Effects of Dietary Zinc Level on Growth Performance, Carcass Characteristics, Serum Immune Indices and Zinc Excretion in Broiler Chickens (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary zinc levels on growth performance, slaughter performance, serum immune indices, and zinc excretion in broiler chickens. A total of 600 healthy 1-day-old Arbor Acres (AA) broiler chickens were selected and randomly divided into 5 groups with 6 replicates per group and 20 birds per replicate (half male and half female). The dietary zinc levels for each group were 30 (basal diet group), 60, 90, 120, and 150 mg/kg, respectively. The experimental period lasted 42 days. The results showed that: 1) During days 1-42, the average daily gain (ADG) of males in the 60, 90, 120, and 150 mg/kg zinc level groups was significantly higher than that in the basal diet group ($P<0.05$), while no significant difference was observed in ADG of females among all groups ($P>0.05$). 2) Dietary zinc levels had no significant effect on immune organ indices and slaughter performance of broiler chickens at 21 and 42 days of age ($P>0.05$). 3) At 21 days of age, the serum antibody titer against Newcastle disease in males from the 120 and 150 mg/kg zinc level groups was significantly higher than that in the basal diet group and the 60 and 90 mg/kg zinc level groups ($P<0.05$); at 42 days of age, no significant difference in serum antibody titer against Newcastle disease was observed among all groups ($P>0.05$). In females at 21 days of age, no significant difference in serum antibody titer against Newcastle disease was observed among all groups ($P>0.05$); at 42 days of age, the serum antibody titer against Newcastle disease in the 120 mg/kg zinc level group was significantly higher than that in all other groups ($P<0.05$). 4) Dietary zinc levels had significant effects on fecal zinc content and apparent zinc utilization in broiler chickens at 21 and 42 days of age ($P<0.05$). At different growth stages, fecal zinc content at 42 days of age was significantly higher than that at 21 days of age, while apparent zinc utilization at 21 days of age was significantly higher than that at 42 days of age. It can be concluded that under the conditions of this experiment, a dietary zinc level of 60 mg/kg could meet the growth requirements of broiler chickens, while a dietary zinc level of 120 mg/kg could maintain better immune function in broiler chickens.

Full Text

Effects of Dietary Zinc Level on Growth Performance, Slaughter Performance, Serum Immune Indices, and Zinc Excretion in Broilers

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Abstract: This experiment was conducted to investigate the effects of dietary zinc level on growth performance, slaughter performance, serum immune indices, and zinc excretion in broilers. A total of 600 healthy 1-day-old Arbor Acres (AA) broilers were randomly allocated to 5 groups with 6 replicates per group and 20 broilers per replicate (half male and half female). The dietary zinc levels were 30 (basal diet group), 60, 90, 120, and 150 mg/kg. The experiment lasted for 42 days. The results showed: 1) During days 1–42, the average daily gain (ADG) of male broilers in the 60, 90, 120, and 150 mg/kg zinc groups was significantly higher than that in the basal diet group ($P<0.05$), while no significant differences were observed in ADG of female broilers among all groups ($P>0.05$). 2) Dietary zinc level had no significant effect on immune organ index or slaughter performance of broilers at 21 and 42 days of age ($P>0.05$). 3) In male broilers at 21 days of age, the Newcastle disease serum antibody titer in the 120 and 150 mg/kg zinc groups was significantly higher than that in the basal diet group and the 60 and 90 mg/kg zinc groups ($P<0.05$); at 42 days of age, no significant differences in antibody titer were observed among groups ($P>0.05$). In female broilers at 21 days of age, no significant differences in antibody titer were observed among groups ($P>0.05$); however, at 42 days of age, the antibody titer in the 120 mg/kg zinc group was significantly higher than that in all other groups ($P<0.05$). 4) Dietary zinc level had significant effects on fecal zinc content and apparent zinc utilization rate in broilers at both 21 and 42 days of age ($P<0.05$). Across different growth stages, fecal zinc content at 42 days was significantly higher than at 21 days, while apparent zinc utilization rate at 21 days was significantly higher than at 42 days. In conclusion, under the conditions of this experiment, a dietary zinc level of 60 mg/kg met the growth requirements of broilers, while a level of 120 mg/kg maintained better immune function.

