Effects of Choline Chloride on Production Performance and Egg Quality in 19- to 42-Week-Old Laying Hens (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary choline chloride supplementation on production performance and egg quality of laying hens aged 19-42 weeks. A total of 990 Jinghong No. 1 laying hens aged 18 weeks were randomly divided into 6 groups with 12 replicates per group and 15 hens per replicate. The hens were fed experimental diets supplemented with 0, 1,000, 2,000, 4,000, 8,000, and 16,000 mg/kg choline chloride in the basal diet. The experimental period lasted 24 weeks. The results showed: 1) During 19-42 weeks of age, the egg production rate in the 2,000, 4,000, 8,000, and 16,000 mg/kg groups was significantly lower than that in the 0 mg/kg group ($P<0.05$); the egg production rate in the 8,000 and 16,000 mg/kg groups was significantly lower than that in the 1,000 mg/kg group ($P<0.05$). The egg weight and feed intake in the 16,000 mg/kg group were significantly lower than those in the 0 mg/kg group ($P<0.05$), and there was no significant difference in feed-to-egg ratio among all groups ($P>0.05$). 2) The eggshell thickness in the 2,000 mg/kg group at 20 and 22 weeks of age was significantly higher than that in the 0 mg/kg group ($P<0.05$); the eggshell strength in the 16,000 mg/kg group at 22 and 38 weeks of age was significantly lower than that in the 0 mg/kg group ($P<0.05$). The albumen height and Haugh unit in the 16,000 mg/kg group at 26 weeks of age were significantly lower than those in the 0 mg/kg group ($P<0.05$); the Haugh unit in the 8,000 mg/kg group at 22 weeks of age was significantly higher than that in the 0 mg/kg group ($P<0.05$). At 20, 21, 22, and 38 weeks of age, the yolk color in the 1,000, 2,000, 4,000, 8,000, and 16,000 mg/kg groups was significantly higher than that in the 0 mg/kg group ($P<0.05$). 3) The whole egg phosphatidylcholine content in the 1,000, 2,000, 4,000, 8,000, and 16,000 mg/kg groups at 24 weeks of age was significantly higher than that in the 0 mg/kg group ($P<0.05$); the whole egg phosphatidylcholine content in the 16,000 mg/kg group at 27 and 38 weeks of age was significantly higher than that in the 0 mg/kg group ($P<0.05$). The yolk phosphatidylcholine content in the

16,000 mg/kg group at 38 weeks of age was significantly higher than that in the 0 mg/kg group (P<0.05). It can be concluded that dietary supplementation with appropriate amounts of choline chloride can improve eggshell thickness, eggshell strength, and yolk color, with a suitable supplementation level of 1,000 mg/kg. However, dietary supplementation with choline chloride at levels higher than 1,000 mg/kg can reduce egg production rate; supplementation at levels higher than 4,000 mg/kg can reduce egg weight and feed intake.

Full Text

Effects of Choline Chloride on Performance and Egg Quality of Laying Hens Aged from 19 to 42 Weeks

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Abstract

This experiment was conducted to investigate the effects of dietary choline chloride supplementation on the performance and egg quality of laying hens aged 19–42 weeks. Nine hundred and ninety 18-week-old Jinghong No. 1 laying hens were randomly allocated into six groups with 12 replicates per group and 15 hens per replicate. The hens were fed basal diets supplemented with 0, 1,000, 2,000, 4,000, 8,000, and 16,000 mg/kg choline chloride for a 24-week experimental period.

The results showed: 1) During weeks 19–42, the egg production rate in the 2,000, 4,000, 8,000, and 16,000 mg/kg groups was significantly lower than that in the 0 mg/kg group (P<0.05), while the 8,000 and 16,000 mg/kg groups had significantly lower production than the 1,000 mg/kg group (P<0.05). The 16,000 mg/kg group exhibited significantly lower egg weight and feed intake compared to the 0 mg/kg group (P<0.05), though no significant differences were observed in feed-to-egg ratio among all groups (P>0.05). 2) The 2,000 mg/kg group showed significantly greater shell thickness at weeks 20 and 22 compared to the control (P<0.05), whereas the 16,000 mg/kg group had significantly lower shell strength at weeks 22 and 38 (P<0.05). The 16,000 mg/kg group also displayed significantly reduced albumen height and Haugh unit at week 26 (P<0.05), while the 8,000 mg/kg group showed significantly higher Haugh unit at week 22 (P<0.05). Yolk color in all supplemented groups (1,000–16,000 mg/kg) was significantly higher than the control at weeks 20, 21, 22, and 38 (P<0.05). 3) Whole egg phosphatidylcholine content in the 1,000–16,000 mg/kg groups was significantly elevated at week 24 (P<0.05), with the 16,000 mg/kg group maintaining significantly higher levels at weeks 27 and 38 (P<0.05). Additionally, the 16,000 mg/kg group exhibited significantly higher yolk phosphatidylcholine content at week 38 (P<0.05).

