# Effects of Phytase Supplementation in Low-Zinc Straw-Based Diets on Growth Performance, Tibial Development, and Antioxidant Capacity of 5- to 16-Week-Old Wulong Geese (Postprint)

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## Abstract

This study aimed to investigate the effects of phytase supplementation in low-zinc straw-based diets on growth performance, tibial development, and antioxidant capacity of Wulong geese aged 5 to 16 weeks. A total of 360 five-week-old Wulong geese were selected and randomly divided into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group I served as the control group, fed a basal diet (supplemented with 75 mg/kg zinc sulfate, without phytase); Groups II to VI were fed the basal diet supplemented with 0, 15, 30, 45, and 60 mg/kg zinc sulfate, respectively, and all received 1,200 U/kg phytase. The experimental period lasted 12 weeks. The results showed: 1) Body weight and average daily gain of Groups IV and V were extremely significantly higher than those of Groups I and II ($P<0.01$), average daily feed intake of Group IV was significantly or extremely significantly higher than that of Groups I and II ($P<0.05$ or $P<0.01$), and feed-to-gain ratio of Groups IV and V was significantly or extremely significantly lower than that of Groups I and II ($P<0.05$ or $P<0.01$). Based on the curvilinear regression equation, the maximum average daily gain was achieved when dietary zinc sulfate supplementation level was 60.50 mg/kg; the lowest feed-to-gain ratio was obtained at 43.44 mg/kg dietary zinc sulfate supplementation. 2) Dressing percentage of Groups III to VI was significantly or extremely significantly higher than that of Groups I and II ($P<0.05$ or $P<0.01$), and semi-eviscerated yield of Group II was significantly lower than that of other groups ($P<0.05$). 3) Bone mineral density (BMD) of Groups IV to VI was significantly or extremely significantly higher than that of Group II ($P<0.05$ or $P<0.01$), and calcium and phosphorus contents in bone of Groups IV to VI were significantly or extremely significantly higher than those of Groups I and II ($P<0.05$ or $P<0.01$). 4) Total antioxidant capacity (T-AOC) in serum and liver of Groups IV to VI was significantly or extremely significantly higher than that of Group II (P<0.05 or P<0.01), and total antioxidant capacity in serum and liver was highest when dietary zinc sulfate supplementation level was 45 mg/kg. These results indicate that phytase supplementation in low-zinc straw-based diets improved growth performance, promoted tibial development, and enhanced antioxidant capacity of geese aged 5 to 16 weeks. Dietary phytase supplementation can improve zinc bioavailability and reduce dietary zinc sulfate supplementation levels. It is recommended that under the condition of 1,200 U/kg phytase supplementation in diets, the appropriate dietary zinc sulfate supplementation level is 40 to 60 mg/kg.

## Full Text

## Effects of Phytase Supplementation in Low-Zinc Straw-Based Diets on Growth Performance, Tibia Development, and Antioxidant Capacity of Wulong Geese Aged 5-16 Weeks

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### Abstract

This experiment investigated the effects of phytase supplementation in low-zinc straw-based diets on growth performance, tibia development, and antioxidant capacity of Wulong geese aged 5-16 weeks. A total of 360 five-week-old Wulong geese with similar body condition were randomly allocated into six groups, each consisting of six replicates of ten geese (half male, half female). Group I served as the control, receiving a basal diet supplemented with 75 mg/kg zinc sulfate without phytase. Groups II–VI received the basal diet supplemented with 0, 15, 30, 45, and 60 mg/kg zinc sulfate, respectively, all with 1,200 U/kg phytase. The 12-week trial yielded the following results: (1) Body weight and average daily gain in groups IV and V were significantly higher than those in groups I and II (P<0.01). Average daily feed intake in group IV was significantly or extremely significantly higher than in groups I and II (P<0.05 or P<0.01), while feed-to-gain ratio in groups IV and V was significantly or extremely significantly lower than in groups I and II (P<0.05 or P<0.01). Quadratic regression analysis indicated that maximum average daily gain occurred at 60.50 mg/kg zinc sulfate supplementation, and the lowest feed-to-gain ratio occurred at 43.44 mg/kg. (2) Dressing percentage in groups III–VI was significantly or extremely significantly higher than in groups I and II (P<0.05 or P<0.01), while group II showed significantly lower half-eviscerated yield than other groups (P<0.05).