Keywords: zinc; broilers; growth performance; immunity; zinc excretion
Classification: S831.5

Zinc is an essential trace element for broiler growth and development, possessing the most diverse physiological functions among the 15 confirmed essential trace elements. Zinc participates not only in the metabolism of proteins, amino acids, nucleic acids, lipids, carbohydrates, vitamins, and other trace elements, but also is associated with the activity of insulin, glucagon, prostaglandins, and gonadotropins. It plays crucial roles in physiological functions including bone development, reproduction, immunity, blood coagulation, biomembrane stability, and gene expression. Additionally, zinc is a component of many functional proteins such as metallothionein, nucleoproteins, and receptors, earning it the designation "element of life" due to its extensive physiological and biochemical functions. Zinc deficiency in livestock and poultry not only impairs growth and development but also causes metabolic disorders and atrophy of immune organs, leading to compromised immune function and increased susceptibility to bacterial, viral, and fungal infections. Currently, excessive zinc supplementation is common in broiler production, which not only fails to improve growth performance and health status but also reduces overall trace element utilization efficiency, resulting in waste of trace element resources and environmental pollution [1]. This experiment aimed to investigate the effects of dietary zinc level on growth performance, slaughter performance, serum immune indices, and zinc excretion in broilers, and to determine the appropriate supplementation level to provide reference for establishing zinc addition limits in broiler diets.

1.1 Experimental Materials

Zinc sulfate monohydrate (feed grade) containing 35.5% zinc was purchased from Beijing Precision Animal Nutrition Research Center.

1.2 Experimental Design

The experiment was conducted at the Nankou Experimental Base of the Feed Research Institute, Chinese Academy of Agricultural Sciences. A total of 600 healthy 1-day-old Arbor Acres (AA) broilers were randomly allocated to 5 groups with 6 replicates per group and 20 broilers per replicate (10 males and 10 females, housed in 2 cages). The groups were fed diets with zinc levels of 30 (basal diet), 60, 90, 120, and 150 mg/kg. The basal diet was a corn-soybean meal-miscellaneous meal type diet formulated as pelleted feed with a basal zinc level of approximately 30 mg/kg. Its composition and nutrient levels are shown in Table 1 . The other four experimental diets were prepared by adding 30, 60, 90, and 120 mg/kg zinc as zinc sulfate monohydrate to the basal diet. The experimental design is presented in Table 2 .

1.3 Management Practices

Broilers were housed in three-tier stainless steel cages with 23 hours of lighting and provided water via nipple drinkers and ad libitum access to feed. House temperature was maintained at 33°C during days 1-3, then gradually decreased until reaching ambient temperature. Vaccination against Marek' s disease and Newcastle disease was performed at 1 day of age, and infectious bursal disease vaccine was administered via drinking water at 21 days of age. Routine hygiene management was implemented. Daily observations were made regarding bird mental state, appetite, fecal condition, and mortality.

1.4 Measurement Indicators

1.4.1 Growth Performance Body weight and feed consumption were measured on days 1, 21, and 42. Average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated for the early growth stage (days 1–21), late growth stage (days 22–42), and overall period (days 1–42) on a replicate basis. Overall mortality rate was also calculated.

1.4.2 Slaughter Performance On days 21 and 42, one male and one female broiler were randomly selected from each replicate, slaughtered after bloodletting, and evaluated for eviscerated yield, semi-eviscerated yield, leg muscle percentage, breast muscle percentage, and abdominal fat percentage according to "Poultry Production Performance Terminology and Measurement Methods" (NY/T 823-2004).