In conclusion, appropriate choline chloride supplementation can improve shell thickness, shell strength, and yolk color, with an optimal level of 1,000 mg/kg. However, supplementation exceeding 1,000 mg/kg adversely affects egg production rate, while levels above 4,000 mg/kg reduce egg weight and feed intake.

Keywords: laying hens; choline chloride; performance; egg quality

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Introduction

Choline, also known as vitamin B4, exists in animals primarily as lecithin, lysolecithin, phosphocholine, acetylcholine, and neuronal choline [1-4]. It serves three major metabolic functions: 1) as a precursor for the neurotransmitter acetylcholine released at parasympathetic nerve terminals, maintaining normal nervous system function [5-6]; 2) as a structural component of phosphatidylcholine and sphingomyelin, participating in biological membrane and lipoprotein formation [7-8] and preventing conditions such as chicken tibial chondrodysplasia [9-10]; and 3) through oxidation to betaine, acting as an active methyl donor in the synthesis of methionine from homocysteine and creatine from guanidinoacetate [3]. The NRC (1994) recommends choline requirements of 105 and 115 mg/(bird · day) for white-egg and brown-egg laying hens, respectively [11], while Chinese agricultural industry standards (NY/T 33-2004) "Feeding Standard of Chicken" recommend 500 mg/kg for laying hens [12]. Notably, the NRC (1994) recommendation for brown-egg hens was not derived from direct experimental evidence but extrapolated from white-egg hen requirements plus 10% based solely on larger body weight and potentially higher daily egg production. Moreover, the NRC (1994) estimation criteria considered only egg production and weight, ignoring choline' s effects on egg quality. Research on choline in laying hens is primarily over two decades old and yields inconsistent results, creating substantial controversy regarding whether practical production requires additional choline supplementation and what constitutes effective and safe dosage levels [13-16]. As choline chloride is commonly used to supplement choline in livestock diets, and few studies have examined long-term supplementation in brown-egg laying hens, this research aims to provide theoretical reference for choline chloride application in laying hen diets by investigating its effects on performance and egg quality.

1. Materials and Methods

1.1 Experimental Material Choline chloride (white crystals) with purity 98.0% was provided by Sinopharm Chemical Reagent Beijing Co., Ltd.

1.2 Experimental Design and Management Nine hundred and ninety Jinghong No. 1 laying hens at 18 weeks of age were randomly divided into six groups with 12 replicates per group and 15 hens per replicate. Hens were fed corn-soybean meal basal diets supplemented with 0, 1,000, 2,000, 4,000, 8,000, and 16,000 mg/kg choline chloride for 24 weeks. The basal diet was formulated according to NRC (1994) requirements for laying hens. Diet composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (air-dry basis), %

The premix provided per kilogram of diet: VA 3,700 IU, VD 860 mg, VE 14 mg, VK 1 mg, VB 4.6 mg, VB 8 mg, VB 8.6 mg, VB 0.02 mg, calcium pantothenate 14 mg, nicotinic acid 40 mg, folic acid 2.3 mg, biotin 0.17 mg, Mn 87 mg, Zn 34 mg, Fe 21 mg, Cu 7.5 mg, Co 0.2 mg, I 0.5 mg, Se 0.1 mg. Crude protein and calcium were measured values, while others were calculated using *Feed Ingredients and Nutritional Value of China* (23rd edition, 2012).

The feeding trial was conducted at Changping Experimental Base of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. Hens were housed in three-tier step cages (3 hens per cage) and fed three times daily with ad libitum access to feed and water. Lighting was controlled automatically at 16 h/day. Temperature and humidity were monitored using dry-wet bulb thermometers, maintaining 20–25°C and 60–70% relative humidity.

