ME, CP, Met and Lys were calculated to be 11.90 MJ/kg, 21.08%, 0.51% and 1.05%, respectively.

## Full Text

# Study on the Optimal Levels of Metabolic Energy, Crude Protein, Methionine and Lysine in Diets for Arbor Acres×Roman Hybrid Broilers Aged 1 to 21 Days

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**Abstract:** This experiment was conducted to investigate the optimal dietary levels of metabolic energy (ME), crude protein (CP), methionine (Met) and lysine (Lys) for Arbor Acres (AA)×Roman hybrid broilers aged 1 to 21 days. An $L_9(3^4)$ orthogonal experimental design was employed with three levels for each factor: ME at 11.70, 12.12 and 12.54 MJ/kg; CP at 19%, 20% and 21%; Met at 0.45%, 0.50% and 0.55%; and Lys at 1.00%, 1.10% and 1.20%. A total of 864 one-day-old AA×Roman hybrid broilers were randomly allocated to 9 groups with 6 replicates per group and 16 birds per replicate. The feeding effects of different nutrient levels were evaluated through feeding trials and comparative slaughter experiments over a 21-day period.

The results showed: 1) Dietary CP, Met and Lys levels significantly affected average daily gain (ADG) (P<0.05). The 21% CP group exhibited significantly higher ADG than the 20% CP group (P<0.05). The 0.50% Met group showed significantly higher ADG than the 0.55% Met group (P<0.05). The 1.20%

Lys group demonstrated significantly higher ADG than the 1.10% Lys group ($P<0.05$). 2) Dietary ME level significantly influenced body energy content at 21 days ($P<0.05$), with the 12.12 MJ/kg ME group being significantly higher than the 12.54 MJ/kg ME group ($P<0.05$). Dietary Met level significantly affected body crude fat content ($P<0.05$), with both 0.50% and 0.55% Met groups showing significantly higher values than the 0.45% Met group ($P<0.05$). 3) Dietary ME and CP levels significantly impacted body energy deposition rate at 21 days ($P<0.05$). The 11.70 MJ/kg ME group exhibited significantly higher energy deposition rate than the 12.54 MJ/kg ME group ($P<0.05$), while the 21% CP group showed significantly higher energy deposition rate than both 20% and 19% CP groups ($P<0.05$). The 1.10% Lys group demonstrated significantly higher Lys deposition rate than the 1.20% Lys group ($P<0.05$). 4) Dietary Lys level significantly affected serum glucose, uric acid and urea nitrogen levels ($P<0.05$). The 1.00% Lys group had the highest serum glucose level, significantly exceeding the 1.20% Lys group ($P<0.05$). The 1.10% Lys group showed the highest serum uric acid level, significantly higher than the 1.20% Lys group ($P<0.05$). The 1.20% Lys group exhibited the highest serum urea nitrogen level, significantly surpassing the 1.10% Lys group ($P<0.05$). 5) Regression analysis yielded the following formulas for optimal nutrient levels: $ME=45.33W^{0.75}+183.84\Delta W$, $CP=19.77W^{0.75}+626.47\Delta W$, $Met=1.44W^{0.75}+10.31\Delta W$, $Lys=3.01W^{0.75}+21.28\Delta W$ (where $W^{0.75}$ represents metabolic body weight and $\Delta W$ represents average daily gain). Based on these formulas, the optimal dietary levels were calculated to be 11.90 MJ/kg ME, 21.08% CP, 0.51% Met and 1.05% Lys.

