Postprint of a Study on Dietary Sulfur Amino Acid Requirements for 7-12 Week-Old Commercial Jinghong Laying Hens

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Abstract

This study investigated the effects of varying dietary sulfur amino acid levels on growth performance and serum biochemical parameters in Jinghong commercial layer pullets aged 7–12 weeks to determine their sulfur amino acid requirement. Seven hundred fifty Jinghong commercial layer pullets with uniform body weight and shank length at the end of week 6 were randomly allocated to 5 groups, each consisting of 5 replicates of 30 birds. The control group (Group A) received a basal diet containing 0.42% sulfur amino acids, while experimental groups B, C, D, and E were fed the basal diet supplemented with 0.08%, 0.16%, 0.24%, and 0.32% methionine, respectively, replacing equivalent amounts of bentonite to achieve sulfur amino acid levels of 0.50%, 0.58%, 0.66%, and 0.74%. The trial duration was 6 weeks. The results demonstrated: 1) Final body weight and average daily gain were significantly greater in the 0.58%, 0.66%, and 0.74% sulfur amino acid groups compared with the 0.42% and 0.50% groups ($P<0.05$). Additionally, the 0.66% sulfur amino acid group exhibited a significantly lower feed-to-gain ratio than the 0.50% group ($P<0.05$), along with significantly higher serum total protein and globulin concentrations relative to other treatment groups ($P<0.05$). 2) Single-slope broken-line regression analysis based on final body weight and average daily gain indicated that the optimal sulfur amino acid requirement for 7–12-week-old Jinghong commercial layer pullets was 0.62%. In summary, the dietary sulfur amino acid requirement for Jinghong commercial layer pullets during the 7–12-week period ranges from 0.62% to 0.66%.

Full Text

Dietary Sulphur Amino Acids Requirement of Jinghong Commercial Layers from 7 to 12 Weeks of Age

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Abstract

This experiment was conducted to investigate the effects of different dietary sulphur amino acid levels on growth performance and serum biochemical indices of Jinghong commercial layers aged 7–12 weeks and to determine their sulphur amino acid requirement. A total of 750 Jinghong layers at 6 weeks of age with uniform body weight and tibia length were randomly allocated into 5 groups, each consisting of 5 replicates of 30 hens. The control group (A) received a basal diet containing 0.42% sulphur amino acids, while experimental groups B, C, D, and E were fed diets in which 0.08%, 0.16%, 0.24%, and 0.32% methionine replaced equivalent amounts of bentonite, resulting in sulphur amino acid levels of 0.50%, 0.58%, 0.66%, and 0.74%, respectively. The trial lasted for 6 weeks. The results showed: (1) Final body weight and average daily gain in the 0.58%, 0.66%, and 0.74% sulphur amino acid groups were significantly higher than those in the 0.42% and 0.50% groups (P<0.05). The feed-to-gain ratio in the 0.66% group was significantly lower than that in the 0.50% group (P<0.05), and serum total protein and globulin contents were also significantly higher than in other groups (P<0.05). (2) Single-slope nonlinear broken-line regression analysis based on final body weight and average daily gain indicated that the recommended sulphur amino acid requirement for 7–12-week-old Jinghong commercial layers was 0.62%. In conclusion, the dietary sulphur amino acid requirement for Jinghong commercial layers aged 7–12 weeks is 0.62–0.66%.

Keywords: Jinghong commercial layers; sulphur amino acids; growth performance; serum biochemical indices; requirement

Jinghong layers are a brown-shell layer breed independently developed in China, characterized by early sexual maturity, strong adaptability, high egg production, and low feed consumption. Sulphur amino acids serve as the primary limiting amino acids in poultry diets, participating in the synthesis of choline, creatine, and other compounds, while also playing crucial roles in immune function, detoxification and liver protection, methyl group transfer, and coccidiosis resistance [1]. The 7–12 week period is critical for skeletal and muscular development in layers, directly influencing subsequent production performance. Current literature reports varying sulphur amino acid requirements for layers during this phase: D'Agostini et al. [2] determined that 7–12-week-old Lohmann Pink and Lohmann Brown pullets require 0.710% and 0.706% total methionine + cystine

