

## Postprint: Optimal Dietary Metabolizable Energy and Crude Protein Levels for Growing Taihang Chickens

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### Abstract

This experiment was conducted to investigate the effects of dietary metabolizable energy and crude protein levels on growth performance and serum biochemical indices of growing Taihang chickens, so as to determine appropriate dietary metabolizable energy and crude protein levels and provide a basis for establishing feeding standards for Taihang chickens. Using metabolizable energy (11.0, 11.5, 12.0 MJ/kg) and crude protein (14%, 15%, 16%) as factors, a 2-factor 3-level experimental design was adopted. Three hundred and sixty healthy 77-day-old Taihang chicken hens with uniform body weight were randomly divided into 9 groups, with 4 replicates per group and 10 chickens per replicate. The preliminary period was 3 days, and the experimental period was 42 days. The results showed that: 1) Dietary metabolizable energy level had a significant effect ( $P < 0.05$ ) on average daily feed intake and feed conversion ratio of growing Taihang chickens; with increasing dietary metabolizable energy level, the average daily feed intake and feed conversion ratio of growing Taihang chickens decreased. Dietary crude protein level had a significant effect ( $P < 0.05$ ) on feed conversion ratio of growing Taihang chickens; as dietary crude protein level increased, the average daily feed intake of growing Taihang chickens first increased and then decreased, while the feed conversion ratio first decreased and then increased. Dietary metabolizable energy and crude protein levels showed a significant interaction effect ( $P < 0.05$ ) on average daily feed intake, average daily gain, and feed conversion ratio of growing Taihang chickens. Based on comprehensive analysis of growth performance indicators, Taihang chickens showed better growth performance when dietary metabolizable energy level was 11.5-12.0 MJ/kg and crude protein level was 14%-15%. 2) Dietary metabolizable energy level had a significant effect ( $P < 0.05$ ) on average daily metabolizable energy intake (ADMEI) and average daily crude protein intake (ADCPI) of growing Taihang chickens; with increasing dietary metabolizable energy level,

ADMEI of growing Taihang chickens increased. Dietary crude protein level had a significant effect ( $P < 0.05$ ) on ADCPI of growing Taihang chickens; as dietary crude protein level increased, ADCPI of growing Taihang chickens increased. Dietary metabolizable energy and crude protein levels showed a significant interaction effect ( $P < 0.05$ ) on ADMEI and ADCPI of growing Taihang chickens. 3) Dietary metabolizable energy level had a significant effect ( $P < 0.05$ ) on serum triglyceride content of growing Taihang chickens. Dietary metabolizable energy and crude protein levels showed a significant interaction effect ( $P < 0.05$ ) on serum triglyceride content of growing Taihang chickens. Based on comprehensive analysis of serum biochemical indicators, a dietary metabolizable energy level of 11.5 MJ/kg could meet the energy requirement of Taihang chickens. Taking all indicators into consideration, the appropriate dietary metabolizable energy level for growing Taihang chickens was 11.5 MJ/kg, and the appropriate crude protein level was 14%-15%.

## Full Text

### Study on Optimum Dietary Metabolizable Energy and Crude Protein Levels of Taihang Chickens during Growing Period

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**Abstract:** This experiment was conducted to investigate the effects of dietary metabolizable energy and crude protein levels on growth performance and serum biochemical indices of Taihang chickens during the growing period, and to determine the optimum dietary metabolizable energy and crude protein levels for formulating feeding standards for Taihang chickens. Using metabolizable energy (11.0, 11.5, and 12.0 MJ/kg) and crude protein (14%, 15%, and 16%) as factors, a 2×3 factorial design was employed. A total of 360 healthy 77-day-old Taihang hens with uniform body weight were randomly divided into 9 groups, with 4 replicates per group and 10 chickens per replicate. The pre-feeding period lasted 3 days, and the formal experimental period lasted 42 days. The results showed: (1) Dietary metabolizable energy level had significant effects on average daily feed intake and feed-to-gain ratio of growing Taihang chickens ( $P < 0.05$ ). As dietary metabolizable energy level increased, average daily feed intake and feed-to-gain ratio decreased. Dietary crude protein level had significant effects on feed-to-gain ratio ( $P < 0.05$ ). With increasing crude protein level, average daily feed intake first increased then decreased, while feed-to-gain ratio

