Effects of Different Zinc Sources and Levels on Growth Performance, Serum Antioxidant Indices, and Trace Element Utilization in Growing-Finishing Pigs: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with low levels of zinc methionine (Zn-Met) or zinc sulfate (ZnSO4) on growth performance, serum antioxidant indices, and trace element utilization in growing-finishing pigs, determine the appropriate zinc supplementation level in diets for growing-finishing pigs, achieve efficient resource utilization, and reduce the environmental impact of zinc in excreta. Thirty-two growing-finishing pigs (Duroc × Landrace × Large White) with a body weight of (33.70±2.76) kg were selected and randomly divided into 4 groups, with 8 replicates per group and 1 pig per replicate. The control group was fed a basal diet, while the experimental groups were supplemented with 40 mg/kg Zn-Met, 40 mg/kg ZnSO4, and 80 mg/kg ZnSO4 (as zinc element) in the basal diet, respectively. The experiment consisted of a 7-day preliminary period and a 72-day formal experimental period, which was divided into two stages: the growing period and the finishing period. The results showed that: 1) Compared with the control group, dietary supplementation with different sources and levels of zinc had no significant effects on average daily gain, average daily feed intake, and feed-to-gain ratio in growing-finishing pigs ($P>0.05$). 2) Compared with the control group, dietary supplementation with different sources and levels of zinc significantly increased serum total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and copper-zinc superoxide dismutase (CuZn-SOD) activities ($P<0.05$), and significantly decreased serum malondialdehyde (MDA) content ($P<0.05$); the 40 mg/kg Zn-Met group exhibited the highest serum T-SOD and GSH-Px activities and the lowest serum MDA content; while serum CuZn-SOD activity increased with increasing ZnSO4 supplementation, with the highest value in the 80 mg/kg ZnSO4 group. 3) Compared with the control group, dietary Zn-Met supplemen-

tation significantly increased the apparent absorption rates of zinc, copper, and manganese (P<0.05), whereas dietary ZnSO4 supplementation only significantly increased the apparent absorption rate of zinc (P<0.05), which decreased with increasing ZnSO4 supplementation; the 40 mg/kg Zn-Met group showed the highest apparent absorption rate of zinc. In conclusion, dietary supplementation with Zn-Met or ZnSO4 did not significantly improve growth performance in growing-finishing pigs, but significantly improved serum antioxidant indices and apparent absorption rates of trace elements; under the conditions of this experiment, the ZnSO4 supplementation level in diets for growing-finishing pigs should be lower than 80 mg/kg, while 40 mg/kg Zn-Met was more appropriate.

Full Text

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Abstract

This study investigated the effects of dietary supplementation with low levels of zinc methionine (Zn-Met) or zinc sulfate (ZnSO) on growth performance, serum antioxidant indices, and trace element utilization in growing-finishing pigs to determine the appropriate zinc supplementation level for efficient resource utilization and reduced environmental zinc excretion. Thirty-two "Duroc × Landrace × Large White" growing-finishing pigs with an initial body weight of (33.70 ± 2.76) kg were randomly allocated to four groups with eight replicates per group (one pig per replicate). The control group received a basal diet, while the experimental groups received the basal diet supplemented with 40 mg/kg Zn-Met, 40 mg/kg ZnSO , or 80 mg/kg ZnSO (calculated as zinc element). The experiment consisted of a 7-day adaptation period followed by a 72-day formal trial divided into growing and finishing phases. The results showed that: (1) Compared with the control group, different zinc sources and levels had no significant effects on average daily gain (ADG), average daily feed intake (ADFI), or feed-to-gain ratio (F/G) (P > 0.05). (2) Dietary zinc supplementation significantly increased serum total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and copper-zinc superoxide dismutase (CuZn-SOD) activities (P < 0.05) while significantly decreasing serum malondialdehyde (MDA) content (P < 0.05). The 40 mg/kg Zn-Met group exhibited the highest T-SOD and GSH-Px activities and the lowest MDA content, whereas serum CuZn-SOD activity increased with ZnSO supplementation, reaching its highest value in the 80 mg/kg ZnSO group. (3) Compared with the control group, Zn-Met supplementation significantly im-

proved the apparent absorption rates of zinc, copper, and manganese ($P < 0.05$), whereas ZnSO supplementation only significantly enhanced zinc apparent absorption rate ($P < 0.05$), which decreased with increasing ZnSO levels. The 40 mg/kg Zn-Met group achieved the highest zinc apparent absorption rate. In conclusion, dietary supplementation with Zn-Met or ZnSO did not significantly improve growth performance but significantly enhanced serum antioxidant indices and trace element apparent absorption rates. Under the conditions of this experiment, the ZnSO supplementation level should be below 80 mg/kg, while 40 mg/kg Zn-Met is more appropriate for growing-finishing pigs.

