# Effect of Dietary Zinc Sulfate Supplementation on Laying Performance of Jinghong Laying Hens (Postprint)

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## Abstract

This experiment investigated the effects of supplementing basal diets with different levels of zinc sulfate on the laying performance and tissue metallothionein content of Jinghong laying hens during peak production. A total of 540 healthy Jinghong laying hens at 20 weeks of age during peak production were randomly allocated into 6 groups with 6 replicates per group and 15 birds per replicate. The control group was fed a basal diet containing 25 mg/kg Zn, while the treatment groups were fed the basal diet supplemented with 25, 50, 75, 100, and 125 mg/kg zinc sulfate (as Zn), respectively. The experiment consisted of a 2-week preliminary period followed by a 24-week formal experimental period. The results showed: 1) Dietary supplementation with different levels of zinc sulfate had no significant effects on laying rate, average daily feed intake, average egg weight, feed-to-egg ratio, or mortality throughout the experimental period ($P>0.05$); 2) Dietary supplementation with different levels of zinc sulfate significantly increased metallothionein content in breast muscle ($P<0.05$), but had no significant effect on metallothionein content in cardiac muscle ($P>0.05$); 3) Quadratic regression analysis predicted that the lowest average daily feed intake during the entire peak production period would occur at a dietary zinc sulfate supplementation level of 75 mg/kg for Jinghong laying hens. These results suggest that dietary supplementation with different levels of zinc sulfate had no significant effect on the laying performance of Jinghong laying hens during peak production.

## Full Text

# Effects of Dietary Zinc Sulfate on Egg Production Performance of Jinghong Laying Hens

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**Abstract:** This experiment investigated the effects of different supplemental levels of zinc sulfate in basal diet on egg production performance and metallothionein content in tissues of Jinghong laying hens during peak egg production period. Five hundred and forty healthy 20-week-old Jinghong laying hens were randomly allocated to six groups with six replicates per group and 15 hens per replicate. The control group was fed a basal diet containing 25 mg/kg zinc, while the experimental groups were fed diets supplemented with 25, 50, 75, 100, and 125 mg/kg zinc sulfate (calculated as zinc) in the basal diet. The pre-feeding period lasted for 2 weeks, followed by a formal experimental period of 24 weeks. The results showed that: 1) Different supplemental levels of zinc sulfate in the basal diet had no significant effects on laying rate, average daily feed consumption, average egg weight, feed-to-egg ratio, or mortality throughout the entire experimental period ($P>0.05$). 2) Different supplemental levels of zinc sulfate in the basal diet significantly increased metallothionein content in breast muscle ($P<0.05$), but had no significant effect on metallothionein content in heart muscle ($P>0.05$). 3) Quadratic curve regression prediction indicated that when the supplemental level of zinc sulfate in the diet of Jinghong laying hens was 75 mg/kg, the average daily feed consumption during the entire peak egg production period was minimized. These results suggest that dietary supplementation with different levels of zinc sulfate has no significant effect on egg production performance of Jinghong laying hens during peak egg production period.

**Keywords:** zinc sulfate; laying hen; egg production performance; metallothionein
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## Introduction

Determining the appropriate supplemental dose of zinc in the diet of Jinghong laying hens during peak egg production period is of great significance for improving their production performance and promoting eco-friendly farming practices. Zinc is one of the essential trace elements for avian growth and life activities, being associated with the structure or activation of more than 300 enzymes and proteins, such as lactate dehydrogenase, alkaline phosphatase, copper-zinc superoxide dismutase, carbonic anhydrase, and DNA polymerase [2]. Zinc participates in the synthesis and function of hormones like insulin, and has close relationships with nucleic acids, lipids, proteins, vitamins, and other mineral trace elements [3]. As a component of carbonic anhydrase, zinc affects carbonate supply and thus influences eggshell formation [4]. Therefore, zinc has significant effects on poultry growth, production performance, reproduction, and immune function [5]. Research reports indicate that dietary zinc content below 10 mg/kg leads to reduced laying rate and hatchability [6], while 28 mg/kg zinc in the diet can meet the needs of hens for egg production, incubation, and chick growth [7]. The NRC (1994) recommends 35.5 mg/kg zinc in the diet to meet the requirements of brown-egg laying hens, and studies have shown that adding 48 mg/kg zinc does not affect laying rate [8]. Synthesizing previous research findings, the traditional method evaluates the zinc requirement of laying hens as 30-50 mg/kg [9]. However, with deeper research on trace element nutrition in recent years, more sensitive indicators such as tibial zinc have been used to evaluate zinc requirements, yielding values higher than those assessed by traditional methods, specifically 72 mg/kg [10]. Meanwhile, to avoid subclinical symptoms, excessive supplementation has become a common practice in production, which not only causes metabolic disorders in laying hens but also wastes resources and pollutes the environment. Adding appropriate amounts of zinc during peak egg production period is crucial for maintaining laying performance and immune function. One of the sensitive indicators for evaluating zinc requirements is metallothionein (MT), a cysteine-rich metal-binding protein involved in the storage, transport, and regulation of essential metal elements in the body to achieve optimal physiological status [11]. Zinc can induce MT synthesis in various tissues, and MT is sensitive to dietary zinc changes [12]. This experiment investigated the effects of dietary zinc levels on egg production performance and tissue MT content in laying hens under practical feeding conditions to evaluate the appropriate supplemental level and provide scientific guidance for rational zinc supplementation in farming production.