**Keywords:** hybrid broilers; metabolic energy; crude protein; methionine; lysine; nutrient requirements

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Arbor Acres (AA)×Roman hybrid broiler is a crossbreed developed by mating white-feathered meat-type breeds (AA broiler parent stock roosters) with egg-type breeds (Roman layer commercial hens), representing one of the "817" broiler hybrid lines. This hybrid exhibits advantages including lower production costs, superior meat quality, and moderate body size, making it particularly suitable for processing into braised, roasted, and smoked chicken products. Currently, annual "817" broiler production in China exceeds 1 billion birds, with increasing utilization in processed products such as white-feathered carcasses, dressed chickens, prepared chicken, and roasted chicken. This segment has become an important component of China's broiler industry, delivering significant socioeconomic benefits. The "817" broiler differs from both fast-growing white-feathered broilers and local high-quality broilers in China, as it represents a hybrid line between layer and broiler breeds first raised in Liaocheng, Shandong Province in 1988. Systematic investigation is required regarding its nutrient requirements, growth patterns, and breed standards, while research on the ME, CP, Met and Lys requirements for AA×Roman hybrid broilers remains limited. Therefore, this study investigated the optimal dietary levels of ME, CP, Met and Lys for

AA×Roman hybrid broilers aged 1 to 21 days to provide theoretical basis for rational diet formulation.

### 1.1.1 Experimental Animals

A total of 864 healthy one-day-old AA×Roman hybrid broilers (AA broiler parent stock rooster × Roman brown layer commercial hen) were purchased from Jinan Limin Breeding Farm.

### 1.1.2 Main Equipment

The experiment utilized a Parr6200 automatic oxygen bomb calorimeter, KND-102C nitrogen analyzer, Hitachi L-8900 automatic amino acid analyzer, drying oven, XD811 semi-automatic biochemical analyzer, electronic analytical balance, and low-temperature high-speed centrifuge.

The 864 one-day-old AA×Roman hybrid broilers were randomly divided into 9 groups with 6 replicates per group and 16 birds per replicate. All broilers were raised in cages with ad libitum access to feed and water under conventional management practices throughout the 21-day experimental period. Dietary formulation followed a 4-factor, 3-level orthogonal experimental design , with ME levels of 11.70, 12.12 and 12.54 MJ/kg; CP levels of 19%, 20% and 21%; Met levels of 0.45%, 0.50% and 0.55%; and Lys levels of 1.00%, 1.10% and 1.20%. Except for ME, CP, Met and Lys, all other nutrient levels were identical across groups. Dietary composition and nutrient levels are presented in .

**Table 1** Experimental Design

| Groups | ME/(MJ/kg) | CP/% | Met/% | Lys/% |
|---|---|---|---|---|
| 1 | 11.70 | 19 | 0.45 | 1.00 |
| 2 | 11.70 | 20 | 0.50 | 1.10 |
| 3 | 11.70 | 21 | 0.55 | 1.20 |
| 4 | 12.12 | 19 | 0.50 | 1.20 |
| 5 | 12.12 | 20 | 0.55 | 1.00 |
| 6 | 12.12 | 21 | 0.45 | 1.10 |
| 7 | 12.54 | 19 | 0.55 | 1.10 |
| 8 | 12.54 | 20 | 0.45 | 1.20 |
| 9 | 12.54 | 21 | 0.50 | 1.00 |

**Table 2** Dietary Composition and Nutrient Levels

| Items | Groups 1 | Groups 2 | Groups 3 |
|---|---|---|---|
| **Ingredients** | | | |
| Corn | 57.80 | 55.20 | 52.60 |
| Wheat middling | 5.00 | 5.00 | 5.00 |