first decreased then increased. Dietary metabolizable energy and crude protein levels showed significant interactive effects on average daily feed intake, average daily gain, and feed-to-gain ratio ( $P < 0.05$ ). Based on comprehensive growth performance indicators, Taihang chickens exhibited optimal performance when dietary metabolizable energy level was 11.5–12.0 MJ/kg and crude protein level was 14%–15%. (2) Dietary metabolizable energy level had significant effects on average daily metabolizable energy intake (ADMEI) and average daily crude protein intake (ADCPI) ( $P < 0.05$ ). ADMEI increased with higher dietary metabolizable energy levels. Dietary crude protein level had significant effects on ADCPI ( $P < 0.05$ ), which increased with higher crude protein levels. Metabolizable energy and crude protein levels showed significant interactive effects on ADMEI and ADCPI ( $P < 0.05$ ). (3) Dietary metabolizable energy level had significant effects on serum triglyceride content ( $P < 0.05$ ). Metabolizable energy and crude protein levels showed significant interactive effects on serum triglyceride content ( $P < 0.05$ ). Based on comprehensive serum biochemical indicators, a dietary metabolizable energy level of 11.5 MJ/kg could meet the energy requirements of Taihang chickens. Considering all indicators, the optimum dietary metabolizable energy level for growing Taihang chickens is 11.5 MJ/kg, and the optimum crude protein level is 14%–15%.

**Keywords:** Taihang chickens; growing period; metabolizable energy; crude protein; growth performance; serum biochemical indexes

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Taihang chicken is a high-quality local breed in Hebei Province, approved by the National Animal Genetic Resources Committee in 2015. It is favored by farmers and consumers for its small body size, tolerance to roughage, strong adaptability, excellent meat and egg quality, and rich nutritional value. Appropriate metabolizable energy and crude protein levels are essential for healthy poultry growth. Research indicates that nutrient requirements vary among different breeds, sexes, and ages. Studies have reported optimal nutrient levels for various breeds: Xinyang blue-shell layers require 11.40 MJ/kg metabolizable energy and 14.45% crude protein during the growing period [1]; Hy-Line Gray layers in plateau regions need 11.6 MJ/kg metabolizable energy and 17.13% crude protein at 91–128 days of age [2]; Yinxiangma chickens require 12.54 MJ/kg metabolizable energy and 18.0% crude protein at 9–15 weeks [3]; Huainan partridge chickens need 11.73 MJ/kg metabolizable energy and 15.0% crude protein at 7–11 weeks, and 11.69 MJ/kg metabolizable energy and 14.0% crude protein at 12–18 weeks [4]; Lihua pheasant hens require 21.00% crude protein at 5–7 weeks, and roosters need 21.00% crude protein at 5–10 weeks [5]; Huaibei partridge chickens need 11.9 MJ/kg metabolizable energy and 17.5% crude protein, or 10.9 MJ/kg metabolizable energy and 16% crude protein at 1–4 weeks [6]; black-bone chickens require 11.72 MJ/kg metabolizable energy and 20% crude protein for roosters, and 11.30 MJ/kg metabolizable energy and 20% crude protein for hens at 1–4 weeks [7]; golden chickens need 12.13 MJ/kg metabolizable energy and 19% crude protein at 1–6 weeks, and 12.13 MJ/kg metabolizable

energy and 15% crude protein at 7-14 weeks [8]; Taihang roosters require 12.15 MJ/kg metabolizable energy and 17.6% crude protein for fattening at 0-6 weeks [9]; and Taihang chickens need 12.0-12.3 MJ/kg metabolizable energy and 17%-20% crude protein during the brooding period [10]. Since large-scale farming of Taihang chickens started relatively late, most research has focused on breed selection and growth patterns [11-13], with limited studies on nutrient requirements, and no standardized feeding guidelines have been established. Therefore, this study aims to determine the optimum dietary metabolizable energy and crude protein levels for Taihang chickens to provide a basis for developing feeding standards and standardized farming practices.

