Effects of Phytase Supplementation in Low-Zinc Diets on Growth Performance, Tibial Development, Immune Function, and Antioxidant Capacity of 1- to 4-Week-Old Geese: A Postprint

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Abstract

This study was conducted to investigate the effects of phytase supplementation in low-zinc diets on growth performance, tibial development, immune function, and antioxidant capacity of 1- to 4-week-old geese, and to explore whether dietary phytase supplementation could improve zinc bioavailability and effectively reduce zinc supplementation levels. Three hundred sixty 1-day-old Wulong geese were randomly allocated to 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group I was the positive control group, fed the basal diet supplemented with 80 mg/kg zinc sulfate without phytase; Group II was the negative control group, fed the basal diet supplemented with 1,200 U/kg phytase without zinc; Groups III-VI were fed the basal diet supplemented with 1,200 U/kg phytase and 16, 32, 48, and 64 mg/kg zinc sulfate, respectively. The experimental period lasted 28 days. The results showed: 1) Quadratic regression analysis indicated that dietary zinc supplementation levels of 34.00-44.70 mg/kg yielded optimal average daily gain and feed-to-gain ratio when the diet was supplemented with 1,200 U/kg phytase. 2) Bone mineral density (BMD) in Groups IV and V was significantly higher than that in Group II ($P<0.05$); bone mineral content (BMC) and serum alkaline phosphatase (AKP) activity in Groups IV and V were significantly or extremely significantly higher than those in Groups I and II ($P<0.05$ or $P<0.01$). Quadratic regression analysis showed that serum AKP activity reached its maximum at a dietary zinc supplementation level of 51.38 mg/kg. 3) Serum and hepatic total antioxidant capacity (T-AOC) in Group IV was significantly higher than that in Group II ($P<0.05$), with no significant difference from Group I ($P>0.05$). Quadratic regression analysis revealed that serum and hepatic T-AOC reached their maximum at dietary zinc supplementation levels of 50.24 and 47.49 mg/kg, respectively. 4) Thymus index and bursa of Fabricius index in Groups III-VI were significantly or extremely significantly higher than those in Groups I and II ($P<0.05$ or $P<0.01$); Newcastle disease antibody titers before and after immunization in Groups IV-VI were significantly or extremely significantly higher than those in Groups I and II ($P<0.05$ or $P<0.01$). These results demonstrate that dietary phytase supplementation can improve growth performance, promote tibial development, enhance antioxidant capacity and immune function, effectively increase zinc utilization, reduce dietary zinc supplementation levels, and improve zinc bioavailability in 1- to 4-week-old Wulong geese. It is recommended that under the condition of dietary supplementation with 1,200 U/kg phytase, the appropriate zinc supplementation level is 44.70-51.38 mg/kg.

Full Text

Effects of Phytase Supplementation in a Low Zinc Diet on Growth Performance, Tibial Development, Immune Performance and Antioxidant Capacity of Geese Aged from 1 to 4 Weeks

