

## Effects of Dietary Lysine Level on Production Performance, Egg Quality, Serum Biochemical Indices, and Nitrogen Metabolism in Linwu Ducks during Peak Laying Period: Postprint

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### Abstract

This experiment aimed to investigate the effects of dietary lysine levels on production performance, egg quality, serum biochemical indices, and nitrogen metabolism in Linwu ducks during peak laying period (30–38 weeks of age), and to determine the lysine requirement for Linwu ducks at 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 selected and randomly divided into 5 groups with 5 replicates per group and 8 ducks per replicate. Dietary lysine levels were 0.65%, 0.75%, 0.85%, 0.95%, and 1.05%, respectively, and the experimental period lasted 63 days. After the feeding trial, 10 ducks with body weight close to the group average were selected from each group for a metabolism trial. The results showed: 1) The daily egg weight in 0.95% and 1.05% lysine groups was significantly higher than that in 0.65% and 0.75% lysine groups ( $P < 0.05$ ), while the feed-to-egg ratio in 0.95% and 1.05% lysine groups was significantly lower than that in 0.65% and 0.75% lysine groups ( $P < 0.05$ ). 2) Dietary lysine level had no significant effect on egg quality ( $P > 0.05$ ). 3) Serum total protein content in 0.85%, 0.95%, and 1.05% lysine groups was significantly higher than that in 0.65% lysine group ( $P < 0.05$ ). The triiodothyronine content in serum of 0.95% lysine group was significantly higher than that in 0.65%, 0.75%, and 1.05% lysine groups ( $P < 0.05$ ). 4) Net protein utilization and nitrogen retention in 0.95% lysine group were significantly or extremely significantly higher than those in 0.65% lysine group ( $P < 0.05$  or  $P < 0.01$ ). 5) Quadratic curve analysis indicated that based on total protein, net protein utilization, and nitrogen retention as evaluation indices, the dietary lysine requirements for Linwu ducks during peak laying period were 0.92%, 0.96%, and 0.95%, respectively. Therefore, daily egg weight, feed-to-egg ratio, total protein, net

protein utilization, and nitrogen retention of 30–38-week-old Linwu ducks were relatively sensitive to changes in dietary lysine level, and the appropriate lysine level for obtaining better production performance, serum biochemical indices, and nitrogen balance was 0.92%–0.96%.

## Full Text

### Effects of Dietary Lysine Level on Performance, Egg Quality, Serum Biochemical Indices and Nitrogen Metabolism of Linwu Ducks in Peak Laying Period

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## Abstract

This experiment was conducted to investigate the effects of dietary lysine level on production performance, egg quality, serum biochemical indices, and nitrogen metabolism of Linwu ducks during peak laying period (30–38 weeks of age), and to determine the lysine requirement for this production stage. Two hundred Linwu ducks with similar body weight, good health status, and comparable laying rates ( $P > 0.05$ ) were randomly allocated to five groups with five replicates per group and eight ducks per replicate. Dietary lysine levels were 0.65%, 0.75%, 0.85%, 0.95%, and 1.05%, with a 63-day experimental period. Following the feeding trial, ten ducks per group with body weights close to their respective group means were selected for a metabolism trial. The results showed: 1) Daily egg weight in the 0.95% and 1.05% lysine groups was significantly higher than in the 0.65% and 0.75% lysine groups ( $P < 0.05$ ), while feed-to-egg ratio in the 0.95% and 1.05% lysine groups was significantly lower ( $P < 0.05$ ). 2) Dietary lysine level had no significant effect on egg quality ( $P > 0.05$ ). 3) Serum total protein content in the 0.85%, 0.95%, and 1.05% lysine groups was significantly higher than in the 0.65% lysine group ( $P < 0.05$ ). Serum triiodothyronine content in the 0.95% lysine group was significantly higher than in the 0.65%, 0.75%, and 1.05% lysine groups ( $P < 0.05$ ). 4) Net protein utilization and nitrogen deposition in the 0.95% lysine group were significantly or extremely significantly higher than in the 0.65% lysine group ( $P < 0.05$  or  $P < 0.01$ ). 5) Quadratic curve analysis indicated that based on total protein, net protein utilization, and nitrogen deposition as evaluation criteria, the optimal dietary lysine requirements for peak-period Linwu ducks were 0.92%, 0.96%, and 0.95%, respectively. These findings demonstrate that daily egg weight, feed-to-egg ratio, total protein, net protein utilization, and nitrogen deposition in 30–38 week-old Linwu ducks are sensitive to dietary lysine level changes, with 0.92%–0.96% being the appropriate lysine level for optimal production performance, serum biochemical indices, and nitrogen balance.