1.3 Measurements 1.3.1 Production Performance

Daily egg number and weight were recorded per replicate; feed consumption was recorded weekly to calculate egg production rate, egg weight, feed intake, and feed-to-egg ratio.

1.3.3 Egg Quality

All eggs collected at weeks 19, 20, 21, 22, 26, 30, and 38 were analyzed within 12 h. Shell strength was measured using a Model- shell strength tester (Robotmation, Japan). Shell thickness was measured using a Model P-1 shell thickness gauge (Ozaki MFG, Japan). Albumen height, Haugh unit, and yolk color were determined using an EMT-2500 egg quality tester (Robotmation, Japan).

1.3.4 Egg Phosphatidylcholine Content

Eggs collected at weeks 19, 20, 21, 22, 24, 27, 30, and 38 were analyzed for whole egg phosphatidylcholine content using high-performance liquid chromatography-evaporative light scattering detection. At week 38, yolk and albumen from the 0, 1,000, and 16,000 mg/kg groups were separated and analyzed for phosphatidylcholine content using the same method.

1.4 Statistical Analysis Data were analyzed using SAS 9.2 software ANOVA procedure with Duncan' s multiple comparison. Significance was set at $P<0.05$. Results are expressed as means±SD, with egg production rate subjected to arcsine transformation before analysis.

2. Results

2.1 Dietary Choline Chloride Content Supplemental levels, calculated values, and measured values of choline chloride in experimental diets are shown in Table 2 . The small discrepancy between calculated and measured values in the 0 mg/kg group indicates accurate diet formulation; the difference likely originated from choline naturally present in corn and soybean meal.

Table 2 Supplemental levels, calculated values, and measured values of choline chloride in experimental diets, mg/kg

2.2.1 Effects on Egg Production Rate Effects of choline chloride on egg production are presented in Table 3 . At weeks 19-23, the 8,000 mg/kg group showed significantly lower production than the 0 mg/kg group ($P<0.05$). At weeks 25-27, the 4,000 mg/kg group had significantly lower production than the 1,000 mg/kg group ($P<0.05$). During weeks 28-36, groups supplemented with 1,000-8,000 mg/kg exhibited significantly lower production than the control ($P<0.05$). At weeks 37-42, the 8,000 and 16,000 mg/kg groups showed significantly lower production than the control ($P<0.05$). Overall (weeks 19-42), the 2,000, 4,000, 8,000, and 16,000 mg/kg groups had significantly lower production than the control ($P<0.05$), while the 8,000 and 16,000 mg/kg groups were significantly lower than the 1,000 mg/kg group ($P<0.05$).

Table 3 Effects of choline chloride on egg production of laying hens, %

In the same row, values with different small letter superscripts differ significantly ($P<0.05$); same or no letters indicate no significant difference ($P>0.05$). This applies to all tables.

2.2.2 Effects on Egg Weight Effects of choline chloride on egg weight are shown in Table 4 . The 16,000 mg/kg group had significantly lower egg weight than the control at weeks 19-27, 28-30, 31-33, 37-39, and 40-42 ($P<0.05$). At week 24, the 8,000 mg/kg group also showed significantly lower egg weight ($P<0.05$). During weeks 31-33, the 4,000 mg/kg group had significantly lower egg weight than the control ($P<0.05$). Overall (weeks 19-42), the 16,000 mg/kg group exhibited significantly lower egg weight than all other groups ($P<0.05$).

Table 4 Effects of choline chloride on egg weight of laying hens, g

2.2.3 Effects on Feed Intake Effects of choline chloride on feed intake are presented in Table 5 . At week 25, the 4,000 and 16,000 mg/kg groups had significantly lower intake than the 1,000 mg/kg group ($P<0.05$). At week 26, the 16,000 mg/kg group showed significantly lower intake than the 1,000 mg/kg group ($P<0.05$). During weeks 28-30, the 4,000 mg/kg group had significantly lower intake than the control ($P<0.05$). At weeks 31-33, groups supplemented with 1,000-8,000 mg/kg showed significantly lower intake than the control ($P<0.05$). During weeks 37-42, the 8,000 and 16,000 mg/kg groups exhibited significantly lower intake than the control and 1,000 mg/kg group

(P<0.05). Overall (weeks 19-42), the 8,000 and 16,000 mg/kg groups had significantly lower feed intake than the control (P<0.05).

