Threonine Requirement of Linwu Ducks During Peak Laying Period: Postprint

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Abstract

The present study was conducted to investigate the effects of dietary threonine levels on laying performance, egg quality, and serum biochemical indices of Linwu ducks aged 30-38 weeks, and to determine the threonine requirement during the peak laying period. Two hundred Linwu ducks with similar body weight, good health status, and no significant difference in laying rate ($P>0.05$) were randomly allocated into 5 groups with 5 replicates per group and 8 ducks per replicate. The dietary threonine levels for each group were 0.55%, 0.60%, 0.65%, 0.70%, and 0.75%, respectively. The experimental period lasted 63 days. The results showed: 1) The feed-to-egg ratio of the 0.65% threonine group was significantly lower than that of the 0.55% threonine group ($P<0.05$). Dietary threonine levels had no significant effects on average daily feed intake, laying rate, average egg weight, or daily egg production ($P>0.05$). 2) The albumen height and Haugh unit of the 0.65%, 0.70%, and 0.75% threonine groups were significantly higher than those of the 0.55% threonine group ($P<0.05$). Dietary threonine levels had no significant effects on egg shape index, shell thickness, yolk color, yolk ratio, shell ratio, or albumen ratio ($P>0.05$). 3) The serum total protein content of the 0.70% threonine group was significantly higher than that of the 0.55% threonine group ($P<0.05$). The serum alanine aminotransferase activity of the 0.65% threonine group was significantly higher than that of the 0.55% threonine group ($P<0.05$). The serum urea nitrogen content of the 0.65%, 0.70%, and 0.75% threonine groups was significantly lower than that of the 0.55% and 0.60% threonine groups ($P<0.05$). Dietary threonine levels had no significant effects on serum albumin, total cholesterol, triglyceride, uric acid, or creatinine contents, nor on alkaline phosphatase or aspartate aminotransferase activities ($P>0.05$). 4) Quadratic regression analysis indicated that, using feed-to-egg ratio and serum urea nitrogen content as evaluation criteria, the threonine requirements of Linwu ducks during the peak laying period were 0.67% and 0.69%, respectively. In conclusion, appropriate dietary threonine levels can

improve laying performance, egg quality, and protein utilization in Linwu ducks. Based on these indices, the appropriate dietary threonine level for Linwu ducks during the peak laying period (30-38 weeks of age) is 0.67%-0.69%.

Full Text

Threonine Requirement of Linwu Ducks in Peak Laying Period

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Abstract: This experiment was conducted to investigate the effects of dietary threonine level on laying performance, egg quality, and serum biochemical indices of Linwu ducks aged 30–38 weeks, and to determine the threonine requirement during their peak laying period. Two hundred healthy Linwu ducks with similar body weight and laying rate (P>0.05) were randomly allocated to 5 groups with 5 replicates per group and 8 ducks per replicate. The dietary threonine levels were 0.55%, 0.60%, 0.65%, 0.70%, and 0.75%, respectively. The experiment lasted for 63 days. The results showed: 1) The feed-to-egg ratio in the 0.65% threonine group was significantly lower than that in the 0.55% threonine group (P<0.05). Dietary threonine level had no significant effects on average daily feed intake, laying rate, average egg weight, or daily egg yield (P>0.05). 2) Albumen height and Haugh unit in the 0.65%, 0.70%, and 0.75% threonine groups were significantly higher than those in the 0.55% threonine group (P<0.05). Dietary threonine level had no significant effects on egg shape index, eggshell thickness, yolk color, or the percentages of yolk, eggshell, and albumen (P>0.05). 3) Serum total protein content in the 0.70% threonine group was significantly higher than that in the 0.55% threonine group (P<0.05). Serum alanine aminotransferase activity in the 0.65% threonine group was significantly higher than that in the 0.55% threonine group (P<0.05). Serum urea nitrogen content in the 0.65%, 0.70%, and 0.75% threonine groups was significantly lower than that in the 0.55% and 0.60% threonine groups (P<0.05). Dietary threonine level had no significant effects on serum albumin, total cholesterol, triglyceride, uric acid, creatinine contents, or alkaline phosphatase and aspartate aminotransferase activities (P>0.05). 4) Quadratic curve analysis indicated that based on feed-to-egg ratio and serum urea nitrogen content, the threonine requirements for Linwu ducks during peak laying period were 0.67% and 0.69%, respectively. These results demonstrate that optimal dietary threonine level can improve laying performance, egg quality, and protein utilization efficiency in Linwu ducks.