**Keywords:** lysine; laying ducks; performance; nitrogen metabolism

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The efficiency of protein utilization in poultry depends on amino acid content, composition, and availability in the diet. Lysine, as the second limiting amino acid in poultry diets, is primarily used for protein synthesis and is therefore referred to as a “growth amino acid.” Dietary lysine content not only reflects protein quality but also significantly influences poultry production performance and feed conversion efficiency. Previous studies have reported varying lysine requirements for laying ducks due to differences in breed, production stage, diet type, and nutritional levels. Research indicates that the appropriate lysine level for laying ducks ranges between 0.80%-0.95% during the laying period. The Chinese Feeding Standard for Meat Ducks (2012) recommends lysine requirements of 0.80%, 0.95%, and 1.00% for Peking ducks in early, middle, and late laying periods, respectively; 0.80% for Muscovy ducks; and 0.80%, 0.85%, and 0.85% for dual-purpose ducks in early, middle, and late laying periods. Linwu duck is a renowned local dual-purpose duck breed in China. To fully exploit its production potential and improve feed utilization, research on lysine requirements across different production stages is necessary. Therefore, this study used 30-38 week-old Linwu ducks to investigate the effects of varying lysine supplementation levels on production performance, egg quality, serum biochemical indices, and nitrogen metabolism, providing theoretical basis and data support for establishing feeding standards for Linwu ducks.

### 1.1 Experimental Design and Diets

Two hundred 29-week-old Linwu ducks in peak laying period with good health status and similar body weight were randomly divided into five groups with five replicates per group and eight ducks per replicate for a 63-day feeding trial, including a 7-day pre-trial period and a 56-day formal trial period. The basal diet was formulated according to NRC (1994) and Chinese Feeding Standard for Meat Ducks (2012), combined with other nutrient requirement parameters previously determined by our laboratory. A corn-soybean meal basal diet was used with lysine levels of 0.65%, 0.75%, 0.85%, 0.95%, and 1.05%. Other nutrient levels were consistent across all experimental diets, with lysine levels adjusted by adding synthetic L-lysine hydrochloride (purity 78.5%). All diets were pelleted. The composition and nutrient levels of the experimental diets for 30-38 week-old Linwu ducks are shown in Table 1 .

**Table 1. Composition and Nutrient Levels of Experimental Diets (Air-Dry Basis), %**

*Note: 1) The premix provided the following per kg of diet: VA 5,000 IU, VB1 2 mg, VB2 15 mg, VB6 4 mg, VB12 0.02 mg, VD3 800 IU, VE 20 IU, VK3 0.5 mg, biotin 0.2 mg, folic acid 0.6 mg, D-pantothenic acid 60 mg, nicotinic*

acid 60 mg, choline 1,500 mg, antioxidant 100 mg, Cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 50 mg, Zn (as zinc sulfate) 60 mg, I (as potassium iodide) 0.40 mg, Se (as sodium selenite) 0.20 mg. 2) CP and Lys were measured values, while other nutrient levels were calculated values.

## 1.2 Husbandry Management

The feeding trial was conducted at the Waterfowl Experimental Duck Farm of Hunan Institute of Animal Science and Veterinary Medicine. Ducks were housed in a closed duck house with two-tier metal cages in a three-dimensional cage system, with individual cage housing. Throughout the trial, ducks had free access to feed and water (measured but not limited) and were managed and immunized according to conventional methods.

### 1.3.1 Production Performance

During the trial, daily records were kept by replicate for total egg number, total egg weight, feed intake, number of unqualified eggs (including soft-shelled, cracked, misshapen, and sand-shelled eggs), and mortality. Group statistics were calculated for average egg weight, daily egg weight, laying rate, qualified egg rate, mortality rate, average daily feed intake, and feed-to-egg ratio.