Table 5 Effects of choline chloride on feed intake of laying hens, g/d

2.2.4 Effects on Feed-to-Egg Ratio Effects of choline chloride on feed-to-egg ratio are shown in Table 6 . At weeks 22-25, the 1,000 mg/kg group had significantly higher ratio than the control (P<0.05). During weeks 28-30, the 16,000 mg/kg group showed significantly higher ratio than all other groups (P<0.05), while the 4,000 mg/kg group had significantly lower ratio than the control (P<0.05). At weeks 31-33, groups supplemented with 1,000-8,000 mg/kg exhibited significantly lower ratio than the control (P<0.05). During weeks 37-39, the 8,000 and 16,000 mg/kg groups had significantly lower ratio than the 1,000 and 4,000 mg/kg groups (P<0.05). At weeks 40-42, the 16,000 mg/kg group showed significantly lower ratio than the control (P<0.05), with both the 8,000 and 16,000 mg/kg groups lower than the 1,000 and 4,000 mg/kg groups (P<0.05). Overall (weeks 19-42), no significant differences were observed among groups (P>0.05).

Table 6 Effects of choline chloride on feed/egg ratio of laying hens

2.3.1 Effects on Shell Thickness Effects of choline chloride on shell thickness are presented in Table 7 . At week 20, all supplemented groups (1,000-16,000 mg/kg) showed significantly greater shell thickness than the control (P<0.05). At week 21, the 1,000, 4,000, and 16,000 mg/kg groups had significantly greater thickness than the control (P<0.05). At week 22, the 2,000, 4,000, 8,000, and 16,000 mg/kg groups exhibited significantly greater thickness than the control (P<0.05).

Table 7 Effects of choline chloride on shell thickness of laying hens, mm

2.3.2 Effects on Shell Strength Effects of choline chloride on shell strength are shown in Table 8 . At week 19, the 4,000 mg/kg group had significantly higher strength than the 8,000 and 16,000 mg/kg groups (P<0.05). At week 20, the 4,000 mg/kg group showed significantly higher strength than the control (P<0.05). At week 22, the 8,000 mg/kg group exhibited significantly lower strength than the control and the 1,000, 2,000, and 4,000 mg/kg groups (P<0.05). At week 38, the 4,000 and 16,000 mg/kg groups had significantly lower strength than the control and the 1,000, 2,000, and 8,000 mg/kg groups (P<0.05), while the 1,000 and 8,000 mg/kg groups were significantly lower than the 2,000 mg/kg group (P<0.05).

Table 8 Effects of choline chloride on shell strength of laying hens, N

2.3.3 Effects on Albumen Height Effects of choline chloride on albumen height are presented in Table 9 . At week 26, the 16,000 mg/kg group showed significantly lower albumen height than the control and 1,000 mg/kg group

(P<0.05). No significant effects were observed at other timepoints (data not shown, P>0.05).

Table 9 Effects of choline chloride on albumen height of laying hens, mm

2.3.4 Effects on Haugh Unit Effects of choline chloride on Haugh unit are shown in Table 10 . At week 20, the 16,000 mg/kg group had significantly higher Haugh unit than the 2,000 mg/kg group (P<0.05). At week 22, the 8,000 and 16,000 mg/kg groups exhibited significantly higher Haugh unit than the control (P<0.05). At week 26, the 16,000 mg/kg group showed significantly lower Haugh unit than the control and 1,000 mg/kg group (P<0.05). No significant effects were observed at other timepoints (data not shown, P>0.05).

Table 10 Effects of choline chloride on Haugh unit of laying hens

2.3.5 Effects on Yolk Color Effects of choline chloride on yolk color are presented in Table 11 . At weeks 20–22, all supplemented groups (1,000–16,000 mg/kg) showed significantly higher yolk color than the control (P<0.05). At week 26, the 1,000, 4,000, 8,000, and 16,000 mg/kg groups had significantly lower yolk color than the control (P<0.05). At week 38, the 1,000, 2,000, and 4,000 mg/kg groups exhibited significantly higher yolk color than the control (P<0.05), while the 16,000 mg/kg group was significantly lower than these three groups (P<0.05).