| Items | Groups 1 | Groups 2 | Groups 3 |
|---|---|---|---|
| Wheat bran | 3.00 | 3.00 | 3.00 |
| Soybean meal | 25.00 | 27.60 | 30.20 |
| Corn protein meal | 3.00 | 3.00 | 3.00 |
| Fish meal | 2.00 | 2.00 | 2.00 |
| Fish oil | 1.50 | 1.50 | 1.50 |
| L-Lys | 0.20 | 0.30 | 0.40 |
| DL-Met | 0.10 | 0.20 | 0.30 |
| Thr | 0.10 | 0.10 | 0.10 |
| $CaHPO_4$ | 1.20 | 1.20 | 1.20 |
| Limestone | 0.80 | 0.80 | 0.80 |
| Premix[1]) | 0.30 | 0.30 | 0.30 |
| NaCl | 0.30 | 0.30 | 0.30 |
| **Total** | 100.00 | 100.00 | 100.00 |
| **Nutrient levels[2])** | | | |
| ME/(MJ/kg) | 11.70 | 12.12 | 12.54 |
| CP | 19.00 | 20.00 | 21.00 |
| Met | 0.45 | 0.50 | 0.55 |
| Lys | 1.00 | 1.10 | 1.20 |
| Ca | 1.00 | 1.00 | 1.00 |
| AP | 0.45 | 0.45 | 0.45 |
| TP | 0.70 | 0.70 | 0.70 |

[1])The premix provided the following per kg of diet: VA 16,000 IU, VD 8,000 IU, VE 44 IU, $VK_3$ 2.67 mg, $VB_1$ 3.24 mg, $VB_2$ 12.80 mg, $VB_6$ 5.12 mg, $VB_{12}$ 20.48 mg, biotin 0.32 mg, Fe 100 mg, Cu 8.33 mg, Zn 74.67 mg, Mn 82.67 mg, I (10%) 2.5 mg, Se 0.86 mg.
[2])CP, Ca and TP were measured values, while the others were calculated values.

### 1.3 Measurement Indicators and Methods

**1.3.1 Growth Performance** On a per-replicate basis, the fasting body weight of day-old chicks was measured, and fasting body weight was again recorded at 08:00 on day 21. Daily feed intake was recorded by replicate. Average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio (F/G) were calculated for the 1-21 day period.

**1.3.2 Body Nutrients and Nutrient Deposition Rate** Comparative slaughter experiments were conducted to determine body nutrient content and nutrient deposition rates. Six day-old chicks were weighed, euthanized by asphyxiation, and stored at -20°C for subsequent analysis. On day 21, one broiler per replicate with body weight close to the replicate mean was selected (after fasting), euthanized by asphyxiation, and stored at -20°C. Carcasses were thawed at room temperature, emptied of gastrointestinal contents, and weighed

to obtain empty body weight. Fresh samples were processed into air-dried samples using the method described in reference [4].

Moisture, crude protein and crude fat contents were determined according to reference [5]. Gross energy was measured using a Parr6200 automatic oxygen bomb calorimeter, and amino acid content was analyzed using a Hitachi L-8900 automatic amino acid analyzer.

Body nutrient content was calculated by converting measured nutrient levels in air-dried samples to fresh weight basis: - Body nutrient content (%) = Nutrient content in air-dried sample × [100 - moisture content (%)] - Average daily nutrient intake (g) = Nutrient content in feed × Feed intake / Experimental days - Average daily nutrient deposition (g) = (Nutrient content in 21-day empty body × Air-dried weight of 21-day empty body - Nutrient content in 1-day-old body × Weight of 1-day-old body) / Experimental days - Nutrient deposition rate (%) = (Nutrient deposition amount / Nutrient intake amount) × 100

On day 21, one bird per replicate was randomly selected for cardiac blood collection (4-5 mL). Serum was separated by centrifugation and stored at -20°C for analysis. All serum biochemical indices were measured using an XD811 semi-automatic biochemical analyzer.

### 1.4 Data Processing and Statistical Analysis

Experimental data were initially processed using Excel software for regression analysis. All data were subjected to orthogonal experimental design analysis of variance using SPSS 17.0 statistical software, with multiple comparisons performed using LSD (Least Significant Difference) method.

