Effects of Dietary Zinc Supplementation Levels on Zinc Deposition in Tissues and Organs and Tibial Indices of Jinghong No.1 Laying Hens during Peak Laying Period: Postprint

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Abstract

This study investigated the effects of dietary zinc supplementation at different levels on zinc deposition in tissues and organs and tibial indices of Jinghong No. 1 laying hens during peak production. A total of 540 healthy Jinghong No. 1 laying hens at 20 weeks of age during peak production were randomly allocated into 6 groups with 6 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 experimental diets supplemented with 25, 50, 75, 100, and 125 mg/kg zinc (using zinc sulfate monohydrate as the zinc source) based on the basal diet. The experiment consisted of a 2-week preliminary period followed by a 24-week formal experimental period. The results showed: 1) Dietary zinc supplementation at different levels significantly or extremely significantly increased serum zinc content in Jinghong No. 1 laying hens at the end of 33 weeks of age ($P<0.01$ or $P<0.05$), but had no significant effect on serum zinc content at the end of 46 weeks of age ($P>0.05$). 2) Dietary zinc supplementation at different levels extremely significantly affected liver zinc content in Jinghong No. 1 laying hens at the end of 46 weeks of age ($P<0.01$), with liver zinc content increasing as zinc supplementation levels increased, but had no significant effect on zinc content in skeletal muscle and pancreas ($P>0.05$). 3) Dietary zinc supplementation at different levels had no significant effect on tibial indices (tibia weight, fat-free tibia weight, tibia ash weight, and zinc content in tibia and tibia ash) in Jinghong No. 1 laying hens at the end of 46 weeks of age ($P>0.05$). 4) Dietary zinc supplementation at different levels extremely significantly affected fecal zinc content and apparent zinc utilization rate in Jinghong No. 1 laying hens at the end of 46 weeks of age ($P<0.01$), with fecal zinc content increasing as zinc supplementation levels increased, while apparent zinc utilization rate decreased

as zinc supplementation levels increased. Taking all factors into consideration, using zinc sulfate monohydrate as the zinc source, the appropriate zinc supplementation level in the basal diet (containing 25 mg/kg zinc) for Jinghong No. 1 laying hens during peak production is 75 mg/kg.

Full Text

Effects of Zinc Supplemental Level on Tissue and Organ Zinc Deposition and Tibia Indexes of Jinghong No. 1 Layer Hens during Peak Laying Period

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Abstract

This study investigated the effects of dietary zinc supplementation on zinc deposition in tissues and organs and tibia characteristics of Jinghong No. 1 layer hens during peak laying period. Five hundred and forty healthy 20-week-old Jinghong No. 1 layer hens were randomly allocated to 6 groups with 6 replicates per group and 15 hens per replicate. The control group received a basal diet containing 25 mg/kg zinc, while treatment groups received the basal diet supplemented with 25, 50, 75, 100, or 125 mg/kg zinc (as zinc sulfate monohydrate). The experiment consisted of a 2-week pre-feeding period followed by a 24-week formal experimental period. The results showed: (1) Dietary zinc supplementation significantly or extremely significantly increased serum zinc content at 33 weeks of age ($P < 0.01$ or $P < 0.05$), but had no significant effect on serum zinc content at 46 weeks of age ($P > 0.05$). (2) Zinc supplementation extremely significantly affected liver zinc content at 46 weeks of age ($P < 0.01$), with liver zinc content increasing as supplementation levels rose, but did not significantly affect zinc content in skeletal muscle or pancreas ($P > 0.05$). (3) Zinc supplementation had no significant influence on tibia indexes (tibia weight, fat-free tibia weight, tibia ash weight, tibia zinc content, or tibia ash zinc content) at 46 weeks of age ($P > 0.05$). (4) Zinc supplementation extremely significantly affected fecal zinc content and zinc apparent availability ($P < 0.01$), with fecal zinc content increasing and apparent availability decreasing as supplementation levels increased. Based on comprehensive evaluation of all factors, the optimal supplemental level of zinc as zinc sulfate monohydrate in the basal diet (containing 25 mg/kg zinc) for Jinghong No. 1 layer hens during peak laying period is 75 mg/kg.