Table 11 Effects of choline chloride on yolk color of laying hens

2.4.1 Effects on Whole Egg Phosphatidylcholine Content Effects of choline chloride on whole egg phosphatidylcholine content are shown in Table 12 . At weeks 21 and 38, the 8,000 and 16,000 mg/kg groups had significantly higher content than the control (P<0.05). At week 22, the 16,000 mg/kg group exhibited significantly higher content than all other groups (P<0.05). At week 24, all supplemented groups (1,000–16,000 mg/kg) showed significantly higher content than the control (P<0.05). At week 27, the 4,000 and 16,000 mg/kg groups had significantly higher content than the control (P<0.05). At week 30, the 4,000 and 8,000 mg/kg groups exhibited significantly higher content than the control (P<0.05).

Table 12 Effects of choline chloride on egg phosphatidylcholine content of laying hens, mg/g

2.4.2 Effects on Yolk and Albumen Phosphatidylcholine Content at Week 38 Effects of choline chloride on phosphatidylcholine content in yolk and albumen at week 38 are presented in Table 13 . The 16,000 mg/kg group showed significantly higher yolk phosphatidylcholine content than the control (P<0.05), representing a 26.5% increase, while no significant difference was observed between the 1,000 mg/kg group and the control (P>0.05). Phosphatidylcholine was not detected in albumen from any group.

Table 13 Effects of choline chloride on phosphatidylcholine content in yolk and albumen of laying hens at week 38, mg/kg

−Means not detected.

3. Discussion

3.1 Effects of Choline Chloride on Performance The results indicate that supplementation with 1,000, 2,000, and 4,000 mg/kg choline chloride did not significantly affect egg production during weeks 19-27. However, as hen age increased, tolerance to choline chloride decreased. During weeks 31-36, no additional dietary choline was needed to maintain production, and supplementation at 1,000 mg/kg or above adversely affected production rate. During weeks 36-42, supplementation with 1,000-4,000 mg/kg showed no significant effects. Nesheim et al. [17] reported that 1,400 mg/kg choline chloride did not significantly affect production in Leghorn hens aged 31-42 weeks. March [14] found similar results with 1,340 mg/kg supplementation in Leghorn hens aged 28-49 weeks. Zhai et al. [18] reported that additional choline chloride at 1,000-8,000 mg/kg in corn-soybean diets did not significantly affect production in Hy-Line brown hens aged 19-68 weeks. Unlike Leghorn and Hy-Line hens, Jinghong No. 1 hens showed significantly reduced production at 1,000 mg/kg during weeks 31-36, suggesting breed-specific differences in choline tolerance.

Regarding egg weight, supplementation 4,000 mg/kg did not significantly affect Jinghong No. 1 hens during weeks 18-30. Tolerance decreased during weeks 31-33, though 1,000 and 2,000 mg/kg remained without significant effect. During weeks 34-36, 16,000 mg/kg showed no adverse effect, possibly due to rapid egg weight increase and elevated choline requirements in Jinghong No. 1 hens at this stage. After week 36, as egg weight gain slowed, 16,000 mg/kg significantly reduced egg weight. Zhai et al. [18] investigated long-term choline chloride supplementation in Hy-Line brown hens and found 8,000 mg/kg did not significantly affect egg weight during weeks 20-68, consistent with our findings during weeks 34-42.

Supplementation with 1,000-4,000 mg/kg did not significantly affect feed intake during weeks 18-30. During weeks 31-33, no additional choline was required for normal intake, and levels 1,000 mg/kg adversely affected consumption. After week 36, 1,000-4,000 mg/kg showed no significant effects. Zhai et al. [18] reported slightly different results, finding no significant effects on intake with 500-8,000 mg/kg supplementation in Hy-Line brown hens aged 20-68 weeks. Choline metabolism in laying hens is closely related to dietary crude protein (CP) and total sulfur-containing amino acids (TSAA) content [15,19-20], and differences in dietary CP and TSAA may explain variations among studies. Some researchers suggest young hens can utilize choline to partially meet methionine requirements better than older hens, thus requiring more choline [17,21-22]. This implies age-related differences in choline requirements and tolerance, consistent with our findings.