## 2.1 Effects of Dietary ME, CP, Met and Lys Levels on Growth Performance of AA×Roman Hybrid Broilers Aged 1-21 Days

As shown in , dietary ME level showed no significant effects on any growth performance indices ($P>0.05$). Dietary CP level did not significantly affect ADFI or F/G ($P>0.05$), but ADG was highest in the 21% CP group, which was significantly higher than the 20% CP group ($P<0.05$) but not significantly different from the 19% CP group ($P>0.05$). Dietary Met level did not significantly affect ADFI or F/G ($P>0.05$), but ADG was highest in the 0.50% Met group, which was significantly higher than the 0.55% Met group ($P<0.05$) but not significantly different from the 0.45% Met group ($P>0.05$). Dietary Lys level did not significantly affect ADFI or F/G ($P>0.05$), but ADG was highest in the 1.20% Lys group, which was significantly higher than the 1.10% Lys group ($P<0.05$) but not significantly different from the 1.00% Lys group ($P>0.05$).

**Table 3** Growth Performance of AA×Roman Hybrid Broilers Aged 1-21 Days

| Items | Level | ADG (g/d) | ADFI (g/d) | F/G |
|---|---|---|---|---|
| **ME/(MJ/kg)** | 11.70 | 17.42$±1.65\|29.96±3.35\|1.73±0.18\|\|\|12.12\|17.42±1.92\|29.96±3.56\|1.73±0.17\|\|\|12.5 | | |
| | | $*CP/$±1.15$ab$\|30.84±2.67\|1.81±0.12\|\|\|20\|16.63±2.06$b$\|28.14±2.86\|1.70±0.14\|\|\|21\|18 | | |
| | | $*Met/$±1.65$ab$\|30.24±3.89\|1.71±0.15\|\|\|0.50\|18.53±1.89$a$\|31.20±2.28\|1.70±0.18\|\|\|0. | | |
| | | $*Lys/$±2.31$ab$\|30.47±3.45\|1.72±0.14\|\|\|1.10\|16.33±0.73$b$\|28.85±4.05\|1.77±0.23\|\|\|1.2 | | |

*In the same column and item, values with different lowercase letter superscripts differ significantly (P<0.05), while values with different uppercase letter superscripts differ extremely significantly (P<0.01). Values with no superscript or the same superscript do not differ significantly (P>0.05). This applies to all tables.*

## 2.2 Effects of Dietary ME, CP, Met and Lys Levels on Body Nutrient Content of AA×Roman Hybrid Broilers at 21 Days

As shown in , dietary ME level showed no significant effects on body moisture, crude protein, crude fat, Met or Lys content (P>0.05). However, body energy content was highest in the 12.12 MJ/kg ME group, which was significantly higher than the 12.54 MJ/kg ME group (P<0.05) but not significantly different from the 11.70 MJ/kg ME group (P>0.05). Dietary CP and Lys levels showed no significant effects on any body nutrient content indices (P>0.05). Dietary Met level showed no significant effects on body moisture, energy, crude protein, Met or Lys content (P>0.05), but significantly affected body crude fat content (P<0.05), with both 0.50% and 0.55% Met groups showing significantly higher values than the 0.45% Met group (P<0.05).

**Table 4** Body Nutrient Content of AA×Roman Hybrid Broilers at 21 Days

| Items | Level | Water/% | Energy/(MJ/kg) | CP/% | Crude Fat/% | Met/% | Lys/% |
|---|---|---|---|---|---|---|---|
| **ME/(MJ/kg)** | 11.70 | 69.39$±0.51\|6.76±0.21$ab$\|21.30±0.73\|6.88±0.57\|0.19±0.03\|0.55±0.08\|\|\|12.12\|68.96±0.34\|6.96± | | | | | |
| | | $*CP/$±0.41\|6.75±0.36\|21.38±1.13\|6.87±0.61\|0.21±0.02\|0.59±0.06\|\|\|20\|69.01±0.38\|6.63±0.20\| | | | | | |
| | | $*Met/$±0.37\|6.72±0.36\|21.44±0.69\|6.60±0.34$b$\|0.19±0.03\|0.53±0.07\|\|\|0.50\|69.19±0.48\|6.93±0 | | | | | |
| | | $*Lys/$±0.43\|6.71±0.17\|21.63±0.73\|7.24±0.93\|0.19±0.01\|0.53±0.05\|\|\|1.10\|69.35±0.48\|6.80±0.4 | | | | | |