Keywords: organic zinc; growing-finishing pig; antioxidant; apparent absorptivity

Introduction

Zinc is an essential trace element for animals and a component of over 200 enzymes involved in various metabolic reactions critical for animal growth and development. Since Danish scientists discovered in 1989 that high-dose inorganic zinc supplementation reduced diarrhea and improved daily gain in weaned piglets, dietary zinc levels have progressively increased. Research indicates that zinc methionine (Zn-Met), an amino acid chelated form of zinc, offers superior biological availability, stability, and efficacy compared to inorganic zinc sulfate (ZnSO), while reducing environmental pollution and effectively promoting animal growth. Studies have shown that trace element requirements in growing pigs decrease with increasing body weight, with high zinc levels being necessary only during early developmental stages, while lower levels suffice for later stages to maintain serum zinc concentration and stability while reducing fecal zinc excretion.

Gowanlock et al. reported that reducing mineral element supplementation by 50% based on NRC (2012) standards did not affect pig growth performance or meat quality. Wen Chaoyue et al. similarly found that reducing dietary mineral elements by 30% and 60% had no significant impact on finishing pig growth performance or meat quality. However, excessive dietary zinc use has become increasingly prevalent, posing growing environmental risks. Japanese experts estimate that if all zinc from pig manure remained in soil, zinc levels would exceed safe limits (120 mg/kg) within 17 years, rendering contaminated land unsuitable for cultivation. In response to these hazards, Japan has set maximum zinc oxide supplementation levels at 120 mg/kg for different pig stages, while the European Union permits no more than 250 mg/kg. The Dutch SFR Feed Research Institute recommends 80 mg/kg zinc for growing pigs (including both basal diet and supplemental zinc). Nevertheless, research on low zinc levels during both growing and finishing phases remains limited. Therefore, this study aimed to investigate the effects of low zinc levels on growth performance, serum antioxidant indices, and trace element utilization in growing-finishing pigs to

provide a theoretical basis for appropriate zinc supplementation that ensures efficient resource utilization, reduced excretion, and minimized environmental impact.

1.1 Experimental Design and Diets

Thirty-two healthy "Duroc × Landrace × Large White" crossbred growing-finishing barrows with an initial body weight of (33.70 ± 2.76) kg were randomly divided into four groups with eight replicates per group (one pig per replicate), housed individually. The control group received a basal diet, while experimental groups received the basal diet supplemented with 40 mg/kg Zn-Met, 40 mg/kg ZnSO , or 80 mg/kg ZnSO (calculated as zinc element). The basal diet was formulated according to NRC (2012) nutrient requirements for pigs, with composition and nutrient levels shown in . The trial included a 7-day adaptation period followed by a 72-day experimental period, comprising a 32-day growing phase and a 40-day finishing phase.

1.2 Experimental Materials

Feed-grade Zn-Met (17.20% zinc content) and feed-grade zinc sulfate monohydrate (34.50% zinc content) were provided by Shandong Longxin Feed Co., Ltd.

1.3 Measurement Indicators and Methods

1.3.1 Growth Performance Feed intake and residual feed were recorded weekly to calculate average daily feed intake (ADFI) for each pig. Body weight was measured biweekly after overnight fasting to calculate average daily gain (ADG). Feed-to-gain ratio (F/G) was calculated based on ADFI and ADG.

1.3.2 Serum Antioxidant Indices On day 15 of the growing phase and day 20 of the finishing phase, 5 mL blood samples were collected from the anterior vena cava of all 32 pigs before morning feeding. Serum was separated by centrifugation at 3,000 rpm for 10 minutes at 4°C and stored at -20°C for analysis. Serum antioxidant indices were measured using assay kits from Nanjing Jiancheng Bioengineering Institute, including malondialdehyde (MDA) content, total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, and copper-zinc superoxide dismutase (CuZn-SOD) activity.

1.3.3 Determination of Zinc, Copper, Iron, and Manganese in Feces Pigs were housed individually in cages (1.0 m × 1.2 m) and fed three times daily at 07:00, 12:00, and 17:00 with free access to water. Total fecal collection was conducted on days 28–32 of the growing phase and days 36–40 of the finishing phase. Feces were collected during four periods (06:30–07:00, 11:30–12:00, 16:30–17:00, and 21:00–21:30), thoroughly mixed, and sampled at 10% of total weight before storage at -20°C. After collection, fecal samples from the same replicate were pooled, air-dried, ground with a mortar, and passed through a 40-mesh

sieve. Samples were processed using a MARS 6 microwave digestion system (CEM, USA), and zinc, copper, iron, and manganese concentrations were simultaneously determined using an Agilent 7900 inductively coupled plasma mass spectrometer (ICP-MS). Apparent absorption rate was calculated as:

Trace element intake = feed intake × dietary trace element content
Trace element excretion = fecal output × fecal trace element content
Apparent absorption rate = 100 × (trace element intake -trace element excretion) / trace element intake

1.4 Data Processing and Analysis

Experimental data were analyzed using one-way ANOVA with SAS 9.2 software. Duncan' s multiple range test was used for post-hoc comparisons, with $P < 0.05$ considered statistically significant.