## 1. Materials and Methods

**1.1 Experimental Material** Zinc sulfate monohydrate (feed grade) containing 35.5% zinc.

**1.2 Experimental Design** Five hundred and forty 20-week-old replacement laying hens were randomly divided into six groups with six replicates per group and 15 hens per replicate (housed in five cages with three hens per cage). A corn-soybean meal basal diet containing 25 mg/kg zinc was formulated according to the breeding standards recommended by the Jinghong Laying Hen Company, with composition and nutrient levels shown in Table 1 . Six experimental diets were prepared by supplementing the basal diet with different levels of zinc sulfate (Table 2 ), including T1 (basal diet), T2 (basal diet + 25 mg/kg zinc sulfate, calculated as zinc, the same below), T3 (basal diet + 50 mg/kg zinc sulfate), T4 (basal diet + 75 mg/kg zinc sulfate), T5 (basal diet + 100 mg/kg zinc sulfate), and T6 (basal diet + 125 mg/kg zinc sulfate).

**1.3 Management** The experiment was conducted from January 2017 to August 2017 at the Tongzhou Experimental Base of the Feed Research Institute, Chinese Academy of Agricultural Sciences. Hens were housed in three-tier stacked cages with 16 hours of lighting, provided water through nipple drinkers, and fed experimental diets (powder form) ad libitum. Avian influenza vaccine was administered once during the period, and routine hygiene management was practiced. The pre-feeding period lasted for two weeks during which the basal diet was fed for health observation. Egg production performance was analyzed for each group, and experimental hens were selected and adjusted to ensure no significant differences between groups ($P>0.05$) before commencing the formal experiment. During the 24-week formal experimental period, hens were fed the experimental diets.

### 1.4 Measurement Indicators

**1.4.1 Egg Production Performance** Eggs were collected twice daily at 12:00 and 21:00. Daily egg number and weight were recorded, and average egg weight and laying rate were calculated for each replicate. Feed remaining was weighed every four weeks to calculate average daily feed consumption and feed-to-egg ratio. Mortality and culling were recorded during the experimental period to calculate overall mortality.

**1.4.2 Tissue MT Content** At the end of 45 weeks of age, three hens were randomly selected from each replicate, and breast muscle and heart muscle were sampled to determine MT content. Tissues were ground with phosphate buffer solution (PBS) at a 1:10 ratio for analysis. MT content was determined by enzyme-linked immunosorbent assay using kits purchased from Nanjing Jiancheng Bioengineering Institute, with detection performed using a Multiskan MK3 microplate reader.

**1.5 Statistical Analysis** Data were analyzed using one-way ANOVA in SPSS 17.0 software, with significance of factors tested by F-test. LSD multiple comparisons were performed for indicators with significant main effects. Linear and quadratic model regression analyses between zinc supplemental levels and zinc-sensitive indicators were conducted using the curve estimate module, with significance set at $P<0.05$.

## 2. Results

**2.1.1 Effects of Different Supplemental Levels of Zinc Sulfate on Average Daily Feed Consumption of Jinghong Laying Hens During Peak**

**Egg Production Period** As shown in Table 3 , supplementation with different levels of zinc sulfate had no significant effect on average daily feed consumption at different feeding stages compared with the control group ($P>0.05$). There was no significant linear or quadratic regression relationship between average daily feed consumption at various feeding stages from 22 to 41 weeks of age and zinc sulfate supplemental level ($P>0.05$). However, during the entire experimental period (22-45 weeks of age), there was a significant linear and quadratic regression relationship between average daily feed consumption and zinc sulfate supplemental level ($P<0.05$), with regression equations: $Y=0.001X^2-0.150X+121.031(R^2=0.187)$ and $Y=-0.045X+119.275(R^2=0.127)$. As shown in Figure 1 [Figure 1: see original paper], quadratic curve regression predicted that average daily feed consumption reached its minimum when zinc sulfate supplemental level was 75 mg/kg during the entire experimental period.