## 2.3 Effects of Dietary ME, CP, Met and Lys Levels on Body Nutrient Deposition Rate of AA×Roman Hybrid Broilers at 21 Days

As shown in , dietary ME level significantly affected body energy deposition rate (P<0.05), with the 11.70 MJ/kg ME group showing the highest value, significantly exceeding the 12.54 MJ/kg ME group (P<0.05) but not significantly

different from the 12.12 MJ/kg ME group (P>0.05). However, ME level showed no significant effects on crude protein, Met or Lys deposition rates (P>0.05). Dietary CP level significantly influenced body energy deposition rate (P<0.05), with the 21% CP group being significantly higher than both 20% and 19% CP groups (P<0.05), but showed no significant effects on crude protein, Met or Lys deposition rates (P>0.05). Dietary Met level showed no significant effects on any body nutrient deposition rates (P>0.05). Dietary Lys level significantly affected body Lys deposition rate (P<0.05), with the 1.10% Lys group being significantly higher than the 1.20% Lys group (P<0.05) but not significantly different from the 1.00% Lys group (P>0.05), while showing no significant effects on energy, crude protein or Met deposition rates (P>0.05).

**Table 5** Body Nutrient Deposition Rate of AA×Roman Hybrid Broilers at 21 Days

| Items | Level | Energy Deposition Rate | CP Deposition Rate | Met Deposition Rate | Lys Deposition Rate |
|---|---|---|---|---|---|
| **ME/(MJ/kg)** | 11.70 | 34.45$±3.87*a* | 62.32±6.36 | 20.16±3.71 | 25.59±4.41 |
| | 12.12 | 33.07±3.31*a* | 60.43±5.37 | 19.92±2.39 | 26. |
| | *CP/% | | | | |
| | 19 | 31.80±3.36*b* | 61.45±4.59 | 20.22±3.53 | 25.94± |
| | 20 | | | | |
| | 21 | | | | |
| | *Met/% | | | | |
| | 0.47 | | | | |
| | 0.50 | 34.63±3.15 | 61.34±5.89 | 20.51±3.12 | 25.93± |
| | 0.53 | | | | |
| | *Lys/% | | | | |
| | 1.00 | | | | |
| | 1.10 | 32.64±4.40 | 59.59±5.80 | 21.01±3.28 | 27.7 |
| | 1.20 | | | | |

## 2.4 Effects of Dietary ME, CP, Met and Lys Levels on Serum Biochemical Indices of AA×Roman Hybrid Broilers at 21 Days

As shown in , dietary ME, CP and Met levels showed no significant effects on serum total protein, glucose, triglycerides, total cholesterol, uric acid or urea nitrogen levels (P>0.05). Dietary Lys level significantly affected serum glucose level (P<0.05), with the 1.00% Lys group showing the highest value, significantly exceeding the 1.20% Lys group (P<0.05) but not significantly different from the 1.10% Lys group (P>0.05). Dietary Lys level also significantly influenced serum uric acid level (P<0.05), with the 1.10% Lys group being highest, significantly higher than the 1.20% Lys group (P<0.05) but not significantly different from the 1.00% Lys group (P>0.05). Additionally, dietary Lys level significantly affected serum urea nitrogen level (P<0.05), with the 1.20% Lys group showing the highest value, significantly surpassing the 1.10% Lys group (P<0.05) but not significantly different from the 1.00% Lys group (P>0.05).