(0.639% and 0.635% digestible), respectively, while Chen et al. [3] recommended 0.55% sulphur amino acids for 7–12-week-old Cenxi Sanhuang chickens. However, due to substantial differences between Jinghong layers and foreign breeds, their nutritional requirements also differ. Continuing to formulate diets based on standards for Hy-Line Brown, Lohmann, Nick, and Isa Brown layers may result in feed resource waste or suboptimal economic returns. Therefore, this study investigated the effects of varying dietary sulphur amino acid levels on growth performance and serum biochemical indices in 7–12-week-old Jinghong commercial layers to estimate their sulphur amino acid requirement and provide a theoretical basis for establishing feeding standards.

1.1 Experimental Design

Seven hundred fifty Jinghong commercial layers with uniform body weight and tibia length at 6 weeks of age were randomly assigned to 5 groups following a single-factor experimental design, with 5 replicates per group and 30 hens per replicate. The control group (A) received a basal diet containing 0.42% sulphur amino acids (Table 1), with other essential amino acid levels (except sulphur amino acids) determined based on factorial-derived net requirements and dietary amino acid utilization rates. All other nutritional parameters met the requirements of NY/T 33—2004. Experimental groups B, C, D, and E were fed diets in which 0.08%, 0.16%, 0.24%, and 0.32% methionine replaced equivalent amounts of bentonite, achieving sulphur amino acid levels of 0.50%, 0.58%, 0.66%, and 0.74%, respectively. Hens were housed in a closed chicken coop with three-tier full-step cage systems, maintaining temperatures of 18–27°C with longitudinal negative-pressure ventilation. Dry mash feed was provided ad libitum with twice-daily feeding; feed amounts were adjusted based on consumption to stimulate appetite. Nipple drinkers provided water ad libitum. Management followed the *Jinghong Commercial Layer Breeding Manual* throughout the 6-week experimental period.

1.2 Experimental Materials

DL-methionine (purity $ $99%) used in the experiment was provided by Sumitomo Chemical Co., Ltd.

1.3.1 Growth Performance Indicators

At the end of week 6, initial body weight (IBW) was recorded and tibia length measured for each replicate. Feed consumption and body weight were recorded every two weeks, with final body weight (FBW) obtained at the end of week 12. Average daily gain (ADG), average daily feed intake (ADFI), feed-to-gain ratio (F/G), and tibia length were calculated for each group.

1.3.2 Serum Biochemical Indices

At the conclusion of the experiment, one hen per replicate was randomly selected for blood collection from the wing vein. Blood samples were allowed to clot at room temperature, then centrifuged at 3,000 r/min for 15 minutes. Serum was collected and stored at –20°C. Serum total protein, albumin, uric acid, and ammonia contents, as well as alkaline phosphatase activity, were measured using a Hitachi automatic biochemical analyzer (Hitachi-7600). Globulin content and albumin-to-globulin ratio were calculated from total protein and albumin values.

1.4 Data Processing and Analysis

Experimental data were compiled using Excel and analyzed using SAS 9.2 software. One-way ANOVA was performed using the General Linear Model (GLM) procedure, with Duncan's multiple range test for pairwise comparisons. Contrast statements were used for linear and quadratic trend analysis. Single-slope nonlinear broken-line regression models were applied to determine sulphur amino acid requirements, with the breakpoint corresponding to the X-axis intercept representing the requirement. Growth performance data were analyzed with replicate as the experimental unit, while serum biochemical indices were analyzed with individual hens as the experimental unit. Results are expressed as means and standard errors, with $P<0.05$ considered statistically significant.