Based on these indicators, the suitable dietary threonine level for Linwu ducks during peak laying period (30–38 weeks of age) is 0.67%–0.69%.

Keywords: threonine; Linwu ducks; requirement; serum biochemical indices

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Introduction

In recent years, the appropriate supplementation levels of essential amino acids in livestock and poultry diets have become a research hotspot. Threonine is the third limiting amino acid in corn-soybean meal-based diets after methionine and lysine [1], and possesses biological functions such as promoting growth and enhancing immunity [2]. Consequently, it has gradually become a limiting factor affecting poultry production performance. Studies have shown that appropriate dietary threonine levels can promote amino acid balance, improve feed utilization and protein deposition, and reduce nitrogen emissions [3]. Niemeyer [4] reported that a dietary threonine level of 0.76% significantly improved laying performance in 42-week-old commercial laying hens and reduced their crude protein requirement. Liu et al. [5] found that under low protein level (14.56%) diets, a threonine level of 0.76% yielded optimal laying rate and feed efficiency in 56-week-old Jinghong No. 1 laying hens. Additionally, the Duck Feeding Standard (2012) recommends threonine levels for laying ducks as follows: 0.65% for Pekin ducks during laying period (27–70 weeks), 0.60% for Muscovy and mule ducks during laying period (27–65 weeks), and 0.60% for dual-purpose meat-type ducks during laying period (24–70 weeks) [6]. These studies have primarily focused on laying hens and broilers. Linwu duck, as a high-quality local dual-purpose breed, has experienced rapid development in production; however, no studies have reported the threonine requirement for this breed. Therefore, this experiment used peak-laying Linwu ducks to investigate the effects of dietary threonine levels on laying performance, egg quality, and serum biochemical indices from 30 to 38 weeks of age, providing a theoretical basis for determining the optimal dietary threonine level.

1.1 Experimental Design and Diets

Two hundred 29-week-old Linwu ducks with good health, similar body weight, and no significant difference in laying rate ($P>0.05$) were randomly divided into 5 groups with 5 replicates per group and 8 ducks per replicate. A 63-day feeding trial was conducted. The dietary crude protein, metabolizable energy, and amino acid parameters were formulated according to the Linwu Duck Nutrient Requirements Standard (2014) [7]. The composition and nutrient levels of experimental diets are shown in Table 1 . The threonine supplementation gradient was 0.05%, resulting in dietary threonine levels of 0.55%, 0.60%, 0.65%, 0.70%, and 0.75% across groups. Ducks were housed in a closed duck house with double-layer metal cages in a three-dimensional cage system, one duck per cage, with

ad libitum access to feed and water throughout the trial period. Conventional feeding management and immunization procedures were followed.

1.2.2 Egg Quality

At the end of weeks 5 and 9 of the experiment, 15 eggs (3 per replicate) with weights close to the average were collected from each group and stored at 4°C. Within 24 hours, the percentages of yolk, eggshell, and albumen, eggshell thickness (measured with eggshell thickness gauge), egg shape index (measured with vernier caliper), yolk color (measured with yolk color fan), and albumen height (measured with albumen height gauge) were determined, and Haugh unit was calculated using the formula:

$$\text{Haugh unit} = 100 \times \log(H - 1.7W^{0.37} + 7.57)$$

where H is albumen height (mm) and W is egg weight (g).