Keywords: zinc sulfate; layer hens; zinc deposition; tibia indexes

Introduction

Zinc is an essential trace element for avian growth, development, and metabolic activities. It serves as a component or activator of numerous enzymes and participates in the synthesis and function of various hormones. Zinc influences not only cell division, growth, and regeneration but also the metabolism of nucleic acids, lipids, proteins, vitamins, and other mineral elements. Consequently, zinc plays a vital role in poultry growth, production performance, reproduction, and immune function. In modern poultry production, zinc is routinely added to diets to promote growth, enhance immune function, and even produce zinc-enriched health products through high-dose supplementation. However, excessive supplementation of trace minerals in layer diets leads to accumulation in eggs and excreta after meeting production requirements. Prolonged consumption of high-zinc diets can cause zinc accumulation in the liver, resulting in decreased production performance and environmental pollution through excretion. Research indicates that zinc concentration in broiler excreta is approximately four times that in the diet. Dietary trace mineral content and poultry age are important factors affecting trace mineral excretion. Appropriate zinc levels in layer diets can mitigate the increased excretion caused by excessive supplementation. High-dose trace mineral usage not only increases production costs and wastes resources but also causes environmental pollution and metabolic disorders that affect production performance and product quality. Therefore, determining optimal dietary zinc levels that meet the needs for egg production and immune function while ensuring ecological sustainability is urgently needed.

Previous studies suggest that bone zinc content is the most sensitive indicator for assessing zinc status. Broiler experiments have shown that zinc content in bone, liver, and kidney are the most sensitive indicators. Tibia zinc content is not only sensitive to dietary zinc levels but also significantly correlated with zinc content in liver, pancreas, and muscle. Research has demonstrated that adding 20–40 mg/kg zinc to diets significantly improves zinc content in broiler liver, pancreas, and tibia. Dewar et al. reported that tibia zinc content reached saturation when dietary zinc was 30 mg/kg in turkeys. However, few studies have investigated optimal zinc supplementation levels and tissue zinc metabolism in Jinghong No. 1 layer hens during peak laying period. This study used zinc sulfate monohydrate as the zinc source to examine the effects of zinc supplementation on tissue and organ zinc deposition and tibia indexes in Jinghong No. 1 layer hens under practical feeding conditions, providing scientific guidance for rational zinc supplementation in layer production.

Materials and Methods

1.1 Zinc Source The zinc source used in this experiment was feed-grade zinc sulfate monohydrate containing 35.5% zinc, purchased from Beijing Precision Animal Nutrition Research Center.

1.2 Experimental Design Five hundred and forty 20-week-old Jinghong No. 1 layer hens were randomly divided into 6 groups with 6 replicates per group and 15 hens per replicate. The control group received a corn-soybean meal basal diet containing 25 mg/kg zinc (T1, no supplemental zinc). The composition and nutrient levels of the basal diet are shown in Table 1 . Five experimental diets were formulated by supplementing the basal diet with different zinc levels to feed the treatment groups. The experimental design is detailed in Table 2 .

1.3 Management The experiment was conducted from January 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 light at 10 lx intensity. Nipple drinkers provided water ad libitum, and hens had free access to feed. Hens were vaccinated once for avian influenza during the trial. Routine hygiene management was practiced. The 2-week pre-feeding period involved health observation, and the formal 24-week experimental period began when the average laying rate reached 94% across groups.

1.4 Sample Collection and Analysis

1.4.1 Serum Zinc Content Determination At the end of the mid-experimental period (33 weeks of age) and the end of the experimental period (46 weeks of age), one hen per replicate was selected for blood collection from the wing vein using dry vacuum tubes. Serum was prepared and serum zinc content was determined using the PAPS colorimetric method with kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.2 Tissue and Organ Zinc Content Determination At 46 weeks of age, three hens per replicate were randomly selected. Skeletal muscle (pectoral muscle), liver, pancreas, and tibia were collected to determine zinc content. Skeletal muscle, pancreas, and liver were homogenized with buffer solution at a 1:10 mass-to-volume ratio, and zinc content was measured using the PAPS colorimetric method on a Hitachi 7600 automatic analyzer. Tibia samples were dried at 105°C for 24 hours, soaked in anhydrous ethanol for 48 hours, extracted with anhydrous ether in a Soxhlet apparatus for 48 hours, then dried again (105°C, 10 hours) to obtain fat-free dry weight. Samples were ashed in a muffle furnace at 550°C for 18 hours, cooled, dissolved in 1:60 (volume ratio) dilute hydrochloric acid, filtered through quantitative filter paper, brought to

50 mL with dilute hydrochloric acid, and further diluted to appropriate concentration. Tibia ash zinc content was determined by flame atomic absorption spectrophotometry and converted to tibia zinc content.