**2.1.2 Effects of Different Supplemental Levels of Zinc Sulfate on Laying Rate of Jinghong Laying Hens During Peak Egg Production Period** As shown in Table 4 , supplementation with different levels of zinc sulfate had no significant effect on laying rate at various feeding stages during peak egg production period ($P>0.05$). There was no significant linear or quadratic regression relationship between laying rate at various feeding stages and zinc sulfate supplemental level ($P>0.05$).

**2.1.3 Effects of Different Supplemental Levels of Zinc Sulfate on Average Egg Weight of Jinghong Laying Hens During Peak Egg Production Period** As shown in Table 5 , supplementation with different levels of zinc sulfate had no significant effect on average egg weight at various feeding stages during peak egg production period ($P>0.05$). However, there were significant quadratic regression relationships between average egg weight and zinc sulfate supplemental level at 22-25 weeks of age and 22-29 weeks of age ($P<0.05$), with regression equations: $Y=-0.000\ 265X^2+0.032X+60.012(R^2=0.173)$ and $Y=-0.000\ 246X^2+0.040X+59.805(R^2=0.181)$. As shown in Figure 2 [Figure 2: see original paper] and Figure 3 [Figure 3: see original paper], quadratic curve regression predicted that average egg weight reached its maximum at zinc sulfate supplemental levels of 60.38 mg/kg and 81.30 mg/kg for hens at 22-25 weeks and 22-29 weeks of age, respectively. No significant linear or quadratic regression relationships were observed between average egg weight and zinc sulfate supplemental level at other feeding stages ($P>0.05$).

**2.1.4 Effects of Different Supplemental Levels of Zinc Sulfate on Feed-to-Egg Ratio of Jinghong Laying Hens During Peak Egg Production Period** As shown in Table 6 , supplementation with different levels of zinc sulfate significantly increased the feed-to-egg ratio at 22-29 weeks of age ($P<0.05$), though there were no significant differences among experimental groups ($P>0.05$). However, supplementation with different levels of zinc sulfate had no significant effect on feed-to-egg ratio at other feeding stages ($P>0.05$).

There was a significant quadratic regression relationship between feed-to-egg ratio and zinc sulfate supplemental level at 22–29 weeks of age ($P<0.05$), with the regression equation: $Y=0.000\ 0237X^2 - 0.005X+2.287(R^2=0.237)$. As shown in Figure 4 [Figure 4: see original paper], quadratic curve regression predicted that the feed-to-egg ratio reached its minimum when zinc sulfate supplemental level was 105 mg/kg for hens at 22–29 weeks of age.

**2.1.5 Effects of Different Supplemental Levels of Zinc Sulfate on Overall Mortality of Jinghong Laying Hens During Peak Egg Production Period** As shown in Table 7 , supplementation with different levels of zinc sulfate had no significant effect on overall mortality during peak egg production period ($P>0.05$). There was no significant linear or quadratic regression relationship between overall mortality and zinc sulfate supplemental level ($P>0.05$).

**2.2 Effects of Different Supplemental Levels of Zinc Sulfate on MT Content in Different Tissues of Jinghong Laying Hens at 45 Weeks of Age** As shown in Table 8 , supplementation with different levels of zinc sulfate had no significant effect on MT content in heart muscle of 45-week-old Jinghong laying hens ($P>0.05$). The group supplemented with 125 mg/kg zinc sulfate had significantly higher MT content in breast muscle compared with the control group and the 25 mg/kg zinc sulfate group ($P<0.05$), though no significant differences were observed among other supplemental level groups ($P>0.05$). There were highly significant linear and quadratic regression relationships between zinc sulfate supplemental level and MT content in breast muscle of 45-week-old hens ($P<0.01$), with regression equations: $Y=0.000\ 008X^2+0.001X+0.629(R^2=0.300)$ and $Y=-0.002X+0.612(R^2=0.293)$. As shown in Figure 5 [Figure 5: see original paper], linear regression predicted that MT content in breast muscle gradually increased with increasing dietary zinc sulfate levels (25–125 mg/kg).