### 1.1 Experimental Animals and Materials

Experimental animals consisted of 360 healthy 77-day-old Taihang hens with uniform body weight, selected from the experimental farm of Hebei Agricultural University. Feed ingredients were provided by the feed factory at the same farm.

### 1.2 Experimental Design and Diets

The 360 Taihang chickens were randomly divided into 9 groups with 4 replicates per group and 10 chickens per replicate. A 2×3 factorial design was employed using metabolizable energy and crude protein as factors. The selected levels were based primarily on China's "Feeding Standard of Chickens" (NY/T 33-2004) and practical farming experience. Formulation data were referenced from the "Feed Composition and Nutritional Value Table in China" (26th edition, 2015). Diet composition and nutrient levels are shown in .

### 1.3 Management Practices

Before the experiment, the chicken house floor, walls, and cages were thoroughly disinfected. Chickens were raised in 4-tier half-step cages with uniform distribution of groups across tiers. Nipple drinkers provided ad libitum water. Manure was cleaned daily, and the house was disinfected every two days. Temperature and humidity were maintained through regular water spraying. Chickens had free access to feed and water throughout the experimental period according to conventional management practices.

### 1.4 Measurement Indices

#### 1.4.1 Growth Performance

On a replicate basis, weekly records of fasting body weight, feed intake, and bird numbers were collected to calculate average daily gain, average daily feed intake, and feed-to-gain ratio.

#### 1.4.2 Serum Biochemical Indices

At the end of the experiment, 3 chickens were randomly selected from each replicate for blood collection from the wing vein. Blood samples were centrifuged

at 300 r/min for 10 minutes at 4°C, and serum was separated and stored at -20°C for later analysis. Reagent kits from Nanjing Jiancheng Bioengineering Institute were used with a biochemical analyzer to determine serum biochemical indices .

## 1.5 Statistical Analysis

Data were processed and analyzed using SPSS 19.0 statistical software. General multivariate analysis was used for main effects and interaction variance analysis, followed by Duncan' s multiple comparison test and LSD test for significance between groups. Data are expressed as means±SD, with  $P<0.05$  considered statistically significant.

### 2.1 Effects of Dietary Metabolizable Energy and Crude Protein Levels on Growth Performance of Growing Taihang Chickens

As shown in , main effect analysis revealed that final weight and average daily gain of growing Taihang chickens increased while average daily feed intake and feed-to-gain ratio decreased with higher dietary metabolizable energy levels. Metabolizable energy level had significant effects on average daily feed intake and feed-to-gain ratio ( $P<0.05$ ). The low metabolizable energy group had the highest average daily feed intake, significantly higher than the medium and high groups by 0.96% ( $P<0.05$ ) and 0.77% ( $P<0.05$ ), respectively, with no significant difference between medium and high groups ( $P>0.05$ ). The high metabolizable energy group had the lowest feed-to-gain ratio, decreasing by 3.10% ( $P>0.05$ ) and 8.08% ( $P<0.05$ ) compared to medium and low groups, respectively, while the medium group decreased by 5.14% compared to the low group ( $P<0.05$ ). Average daily gain in the high metabolizable energy group was 7.34% ( $P>0.05$ ) and 12.78% ( $P<0.05$ ) higher than medium and low groups, respectively, with the medium group 5.07% higher than the low group ( $P>0.05$ ).

With increasing dietary crude protein level, final weight, average daily gain, and average daily feed intake first increased then decreased, while feed-to-gain ratio first decreased then increased. Crude protein level had significant effects on feed-to-gain ratio ( $P<0.05$ ). The medium protein group showed a feed-to-gain ratio 3.53% ( $P>0.05$ ) and 8.85% ( $P<0.05$ ) lower than low and high protein groups, respectively, while the low protein group was 5.52% lower than the high protein group ( $P<0.05$ ). Average daily gain in the medium protein group was 5.65% ( $P>0.05$ ) and 11.97% ( $P<0.05$ ) higher than low and high protein groups, respectively, with the low protein group 5.99% higher than the high protein group ( $P>0.05$ ).