1.4.3 Zinc Apparent Availability Determination of acid-insoluble ash in diet and feces: Approximately 3 g of diet or feces sample was placed in a 500 mL beaker, 50 mL of 4 mol/L hydrochloric acid was added, and after foam disappeared, the sample was slowly boiled on a hot plate for 30 minutes. The mixture was filtered through quantitative filter paper and washed with hot distilled water until neutral. The residue and filter paper were transferred to a pre-weighed crucible, dried at 105°C, carbonized on an electric furnace until smokeless, then ashed in a muffle furnace to gray-white color. After cooling in a desiccator for 30 minutes, the sample was weighed, reheated in the muffle furnace for 30 minutes to constant weight, and the acid-insoluble ash weight was obtained by subtracting the empty crucible weight.

Determination of zinc in diet and feces: Accurately weighed diet or feces samples were carbonized on an electric furnace until smokeless, then ashed to constant weight in a muffle furnace, dissolved in dilute hydrochloric acid, and analyzed following the same procedure as for tibia ash zinc content.

Zinc apparent availability (%) = [1 - (acid-insoluble ash in diet / acid-insoluble ash in feces) × (zinc in feces / zinc in diet)] × 100.

1.5 Statistical Analysis Data were analyzed using one-way ANOVA in SPSS 17.0, with factor significance expressed as P-values. LSD multiple comparisons were performed for significant main effects. Curve estimate module was used for linear and quadratic regression analysis between zinc supplemental level (X) and tissue/organ zinc content, fecal zinc content, and zinc apparent availability. $P < 0.05$ was considered significant and $P < 0.01$ extremely significant. The quadratic curve inflection point was used to estimate the optimal zinc supplemental level.

Results

2.1 Effects of Zinc Supplemental Level on Serum Zinc Content As shown in Table 3 , dietary zinc supplementation above 25 mg/kg significantly increased serum zinc content at 33 weeks of age. The 125 mg/kg zinc group (T6) had the highest serum zinc content, which was extremely significantly higher than the control group (T1) and the 25 mg/kg zinc group (T2) ($P < 0.01$), but not significantly different from the 50 mg/kg (T3), 75 mg/kg (T4), and 100 mg/kg (T5) groups ($P > 0.05$). Dietary zinc supplementation had no significant effect on serum zinc content at 46 weeks of age ($P > 0.05$).

There was an extremely significant linear or quadratic relationship between zinc supplemental level (x) and serum zinc content (y) at 33 weeks of age ($P < 0.01$),

with regression equations: $y = 0.151x + 52.715$ ($R^2 = 0.332$) and $y = -0.001x^2 + 0.287x + 50.452$ ($R^2 = 0.355$). Quadratic regression predicted maximum serum zinc content at 143.5 mg/kg zinc supplementation. No significant linear or quadratic relationship existed between zinc supplemental level and serum zinc content at 46 weeks of age ($P > 0.05$).

2.2 Effects of Zinc Supplemental Level on Tissue and Organ Zinc Content As shown in Table 4 , dietary zinc supplementation had no significant effect on skeletal muscle or pancreas zinc content at 46 weeks of age ($P > 0.05$), but extremely significantly affected liver zinc content ($P < 0.01$). Compared with the control group, zinc supplementation at 25–125 mg/kg extremely significantly increased liver zinc content ($P < 0.01$). No significant differences were observed among the 25, 50, 75, and 100 mg/kg groups ($P > 0.05$), but the 125 mg/kg group had significantly higher liver zinc content than other supplementation groups ($P < 0.05$).

There was an extremely significant linear or quadratic relationship between zinc supplemental level (x) and liver zinc content (y) at 46 weeks of age ($P < 0.01$), with regression equations: $y = 0.038x + 4.056$ ($R^2 = 0.318$) and $y = 0.0001x^2 + 0.024x + 4.279$ ($R^2 = 0.322$). Linear regression predicted that liver zinc content increased gradually with dietary zinc supplementation. A significant quadratic relationship existed between zinc supplemental level (x) and skeletal muscle zinc content (y) ($P < 0.05$): $y = 0.0003x^2 - 0.048x + 4.772$ ($R^2 = 0.207$), predicting minimum skeletal muscle zinc content at 80 mg/kg zinc supplementation.

2.3 Effects of Zinc Supplemental Level on Tibia Indexes As shown in Table 5 , dietary zinc supplementation had no significant effect on tibia weight, fat-free tibia weight, tibia ash weight, tibia zinc content, or tibia ash zinc content at 46 weeks of age ($P > 0.05$). No significant linear or quadratic relationships were found between zinc supplemental level and these tibia indexes ($P > 0.05$).

