Effects of Different Zinc Sources and Levels on Serum Enzyme Activity and Zinc Deposition in Tissues and Organs of Growing-Finishing Pigs (Postprint)

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Abstract

This experiment aimed to investigate the effects of different zinc sources [zinc methionine (Zn-Met) or zinc sulfate (ZnSO4)] and levels on serum enzyme activities and zinc deposition in tissues and organs of growing-finishing pigs. Thirty-two growing-finishing pigs of "Duroc × Landrace × Large White" with similar body weight [(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, and the experimental groups were supplemented with 40 mg/kg Zn-Met, 40 mg/kg ZnSO4, and 80 mg/kg ZnSO4 (as zinc element) to the basal diet, respectively. There was a 7-day pre-trial period and a 72-day formal trial period, divided into two stages: 30-60 kg and 61-90 kg. The results showed: 1) Compared with the control group, dietary supplementation with 40 mg/kg Zn-Met significantly decreased the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) ($P<0.05$), and significantly increased the activity of serum alkaline phosphatase (ALP) ($P<0.05$) in growing-finishing pigs; dietary supplementation with 40 mg/kg ZnSO4 significantly decreased serum ALT and LDH activities ($P<0.05$); dietary supplementation with 80 mg/kg ZnSO4 significantly decreased serum ALT activity ($P<0.05$) and significantly increased serum ALP activity ($P<0.05$). The 40 mg/kg Zn-Met group had the lowest serum ALT, AST, and LDH activities and the highest serum ALP activity in growing-finishing pigs. 2) Compared with the control group, dietary supplementation with 40 mg/kg Zn-Met significantly increased serum zinc content ($P<0.05$), and the 40 mg/kg Zn-Met group had the highest serum zinc content. 3) Compared with the control group, dietary supplementation with 40 mg/kg Zn-Met or 40, 80 mg/kg ZnSO4 had no significant effect on zinc deposition in

spleen, pancreas, hair, and muscle (P>0.05), but significantly increased zinc deposition in liver and bone (P<0.05) of growing-finishing pigs. In conclusion, dietary supplementation with 40 mg/kg Zn-Met or 40, 80 mg/kg ZnSO4 can improve serum enzyme activities, serum zinc content, and zinc deposition in liver and bone of growing-finishing pigs. Under the conditions of this experiment, supplementation with 40 mg/kg Zn-Met in the diet of growing-finishing pigs is recommended.

Full Text

Effects of Different Zinc Sources and Levels on Serum Enzyme Activities and Tissue and Organ Zinc Accumulations of Growing-Finishing Pigs

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Abstract

This study investigated the effects of different zinc sources [zinc methionine (Zn-Met) or zinc sulfate (ZnSO)] and supplementation levels on serum enzyme activities and zinc deposition in tissues and organs of growing-finishing pigs. Thirty-two "Duroc × Landrace × Large White" crossbred growing-finishing pigs with similar initial body weight [(33.70 ± 2.76) kg] were randomly allocated to four groups, each consisting of eight replicates with 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 (as zinc element). The trial included a 7-day adaptation period followed by a 72-day experimental period, which was divided into two phases: 30–60 kg and 61–90 kg body weight ranges. The results demonstrated: (1) Compared with the control group, dietary supplementation with 40 mg/kg Zn-Met significantly reduced serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities ($P < 0.05$), while significantly increasing serum alkaline phosphatase (ALP) activity ($P < 0.05$). Supplementation with 40 mg/kg ZnSO significantly decreased serum ALT and LDH activities ($P < 0.05$), whereas 80 mg/kg ZnSO significantly reduced serum ALT activity ($P < 0.05$) and increased serum ALP activity ($P < 0.05$). The 40 mg/kg Zn-Met group exhibited the lowest serum ALT, AST, and LDH activities and the highest ALP activity. (2) Dietary supplementation with 40 mg/kg Zn-Met significantly elevated serum zinc content compared with the control group ($P < 0.05$), with this group showing the highest serum zinc concentration. (3) While supplementation with 40 mg/kg Zn-Met or 40 and 80 mg/kg ZnSO had no significant effects on zinc accumulation in the spleen, pancreas, hair, or muscle ($P > 0.05$), it significantly increased zinc deposition in the liver and bone (P < 0.05). In conclusion, dietary supplementation with 40 mg/kg Zn-Met or 40 and 80 mg/kg ZnSO can improve serum enzyme activities, serum zinc content, and zinc deposition in the liver and bone of growing-finishing pigs. Under the conditions of this experiment, 40 mg/kg Zn-Met is recommended as the appropriate supplementation level for growing-finishing pigs.