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Abstract

This experiment was conducted to investigate the effects of phytase supplementation in a low zinc diet on growth performance, tibial development, immune performance, and antioxidant capacity of geese aged from 1 to 4 weeks, and to explore whether dietary phytase could improve zinc bioavailability and effectively reduce zinc supplementation levels. A total of 360 one-day-old Wulong geese were randomly allocated into six groups with six replicates per group and ten geese per replicate (half male and half female). Group I served as the positive control, receiving a basal diet supplemented with 80 mg/kg zinc sulfate without phytase; Group II was the negative control, receiving a basal diet supplemented with 1,200 U/kg phytase without zinc; Groups III–VI were fed the negative control diet supplemented with 16, 32, 48, and 64 mg/kg zinc sulfate, respectively. The experiment lasted for 28 days. The results showed: 1) Based on quadratic curve fitting analysis, under the condition of 1,200 U/kg phytase supplementation, optimal average daily gain and feed-to-gain ratio were achieved when dietary zinc supplementation ranged from 34.00 to 44.70 mg/kg. 2) Bone mineral density (BMD) in Groups IV and V was significantly higher than that in Group II ($P<0.05$); bone mineral content (BMC) and serum alkaline phosphatase (AKP) activity in Groups IV and V were significantly or extremely significantly higher than those in Groups I and II (P<0.05 or P<0.01). Quadratic curve fitting analysis indicated that serum AKP activity peaked at a dietary zinc level of 51.38 mg/kg. 3) Serum and hepatic total antioxidant capacity (T-AOC) in Group IV were significantly higher than those in Group II (P<0.05), with no significant differences compared to Group I (P>0.05). Quadratic curve fitting analysis revealed that serum and hepatic T-AOC reached maximum values at dietary zinc levels of 50.24 and 47.49 mg/kg, respectively. 4) Thymus index and bursa of Fabricius index in Groups III–VI were significantly or extremely significantly higher than those in Groups I and II (P<0.05 or P<0.01); serum Newcastle disease antibody levels before and after immunization in Groups IV–VI were significantly or extremely significantly higher than those in Groups I and II (P<0.05 or P<0.01). These findings demonstrate that dietary phytase supplementation can improve growth performance, promote tibial development, enhance antioxidant capacity and immune performance, effectively increase zinc utilization, reduce dietary zinc supplementation levels, and improve zinc bioavailability in 1–4 week-old Wulong geese. It is recommended that under the condition of 1,200 U/kg phytase supplementation, the optimal dietary zinc level should be 44.70–51.38 mg/kg.

Keywords: zinc; phytase; geese; growth performance; tibial development; antioxidant capacity; immune performance

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Introduction

Zinc is an essential trace element for animals that promotes bone development and enhances immune performance [1], participates in the synthesis of various enzymes including alkaline phosphatase (AKP), and is closely associated with antioxidant capacity [2]. Phytase is a class of phosphomonoesterases that hydrolyzes phosphate groups from phytic acid and phytates, ultimately releasing inositol and inorganic phosphorus. It can improve the bioavailability of trace elements and promote growth performance in monogastric animals [4]. Therefore, investigating the effects of phytase on zinc utilization and exploring the beneficial roles of zinc and phytase on growth, immune organ indices, and antioxidant capacity are important for scientifically reducing zinc supplementation levels in goose diets. Zinc is the trace element most affected by dietary phytic acid content [5]. Research has confirmed that zinc can form extremely insoluble phytate salts with phytase in the upper small intestine, which cannot be digested or absorbed by animals, thereby hindering zinc bioavailability [6].

According to China' s Ministry of Agriculture Announcement No. 1224, the recommended zinc sulfate ($ZnSO_4$) supplementation level in goose diets is 60 mg/kg. However, Chen et al. [7] reported that the optimal zinc level for Wulong geese to achieve maximum growth performance was 106.45 mg/kg, exceeding the national recommendation. Brnić et al. [8] found that phytase supplementation improved the utilization of zinc oxide and zinc sulfate by approximately 80%.

Revy et al. [9] demonstrated that zinc bioavailability was superior in piglets receiving both zinc and phytase compared to zinc alone. Zhu et al. [10] investigated the effects of different phytases on trace element deposition in laying hens and found that 400 U/kg phytase effectively increased zinc tissue deposition. This experiment aimed to explore the synergistic effects of phytase and zinc on growth performance, tibial development, immune performance, and antioxidant capacity in 1–4 week-old geese, determine the effect of phytase on zinc utilization, and provide a theoretical basis for the rational use of phytase in goose diets.