1.4.3 Immune Organ Index On days 21 and 42, one male and one female broiler were randomly selected from each replicate. The spleen, bursa of Fabricius, and thymus were collected and weighed to calculate the immune organ index using the formula: Immune organ index (g/kg) = immune organ weight (g) / carcass weight (kg).

1.4.4 Serum Immune Indices On days 21 and 42, one male and one female broiler were randomly selected from each replicate for cardiac blood collection. Serum was prepared for measurement of Newcastle disease virus antibody titer using the hemagglutination inhibition test (antigens, negative and positive sera purchased from Beijing Zhonghai Biotechnology Co., Ltd.). Serum immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) concentrations were determined using a Hitachi 7600 automatic biochemical analyzer.

1.4.5 Apparent Zinc Utilization Rate The titanium dioxide indicator method was used. Titanium dioxide was added to all diets at 0.4% and thoroughly mixed before cold-pelleting. Fecal samples were collected for 3 consecutive days during days 17-21 and days 37–42, mixed uniformly, dried at 65°C, and analyzed for zinc and titanium dioxide content to calculate apparent zinc utilization rate using the formula: Apparent zinc utilization rate (%) = [1 - (TiO content in diet / TiO content in feces) × (Zn content in feces / Zn content in diet)] × 100.

1.4.6 Zinc Content in Diets and Feces Samples were dried at 105°C to constant weight, then equilibrated in air for 24 hours. Weighed diet and fecal samples were ashed for 12 hours. If carbon particles remained, concentrated nitric acid was added dropwise to moisten the residue, which was then heated, dried, and re-ashed until no carbon particles remained. The ash was dissolved in hydrochloric acid and standardized. The prepared samples were analyzed for zinc content using flame atomic absorption spectrophotometry (Hitachi Z-2000).

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA and the General Linear Model (GLM) procedure in SPSS 17.0 for two-factor analysis of variance, with significance of factors tested using F-test. LSD multiple comparisons were performed for indicators with significant main effects. Curve estimation procedures were used for linear and quadratic regression analysis between dietary zinc level (x) and sensitive indicators (y). $P < 0.05$ was considered statistically significant.

2.1 Calculated and Measured Dietary Zinc Levels

The calculated and measured dietary zinc levels are shown in Table 3 . The results indicate that the calculated and measured values were basically consistent.

2.2 Effects of Dietary Zinc Level on Broiler Growth Performance

The effects of dietary zinc level on broiler growth performance are presented in Tables 4 , 5 , and 6 . As shown in Table 4, during the early growth stage, the ADG and ADFI of male broilers in the 90 mg/kg zinc group were significantly higher than those in the basal diet group and 60 mg/kg zinc group ($P<0.05$), while no significant differences were observed in F/G among male groups ($P>0.05$). No significant differences were observed in ADG, ADFI, or F/G among female groups ($P>0.05$).

As shown in Table 5, during the late growth stage, the ADG and ADFI of male broilers in the 60, 90, 120, and 150 mg/kg zinc groups were significantly higher than those in the basal diet group ($P<0.05$), while no significant differences were observed in F/G among male groups ($P>0.05$). No significant differences were observed in ADG, ADFI, or F/G among female groups ($P>0.05$).

As shown in Table 6, during the overall period, the ADG of male broilers in the 60, 90, 120, and 150 mg/kg zinc groups was significantly higher than that in the basal diet group ($P<0.05$), while no significant differences were observed in ADG of female broilers among groups ($P>0.05$). No significant differences were observed in ADFI, F/G, or mortality rate among all groups for either sex ($P>0.05$). No significant linear or quadratic regression relationships were observed between dietary zinc level and ADG, ADFI, or F/G at any growth stage (P>0.05).

2.3 Effects of Dietary Zinc Level on Immune Organ Index and Slaughter Performance

The effects of dietary zinc level on immune organ index and slaughter performance are shown in Tables 7 and 8 . Dietary zinc level had no significant effect on immune organ index or slaughter performance of broilers at 21 or 42 days of age (P>0.05). No significant linear or quadratic regression relationships were observed between dietary zinc level and immune organ index or slaughter performance at either age (P>0.05).