### 1.3.2 Egg Quality

Each month, 15 eggs per group (3 eggs per replicate) with weights close to the average egg weight were collected and stored at 4°C. Within 24 hours, measurements were taken for yolk ratio, albumen ratio, shell thickness (using shell thickness gauge), egg shape index (measured with vernier calipers), yolk color (using yolk color fan), and albumen height (using albumen height gauge). 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).

### 1.3.3 Serum Biochemical Indices

On the final day of the trial, two ducks per replicate with similar body weight were randomly selected. After 12 hours of fasting, 5 mL of blood was collected from the wing vein, allowed to stand for 30 minutes, then centrifuged at 3,000 r/min for 15 minutes to separate serum, which was stored at -20°C. Serum total protein (TP), albumin (ALB), urea nitrogen (UN), and uric acid (UA) were measured using an automatic biochemical analyzer (URIT-8000, Urit, USA). Growth hormone (GH), cortisol, triiodothyronine (T3), and thyroxine (T4) were determined by radioimmunoassay using a GC-300 -radioimmunoassay instrument, and T3/T4 ratio was calculated.

### 1.3.4 Nitrogen Metabolism Indices

After the feeding trial, two ducks per replicate with average body weight were selected and housed in metabolism cages, fed their respective experimental diets with free access to water and feed. After a 3-day pre-feeding period, daily feed intake was recorded and total excreta were collected daily during the formal period. Feathers, scurf, and other contaminants were removed from the feces, which were then fixed with 10% hydrochloric acid, mixed thoroughly, dried in a 65°C oven, rehydrated naturally for 24 hours, weighed accurately, and ground using a small universal grinder for subsequent analysis. Nitrogen content was determined according to *Feed Analysis and Feed Quality Detection Technology* [6]. Net protein utilization and nitrogen deposition were calculated using the following formulas:

$$\text{Net protein utilization (\%)} = (\text{deposited nitrogen} / \text{nitrogen intake}) \times 100$$
$$\text{Nitrogen deposition} = \text{nitrogen intake} - (\text{fecal nitrogen} + \text{urinary nitrogen})$$

### 1.4 Data Processing

Data were analyzed using SPSS 18.0 software for one-way ANOVA and regression analysis. Results are expressed as “mean ± standard deviation” (mean±SD). Statistical significance was set at  $P < 0.05$ , and extreme significance at  $P < 0.01$ . Duncan’s multiple comparison test was applied when significant differences were detected. Finally, sensitive indicators were subjected to linear and quadratic regression analysis to determine the lysine requirement of Linwu ducks in peak laying period.

### 2.1 Effects of Dietary Lysine Level on Performance of Laying Ducks

As shown in Table 2, dietary lysine level had no significant effects on average daily feed intake, laying rate, average egg weight, or qualified egg rate ( $P > 0.05$ ). Daily egg weight in the 0.95% and 1.05% lysine groups was significantly higher than in the 0.65% and 0.75% lysine groups ( $P < 0.05$ ), while feed-to-egg ratio in the 0.95% and 1.05% lysine groups was significantly lower than in the 0.65% and 0.75% lysine groups ( $P < 0.05$ ).

**Table 2. Effects of Dietary Lysine Level on Performance of Laying Ducks**

*Note: In the same row, values with different small letter superscripts indicate significant difference ( $P < 0.05$ ), different capital letter superscripts indicate extreme significant difference ( $P < 0.01$ ), and same or no letter superscripts indicate no significant difference ( $P > 0.05$ ). The same applies below.*

### 2.2 Effects of Dietary Lysine Level on Egg Quality of Laying Ducks

As shown in Table 3, dietary lysine level had no significant effect on egg quality of laying ducks ( $P > 0.05$ ).

**Table 3. Effects of Dietary Lysine Level on Egg Quality of Laying Ducks****2.3 Effects of Dietary Lysine Level on Serum Biochemical Indices of Laying Ducks**

As shown in Table 4 , dietary lysine level had no significant effects on serum albumin, uric acid, urea nitrogen, cortisol, growth hormone, T4 content, or T3/T4 ratio in laying ducks ( $P>0.05$ ). Total protein content in the 0.85%, 0.95%, and 1.05% lysine groups was significantly higher than in the 0.65% lysine group ( $P<0.05$ ). Serum urea nitrogen content showed a decreasing trend with increasing lysine supplementation ( $P>0.05$ ). Serum T3 content in the 0.95% lysine group was significantly higher than in the 0.65%, 0.75%, and 1.05% lysine groups ( $P<0.05$ ).