2.1 Effects of Different Sulphur Amino Acid Levels on Growth Performance of Jinghong Layers Aged 7–12 Weeks

As shown in Table 2 , with no significant differences in initial body weight ($P>0.05$), dietary sulphur amino acid supplementation significantly affected final body weight, average daily gain, and feed-to-gain ratio ($P<0.05$), but had no significant effect on average daily feed intake or tibia length ($P>0.05$). With increasing dietary sulphur amino acid levels, average daily gain and final body weight increased significantly in a linear manner ($P<0.05$). The 0.58%, 0.66%, and 0.74% sulphur amino acid groups exhibited significantly higher final body weight and average daily gain than the 0.42% and 0.50% groups ($P<0.05$), with no significant differences among the three higher-level groups ($P>0.05$). The feed-to-gain ratio in the 0.66% group was significantly lower than that in the 0.50% group ($P<0.05$), with no significant differences among the other three groups ($P>0.05$).

2.2 Effects of Different Sulphur Amino Acid Levels on Serum Biochemical Indices of Jinghong Layers Aged 7–12 Weeks

As shown in Table 3 , different sulphur amino acid levels significantly affected serum total protein, globulin content, and albumin-to-globulin ratio ($P<0.05$), but had no significant effect on albumin, ammonia content, or alkaline phosphatase activity ($P>0.05$). The 0.66% sulphur amino acid group showed significantly higher serum total protein and globulin contents than the other four groups (P<0.05), which did not differ significantly among themselves (P>0.05). The albumin-to-globulin ratio in the 0.66% group was significantly lower than in the other four groups (P<0.05), with no significant differences among the latter groups (P>0.05).

2.3 Determination of Sulphur Amino Acid Requirement Using Single-Slope Nonlinear Broken-Line Regression Analysis

As illustrated in Figure 1 [Figure 1: see original paper], final body weight increased linearly as sulphur amino acid levels rose from 0.42% to 0.62%, after which it plateaued, reaching maximum value at the breakpoint (x=0.62). The single-slope nonlinear broken-line regression equation fitted between final body weight (y1) and sulphur amino acid level (x) was: y1 = 1,015.00 – 389.80(0.62 – x) (P=0.04, R^2=0.96, x$ $0.62).

[Figure 1: see original paper]

As shown in Figure 2 [Figure 2: see original paper], average daily gain increased linearly as sulphur amino acid levels increased from 0.42% to 0.62%, plateauing beyond 0.62% and reaching maximum value at the breakpoint (x=0.62). The single-slope nonlinear broken-line regression equation fitted between average daily gain (y2) and sulphur amino acid level (x) was: y2 = 14.11 – 9.31(0.62 – x) (P=0.04, R^2=0.96, x$ $0.62).

[Figure 2: see original paper]

3.1 Effects of Different Sulphur Amino Acid Levels on Growth Performance of Jinghong Layers Aged 7–12 Weeks

Dietary sulphur amino acids primarily consist of methionine and cysteine, with methionine being the first limiting amino acid in poultry diets. Methionine participates in numerous synthetic and metabolic processes, producing metabolites such as glutathione, taurine, and homocysteine that exert primary physiological functions [4-5]. Optimal sulphur amino acid requirements vary among different breeds and growth stages. For instance, NRC (1994) recommends 0.49% sulphur amino acids for 6–12-week-old brown-shell layers [6], while China's *Feeding Standard of Chicken* (NY/T 33—2004) suggests 0.74% for 0–8-week-old growing layers and 0.55% for 9–18-week-old layers [7]. Appropriate dietary methionine supplementation can promote growth and reduce feed-to-gain ratio in meat poultry [8-9]. Zhang et al. [10] reported that different methionine levels significantly affected average daily gain and feed intake but not feed-to-gain ratio in 7–12-week-old Lueyang black-bone chickens. Song et al. [11] found that dietary methionine levels significantly affected final body weight and feed-to-gain ratio but not average daily gain or feed intake in 5–8-week-old Jinghong layers, establishing a methionine requirement of 0.44% for that stage. Xi et al. [9] demonstrated that increasing dietary methionine levels caused body weight, average daily gain, and flock uniformity to follow a quadratic trend, initially increasing then decreasing, while significantly affecting feed intake and feed-to-gain ratio in a similar quadratic pattern. In the present study, sulphur amino acid levels significantly affected final body weight, average daily gain, and feed-to-gain ratio, showing linear increasing or decreasing trends with elevated levels. While similar to previous research, our results exhibited a linear broken-line pattern rather than a clear quadratic curve. When sulphur amino acid levels reached a certain point (breakpoint x=0.62), final body weight and average daily gain ceased to increase and entered a plateau phase, with 0.62% representing the requirement.