Results

2.1 Effects of Different Zinc Sources and Levels on Growth Performance

As shown in , different zinc sources and levels had no significant effects on growth performance compared with the control group ($P > 0.05$). Dietary Zn-Met supplementation increased ADFI during the growing phase and ADG during the finishing phase, though these differences were not statistically significant ($P > 0.05$).

2.2 Effects of Different Zinc Sources and Levels on Serum Antioxidant Indices

As shown in , dietary Zn-Met or ZnSO supplementation significantly increased serum T-SOD, GSH-Px, and CuZn-SOD activities ($P < 0.05$) while significantly decreasing serum MDA content ($P < 0.05$) in growing-finishing pigs. At the same supplementation level, the 40 mg/kg Zn-Met group showed significantly higher T-SOD, GSH-Px, and CuZn-SOD activities (except during finishing phase) and significantly lower MDA content compared with the 40 mg/kg ZnSO group ($P < 0.05$). Across different levels, the 80 mg/kg ZnSO group exhibited significantly higher serum CuZn-SOD activity than the 40 mg/kg Zn-Met group ($P < 0.05$). The 40 mg/kg Zn-Met group achieved the highest T-SOD and GSH-Px activities and the lowest MDA content, whereas serum CuZn-SOD activity increased with ZnSO supplementation, reaching its peak in the 80 mg/kg ZnSO group.

2.3.1 Effects of Different Zinc Sources and Levels on Zinc Apparent Absorption Rate

As shown in , dietary Zn-Met or ZnSO supplementation significantly improved zinc apparent absorption rate ($P < 0.05$) and significantly reduced zinc excretion

rate ($P < 0.05$) in growing-finishing pigs. The zinc apparent absorption rates among treatment groups ranked from highest to lowest as follows: 40 mg/kg Zn-Met group, 40 mg/kg ZnSO group, and 80 mg/kg ZnSO group.

2.3.2 Effects of Different Zinc Sources and Levels on Copper Apparent Absorption Rate

As shown in , dietary Zn-Met supplementation significantly improved copper apparent absorption rate in growing-finishing pigs ($P < 0.05$). In contrast, ZnSO supplementation significantly decreased copper apparent absorption rate ($P < 0.05$), except for the 40 mg/kg ZnSO group during the growing phase, which showed a significant increase ($P < 0.05$).

2.3.3 Effects of Different Zinc Sources and Levels on Iron Apparent Absorption Rate

As shown in , dietary Zn-Met or ZnSO supplementation (except 80 mg/kg ZnSO during finishing phase) significantly decreased iron apparent absorption rate in growing-finishing pigs ($P < 0.05$). The iron apparent absorption rates among treatment groups ranked from highest to lowest as follows: 80 mg/kg ZnSO group, 40 mg/kg ZnSO group, and 40 mg/kg Zn-Met group.

2.3.4 Effects of Different Zinc Sources and Levels on Manganese Apparent Absorption Rate

As shown in , dietary ZnSO supplementation significantly decreased manganese apparent absorption rate in growing-finishing pigs ($P < 0.05$), whereas Zn-Met supplementation during the finishing phase significantly increased manganese apparent absorption rate ($P < 0.05$).

Discussion

3.1 Effects of Different Zinc Sources and Levels on Growth Performance and Serum Antioxidant Indices

Reports on the effects of dietary zinc levels on growth performance in growing-finishing pigs have been inconsistent. Numerous studies have shown that organic zinc can improve growth performance to some extent. However, Gowanlock et al. reported that reducing mineral element supplementation by 50% based on NRC (2012) standards did not affect pig growth performance or meat quality. Similar findings by Wen Chaoyue et al. and Liu Wanying et al. demonstrated that zinc supplementation levels had no significant effects on ADFI, ADG, or F/G in finishing pigs, which aligns with our results. These findings suggest that mineral zinc supplementation can be reduced during the growing-finishing phase to save feeding costs, improve feeding efficiency, and decrease environmental pressure from excreta. Our study also found that Zn-Met supplementation increased ADFI during the growing phase and ADG during the finishing phase,

possibly because zinc forms gustin with salivary proteins, which plays an important role in the structure, function, and metabolism of oral mucosal epithelial cells. Additionally, Zn-Met can reduce trace element loss, regulate appetite, and increase feed intake.