(3) Bone mineral density (BMD) in groups IV–VI was significantly or extremely significantly higher than in group II ($P<0.05$ or $P<0.01$), and bone calcium and phosphorus contents in groups IV–VI were significantly or extremely significantly higher than in groups I and II ($P<0.05$ or $P<0.01$). (4) Total antioxidant capacity (T-AOC) in serum and liver in groups IV–VI was significantly or extremely significantly higher than in group II ($P<0.05$ or $P<0.01$), with serum and liver T-AOC reaching maximum levels at 45 mg/kg zinc sulfate supplementation.

These findings demonstrate that phytase supplementation in low-zinc straw-based diets enhances growth performance, promotes tibia development, and improves antioxidant capacity in 5–16-week-old geese. Dietary phytase improves zinc bioavailability, thereby reducing the required zinc sulfate supplementation level. Under conditions of 1,200 U/kg phytase supplementation, the optimal dietary zinc sulfate level for 5–16-week-old geese is recommended at 40–60 mg/kg.

**Keywords:** zinc; phytase; geese; growth and development; tibia development; antioxidant capacity

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## Introduction

Zinc is an essential trace element for animals that promotes bone development, enhances immune function, and participates in the synthesis of various enzymes including alkaline phosphatase. It is closely associated with antioxidant capacity and stimulates skeletal development in animals. Phytase is a phosphomonoesterase that hydrolyzes phosphate groups from phytic acid and phytates, ultimately releasing inositol and inorganic phosphorus. This enzyme enhances the bioavailability of trace elements and improves growth performance in monogastric animals. Therefore, investigating the effects of phytase on zinc utilization is crucial for formulating ecologically balanced diets.

Research has shown that dietary phytase supplementation can increase the utilization of zinc oxide (ZnO) and zinc sulfate (ZnSO ) by approximately 80%. Studies in pigs have demonstrated that combined zinc and phytase supplementation yields superior biological effects compared to zinc alone. In laying hens, dietary supplementation with 400 U/kg phytase effectively increased zinc deposition in tissues. Zinc is particularly susceptible to dietary phytic acid content, as zinc and phytic acid form highly insoluble phytate complexes in the upper small intestine that cannot be absorbed, thereby impairing zinc bioavailability. Corn straw contains high levels of phytic acid, and phytase supplementation can mitigate this inhibition of zinc and other trace elements.

According to Chinese Ministry of Agriculture Bulletin No. 1224, the recommended zinc sulfate level in goose diets is 60 mg/kg. However, research indicates that the optimal zinc level for maximum growth performance in geese is 106.45 mg/kg. This experiment compared low-zinc corn straw diets supplemented with phytase against an adequate zinc level diet without phytase to evaluate their effects on growth performance, tibia development, and antioxidant capacity in 5-16-week-old Wulong geese. The study aimed to identify methods for improving zinc bioavailability and reducing zinc sulfate supplementation while determining the optimal combination of phytase and zinc sulfate in straw-based goose diets.

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## Materials and Methods

**1.1 Experimental Animals and Design** Three hundred sixty five-week-old Wulong geese with similar body condition were randomly divided into six groups, each comprising six replicates of ten geese (half male, half female). Group I served as the control, receiving a basal diet supplemented with 75 mg/kg zinc sulfate without phytase. Groups II–VI received the basal diet with 0, 15, 30, 45, and 60 mg/kg zinc sulfate, respectively, all supplemented with 1,200 U/kg phytase. The experiment lasted 12 weeks. Zinc sulfate was purchased from Zhejiang Xinweipu Additive Co., Ltd., and phytase from Jiangsu Yuanfang Zhonghui Biotechnology Co., Ltd.