Furthermore, our growth performance results demonstrate that increasing sulphur amino acid levels significantly improved average daily gain while reducing feed-to-gain ratio, indicating that transitioning from amino acid deficiency to balance is crucial for growth and development. Therefore, appropriate dietary sulphur amino acid supplementation can promote layer growth, enhance feed efficiency, and improve economic returns. Previous studies have also reported that sulphur amino acid levels slightly exceeding NRC standards benefit animal growth [12], consistent with our findings.

The ultimate goal of nutritional regulation during the rearing period is to achieve high and stable production during the laying period, as rearing development correlates strongly with subsequent production performance. Wu et al. [13] found that final body weight of Hy-Line White pullets at 126 days of age was extremely significantly positively correlated with cumulative egg production, daily egg output, and total egg number during weeks 21–65. However, determining nutritional requirements for rearing layers based solely on growth performance data carries certain risks, and comprehensive evaluation combining serum biochemical indices, body composition analysis, and laying period tracking observations is recommended.

3.2 Effects of Different Sulphur Amino Acid Levels on Serum Biochemical Indices of Jinghong Layers Aged 7–12 Weeks

Serum biochemical indices reflect important metabolic processes and organ functions in animals. Serum total protein, comprising albumin and globulin, transports various metabolites, regulates physiological functions of transported substances, maintains vascular osmotic pressure and acid-base balance, and reflects nutritional status [14]. Albumin primarily transports raw materials for tissue synthesis and metabolic waste, while globulin reflects immune competence, with elevated globulin indicating enhanced humoral immunity [15]. Zhang et al. [10] reported that different methionine levels did not significantly affect serum albumin, globulin, or albumin-to-globulin ratio in 7–12-week-old Lueyang blackbone chickens. In our study, sulphur amino acid levels did not significantly affect albumin content, though albumin tended to increase with higher sulphur amino acid levels, suggesting enhanced amino acid metabolism. Song et al. [16] reported that albumin content positively correlates with growth rate, consistent with our body weight gain results. Sulphur amino acid levels significantly affected total protein and globulin contents, with the 0.66% group showing significantly higher values than the other four groups, likely due to high sulphur amino acid levels stimulating immune system activation.

Alkaline phosphatase activity serves as a sensitive indicator for evaluating layer growth and development, with higher activity indicating faster growth [17]. Although sulphur amino acid levels did not significantly affect alkaline phosphatase activity in our study, the 0.58% and 0.66% groups showed higher activity than other groups, suggesting that dietary sulphur amino acid levels of 0.58% and 0.66% promote layer growth and development, consistent with final body weight and average daily gain results.

Uric acid represents a major end product of protein and amino acid metabolism in poultry, with dietary amino acid imbalance affecting blood uric acid content [18]. Studies have shown that dietary methionine supplementation increases broiler body weight while reducing serum uric acid [19], a pattern inconsistent with our results. This discrepancy may be explained as follows: the 0.42%–0.50% sulphur amino acid levels failed to meet animal requirements, causing essential amino acid imbalance and waste of amino acids other than sulphur amino acids, resulting in higher serum uric acid. As sulphur amino acid levels increased, amino acid composition approached balance, improving utilization and gradually reducing uric acid content. When sulphur amino acid levels became excessive, unused amino acids were degraded to uric acid. This suggests that sulphur amino acid levels between 0.50% and 0.66% represent a transition from gradual amino acid balance to excess.

4 Conclusion

Based on comprehensive evaluation of growth performance and serum biochemical indices, the dietary sulphur amino acid requirement for 7–12-week-old Jinghong commercial layers ranges from 0.58% to 0.66%. Using final body weight and average daily gain as criteria, single-slope nonlinear broken-line regression analysis determined the sulphur amino acid requirement to be 0.62% (equivalent to 314 mg/d per bird).

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