Materials and Methods

1.1 Experimental Animals and Design A total of 360 one-day-old Wulong geese with similar body condition were randomly divided into six groups with six replicates per group and ten geese per replicate (half male and half female). Group I was the positive control, receiving a basal diet supplemented with 80 mg/kg zinc sulfate without phytase; Group II was the negative control, receiving a basal diet supplemented with 1,200 U/kg phytase without zinc; Groups III–VI received the negative control diet supplemented with 16, 32, 48, and 64 mg/kg zinc sulfate, respectively. Zinc sulfate was purchased from Zhejiang Xinweipu Additive Co., Ltd., and phytase was purchased from Jiangsu Yuanfang Zhonghui Biotechnology Co., Ltd.

1.2 Experimental Diets The experimental diets used corn and soybean meal as the main ingredients, with formulation designed according to the nutrient levels recommended by NRC (1994) for poultry. The experiment lasted for 28 days. The composition and nutrient levels of the basal diet are shown in Table 1 . Zinc content in the basal diet was measured using plasma emission spectrometry.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Note: 1) The multi-vitamin and trace elements provided the following per kg of diet: VD_3 200 IU, VA 1,500 mg, VE 12.5 mg, VK 31.5 mg, VB_1 2.2 mg, VB_2 5.0 mg, nicotinic acid 65 mg, pantothenate 15 mg, VB_6 2 mg, biotin 0.2 mg, folic acid 0.5 mg, choline 1,000 mg, Fe 85 mg, Mn 80 mg, Cu 20 mg, I 0.42 mg, Se 0.3 mg, Co 2.5 mg. 2) CF and Zn were measured values, while other nutrient levels were calculated values.

1.3 Management Before the experiment, the goose house and equipment were washed and disinfected with potassium permanganate spray, then fumigated with formaldehyde and potassium permanganate with closed doors and windows for 24 hours. The experiment began one week later. Geese were raised on net floors with ad libitum access to feed and water. Temperature, humidity, and lighting were strictly controlled, and regular disinfection was performed during the experimental period. At 1–3 days of age, goslings were subcutaneously vaccinated with combined inactivated vaccine against gosling plague and goose paramyxovirus; at 10 days of age, they received Newcastle disease H5N1 vaccine subcutaneously; and at 20 days of age, they received Newcastle disease oil emulsion vaccine via intramuscular injection in the chest.

1.4.1 Growth Performance Goslings were weighed at 1 day of age, and at the end of week 4, fasting body weight was recorded by replicate to calculate average daily gain (ADG) from 1 to 4 weeks. Daily feed consumption was recorded to calculate average daily feed intake (ADFI). Mortality was recorded daily to calculate feed-to-gain ratio (F/G) and mortality rate [11].

1.4.2 Tibial Development At the end of week 4, the right tibia was collected from one goose per replicate (36 total). Bone mineral density (BMD) was measured using a digital scintillation cone-scanning bone densitometer (osteocoer 3), and bone mineral content (BMC) was calculated. Tibias were then dried at 105°C and weighed. Tibial ash, calcium (Ca), and phosphorus (P) contents were determined according to GB/T 6438-1992 using atomic spectrophotometry and molybdenum yellow colorimetry. Serum AKP activity was measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.3 Antioxidant Indices At the end of week 4, serum and liver samples were collected from two geese per replicate (72 total). Total antioxidant capacity (T-AOC) was measured by colorimetry, glutathione peroxidase (GSH-Px) activity by colorimetry, copper-zinc superoxide dismutase (Cu-Zn SOD) activity by xanthine oxidase method, and malondialdehyde (MDA) content by thiobarbituric acid reaction (TBA). All indices were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.4 Immune Performance **Immune organ indices:** At the end of week 4, immune organs (thymus, spleen, and bursa of Fabricius) were collected, attached tissues were removed, and fresh weights were recorded after absorbing excess moisture with filter paper. Immune organ index (mg/g) = fresh immune organ weight (mg) / live body weight (g).

Newcastle disease antibody titer: Before vaccination, six geese with similar body weight were selected from each group for blood collection. After Newcastle disease vaccination, blood was collected again from the same geese on days 7 and 14 post-vaccination. Blood samples were processed without anticoagulant, centrifuged at 3,000 r/min for 10 min, and serum was collected for antibody titer determination using hemagglutination and hemagglutination inhibition tests. Antibody titer was expressed as the highest serum dilution showing 100% inhibition of agglutination.