1.2.3 Serum Biochemical Indices

At the end of the experiment, 2 ducks with similar body weight were randomly selected from each replicate. After 12 hours of fasting, 5 mL of blood was collected from the wing vein. After standing for 40 minutes, serum was separated by centrifugation at 3,000 r/min for 15 minutes and stored at -20°C. Serum total protein, albumin, urea nitrogen, uric acid, creatinine, total cholesterol, and triglyceride contents were measured using an automatic biochemical analyzer (URIT-8000, Urit, USA). Serum alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities were measured using colorimetric methods. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.3 Data Processing

Data were analyzed using one-way ANOVA and regression analysis with SPSS 18.0 software. Results are expressed as "mean ± standard deviation"(mean±SD). Differences were considered significant at $P<0.05$, and Duncan' s multiple comparison test was performed for significant differences. Finally, linear or curvilinear regression equations were established between the measured indices and dietary threonine levels for significant indicators, and the optimal threonine level was derived from the curvilinear regression equations.

2.1 Effects of Dietary Threonine Level on Laying Performance of Linwu Ducks

As shown in Table 2 , dietary threonine level had no significant effects on average daily feed intake, laying rate, average egg weight, or daily egg yield ($P>0.05$). The feed-to-egg ratio in the 0.65% threonine group was significantly lower than

that in the 0.55% threonine group (P<0.05), but showed no significant differences compared with the other three groups (P>0.05).

2.3 Effects of Dietary Threonine Level on Serum Biochemical Indices of Linwu Ducks

As shown in Table 4 , serum total protein content exhibited a trend of first increasing then decreasing with increasing dietary threonine levels, with the 0.70% threonine group being significantly higher than the 0.55% threonine group (P<0.05). Serum alanine aminotransferase activity in the 0.65% threonine group was significantly higher than that in the 0.55% threonine group (P<0.05). Serum urea nitrogen content in the 0.65%, 0.70%, and 0.75% threonine groups was significantly lower than that in the 0.55% and 0.60% threonine groups (P<0.05). Dietary threonine level had no significant effects on serum albumin, total cholesterol, triglyceride, uric acid, creatinine contents, or alkaline phosphatase and aspartate aminotransferase activities (P>0.05).

2.4 Threonine Requirement of Linwu Ducks in Peak Laying Period

As shown in Table 5 , albumen height, Haugh unit, and serum total protein content of peak-laying Linwu ducks increased with dietary threonine level (P<0.05). Feed-to-egg ratio and serum urea nitrogen content showed a quadratic trend with increasing dietary threonine level (P<0.05). By establishing quadratic equations, the dietary threonine levels required to achieve the lowest feed-to-egg ratio and serum urea nitrogen content were estimated to be 0.67% and 0.69%, respectively.

3.1 Effects of Dietary Threonine Level on Laying Performance and Egg Quality of Linwu Ducks

Currently, few studies have reported the effects of threonine on laying performance of laying ducks, and results from other poultry species show considerable variation. Some studies suggest that different threonine supplementation levels (0.45%–0.65%) in low-energy diets have no significant effects on feed intake, average egg weight, feed-to-egg ratio, or broken egg rate in peak-laying Hy-Line Brown hens [8]. Zhou [9] reported that threonine levels between 0.55% and 0.75% had no significant effects on growth performance of 1–8-week-old Hepu geese. However, Martínez-Amezcua et al. [10] found that increasing dietary threonine level from 0.47% to 0.52% in corn-soybean meal diets significantly improved average egg weight and feed conversion ratio in 62–72-week-old Isa-Babcock hens, though threonine levels between 0.52% and 0.62% showed no significant effects on laying performance. Jiang et al. [11] reported that a dietary threonine level of 0.74% yielded the fastest growth rate and optimal feed conversion ratio in 1–4-week-old geese, and significantly increased feed intake in 5–8-week-old geese. These discrepancies may be attributed to differences in growth stage, breed, diet composition, and crude protein level. The present study found that 0.65% dietary threonine significantly reduced feed-to-egg ratio

and improved feed utilization efficiency, consistent with the findings of Yamazaki et al. [12].