Dietary metabolizable energy and crude protein levels showed significant interactive effects on average daily feed intake, average daily gain, and feed-to-gain ratio ( $P<0.05$ ), but not on final weight ( $P>0.05$ ). The main effect of average daily feed intake decreased with increasing metabolizable energy level, but at medium protein level, the MM group was 2.23% higher than the LM group ( $P<0.05$ ). The main effect of average daily gain increased with metabolizable

energy level, but at low protein level, the HL group decreased by 16.02% compared to the ML group ( $P>0.05$ ), and at high protein level, the MH group decreased by 6.26% compared to the LH group ( $P>0.05$ ). The main effect of feed-to-gain ratio decreased with metabolizable energy level, but at low protein level, the HL group increased by 20.63% compared to the ML group ( $P<0.05$ ), and at high protein level, the MH group increased by 5.44% compared to the LH group ( $P>0.05$ ).

## 2.2 Effects of Dietary Metabolizable Energy and Crude Protein Levels on Metabolizable Energy and Crude Protein Intake of Growing Taihang Chickens

As shown in , main effect analysis indicated that average daily metabolizable energy intake (ADMEI) of growing Taihang chickens increased with higher dietary metabolizable energy levels. Metabolizable energy level had significant effects on ADMEI and average daily crude protein intake (ADCPI) ( $P<0.05$ ). The high metabolizable energy group had the highest ADMEI, significantly higher than medium and low groups by 4.55% ( $P<0.05$ ) and 8.24% ( $P<0.05$ ), respectively, while the medium group was 3.54% higher than the low group ( $P<0.05$ ). The low metabolizable energy group had the highest ADCPI, significantly higher than high and medium groups by 0.70% ( $P<0.05$ ) and 0.93% ( $P<0.05$ ), respectively, with no significant difference between high and medium groups ( $P>0.05$ ).

ADCPI increased with higher dietary crude protein levels. Crude protein level had significant effects on ADCPI ( $P<0.05$ ). The high protein group had the highest ADCPI, significantly higher than medium and low protein groups by 6.51% ( $P<0.05$ ) and 14.21% ( $P<0.05$ ), respectively, while the medium protein group was 7.33% higher than the low protein group ( $P<0.05$ ).

Dietary metabolizable energy and crude protein levels showed significant interactive effects on ADMEI and ADCPI ( $P<0.05$ ). The main effect of ADMEI showed no significant difference with increasing crude protein level ( $P>0.05$ ), but at low metabolizable energy level, the LL group was 2.32% ( $P<0.05$ ) and 0.76% ( $P<0.05$ ) higher than LM and LH groups, respectively. At medium metabolizable energy level, the MM group was 2.88% ( $P<0.05$ ) and 2.92% ( $P<0.05$ ) higher than MH and ML groups, respectively. At high metabolizable energy level, the HH group was 0.88% ( $P<0.05$ ) and 0.56% ( $P<0.05$ ) higher than HM and HL groups, respectively. The main effect of ADCPI showed a decreasing trend with increasing metabolizable energy level, but at medium protein level, the MM group was 2.23% higher than the LM group ( $P<0.05$ ).

## 2.3 Effects of Dietary Metabolizable Energy and Crude Protein Levels on Serum Biochemical Indices of Growing Taihang Chickens

As shown in , main effect analysis revealed that serum glucose, triglyceride, cholesterol, total protein, and albumin contents increased with higher dietary metabolizable energy levels. Metabolizable energy level had significant effects

on serum triglyceride content ( $P < 0.05$ ). The high metabolizable energy group had the highest serum triglyceride content, significantly higher than medium and low groups by 52.46% ( $P < 0.05$ ) and 86.00% ( $P < 0.05$ ), respectively, while the medium group was 22% higher than the low group ( $P > 0.05$ ). Crude protein level had no significant effects on any serum biochemical indices ( $P > 0.05$ ). Dietary metabolizable energy and crude protein levels showed significant interactive effects on serum triglyceride content ( $P < 0.05$ ).