**Table 6** Serum Biochemical Indices of AA×Roman Hybrid Broilers at 21 Days

| Items | Level | Total Protein (g/L) | Glucose (mmol/L) | Triglycerides (mmol/L) | Total Cholesterol (mmol/L) | Uric Acid ( mol/L) | Urea Nitrogen (mmol/L) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ME/(MJ/kg)** | (1 | 0$ | ±12.29 | 11.03±2.82 | 0.63±0.38 | 5.65±4.70 | 525.11±145.30 | 1.40±0.73 | | | 12.12 | 54.56±17.39 | 9.88±2 |
| | ∗*CP*/ | ±10.51 | 9.80±2.58 | 0.47±0.10 | 3.82±1.45 | 528.56±120.96 | 1.46±0.77 | | | 20 | 56.00±13.21 | 10.60±3.10 | |
| | ∗*Met*/ | ±15.46 | 9.80±2.77 | 0.56±0.19 | 5.53±4.97 | 557.11±177.16 | 1.48±0.64 | | | 0.50 | 53.22±12.66 | 11.14±3 | |
| | ∗*Lys*/ | ±12.28 | 12.26±1.54*a* | 0.52±0.16 | 5.17±4.68 | 558.56±104.40*ab* | 1.55±0.74*ab* | | | 1.10 | 45.78±11.04 | 10 | |

## 2.5 Regression Analysis Results for ME, CP, Met and Lys Requirements

Univariate linear regression analysis was performed with nutrient intake per metabolic body weight ($ME/W^{0.75}$, $CP/W^{0.75}$, $Met/W^{0.75}$ and $Lys/W^{0.75}$) as dependent variables (Y) and corresponding nutrient deposition rates per metabolic body weight ($RE/W^{0.75}$, $RCP/W^{0.75}$, $RMet/W^{0.75}$ and $RLys/W^{0.75}$) as independent variables (X) to derive regression equations Y=a+bX. According to the factorial method in nutrient requirement research: Total nutrient requirement = Maintenance requirement + Production requirement. Therefore, the intercept (a) represents maintenance requirement per kg metabolic body weight, the regression coefficient (b) represents the nutrient intake required per unit of net deposition (energy or protein), and $R^2$ represents the correlation coefficient. Regression results are presented in .

**Table 7** Regression Analysis Results between $ME/W^{0.75}$, $CP/W^{0.75}$, $Met/W^{0.75}$, $Lys/W^{0.75}$ and $RE/W^{0.75}$, $RCP/W^{0.75}$, $RMet/W^{0.75}$, $RLys/W^{0.75}$ for Broilers Aged 1-21 Days

| Item | Regression Equation Y=a+bX | P-value |
|---|---|---|
| ME | Y=1.3379X+45.329 | 0.0001 |
| CP | Y=1.4693X+19.772 | 0.0001 |
| Met | Y=2.9269X+1.4408 | 0.0001 |
| Lys | Y=2.1563X+3.0091 | 0.0001 |

Based on average daily gain and deposition amounts of ME, CP, Met and Lys, the requirements per gram of gain were calculated. Using the b values from the regression equations, formulas for optimal dietary levels were derived:

$ME = 45.33W^{0.75} + 183.84\Delta W$
$CP = 19.77W^{0.75} + 626.47\Delta W$
$Met = 1.44W^{0.75} + 10.31\Delta W$
$Lys = 3.01W^{0.75} + 21.28\Delta W$

Where: $W^{0.75}$ = metabolic body weight (kg); $\Delta W$ = average daily gain (g).

Using these formulas along with average daily gain and metabolic body weight, the optimal dietary levels for 1-21 day-old broilers were calculated to be 11.90 MJ/kg ME, 21.08% CP, 0.51% Met and 1.05% Lys.