2.4 Effects of Dietary Zinc Level on Serum Immune Indices

The effects of dietary zinc level on serum immune indices are presented in Table 9 . No clear patterns were observed in serum IgA, IgM, and IgG concentrations at 21 or 42 days of age. In male broilers at 21 days of age, the Newcastle disease antibody titer in the 120 and 150 mg/kg zinc groups was significantly higher than that in the basal diet group and the 60 and 90 mg/kg zinc groups (P<0.05); at 42 days of age, no significant differences in antibody titer were observed among groups (P>0.05). In female broilers at 21 days of age, no significant differences in antibody titer were observed among groups (P>0.05); however, at 42 days of age, the antibody titer in the 120 mg/kg zinc group was significantly higher than that in all other groups (P<0.05).

At 21 days of age, significant linear and quadratic regression relationships were observed between dietary zinc level (y) and antibody titer in male broilers (x) (P<0.05), with regression equations $y = 0.008x + 5.000$ ($R^2 = 0.285$) and $y = 0.0000794x^2 - 0.002x + 5.143$ ($R^2 = 0.300$), respectively. Quadratic regression analysis indicated that the lowest antibody titer occurred at a dietary zinc level of 42.59 mg/kg. At 42 days of age, a significant quadratic regression relationship was observed between dietary zinc level (y) and serum IgA concentration in male broilers (x) (P<0.05), with the regression equation $y = -0.0000759x^2 + 0.011x + 0.830$ ($R^2 = 0.248$). Quadratic regression analysis indicated that the highest serum IgA concentration occurred at a dietary zinc level of 102.44 mg/kg.

2.5 Effects of Dietary Zinc Level on Fecal Zinc Excretion and Apparent Zinc Utilization

The effects of dietary zinc level on fecal zinc content and apparent zinc utilization are shown in Table 10 . Dietary zinc level had significant effects on fecal zinc content at both 21 and 42 days of age (P<0.05), with fecal zinc content increasing as dietary zinc level increased. Fecal zinc content also differed between growth stages, with content at 42 days being significantly higher than at 21 days. Dietary zinc level significantly affected apparent zinc utilization rate at both ages (P<0.05), with utilization rate tending to increase as dietary zinc level increased. The apparent zinc utilization rate in the 30 mg/kg zinc group was significantly lower than that in the 90 and 150 mg/kg zinc groups at both 21 and 42 days ($P<0.05$). Apparent zinc utilization rate also differed between growth stages, with utilization at 21 days being significantly higher than at 42 days.