### **3.1 Effects of Dietary Metabolizable Energy and Crude Protein Levels on Growth Performance of Growing Taihang Chickens**

Energy and protein are the two most important factors for poultry growth, and excessive or insufficient levels in diets can affect growth and health [2]. Poultry can voluntarily regulate feed intake according to dietary nutrient levels. Studies have shown that increasing dietary metabolizable energy level for laying hens can reduce average daily feed intake, increase average daily gain [14–15], and decrease feed-to-gain ratio [3,16], consistent with our findings. Our results demonstrate that metabolizable energy levels of 11.5–12.0 MJ/kg produced faster weight gain and lower feed-to-gain ratio in growing Taihang chickens, significantly outperforming the 11.0 MJ/kg level. When dietary crude protein level was 14%–15%, Taihang chickens showed higher average daily gain and lower feed-to-gain ratio compared to 16% crude protein, indicating better growth performance. We infer that 14%–15% dietary crude protein meets the requirements of Taihang chickens, and 16% crude protein provides no benefit and may even be detrimental. Zhu et al. [4,17] reported similar results for Huainan partridge chickens at 7–18 weeks, where 14%–15% dietary crude protein met growth requirements. In our study, average daily gain of Taihang chickens first increased then decreased with increasing crude protein level, with the medium protein group showing faster growth, similar to trends observed in growing golden pheasants by Song et al. [18]. Dietary formulation should also consider the protein-to-energy ratio, as studies have shown that excessively high metabolizable energy levels in low-protein diets can disrupt the normal protein-to-energy ratio and inhibit poultry growth [1,19]. In our experiment, when dietary crude protein was 14% and metabolizable energy increased to 12.0 MJ/kg, average daily gain of Taihang chickens decreased instead.

### **3.2 Effects of Dietary Metabolizable Energy and Crude Protein Levels on Metabolizable Energy and Crude Protein Intake of Growing Taihang Chickens**

Due to poultry's ability to eat for energy, Taihang chickens adjust feed intake according to dietary nutrient levels to ensure adequate nutrient intake and avoid waste. As dietary metabolizable energy level increases, chickens can reduce feed intake to balance metabolizable energy intake, resulting in reduced intake of other nutrients such as crude protein, which decreased with increasing metabolizable energy level in our study. However, feed intake cannot be

reduced indefinitely, as it must satisfy satiety, so this regulatory mechanism has limits and is not completely precise. The increase in ADMEI with higher dietary metabolizable energy level in our study likely reflects this mechanism. The dietary protein-to-energy ratio affects poultry growth. In our study, crude protein alone had no significant effect on ADMEI, but the two-factor interaction showed that when metabolizable energy level was constant, chickens with crude protein-to-energy ratios near 13 g/MJ had higher ADMEI.

### **3.3 Effects of Dietary Metabolizable Energy and Crude Protein Levels on Serum Biochemical Indices of Growing Taihang Chickens**

Serum biochemical indices can reflect poultry nutrient metabolism levels and indirectly indicate animal health status [5]. Under normal conditions, serum biochemical indices remain relatively stable without large fluctuations. In our study, no significant differences in serum biochemical indices (except triglycerides) were observed among groups, indicating that all dietary nutrient levels were within normal ranges and did not cause serious harm to Taihang chickens. Serum triglycerides originate from both endogenous synthesis and dietary intake, reflecting lipid metabolism and synthesis. Studies have shown that serum triglyceride content follows the same trend as dietary metabolizable energy level [20-21], consistent with our results showing increased serum triglyceride content with higher metabolizable energy levels, with the high metabolizable energy group significantly higher than medium and low groups. This may be because the 12.0 MJ/kg metabolizable energy level exceeded the energy requirement of Taihang chickens, and the excess energy was converted to lipids stored in the body. Therefore, excessively high dietary metabolizable energy levels not only waste feed but also cause fat accumulation that affects animal health. Our results indicate that a dietary metabolizable energy level of 11.5 MJ/kg can meet the energy requirements of Taihang chickens.

## **4 Conclusion**

Based on comprehensive evaluation of all growth performance indicators, Taihang chickens showed optimal performance when dietary metabolizable energy level was 11.5-12.0 MJ/kg and crude protein level was 14%-15%. Serum biochemical analysis indicated that a dietary metabolizable energy level of 11.5 MJ/kg could meet the energy requirements of Taihang chickens. Considering all indicators comprehensively, the optimum dietary metabolizable energy level for growing Taihang chickens is 11.5 MJ/kg, and the optimum crude protein level is 14%-15%.

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