1.5 Statistical Analysis Data were analyzed using one-way ANOVA with LSD multiple comparison tests in SPSS 19.0 software. Results are expressed as "mean ± standard deviation." Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

Results

2.1 Effects of Phytase Supplementation in a Low Zinc Diet on Growth Performance of Geese Aged 1–4 Weeks As shown in Table 2 , body weight and average daily gain in Groups IV and V were extremely significantly higher than those in Groups I and II ($P<0.01$). Average daily feed intake in Group IV was extremely significantly higher than that in Groups I and II ($P<0.01$). Feed-to-gain ratio in Groups IV, V, and VI was extremely significantly lower than that in Group I ($P<0.05$). No significant differences in mortality rate were observed among groups ($P>0.05$).

These results indicate that dietary phytase supplementation significantly improved growth performance in 1–4 week-old geese, and that average daily gain and feed-to-gain ratio were correlated with dietary zinc level under phytase supplementation. Quadratic curve fitting was performed for average daily gain (Y_1) and feed-to-gain ratio (Y_2) against dietary zinc level (X) for Groups II–VI, yielding the following regression equations:

$$Y_1 = -0.003X^2 + 0.204X + 40.538 \ (R^2 = 0.915, P\_Q = 0.000)$$
$$Y_2 = 2.410 - 0.007X + (7.813E-5)X^2 \ (R^2 = 0.817, P\_Q = 0.000)$$

These equations indicate that maximum average daily gain occurred at 34.00 mg/kg zinc supplementation, while minimum feed-to-gain ratio occurred at 44.70 mg/kg zinc. From a comprehensive benefit perspective, the optimal dietary zinc level for 1–4 week-old geese is recommended to be 34.00–44.70 mg/kg when dietary phytase is supplemented at 1,200 U/kg.

Table 2 Effects of phytase supplementation in a low zinc diet on growth performance of geese aged from 1 to 4 weeks

Note: In the same column, values with the same small letter or no letter superscripts indicate no significant difference (P>0.05), adjacent small letters indicate significant difference (P<0.05), and alternate small letters indicate extremely significant difference (P<0.01). The same applies to Tables 3, 5, 6, and 7.

2.2 Effects of Phytase Supplementation in a Low Zinc Diet on Tibial Development of Geese Aged 1–4 Weeks As shown in Table 3 , bone mineral density in Groups IV and V was significantly higher than that in Group II ($P<0.05$). Bone mineral content in Groups IV and V was significantly or extremely significantly higher than that in Groups I and II ($P<0.05$ or $P<0.01$). Serum AKP activity in Groups IV, V, and VI was significantly higher than that in Group I ($P<0.05$) and extremely significantly higher than that in Group II ($P<0.01$). No significant differences in bone ash content were observed among groups ($P>0.05$). Calcium content in Groups IV and VI was significantly higher than that in Group II ($P<0.05$), and phosphorus content in Groups IV, V, and VI was significantly higher than that in Group II ($P<0.05$).

Quadratic curve fitting analysis for bone mineral density and serum AKP activity in Groups II–VI revealed that the relationship between bone mineral density and dietary zinc level was not significant ($R^2 < 0.700$). The regression equation for serum AKP activity (Y_3) against dietary zinc level (X) was:

$$Y_3 = -0.004X^2 + 0.411X + 90.545 \ (R^2 = 0.911, P\_Q = 0.000)$$

This equation indicates that serum AKP activity peaked at a dietary zinc level of 51.38 mg/kg, suggesting maximal osteoblast activity.

Table 3 Effects of phytase supplementation in a low zinc diet on tibial development of geese aged from 1 to 4 weeks

As shown in Table 4 , bone mineral density and calcium content were extremely significantly positively correlated with serum AKP activity (P<0.01), while serum phosphorus content was significantly positively correlated with serum AKP activity (P<0.05).