## 3.1 Effects of Dietary ME, CP, Met and Lys Levels on Growth Performance of AA×Roman Hybrid Broilers

Dietary ME level directly affects feed intake, body nutrient composition and feed conversion efficiency in broilers. Parsons et al. [6] reported that within appropriate ranges, higher dietary ME levels can improve broiler growth rate and feed conversion efficiency. Adeyemo [7] found that dietary protein levels (14%-17%) had no significant effects on feed intake, daily gain or feed-to-egg ratio in laying hens (8-26 weeks). Chen et al. [8] demonstrated that dietary ME level had highly significant effects on daily gain, feed intake, feed conversion ratio, ME intake and CP intake in Shiqihuang broilers, with daily gain and feed conversion efficiency improving significantly as ME level increased, while feed intake and CP intake decreased. As CP level increased (16%-22%), daily gain and feed conversion efficiency increased significantly and highly significantly, respectively. Jiang et al. [9] reported that dietary ME level had no significant effects on any growth performance indices in Cherry Valley meat ducks. Xu et al. [10] found that dietary CP level had no significant effects on feed intake, daily gain or feed-to-gain ratio in Xianghuang chickens aged 14-21 weeks. Shi [11] reported that the interaction between dietary ME and protein had no significant effects on performance of 1-21 day-old broilers. The present results showed that as dietary ME level increased, ADFI tended to decrease while ADG and F/G remained relatively unchanged in 1-21 day-old AA×Roman hybrid broilers. Dietary CP level significantly affected ADG, with the 21% CP group showing significantly higher ADG than the 20% CP group, consistent with Swennen et al. [12]. This may be because low-energy, high-protein diets promote growth performance in 1-21 day-old AA×Roman hybrid broilers. Differences between these results and previous reports may be attributed to variations in energy and protein levels, physiological characteristics and nutritional requirements of different breeds.

Chen et al. [13] reported that dietary Lys level significantly affected feed intake, ADG, feed conversion ratio, eviscerated yield percentage, breast muscle percentage and abdominal fat percentage in 1-21 day-old male broilers, with optimal Lys levels of 1.10%-1.25% based on rapid growth and carcass quality criteria. Li et al. [14] found that dietary Met levels (0.50%, 0.60%, 0.70%) had no significant effects on weight gain, feed consumption or feed-to-gain ratio in Lingnan Yellow chickens. The current study demonstrated that both dietary Met and Lys levels significantly affected ADG in 1-21 day-old AA×Roman hybrid broilers ($P<0.05$), with lower Met levels (0.50%, 0.45%) and higher Lys level (1.20%) being more beneficial for weight gain. These findings indicate that Met and Lys requirements for 1-21 day-old AA×Roman hybrid broilers are similar to those of other broiler breeds.

### 3.2 Effects of Dietary ME, CP, Met and Lys Levels on Body Nutrient Content and Deposition Rate in AA×Roman Hybrid Broilers

Previous studies have reported varying results regarding the effects of dietary ME, CP and amino acid levels on body nutrient content and deposition rates in chickens. Sklan et al. [15] found that increasing dietary Lys levels (0.7%, 0.9%, 1.1%, 1.3%, 1.5%) significantly improved carcass protein deposition in 7-21 day-old broilers. Li et al. [16] reported that carcass fat content in Fujian Hetian chickens was negatively correlated with dietary energy and protein levels, while carcass protein content was positively correlated with dietary protein level and carcass moisture content was positively correlated with both dietary energy and protein levels. Jiang et al. [17] demonstrated that increasing dietary ME level significantly increased carcass crude fat content and decreased crude protein content in 0-21 day-old Lingnan Yellow broilers. Xi et al. [18] reported that dietary Met levels of 0.35%, 0.40% and 0.45% significantly increased carcass protein content in 43-63 day-old yellow-feathered broilers. Li et al. [19] found that high-nutrient diets increased nutrient intake and excretion of crude protein and Met, but decreased excretion of energy and Lys in silky fowl. The present results indicate that dietary ME and Met levels significantly affected body nutrient composition in 1-21 day-old AA×Roman hybrid broilers. Body energy content did not show a positive correlation with ME level; the medium ME group (12.12 MJ/kg) produced higher body energy content than both low (11.70 MJ/kg) and high (12.54 MJ/kg) ME groups. However, body fat content was positively correlated with dietary Met level. Dietary ME and CP levels significantly affected body energy deposition rate, with low and medium ME groups (11.70, 12.12 MJ/kg) showing higher energy deposition rates than the high ME group (12.54 MJ/kg), and the high CP group (21%) showing higher energy deposition rates than low and medium CP groups (19%, 20%). These results demonstrate that dietary energy, protein and Met levels significantly influence energy metabolism in 1-21 day-old AA×Roman hybrid broilers. The complex metabolic activities in the body are affected by multiple factors, including breed, growth stage, dietary nutrient levels and nutrient interactions, which may explain the inconsistent results among studies.