**1.2 Experimental Diets** The basal diet was formulated using corn, soybean meal, and corn straw as primary ingredients, with nutrient levels designed according to NRC (1994) recommendations for geese. Diet composition and nutrient levels are presented in Table 1 . Basal diet zinc content was measured using plasma emission spectrometry.

**1.3 Management** Before the trial, all goose houses and equipment were thoroughly cleaned, disinfected with sodium hydroxide, fumigated with formaldehyde and potassium permanganate, and sealed for 24 hours. The trial began one week later. Geese were raised on deep litter with ad libitum access to feed and water. Regular disinfection and litter replacement were performed throughout the trial, with strict control of temperature, humidity, and lighting. Vaccinations were administered according to schedule.

**1.4 Measurements** **Growth Performance:** Geese were weighed after fasting at the end of week 5 and week 16. Average daily gain (ADG) from weeks 5–16 was calculated. Daily feed consumption was recorded to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G). Daily mortality was recorded to calculate mortality rate.

**Slaughter Performance:** At week 16, 12 geese per group (two per replicate, half male and half female, 72 total) with body weights close to their group mean were selected, slaughtered by jugular exsanguination, and weighed after wet plucking and draining. Carcass weight, half-eviscerated weight, eviscerated weight, abdominal fat weight, breast muscle weight, and leg muscle weight were recorded to calculate dressing percentage, half-eviscerated yield, eviscerated yield, abdominal fat percentage, breast muscle percentage, and leg muscle percentage.

**Tibia Development:** The right tibia was collected after slaughter. Bone mineral density (BMD) was measured using an Osteocoer 3 digital scintillation cone-scanning densitometer. Tibia strength was determined using a WD-1 electronic universal testing machine. For bone mineral content (BMC), tibias were dried at 105°C and weighed. Ash content and calcium (Ca) and phosphorus (P) concentrations were determined according to GB/T 6438-92 using atomic spectrophotometry and molybdenum yellow colorimetry. Serum alkaline phosphatase (AKP) activity was measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

**Antioxidant Indices:** Serum and liver samples were collected at week 16 for antioxidant analysis. 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) method. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

**1.5 Statistical Analysis** Data were analyzed using one-way ANOVA with LSD multiple comparisons in SPSS 19.0. Results are expressed as "mean ± standard deviation." Significance levels were set at $P<0.05$ and $P<0.01$.

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## Results

**2.1 Effects on Growth Performance** As shown in Table 2 , body weight and average daily gain in groups IV and V were significantly higher than those in groups I and II ($P<0.01$). Average daily feed intake in group IV was significantly or extremely significantly higher than in groups I and II ($P<0.05$ or $P<0.01$), while feed-to-gain ratio in groups IV and V was significantly or extremely significantly lower than in groups I and II ($P<0.05$ or $P<0.01$). No significant differences in mortality rate were observed among groups ($P>0.05$).

The results also revealed correlations between zinc level and growth performance under phytase supplementation. Quadratic curve fitting was performed for average daily gain and feed-to-gain ratio in groups II–VI (phytase-supplemented) against dietary zinc supplementation level (X), yielding the following regression equations:

$$Y \text{ (feed-to-gain ratio)} = 1.857 - 0.003X + (3.453E\text{-}5)X^2 \ (R^2 = 0.840, P = 0.000)$$
$$Y \text{ (average daily gain)} = -0.001X^2 + 0.121X + 40.874 \ (R^2 = 0.796, P = 0.000)$$

These equations indicate that maximum average daily gain occurred at 60.50 mg/kg zinc sulfate supplementation, while the lowest feed-to-gain ratio occurred at 43.44 mg/kg.