These results demonstrate that the combination of dietary phytase and zinc supplementation enhanced serum AKP activity, increased bone mineral density, and promoted calcium and phosphorus deposition in bone, thereby stimulating tibial development.

Table 4 Correlation analysis between serum AKP activity and tibial indicators

Note: indicates significant correlation (P<0.05), ** indicates extremely significant correlation (P<0.05).*

2.3 Effects of Phytase Supplementation in a Low Zinc Diet on Antioxidant Capacity of Geese Aged 1–4 Weeks As shown in Table 5 , in serum, T-AOC in Groups III, IV, and VI was significantly higher than that in Group II (P<0.05), with no significant differences compared to Group I (P>0.05). Cu-Zn SOD activity in Groups III and IV was significantly higher than that in Group II (P<0.05), while Groups V and VI showed extremely significant differences (P<0.01). No significant differences in MDA content were observed among groups (P>0.05). GSH-Px activity in Groups IV–VI was extremely significantly higher than that in Groups I and II (P<0.01).

In liver, T-AOC in Groups V and VI was significantly or extremely significantly higher than that in Groups I and II (P<0.05 or P<0.01). Cu-Zn SOD activity, GSH-Px activity, and MDA content in Groups IV, V, and VI were significantly or extremely significantly higher than those in Groups I and II (P<0.05 or P<0.01). Serum and hepatic antioxidant indices in Groups II–VI showed an improving trend with increasing dietary zinc levels.

Curve fitting analysis for Groups II–VI revealed quadratic relationships between serum T-AOC (Y_4) and hepatic T-AOC (Y_5) with dietary zinc level (X), yielding the following regression equations:

$$Y_4 = 12.179 + 0.042X - (4.18E\text{-}4)X^2 \ (R^2 = 0.702, P\_Q = 0.001)$$
$$Y_5 = 0.853 + 0.017X - (1.79E\text{-}4)X^2 \ (R^2 = 0.748, P\_Q = 0.000)$$

These equations indicate that serum T-AOC peaked at 50.24 mg/kg zinc, while hepatic T-AOC peaked at 47.49 mg/kg zinc. These results demonstrate that phytase supplementation in low zinc diets can enhance antioxidant capacity in both serum and liver of geese.

Table 5 Effects of phytase supplementation in a low zinc diet on antioxidant capacity of geese aged from 1 to 4 weeks

2.4 Effects of Phytase Supplementation in a Low Zinc Diet on Immune Performance of Geese Aged 1-4 Weeks As shown in Table 6 , thymus index and bursa of Fabricius index in Groups III–VI were significantly or extremely significantly higher than those in Groups I and II ($P<0.05$ or $P<0.01$). No significant differences in spleen index were observed among groups ($P>0.05$).

Table 6 Effects of phytase supplementation in a low zinc diet on immune organ index of geese aged from 1 to 4 weeks

Serum Newcastle disease antibody levels before and after immunization in Groups IV–VI were significantly or extremely significantly higher than those in Groups I and II ($P<0.05$ or $P<0.01$), with antibody levels in Groups II–V showing an upward trend as dietary zinc level increased. Quadratic curve fitting analysis for antibody levels in Groups II–VI revealed a quadratic relationship between serum antibody level on day 14 post-immunization (Y_6) and dietary zinc level (X):

Y_6 = 6.365 + 0.045X - (4.53E-4)X^2 (R^2 = 0.882, P_Q = 0.000)

This equation indicates that serum antibody level peaked at a dietary zinc level of 49.67 mg/kg. These results demonstrate that combined phytase and zinc supplementation can improve immune performance in geese, with optimal serum antibody levels achieved at 49.67 mg/kg zinc on day 14 post-immunization.