Egg quality determines egg freshness and is typically evaluated using albumen height and Haugh unit. A higher Haugh unit generally indicates a longer shelf life [13], and albumen height is positively correlated with Haugh unit [14]. Studies by Tian et al. [8] and Okazaki et al. [15] suggest that dietary threonine levels between 0.45% and 0.65% have no significant effects on egg quality of laying hens. However, this experiment showed that dietary threonine level significantly affected albumen height and Haugh unit, consistent with the results of Liu et al. [5]. This may be because appropriate threonine supplementation improved dietary amino acid balance, enhanced protein utilization efficiency, and promoted protein deposition in eggs.

3.2 Effects of Dietary Threonine Level on Serum Biochemical Indices of Linwu Ducks

Protein metabolism in the body includes anabolism and catabolism. Serum total protein and albumin are important indicators reflecting liver protein synthesis capacity and dietary protein utilization efficiency, while serum uric acid and urea nitrogen, as end products of protein and amino acid metabolism, generally indicate lower protein utilization efficiency when their concentrations are higher [16]. Threonine, as an essential amino acid, plays an important role in protein synthesis and uric acid formation [17]. In this experiment, serum total protein content increased linearly with dietary threonine level, with the 0.70% threonine group being significantly higher than the 0.55% threonine group. Threonine levels above 0.65% significantly reduced serum urea nitrogen content, consistent with the findings of Jiang [18] in 1–8-week-old Yangzhou geese. Serum alanine aminotransferase and aspartate aminotransferase activities reflect the conversion rate of α-pyruvate to α-amino acids; higher activities indicate greater protein synthesis and deposition [9]. Wang et al. [19] reported that serum alanine aminotransferase activity in experimental chickens increased with threonine level, while the 0.64% threonine group showed significantly higher aspartate aminotransferase activity than the 0.49% and 0.55% threonine groups. In this experiment, dietary threonine levels between 0.65% and 0.75% increased serum aspartate aminotransferase and alanine aminotransferase activities, indicating that dietary threonine level can affect protein metabolism, though the specific mechanism requires further investigation.

3.3 Threonine Requirement of Linwu Ducks in Peak Laying Period

Current research on threonine requirements in poultry has primarily focused on chickens, with recommended threonine levels ranging from 0.68%–0.79% for broilers [19] and 0.58%–0.75% for laying hens [20–22]. Using broken-line models, Maqbool [23] estimated threonine requirements for early-stage Pekin ducks in low-protein diets to be 0.54%, 0.54%, and 0.50% based on average daily feed intake, average daily gain, and breast muscle rate, respectively, while in high-

protein diets the requirements were 0.62%, 0.61%, and 0.61%. Guo et al. [24] used quadratic models to estimate threonine requirements for early-stage Pekin ducks as 0.794%, 0.733%, and 0.790% based on average daily gain, average daily feed intake, and down length, respectively. Zhang et al. [25] reported threonine requirements of 0.688% and 0.737% for 1–14-day-old Pekin ducks fed high-protein (20%) diets based on average daily feed intake and average daily gain, respectively. No studies have reported threonine requirements for laying ducks. In this experiment, feed-to-egg ratio, albumen height, Haugh unit, and serum total protein and urea nitrogen contents sensitively reflected the threonine nutritional status of experimental ducks. However, only feed-to-egg ratio and serum urea nitrogen content were suitable for establishing quadratic models, from which the threonine requirement for 30–38-week-old Linwu ducks was estimated to be 0.67% and 0.69%. This result is slightly higher than the threonine requirement (0.60%) for dual-purpose meat-type breeder ducks during mid-laying period recommended in the China Duck Feeding Standard (2012).

Under the conditions of this experiment, appropriate dietary threonine level reduced feed-to-egg ratio and improved egg quality and protein utilization efficiency. Based on feed-to-egg ratio and serum urea nitrogen content, the recommended dietary threonine level for Linwu ducks during peak laying period (30–38 weeks of age) is 0.67%–0.69%.

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