Zinc acts as an activator of T-SOD and GSH-Px, and dietary zinc supplementation can enhance T-SOD and GSH-Px activities while improving the ability to scavenge free radicals and strengthening the antioxidant system. MDA, a lipid peroxidation product, is commonly used as an indicator of oxidative stress. Research has shown that dietary zinc supplementation can increase serum T-SOD, CuZn-SOD, and GSH-Px activities while decreasing serum MDA content. Our results demonstrate that dietary zinc significantly improved serum antioxidant indices in growing-finishing pigs, with Zn-Met showing superior effects on T-SOD and GSH-Px compared to ZnSO , consistent with findings by Zhao Xinhong et al., Zhang Caiying et al., Fang Luoyun et al., and Liu Wanying. In pigs, GSH-Px, T-SOD, and MDA work together to scavenge free radicals, reduce lipid peroxidation damage, and enhance antioxidant capacity.

CuZn-SOD is a crucial component of the antioxidant metabolic defense system, with zinc serving as a cofactor. Serum CuZn-SOD activity increases with dietary zinc levels. Our study found that both Zn-Met and ZnSO supplementation significantly improved serum CuZn-SOD activity, which increased with ZnSO dosage, peaking in the 80 mg/kg ZnSO group. This aligns with Xu Zhenhua' s findings in growing rabbits and indicates that dietary zinc significantly enhances serum CuZn-SOD activity. The lower CuZn-SOD activity in the 40 mg/kg Zn-Met group compared to the 80 mg/kg ZnSO group suggests that dietary zinc level may have a more pronounced effect on serum CuZn-SOD activity than zinc source.

In summary, dietary zinc supplementation did not significantly affect growth performance but markedly improved antioxidant capacity in growing-finishing pigs.

3.2 Effects of Different Zinc Sources and Levels on Trace Element Utilization in Growing-Finishing Pigs

Evaluating mineral element absorption is complex and requires consideration of multiple element interactions. Numerous studies have shown that organic zinc demonstrates superior biological efficacy, feed utilization, stability, and environmental benefits compared to inorganic zinc due to its chelated form and absorption via amino acid and small peptide pathways. Our study found that Zn-Met supplementation improved not only zinc apparent absorption rate but also copper and manganese apparent absorption rates, consistent with research showing that organic trace elements are more effective than inorganic forms at reducing fecal trace element excretion. It is generally believed that zinc and copper compete for the same binding sites and metabolic pathways in the intestinal mucosa, with excess zinc reducing copper absorption. However, our

study found that appropriate Zn-Met supplementation increased both copper and manganese apparent absorption rates, suggesting that compared to ZnSO , optimal Zn-Met levels may reduce intraluminal chelation and metal ion interactions, indirectly improving copper and manganese utilization.

Recent studies have shown both synergistic and antagonistic relationships between zinc and iron regarding absorption and excretion. Our study found that zinc supplementation decreased iron apparent absorption rate, possibly due to competitive binding to receptors or carrier proteins during intestinal transport. The highest zinc apparent absorption rate observed with Zn-Met supplementation may be attributed to two factors: first, the form of zinc in the basal diet limited its effective absorption, and second, Zn-Met may enhance utilization by reducing digestive losses or utilizing amino acid absorption pathways. Conversely, when ZnSO supplementation reached 80 mg/kg, zinc apparent absorption rate decreased, likely because the combined zinc from the basal diet and the additional 80 mg/kg ZnSO exceeded the requirements of growing-finishing pigs. Excessive zinc levels do not increase absorption but instead increase metabolic burden. Therefore, under our experimental conditions, ZnSO supplementation should be below 80 mg/kg, while 40 mg/kg Zn-Met is more appropriate.

Conclusion

1. Under the conditions of this experiment, dietary supplementation with different zinc sources and levels did not significantly affect growth performance but significantly improved serum antioxidant indices in growing-finishing pigs, with 40 mg/kg Zn-Met showing the best results.
2. Dietary Zn-Met supplementation significantly improved the apparent absorption rates of zinc, copper, and manganese, whereas ZnSO supplementation only significantly enhanced zinc apparent absorption rate, which decreased with increasing ZnSO levels. The 40 mg/kg Zn-Met group achieved the highest zinc apparent absorption rate.
3. Considering growth performance, serum antioxidant indices, and trace element utilization, ZnSO supplementation should be below 80 mg/kg, while 40 mg/kg Zn-Met is more appropriate under the conditions of this experiment.

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