Table 7 Effects of phytase supplementation in a low zinc diet on serum antibody level of geese aged from 1 to 4 weeks

Discussion

3.1 Effects of Phytase Supplementation in a Low Zinc Diet on Growth Performance of Geese Aged 1-4 Weeks Growth performance is a key indicator of animal development. Young animals have the most vigorous growth, and their growth performance directly affects subsequent development. Both zinc and phytase can positively influence growth performance in starter animals. Su et al. [12] reported that increasing dietary zinc levels improved growth performance in ducklings, with an optimal level of 51.8 mg/kg. Attia et al. [13] found that 500 U/kg microbial phytase significantly improved broiler growth performance. Józefiak et al. [14] reported that enzyme supplementation reduced feed-to-gain ratio from 1.90 to 1.84 in broilers. The present study showed that feed-to-gain ratio in phytase-supplemented Groups III–VI was lower than that in Group I without phytase, while average daily gain was higher. Group II showed significantly lower growth performance than other groups due to insufficient zinc levels, with goslings exhibiting zinc deficiency symptoms. To determine the optimal zinc level under phytase supplementation, quadratic curve fitting was performed for feed-to-gain ratio and average daily gain, suggesting that zinc supplementation should be controlled at 34.00–44.70 mg/kg. This confirms that dietary phytase can effectively reduce zinc supplementation levels.

3.2 Effects of Phytase Supplementation in a Low Zinc Diet on Tibial Development of Geese Aged 1–4 Weeks Both zinc and phytase affect bone development, primarily through influencing AKP activity and calcium-phosphorus metabolism. AKP catalyzes the hydrolysis of phosphate monoesters to release inorganic phosphorus and is an important indicator of bone metabolism and osteoblast activity. Zinc deficiency reduces gastric and serum AKP activity, causing abnormal bone metabolism, altered bone calcification, and decreased bone mineral density. He et al. [15] found that serum AKP activity in broilers increased with dietary zinc levels, peaking at 70 mg/kg. Phytase increases available phosphorus, promotes calcium and phosphorus metabolism in blood and bone, and enhances calcium and phosphorus deposition, thereby stimulating tibial development. Manobhavan et al. [16] reported that 2,500–5,000 U/kg phytase significantly increased mineral content in broiler bones. Lei et al. [17] found that 300 U/kg phytase significantly increased tibial ash and phosphorus content. Zhao et al. [18] demonstrated that phytase improved calcium and phosphorus utilization in broilers. This study first confirmed that serum AKP activity in phytase-supplemented Groups III-VI was significantly higher than in Group I, with a quadratic relationship to dietary zinc level. Curve fitting analysis revealed peak serum AKP activity at 51.38 mg/kg zinc, which was significantly or extremely significantly correlated with bone mineral density and calcium/phosphorus content. Second, the study confirmed that bone phosphorus content in Groups IV–VI was significantly higher than in Groups I and II after phytase supplementation. These findings indicate that dietary phytase improves the biological value of zinc for tibial development and effectively reduces dietary zinc requirements.

3.3 Effects of Phytase Supplementation in a Low Zinc Diet on Antioxidant Capacity of Geese Aged 1–4 Weeks Antioxidant capacity determines the strength of an animal' s defense system. T-AOC is a crucial component of the enzymatic antioxidant system that scavenges continuously produced oxygen free radicals. GSH-Px is an important zinc-containing enzyme that participates in the reduction of hydrogen peroxide by glutathione, preventing lipid peroxidation and enhancing antioxidant capacity. CuZn-SOD is a zinc-dependent enzyme that catalyzes the dismutation of superoxide radicals to eliminate their toxicity and prevent peroxidation. MDA content indirectly reflects oxygen free radical metabolism and lipid peroxidation. Wang et al. [19] reported that increasing dietary zinc levels significantly enhanced serum and hepatic T-AOC and GSH-Px and CuZn-SOD activities while reducing MDA content. Zhong et al. [20] found that higher dietary zinc levels decreased serum and hepatic MDA in geese. Tang et al. [21] showed that zinc supplementation significantly increased SOD and GSH activities in rats with pancreatitis. Liu et al. [22] demonstrated that phytase increased serum T-AOC and SOD activity by 9–16%. Gowanlock et al. [23] found that zinc and phytase synergistically increased hepatic CuZn-SOD activity in piglets. This study found that zinc-deficient Group II had significantly lower serum and hepatic antioxidant indices than other groups. After phytase supplementation, Groups IV–VI showed significantly higher serum and hepatic T-AOC and GSH-Px activity than Group I, with no significant difference in serum MDA but extremely significantly lower hepatic MDA. These results indicate that phytase enhances the biological effect of zinc on antioxidant capacity, and their synergistic action can effectively reduce dietary zinc levels, though zinc deficiency severely impairs antioxidant capacity. Analysis of serum and hepatic T-AOC suggests that optimal antioxidant capacity is achieved at dietary zinc levels of 47.49–50.24 mg/kg.