Esmail [20] reported that at lower dietary protein levels, dietary ME in broilers was primarily deposited as fat, while increasing dietary protein level enhanced protein deposition and reduced relative fat deposition. Esmail [20] and Bikker et al. [21] demonstrated that when protein intake was adequate, body protein deposition in broilers was mainly affected by ME intake, whereas when protein intake was low, excess dietary ME was primarily deposited as body fat. These findings indicate that dietary nutrient levels and their interactions significantly influence body nutrient deposition.

### 3.3 Effects of Dietary ME, CP, Met and Lys Levels on Serum Biochemical Indices in AA×Roman Hybrid Broilers

Serum biochemical indices and their variation patterns represent important biological characteristics that reflect the relationship between physiological functions and phenotypic expression, as well as physiological features under different breeds, sexes, ages and environmental conditions [22]. From a nutritional perspective, serum protein and glucose levels are closely related to nutritional status. Serum urea nitrogen level reflects digestible protein utilization and dietary amino acid balance; when protein utilization decreases, serum urea nitrogen increases first, while balanced amino acid status leads to decreased serum urea nitrogen [23]. Uric acid level is an important indicator of protein nutritional status and metabolism in poultry; differences in dietary protein levels can cause changes in plasma uric acid, with elevated serum uric acid indicating increased nitrogen excretion and affected nitrogen deposition [24]. Fan [25] reported that serum glucose in broilers tended to increase with higher dietary energy levels. Wang [26] found that dietary energy and protein levels had no significant effects on serum urea nitrogen, total cholesterol or triglyceride levels in 70-day-old Yangzhou geese. Li et al. [27] reported that low-protein diets had no significant effects on serum urea nitrogen, uric acid levels or alkaline phosphatase activity in 28-day-old Hetian chickens. The present results demonstrate that dietary ME, CP and Met levels had no significant effects on serum total protein, glucose, triglycerides, total cholesterol, uric acid or urea nitrogen levels in 1-21 day-old AA×Roman hybrid broilers, consistent with Wang [26] and Li et al. [27], indicating that the dietary nutrient levels in this study did not cause abnormal protein, amino acid or lipid metabolism.

## 4 Conclusions

1. From the perspective of growth performance, dietary CP, Met and Lys levels significantly affected ADG in 1-21 day-old AA×Roman hybrid broilers. Optimal levels of 21% CP, 0.50% Met and 1.20% Lys improved ADG, ADFI and F/G. Dietary ME level showed no significant effects on growth performance indices, with 11.70 MJ/kg being appropriate.

2. Regarding nutrient deposition, dietary ME level significantly affected both body energy content and energy deposition rate, with 12.12 MJ/kg significantly increasing body energy content and 11.70 MJ/kg significantly improving energy deposition rate. Dietary CP level of 21% significantly enhanced energy deposition rate. Dietary Met level of 0.55% significantly increased body fat content, while dietary Lys level of 1.10% significantly improved Lys deposition rate.

3. Dietary ME, CP and Met levels showed no significant effects on serum total protein, triglycerides, total cholesterol, uric acid or urea nitrogen levels. However, Lys level significantly affected serum glucose, uric acid and urea nitrogen levels.

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*Note: Figure translations are in progress. See original paper for figures.*