2.4 Effects of Zinc Supplemental Level on Zinc Apparent Availability As shown in Table 6 , the 125 mg/kg zinc group had extremely significantly higher fecal zinc content than the control group ($P < 0.01$) and significantly higher content than the 25, 50, and 75 mg/kg groups ($P < 0.05$), but did not differ significantly from the 100 mg/kg group ($P > 0.05$). Dietary zinc supplementation reduced zinc apparent availability to varying degrees, with the 100 and 125 mg/kg groups being extremely significantly lower than the control and 25 mg/kg groups ($P < 0.01$), though not significantly different from each other ($P > 0.05$).

Significant linear or quadratic relationships existed between zinc supplemental level (x) and both fecal zinc content (y) and zinc apparent availability (y) at 46 weeks of age ($P < 0.05$). The regression equations were: $y = 0.000014x^2 - 0.0002x + 0.015$ ($R^2 = 0.449$) and $y = 0.00049x + 0.026$ ($R^2 = 0.385$) for fecal zinc content; $y = -0.00034x^2 - 0.149x + 45.733$ ($R^2 = 0.814$) and $y = -0.192x$

+ 46.44 ($R^2 = 0.810$) for zinc apparent availability. Linear regression predicted that fecal zinc content increased while zinc apparent availability decreased with rising dietary zinc supplementation.

Discussion

3.1 Effects of Zinc Supplemental Level on Serum Zinc Content After absorption from the diet, zinc enters the bloodstream, complexes with blood proteins, and is transported to various tissues and organs. Zinc absorption in poultry is regulated and controlled by intestinal mucosal cells. Previous studies have shown that serum zinc content in laying hens increases with dietary zinc supplementation but does not readily reach a stable level, suggesting serum zinc as a useful nutritional evaluation index. The present results showed that serum zinc content in Jinghong No. 1 hens generally increased with dietary zinc supplementation at 33 weeks of age, but no significant effects were observed at 46 weeks of age. This may be related to diminished effects of zinc supplementation over extended feeding periods. The 46-week results align with findings from Zhang et al. in 18-week-old commercial Isa Brown pullets and Yu et al. in broilers, where dietary zinc supplementation did not significantly increase serum zinc content.

3.2 Effects of Zinc Supplemental Level on Tissue Zinc Deposition and Tibia Indexes The results demonstrated that dietary zinc supplementation extremely significantly affected liver zinc content at 46 weeks of age, with content increasing as supplementation levels rose. This agrees with Zhang et al.' s findings of increased liver zinc content in commercial Isa Brown pullets fed 60 and 180 mg/kg zinc. However, the present study found no significant effects on tibia zinc, tibia ash zinc, skeletal muscle zinc, or pancreas zinc content at 46 weeks of age, contrasting with Zhang et al.' s observation that 180 mg/kg zinc supplementation significantly increased tibia zinc content in pullets. This discrepancy may be attributed to stronger zinc deposition capacity in tibia during the rearing stage compared to the laying period. Additionally, dietary zinc supplementation had no significant effects on tibia weight, fat-free tibia weight, or tibia ash weight, consistent with previous reports that adding 10–90 mg/kg zinc to a low-zinc basal diet (10.5 mg/kg) did not significantly affect broiler tibia weight. Regression analysis revealed that liver zinc content increased linearly or quadratically with dietary zinc supplementation.

3.3 Effects of Zinc Supplemental Level on Zinc Apparent Availability Dietary zinc is primarily absorbed in the duodenum, with some absorption in the jejunum. Zinc is mainly excreted through feces, with minimal loss via urine. Most fecal zinc represents unabsorbed dietary zinc, with endogenous zinc comprising only a small proportion. Endogenous zinc is primarily excreted through pancreatic fluid, followed by bile. Zinc apparent availability is influenced by

various factors including mineral elements (manganese, iron), vitamin A, and endogenous zinc. The present results showed that dietary zinc supplementation extremely significantly affected fecal zinc content and zinc apparent availability at 46 weeks of age. Linear regression predicted that fecal zinc content increased while zinc apparent availability decreased with rising dietary zinc supplementation.

Conclusion

Based on comprehensive consideration of tissue and organ zinc deposition and tibia indexes, the optimal supplemental level of zinc as zinc sulfate monohydrate in the basal diet (containing 25 mg/kg zinc) for Jinghong No. 1 layer hens during peak laying period is 75 mg/kg.

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