**2.2 Effects on Slaughter Performance** Table 3 shows that dressing percentage in groups III–VI was significantly or extremely significantly higher than in groups I and II ($P<0.05$ or $P<0.01$), with group IV achieving the highest value. Group II exhibited significantly lower half-eviscerated yield than other groups ($P<0.05$). No significant differences were observed in eviscerated yield, breast muscle percentage, leg muscle percentage, or abdominal fat percentage among groups ($P>0.05$), though group II showed lower values for these traits, directly related to zinc deficiency.

**2.3 Effects on Tibia Development** As presented in Table 4 , bone mineral density in groups IV–VI was significantly or extremely significantly higher than in group II ($P<0.05$ or $P<0.01$). Bone calcium and phosphorus contents in groups IV–VI were significantly or extremely significantly higher than in groups I and II ($P<0.05$ or $P<0.01$). Serum AKP activity and ash content in groups IV–VI were significantly higher than in group II ($P<0.05$).

Quadratic curve fitting revealed a quadratic relationship between serum AKP activity and dietary zinc level, with maximum AKP activity occurring at 45.00 mg/kg zinc sulfate supplementation. Furthermore, serum AKP activity showed significant linear relationships with bone mineral density and bone calcium and phosphorus contents. These results indicate that phytase supplementation enhanced the biological efficacy of zinc for tibia development, effectively reducing dietary zinc requirements.

**2.4 Effects on Antioxidant Capacity** **Serum Antioxidant Capacity:** Table 5 shows that serum T-AOC in groups IV–V was significantly or extremely significantly higher than in group II ($P<0.05$ or $P<0.01$), with no significant differences from group I ($P>0.05$). Cu-Zn SOD activity in groups IV–V was significantly or extremely significantly higher than in group II ($P<0.05$ or $P<0.01$). MDA content in groups III and IV was significantly lower than in groups I and II ($P<0.05$), while groups V and VI showed extremely significantly lower MDA content ($P<0.01$). GSH-Px activity in groups IV–VI was extremely significantly higher than in groups I and II ($P<0.01$).

**Liver Antioxidant Capacity:** Table 6 demonstrates that liver T-AOC in groups V and VI was significantly or extremely significantly higher than in groups I and II ($P<0.05$ or $P<0.01$). Cu-Zn SOD and GSH-Px activities in groups IV–VI were significantly or extremely significantly higher than in groups I and II ($P<0.05$ or $P<0.01$). MDA content in groups IV–VI was significantly lower than in group I ($P<0.05$) and extremely significantly lower than in group II ($P<0.01$).

Combined analysis of Tables 5 and 6 reveals that serum and liver T-AOC reached maximum levels at 45.00 mg/kg dietary zinc sulfate, indicating that phytase supplementation in low-zinc straw diets enhances antioxidant capacity in geese.

## Discussion

**3.1 Effects on Growth Performance** Growth performance reflects animal development, and the juvenile period represents the most vigorous growth phase, directly affecting subsequent development. Both zinc and phytase positively influence growth performance. Studies in ducklings have shown that increasing dietary zinc levels improves growth performance, with an optimal zinc sulfate level of 51.8 mg/kg. Research in broiler chickens demonstrated that 500 U/kg microbial phytase significantly improved feed intake and growth performance. Another study reported that enzyme supplementation reduced feed-to-gain ratio from 1.90 to 1.84. Our findings align with these results, showing that phytase-supplemented groups (III–VI) had lower feed-to-gain ratios and higher average daily gains than the control group (I). Group II exhibited inferior growth performance due to insufficient zinc levels, with geese displaying typical zinc deficiency symptoms. Quadratic curve fitting for feed-to-gain ratio and average daily gain under phytase supplementation indicated an optimal zinc sulfate range of 43.44–60.50 mg/kg. Within this range, feed-to-gain ratio decreased by 24.8% and average daily gain increased by 6.7% compared to group I, confirming that phytase effectively reduces dietary zinc requirements.