**Table 4. Effects of Dietary Lysine Level on Serum Biochemical Indices of Laying Ducks****2.4 Effects of Dietary Lysine Level on Nitrogen Metabolism of Laying Ducks**

As shown in Table 5 , dietary lysine level had no significant effect on nitrogen intake ( $P>0.05$ ). Net protein utilization was highest in the 0.95% lysine group, being significantly higher than in the 0.65% lysine group ( $P<0.05$ ). Nitrogen deposition in the 0.95% lysine group was extremely significantly higher than in the 0.65% lysine group ( $P<0.01$ ) and significantly higher than in the 0.75% lysine group ( $P<0.05$ ). Nitrogen deposition in the 1.05% lysine group was significantly higher than in the 0.65% lysine group ( $P<0.05$ ).

**Table 5. Effects of Dietary Lysine Level on Nitrogen Metabolism of Laying Ducks****2.5 Lysine Requirement of Linwu Ducks in Peak Laying Period**

Based on P-values, average daily feed intake, laying rate, average egg weight, qualified egg rate, albumin, uric acid, urea nitrogen, cortisol, growth hormone, T4, T3/T4, and all egg quality parameters showed no significant changes with dietary lysine level ( $P>0.05$ ), and thus were not subjected to regression analysis. Since T3 content did not show linear or quadratic trends with dietary lysine level ( $P>0.05$ ), no appropriate mathematical model could be established. As shown in Table 2, both daily egg weight and feed-to-egg ratio showed linear trends with dietary lysine level ( $P=0.032$ ,  $P=0.041$ ). Regression analysis in Table 6 indicated that serum total protein content, net protein utilization, and nitrogen deposition in Linwu ducks exhibited quadratic trends with increasing dietary lysine level, first increasing then decreasing. Derivation of the quadratic curves yielded peak x-coordinates of 0.92%, 0.96%, and 0.95%, respectively, indicating that dietary lysine levels of 0.92%, 0.96%, and 0.95% would maximize serum total protein content, net protein utilization, and nitrogen deposition.

**Table 6. Lysine Requirement of Linwu Laying Ducks in Peak Laying Period****3.1 Effects of Dietary Lysine Level on Performance and Egg Quality of Laying Ducks**

Numerous studies have shown that appropriate dietary lysine supplementation can increase feed intake and average egg weight while decreasing feed-to-egg ratio in laying hens. This study found that when dietary crude protein level was 18% with low lysine levels, Linwu ducks exhibited lower daily egg weight and higher feed-to-egg ratio, indicating poor feed conversion efficiency. As lysine level increased, daily egg weight increased and feed-to-egg ratio decreased, improving feed utilization, which is consistent with previous reports. Supplementation of 0.95% lysine yielded optimal production performance except for laying rate and qualified egg rate. Regression analysis showed that both daily egg weight and feed-to-egg ratio were linearly correlated with dietary lysine level, suggesting that 0.95% lysine is the appropriate level for peak-period Linwu ducks. This value is slightly higher than the recommendations in Chinese Feeding Standard for Meat Ducks (2012) for Muscovy ducks (0.80%) and dual-purpose ducks (0.85%) during laying period, as well as results from Zhang et al. (0.825% for high-yielding laying ducks in summer) and Chen et al. (0.80% for peak-period Longyan laying ducks), but is consistent with NRC (1994) recommendations for mid-laying Peking ducks (0.95%) and Lin et al. (0.95% for 22–28 week-old Linwu ducks). Yang (2002) suggested that egg weight is primarily affected by genetic factors, body weight, nutrition level, and laying age, with nutritional factors mainly including energy, protein, and lipids. Serum biochemical results from this trial showed that urea nitrogen content in the 0.85%, 0.95%, and 1.05% lysine groups was lower than in the 0.65% and 0.75% lysine groups, indicating that increasing dietary lysine level improved amino acid balance and enhanced protein utilization, which may explain why 0.95% lysine improved daily egg weight and reduced feed-to-egg ratio.