## 3. Discussion

**3.1 Effects of Different Supplemental Levels of Zinc Sulfate on Egg Production Performance and Mortality of Jinghong Laying Hens During Peak Egg Production Period** Based on egg production performance and hatchability indicators, the NRC recommends 35.5 mg/kg zinc in the diet for brown-egg laying hens. The present results showed that supplementing 25–125 mg/kg zinc sulfate in the basal diet significantly reduced the feed-to-egg ratio at 22–29 weeks of age in Jinghong laying hens, but had no significant effects on average daily feed consumption, feed-to-egg ratio, average egg weight, or laying rate at other feeding stages. Tabatabaie et al. [13] reported that feeding 25 and 50 mg/kg zinc sulfate to laying hens did not significantly affect feed consumption, egg weight, laying performance, or feed conversion ratio. Zhang et al. [14] supplemented 52-week-old Hy-Line Gray laying hens with 35, 70, and 115 mg/kg zinc, which did not significantly affect laying rate, average daily feed consumption, average egg weight, or feed-to-egg ratio. Stahl et al. [7] added 10, 20, and 40 mg/kg zinc carbonate to the basal diet of Single Comb White

Leghorn hens (containing 28 or 34 mg/kg zinc) and found no significant improvement in egg production, feed consumption, or feed conversion ratio, concluding that 28 mg/kg zinc in the diet was sufficient to meet the needs for egg production, reproduction, incubation, and offspring growth. Cheng et al. [15] reported that adding 60 mg/kg zinc sulfate to the basal diet of Hy-Line Brown laying hens (containing approximately 31 mg/kg zinc) did not significantly affect laying rate, average egg weight, or feed consumption. These findings are consistent with our results, indicating that 25 mg/kg zinc in the basal diet can meet the requirements of Jinghong laying hens during the 22–45 week peak production period. However, Zhang et al. [16-17] found that adding 60 and 180 mg/kg zinc to the basal diet did not significantly affect egg production, feed consumption, or feed-to-egg ratio in 21-36-week-old hens, but significantly reduced egg weight and slightly decreased broken egg rate. Qu et al. [18-19] reported that adding 120 mg/kg zinc hydroxy-methionine and 80, 120, and 160 mg/kg basic zinc chloride to the basal diet of Dongxiang Black-Bone Green-shell laying hens significantly increased laying rate and decreased feed-to-egg ratio. The improved laying performance observed in Green-shell laying hens may be related to the zinc source or higher zinc requirements in this breed, though the specific mechanism requires further investigation.

Regression analysis showed no significant linear or quadratic regression relationships between mortality or laying rate and zinc sulfate supplemental level at any feeding stage. Regarding laying performance at various stages, significant quadratic regression relationships were observed between average egg weight and zinc sulfate supplemental level at 22–25 weeks and 22–29 weeks, and between feed-to-egg ratio and zinc sulfate supplemental level at 22–29 weeks, though the $R^2$ values were not high. Across the entire experimental period, a significant quadratic regression relationship existed between average daily feed consumption and zinc sulfate supplemental level, with quadratic curve regression predicting minimum average daily feed consumption at a supplemental level of 75 mg/kg.

**3.2 Effects of Different Supplemental Levels of Zinc Sulfate on Tissue MT Content at the End of Peak Egg Production Period** MT is a cysteine-rich metal-binding protein that can coordinate with many metal elements [20]. Zinc can participate in intracellular trace element metabolism and heavy metal detoxification as its ligand, exhibiting anti-radiation, free radical scavenging, and anti-stress effects [21]. MT plays an important role in maintaining cellular zinc homeostasis, directly participating in zinc storage, transport, and biological utilization [22]. Reports show that appropriate zinc supplementation can significantly increase tissue MT content [23]. Zinc supplementation significantly increased MT content or MT gene expression in pancreas [24], kidney [25-26], small intestine [27], and liver [28-29] of broiler chickens. However, few studies have reported the effects of zinc supplementation on MT content and MT gene expression in laying hens. Zhang et al. [16] reported that adding 60 mg/kg zinc sulfate to 28-week-old commercial Isa Brown hens significantly increased liver MT gene expression compared with the control and 180 mg/kg zinc sulfate groups. Qian et al. [30] reported that compared with 40 mg/kg nano-zinc oxide, 80 mg/kg nano-zinc oxide significantly increased MT content in skeletal and cardiac muscle of broilers, while 120 mg/kg nano-zinc oxide did not significantly improve MT content in these tissues. Our results showed that zinc sulfate supplementation significantly increased MT content in skeletal muscle (breast muscle), but did not significantly affect MT content in cardiac muscle. Regression analysis predicted that breast muscle MT content showed an increasing trend with increasing zinc sulfate supplemental level.

## 4. Conclusions

1. Except for significantly reducing the feed-to-egg ratio at 22–29 weeks of age, zinc sulfate supplementation had no significant effects on egg production performance or mortality of Jinghong laying hens during the entire peak production period.
2. Zinc sulfate supplementation significantly increased MT content in breast muscle, which increased with supplemental level, but had no significant effect on MT content in heart muscle.
3. Quadratic curve regression predicted that the average daily feed consumption during the entire peak production period was minimized when the supplemental level of zinc sulfate in Jinghong laying hen diet was 75 mg/kg.

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