**3.2 Effects on Slaughter Performance** Slaughter performance reflects tissue development and carcass economic value. Most studies report that dietary zinc level does not significantly affect slaughter performance. However, research in Peking ducks showed that zinc bacitracin significantly improved both growth and slaughter performance compared to -glucan and control groups. Phytase also enhances slaughter performance, with studies demonstrating that 1,000 U/kg phytase significantly improved growth and slaughter performance in broilers. Limited research exists on phytase effects in geese, though one study found that 600–1,200 U/kg phytase significantly increased dressing and half-eviscerated percentages at 16 weeks. Our results show that phytase-supplemented groups (III–VI) had improved dressing and half-eviscerated percentages compared to groups I and II, with values trending upward as dietary zinc levels increased.

**3.3 Effects on Tibia Development** Both zinc and phytase influence bone development, primarily by affecting AKP activity and calcium-phosphorus metabolism. AKP catalyzes hydrolysis of phosphate monoesters to release inorganic phosphorus, serving as a crucial indicator of bone metabolism and osteoblast activity. Zinc deficiency reduces AKP activity in stomach and blood, causing abnormal bone metabolism, altered bone calcification, and decreased bone mineral density. Studies in broilers have shown that serum AKP activity increases with dietary zinc levels, peaking at 70 mg/kg. Phytase increases dietary available phosphorus, promoting calcium and phosphorus metabolism and deposition in bone, thereby enhancing tibia development. Research in broilers demonstrated that high-dose phytase (2,500–5,000 U/kg) significantly increased bone mineral content, while another study found that 300 U/kg phytase significantly increased tibia ash and phosphorus content. Our study shows that phytase-supplemented groups (III–VI) had significantly increased serum AKP activity compared to group I, with a quadratic relationship to zinc level. Maximum AKP activity occurred at 45.00 mg/kg zinc sulfate, and serum AKP activity showed significant linear relationships with bone mineral density and bone calcium and phosphorus contents. Additionally, bone phosphorus content in groups IV–VI was significantly higher than in groups I and II. These findings demonstrate that phytase enhances zinc' s biological efficacy for tibia development, allowing reduced dietary zinc supplementation.

**3.4 Effects on Antioxidant Capacity** Antioxidant capacity determines the strength of an animal' s defense system. T-AOC is a vital component of the enzymatic antioxidant system, scavenging continuously produced oxygen free radicals. GSH-Px is a zinc-containing enzyme that participates in reducing hydrogen peroxide via glutathione, preventing lipid peroxidation and enhancing antioxidant capacity. Cu-Zn SOD is a zinc-dependent enzyme that catalyzes dismutation of superoxide radicals, eliminating their toxicity and preventing peroxidation. MDA content indirectly reflects free radical metabolism and lipid peroxidation extent. Previous studies have shown that increasing dietary zinc levels significantly increases serum and liver T-AOC and GSH-Px and Cu-Zn SOD activities while decreasing MDA content. Research in pancreatitis rats demonstrated that zinc supplementation significantly increased SOD and GSH-Px activities. Phytase has been shown to increase serum T-AOC and SOD activity by 9–16%, and zinc-phytase synergy can enhance liver Cu-Zn SOD activity in piglets. Our findings reveal that zinc-deficient group II had lower serum and liver antioxidant indices than other groups. Phytase supplementation increased serum and liver T-AOC and GSH-Px activities in groups IV–VI compared to group I, while significantly reducing liver MDA content. This demonstrates that phytase enhances zinc' s biological effect on antioxidant capacity, with synergistic action effectively reducing dietary zinc requirements. However, zinc deficiency severely compromises antioxidant capacity. Analysis of serum and liver T-AOC indicates that 45 mg/kg dietary zinc sulfate provides optimal antioxidant effects.

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## Conclusions

1. Phytase supplementation in low-zinc straw-based diets improves growth performance, promotes tibia development, and enhances serum and liver antioxidant capacity in 5–16-week-old geese.

2. Phytase supplementation improves zinc bioavailability, reducing dietary zinc sulfate requirements. Under conditions of 1,200 U/kg phytase supplementation, the optimal dietary zinc sulfate level for 5–16-week-old geese

is 40-60 mg/kg.

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