3.1 Effects of Dietary Zinc Level on Broiler Growth and Slaughter Performance

Zinc functions in living organisms through various zinc-containing enzymes and participates in multiple metabolic processes, including synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids, thereby affecting broiler growth performance. The effect of dietary zinc supplementation depends not only on the basal zinc level but also on chicken breed, sex, experimental duration, period, and supplementation level. In this experiment, dietary zinc level significantly affected the ADG of male broilers during the overall growth period, consistent with some previous studies. Liao et al. [2] investigated the effects of dietary zinc level on growth performance and serum physiological and biochemical indices, recommending a dietary zinc requirement of 65 mg/kg for broilers aged 22–42 days. Wang [3] reported that while zinc supplementation level did not significantly affect feed conversion ratio at various stages, it significantly affected body weight gain in both Gushi chickens and AA broilers. However, some studies have reported that zinc supplementation within certain ranges does not affect broiler growth performance. Smith et al. [4] found that varying dietary zinc levels from 34 to 181 mg/kg did not significantly affect broiler growth performance. This insensitivity may be attributed to the influence of trace elements in the basal diet, as broilers have relatively low trace element requirements, and the basal diet may already meet their growth needs, thus additional supplementation may not demonstrate improvement effects. Tian et al. [5] reported that dietary zinc levels ranging from NRC recommendations to twice the NRC recommendation did not significantly affect growth performance of 22–42-day-old broilers, and that fecal zinc excretion was affected by other trace element additions. They suggested that a dietary zinc level of 38.23 mg/kg could meet broiler trace element requirements while minimizing fecal zinc excretion. He et al. [6] indicated that dietary zinc levels of 40–50 mg/kg could meet broiler growth requirements. Chen et al. [7] studied the effects of adding 0, 40, or 80 mg/kg zinc to a corn-multiple oilseed meal diet (containing 30 mg/kg zinc) on tissue zinc content, immune organ development, and growth performance in 4-6-week-old broilers. They found that zinc omission did not affect body weight or feed conversion ratio but increased leg abnormality rate, poor feather growth, and mortality, and affected development of some digestive and immune organs. Supplementation with 40 mg/kg zinc improved these adverse conditions, while 80 mg/kg zinc showed no significant additional effects. From nutritional, digestive, and immunological perspectives, they concluded that supplementing the basal diet with 40 mg/kg zinc was appropriate. Sun and Ma [8] determined the optimal zinc requirement for broilers to be 60 mg/kg, with a basal dietary zinc level of 30 mg/kg and a supplementation level of 30 mg/kg. Yan [9] reported that when dietary zinc levels ranged from 47.51 to 87.61 mg/kg, liver and tibia zinc contents remained relatively stable while growth performance and immune function were high, indicating appropriate zinc levels. Further increases in dietary zinc level resulted in high immune function but decreased liver and tibia zinc content and growth performance, indicating zinc excess. At a dietary zinc level of 327.61 mg/kg, liver and tibia zinc content, growth performance, and immune function were all significantly reduced, indicating severe zinc excess. This study emphasized that basal dietary zinc level must be considered when determining appropriate zinc supplementation levels, and that growth performance, immune function, and tissue zinc metabolism indicators should be comprehensively evaluated. Wedekind et al. [10] observed that increasing dietary zinc level from 10 to 30 mg/kg improved chick weight gain. Mohanna and Nys [11] indicated that the zinc requirement for broilers was 40 mg/kg. Zhang et al. [12] suggested that broilers have stage-specific zinc requirements, with 320 mg/kg during the early period and 80–120 mg/kg during the late period providing better growth performance and immune function, though their basal dietary manganese level was low (24.50 mg/kg measured value), which may have affected results. Research on zinc effects on poultry slaughter performance is relatively limited. The current study found no significant differences in slaughter performance among groups, possibly because the basal dietary zinc level of 30 mg/kg was sufficient, and additional supplementation could not further improve slaughter performance.

3.2 Effects of Dietary Zinc Level on Serum Immune Indices

Serum immune indices are sometimes used as criteria for evaluating trace element requirements, as higher immune status and health are important prerequisites for optimal growth performance. Zinc is essential for maintaining normal immune cells and other immune organs. In this experiment, dietary zinc level significantly affected Newcastle disease virus antibody titers, with optimal immune function observed at 90 mg/kg dietary zinc. Prasad and Oberleas [13] demonstrated that zinc is closely related to immune system development and function, and that zinc deficiency impairs immune function, leading to disease susceptibility and growth restriction. Zinc affects immune function through two primary mechanisms: first, zinc deficiency directly causes damage, alteration, or differentiation of immune organs and cells; second, it indirectly alters immune function by affecting nutrition, growth, and metabolism of other tissues. Since zinc influences nutrient metabolism and DNA/RNA function, it necessarily affects immune organs and cells, thereby modifying immunity. Additionally, zinc has close relationships with vitamins A, E, and C in absorption and metabolism, collectively influencing immune function.