Haugh unit is an important indicator of albumen quality; higher values indicate thicker, more viscous albumen, better egg quality, and improved preservation. Li et al. (2011) reported that under low energy conditions, lysine levels of 0.65%–0.85% had no significant effects on egg quality or internal nutrients in Hy-Line Brown hens. Chen et al. (2012) found that lysine had no significant effects on shell strength, Haugh unit, albumen height, or yolk color in Longyan laying ducks. Lin et al. (2014) reported that dietary lysine level had no significant effects on shell thickness, albumen height, Haugh unit, albumen ratio, or yolk ratio in 22–28 week-old Linwu ducks, though 0.95% and 1.05% lysine tended to increase albumen height, Haugh unit, and yolk ratio. This study showed that dietary lysine level had no significant effect on egg quality of 30–38 week-old Linwu ducks, but 0.95% and 1.05% lysine tended to increase albumen height and albumen ratio, suggesting that these lysine levels may increase protein and amino acid deposition in eggs, thereby improving albumen quality and preser-

vation. These results are generally consistent with previous studies, though the underlying mechanism requires further investigation. Thyroid hormones (T3, T4) promote glucose absorption and DNA and protein synthesis, with T3 being the primary physiologically active hormone. The changes in serum T3 content in this trial indicate that ducks in the 0.95% lysine group had vigorous metabolism, with superior absorption of glucose, protein, and amino acids and their deposition in eggs.

### **3.2 Effects of Dietary Lysine Level on Nitrogen Metabolism of Laying Ducks**

Net protein utilization reflects the deposition pattern of dietary protein in the body and effectively indicates crude protein utilization and amino acid balance. Cui et al. (2012) found that 0.70% dietary lysine significantly improved nitrogen deposition and nitrogen biological value in growing rex rabbits, with no further significant effects at higher levels. Ding et al. (2015) studied the effects of different lysine levels on nitrogen metabolism in Xin-yang green-shell laying hens and found that nitrogen deposition and retention rate in the 0.75% lysine group were significantly higher than in the 0.60% lysine group. In this trial, net protein utilization was highest in the 0.95% lysine group, significantly higher than in the 0.65% lysine group. Nitrogen deposition in the 0.95% and 1.05% lysine groups was extremely significantly or significantly higher than in the 0.65% lysine group. Both net protein utilization and nitrogen deposition showed quadratic trends with dietary lysine level. This indicates that in laying duck diets with 18% crude protein, net protein utilization and nitrogen deposition were optimal at 0.95% and 1.05% lysine levels. Combined with production performance and serum biochemical indices, these results suggest that under the conditions of this trial, the 0.95% lysine diet provided superior amino acid balance, with increased protein synthesis and deposition due to improved amino acid balance, consistent with the report by Lin et al. (2014).

### **3.3 Lysine Requirement of Linwu Ducks in Peak Laying Period**

Various methods exist for evaluating amino acid requirements. Traditional nutrition primarily uses dose-response methodology, determining requirements based on the direct relationship between dietary amino acid level and performance. However, with the development of statistical knowledge and SAS software, increasing numbers of scholars use mathematical models to estimate amino acid requirements. Fu et al. (2013) determined the appropriate dietary lysine level for Jinghong I laying breeders to be 0.95% based on comprehensive consideration of egg production, laying rate, and net profit. Ye et al. (2013) reported optimal lysine levels of 1.01%, 0.87%, and 0.72% for Campbell laying ducks at 1-3, 4-6, and 7-9 weeks of age, respectively, using growth performance as the evaluation criterion. Zhang et al. (2008) determined lysine requirements of 1.06%, 1.07%, and 1.12% for early-growing Peking ducks based on daily gain, feed-to-gain ratio, and breast muscle weight using quadratic curve models. These varying re-

sults for poultry lysine requirements are due to differences in research methods, dietary nutrient levels, environmental factors, sensitive indicators, and experimental animals. This trial employed dose-response methodology using sensitive indicators including daily egg weight, feed-to-egg ratio, total protein, net protein utilization, and nitrogen deposition, combined with mathematical model derivation, to determine that the appropriate lysine level for 30-38 week-old Linwu ducks is 0.92%-0.96%.

### Conclusion

Dietary lysine levels of 0.95% and 1.05% significantly increased daily egg weight and nitrogen utilization while decreasing feed-to-egg ratio in experimental ducks. Under the conditions of this trial, the appropriate dietary lysine level for achieving optimal production performance and nitrogen metabolism in 30-38 week-old Linwu ducks is 0.92%-0.96%.

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