Considering effects on production rate, egg weight, and feed intake, choline chloride supplementation exceeding 1,000 mg/kg adversely affected performance, with significant reductions in production rate at 4,000 mg/kg and in egg weight and feed intake at 8,000 mg/kg. However, due to our experimental design, we cannot determine whether supplementation below 1,000 mg/kg would be detrimental during weeks 31-36, necessitating further safety evaluation at lower doses.

3.2 Effects of Choline Chloride on Egg Quality Zhai et al. [18] reported that 500–8,000 mg/kg choline chloride did not significantly affect shell strength or thickness in Hy-Line brown hens aged 19–56 weeks. Our results show that choline chloride increased shell thickness in Jinghong No. 1 hens during weeks 19–21 without affecting shell strength at weeks 19–21, but reduced shell strength at weeks 22 and 38 when supplemented at 4,000–16,000 mg/kg. This indicates breed-specific differences in choline tolerance, with Jinghong No. 1 hens showing higher tolerance during weeks 19–21.

The Haugh unit is a widely accepted egg quality indicator. Tsiagbe et al. [15] reported that adding 500 and 1,000 mg/kg choline chloride to basal diets containing 1,040 mg/kg did not significantly affect Haugh unit in hens at weeks 44 and 64. Zhai [23] found that choline chloride linearly increased albumen height and Haugh unit in Hy-Line brown hens at weeks 59–68 but not at weeks 19–58. In our study, 8,000 and 16,000 mg/kg improved Haugh unit at week 22, while 16,000 mg/kg reduced albumen height at week 24 and Haugh unit at week 26. This suggests breed- and age-specific choline requirements, with 8,000 mg/kg not adversely affecting albumen height or Haugh unit in Jinghong No. 1 hens.

Yolk color is an important quality and marketing trait. Our study found all supplemented groups had significantly higher yolk color than the control at weeks 20–22, but at week 38, while 1,000–4,000 mg/kg maintained higher color, the 16,000 mg/kg group was significantly lower than other supplemented groups. Zhai et al. [18] reported similar results, with choline chloride significantly increasing yolk color at weeks 19–58. However, Danicke et al. [24] reported that 1,000 mg/kg reduced yolk color in Lohmann hens while 4,000 mg/kg had no effect, suggesting different mechanisms for high versus low doses. The mechanism remains unclear, warranting further investigation.

3.3 Effects of Choline Chloride on Egg Phosphatidylcholine Content Phosphatidylcholine in laying hens can be synthesized from choline via the phosphocholine-cytidine diphosphate choline pathway or from cephalin via the phosphatidylethanolamine methyltransferase pathway. During the first two weeks (19–20), choline supplementation did not affect whole egg phosphatidylcholine content. However, supplementation at 1,000–16,000 mg/kg increased whole egg content at week 24, and levels of 4,000–16,000 mg/kg significantly elevated content at weeks 27, 30, and 38. After 20 weeks of supplementation, the 16,000 mg/kg group showed 26.5% higher yolk phosphatidylcholine than the control, while the 1,000 mg/kg group did not differ significantly. This suggests that 1,000 mg/kg meets Jinghong No. 1 requirements, with excess choline converted to phosphatidylcholine for yolk storage. Previous studies reported similar findings: Burns et al. [25] found 3,000 mg/kg increased yolk phosphatidylcholine by 97%, and Tsagbe et al. [26] reported that 1,000 mg/kg added to basal diets containing 16.6% CP, 0.53% TSAA, and 1,041 mg/kg choline significantly increased yolk phosphatidylcholine. Notably, phosphatidylcholine was undetectable in albumen across all groups.

4. Conclusions

1. Dietary choline chloride supplementation exceeding 1,000 mg/kg reduced egg production rate and increased feed-to-egg ratio; levels above 4,000 mg/kg decreased egg weight and feed intake.

2. Supplementation with 1,000–2,000 mg/kg improved shell thickness and strength; levels below 4,000 mg/kg did not significantly affect albumen height or Haugh unit; 1,000–4,000 mg/kg significantly improved yolk color; and choline chloride supplementation increased whole egg phosphatidylcholine content, with 16,000 mg/kg significantly increasing yolk phosphatidylcholine content.

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