Zhang et al. [14] reported that zinc-deficient broilers showed significantly decreased ELISA titers for Marek' s disease and Newcastle disease compared to controls. Stahl et al. [15] reported that broilers fed diets containing 28, 38, 48, 68, or 118 mg/kg zinc showed lowest antibody production at 28 and 118 mg/kg zinc and highest production at 38-48 mg/kg zinc, suggesting that 38 mg/kg dietary zinc was appropriate for chicks, as levels below this reduced antibody response to sheep red blood cells (SRBC), while 150 mg/kg zinc caused marginal immunosuppression without affecting growth. Yan [9] showed that cellular and humoral immune functions were optimal when dietary zinc levels ranged from 44.67 to 84.67 mg/kg, while levels of 4.67 mg/kg (deficient) or 647.61 mg/kg (excessive) significantly reduced both immune functions. Cui et al. [16] reported that high zinc (supraphysiological doses) was detrimental to immune organs such as the thymus, damaging gastric mucosa and causing multiple ulcers, thereby reducing immunity. They also suggested that zinc redistribution during infection helps combat infection, with increased hepatic zinc uptake improving liver function and decreased blood zinc enhancing phagocytic antibacterial activity. Some studies consider immune indices to be less sensitive than growth performance indicators. For example, Stahl et al. [15] found that compared to a basal diet with 37 mg/kg zinc, a diet with 103 mg/kg zinc only showed a trend toward increased phytohemagglutinin titers. Mohanna and Nys [17] found that low-zinc diets did not affect chick immune response, suggesting that immune response may be less sensitive to zinc intake than growth performance.

3.3 Effects of Dietary Zinc Level on Fecal Zinc Excretion and Apparent Zinc Utilization

Environmental pollution caused by excessive trace element excretion has attracted increasing attention, and determining supplementation levels or requirements based on reduced excretion is receiving more focus [18-21]. This study demonstrated that dietary zinc level significantly affected fecal zinc content. Yuan et al. [22] reported that replacing zinc sulfate with 20 mg/kg organic zinc reduced zinc content in air-dried feces by 3.05%. Wang [3] reported that dietary supplementation with 120 mg/kg zinc increased zinc excretion by 335% (Gushi chickens) and 174% (AA broilers) compared to controls. Different growth stages significantly affected apparent zinc utilization rate, with the highest utilization during the early period and significantly lower utilization during the late period, similar to the current findings. Dozier et al. [23] showed that adding 40-120 mg/kg zinc from different sources did not affect broiler growth performance, but the 40 mg/kg level reduced zinc excretion by 50% compared to 120 mg/kg. Similarly, adding 4-12 mg/kg copper from different sources did not affect growth performance but reduced copper excretion by 35%. They concluded that reducing dietary zinc and copper levels could effectively decrease environmental heavy metal accumulation without affecting broiler performance. Mohanna and Nys [11] conducted two experiments investigating the effects of different zinc levels (20-190 mg/kg; basal diet contained 20 mg/kg) and sources (inorganic vs. organic) on growth performance, zinc retention, excretion, and immune response in 5-21-day-old broilers. They found that 45 mg/kg dietary zinc was sufficient for normal growth, while tibia and plasma zinc content increased linearly with dietary zinc level, reaching maximum values at 75 mg/kg. Body zinc content was saturated at 90 mg/kg dietary zinc. Dietary zinc level did not significantly affect immune response or plasma alkaline phosphatase activity. When dietary zinc level was reduced from 190 to 65 mg/kg, zinc retention increased from 8% to 20%, while fecal zinc content decreased by 75%, with no differences between zinc sources. They concluded that reducing dietary zinc level is an effective nutritional strategy to decrease zinc pollution. Therefore, supplementation levels that reduce trace element excretion without affecting animal performance represent important criteria for determining trace element requirements, and identifying effective evaluation indicators under this guidance is a key issue to be addressed.

4 Conclusion

1) Appropriate dietary zinc levels promoted broiler growth and maintained good immune status, but fecal zinc content increased with higher dietary zinc levels.

2) Based on average daily gain as the evaluation criterion, the appropriate dietary zinc level for broilers was 60 mg/kg. A dietary zinc level of 120 mg/kg maintained better immune function in broilers.

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