3.4 Effects of Phytase Supplementation in a Low Zinc Diet on Immune Performance of Geese Aged 1–4 Weeks Immune performance encompasses immune organ indices and serum antibody levels. The main immune organs in poultry are the thymus, spleen, and bursa of Fabricius. The thymus requires zinc for physiological function, the spleen is associated with antigen production and immune response, and the bursa is crucial for humoral immunity in young animals. Their development is measured by corresponding organ indices. Su et al. [24] found that thymus, spleen, and bursa indices increased significantly at 55.7 mg/kg zinc. Cui et al. [25] reported that zinc deficiency severely inhibited bursa development in ducklings. Xu et al. [26] demonstrated that 900 U/kg phytase significantly increased thymus, spleen, and bursa indices in Wulong geese. Zyła et al. [27] found that phytase increased bursa weight in broilers. In this study, phytase supplementation at low zinc levels significantly increased thymus and bursa indices in Groups III–VI, even exceeding those of Groups I and II, with little difference between the two control groups. This may be because Group I lacked phytase, and the high straw content in the diet contained phytic acid that inhibited zinc absorption, while zinc-deficient Group II benefited from phytase supplementation that promoted immune organ development and compensated for zinc deficiency. These results indicate that phytase and zinc synergistically promote immune organ development and can effectively reduce dietary zinc requirements.

Immune antibody titer directly reflects humoral immunity levels and is important for preventing poultry infectious diseases. Wang et al. [28] reported that amino acid-complexed zinc significantly increased Newcastle disease antibody levels in broilers. Chand et al. [29] found that zinc supplementation increased antibody titers against infectious bronchitis and infectious bursal disease. Shaw et al. [30] reported that phytase increased interleukin gene expression in broilers challenged with coccidiosis. Liu et al. [31] demonstrated that high-phytase diets significantly increased Newcastle disease antibody levels in broilers. This study showed that phytase-supplemented Groups III–VI had significantly higher pre- and post-immunization Newcastle disease antibody levels than Group I, while Group II had lower antibody levels, indicating that zinc deficiency adversely affects antibody production. These findings demonstrate that phytase can improve serum antibody levels under low dietary zinc conditions and reduce dietary zinc requirements.

In conclusion, plant-based diets contain high phytate levels, particularly in straw, and monogastric animals like poultry cannot secrete phytase. Supplemental microbial phytase hydrolyzes phytate into inositol and phosphomonoesters, reducing phytate chelation of zinc and other trace elements and releasing them as free inorganic ions for absorption. This improves zinc nutritional utilization, allowing low-zinc diets to achieve satisfactory production performance, which has important implications for sustainable goose production.

Conclusion

1. Phytase supplementation in low-zinc diets can improve growth performance, immune performance, and antioxidant capacity while promoting tibial development and reducing dietary zinc requirements in 1–4 week-old geese.
2. Under the condition of 1,200 U/kg phytase supplementation, the optimal dietary zinc level for 1–4 week-old geese is recommended to be 44.70–51.38 mg/kg.

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