Keywords: organic zinc; growing-finishing pigs; serum enzyme activities; zinc accumulations

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Introduction

Zinc is an essential trace element in animals, serving as a component or activator of numerous enzymes including alkaline phosphatase (ALP), lactate dehydrogenase (LDH), carbonic anhydrase, and carboxypeptidase. Through these enzymatic roles, zinc participates in various metabolic reactions within the body [1-4]. Since Danish scientists discovered in 1989 that high-dose inorganic zinc supplementation could reduce diarrhea and improve daily weight gain in weaned piglets, dietary zinc levels have been progressively increased. However, inorganic zinc readily forms insoluble complexes with antagonistic factors such as calcium, phytic acid, and phytate compounds in the intestinal tract, resulting in poor absorption and utilization by animals and consequently causing environmental pollution [5-6]. Numerous studies have demonstrated that amino acid-chelated zinc exhibits high biological availability, excellent stability, and superior bioefficacy [5,7-9].

Currently, research primarily uses growth performance, serum enzyme activities, and tissue zinc deposition as evaluation criteria to determine optimal dietary zinc levels, with most studies focusing on poultry and meat rabbits [10-13]. As the world' s largest producer and consumer of meat, China produces approximately 65% of its total meat output as pork, making the swine industry economically significant. Reducing excessive zinc supplementation in pig production and decreasing zinc usage during the growing-finishing stage represent urgent challenges for China' s swine industry. This experiment was designed to investigate the effects of different zinc sources [zinc methionine (Zn-Met) or zinc sulfate (ZnSO)] and supplementation levels on serum enzyme activities and tissue zinc deposition in growing-finishing pigs, thereby providing a theoretical basis for establishing appropriate dietary zinc levels during this production phase.

1.1 Experimental Materials

Zn-Met was provided by Shandong Longxin Feed Co., Ltd., containing 17.20% zinc with a chelation degree of 95%. Feed-grade ZnSO was also provided by Shandong Longxin Feed Co., Ltd., containing one crystal water and 34.50% zinc.

1.2 Experimental Design and Diets

The experiment utilized 32 healthy "Duroc × Landrace × Large White"crossbred growing-finishing pigs with similar body weight [(33.70 ± 2.76) kg], randomly divided into four groups with eight replicates per group and one pig per replicate. The control group received a basal diet formulated according to NRC (2012) nutrient requirements, while the experimental groups received the basal diet supplemented with 40 mg/kg Zn-Met, 40 mg/kg ZnSO , or 80 mg/kg ZnSO (as zinc element). The basal diet composition and nutrient levels are presented in Table 1 . The trial included a 7-day adaptation period followed by a 72-day experimental period.

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

Items	30–60 kg	61–90 kg
Ingredients		
Whole plant corn	20.00	30.00
Corn	45.00	40.00
Soybean meal	18.00	14.00
Wheat bran	10.00	10.00
Soybean oil	2.00	2.00
CaHPO	1.50	1.20
Limestone	1.00	1.00
NaCl	0.30	0.30
Premix[1]	2.00	1.50
Met	0.10	0.00
Lys	0.10	0.00
Thr	0.00	0.00
Total	**100.00**	**100.00**
Nutrient levels[2]		
DE (MJ/kg)	13.50	13.50
CP	17.50	15.50
CF	4.50	5.00
TP	0.55	0.50
Zn (mg/kg)	25.00	25.00

[1] The premix provided the following per kg of diet: For 30–60 kg period: VA 1,300 IU, VD 150 IU, VE 11 IU, VK 0.50 mg, biotin 0.05 mg, folic acid 0.30 mg, pantothenic acid 8.00 mg, riboflavin 2.50 mg, VB 1.00 mg, VB 1.00 mg, VB 10.00 g, choline chloride 0.4 g, Cu (as copper sulfate) 4.00 mg, Fe (as ferrous sulfate) 60.00 mg, Mn (as manganese sulfate) 2.00 mg, I (as potassium iodide) 0.14 mg, Se (as sodium selenite) 0.20 mg. For 61–90 kg period: VA 1,300 IU, VD 150 IU, VE 11 IU, VK 0.50 mg, biotin 0.05 mg, folic acid 0.30 mg, pantothenic acid 7.00 mg, riboflavin 2.00 mg, VB 1.00 mg, VB 1.00 mg, VB 5.00 g, choline chloride 0.3 g, Cu (as copper sulfate) 3.50 mg, Fe (as ferrous

sulfate) 50.00 mg, Mn (as manganese sulfate) 2.00 mg, I (as potassium iodide) 0.14 mg, Se (as sodium selenite) 0.15 mg.

[2] Zinc was a measured value, while other nutrient levels were calculated values.

1.3.1 Growth Performance Measurement

Feed intake and residual feed were recorded weekly to calculate average daily feed intake (ADFI) per pig. Body weight was measured every two weeks after overnight fasting to determine average daily gain (ADG). Feed-to-gain ratio (F/G) was calculated based on ADFI and ADG.

1.3.2 Serum Enzyme Activity and Zinc Content Analysis

On day 15 of the 30–60 kg phase and day 20 of the 61–90 kg phase, 5 mL of blood was collected from the anterior vena cava of all 32 pigs at 06:00 h (before morning feeding). Serum was separated by centrifugation at 3,000 rpm for 10 min at 4°C and stored at -20°C for subsequent analysis. Serum ALT, AST, ALP, and LDH activities were measured using an automatic blood analyzer (KX-21, Sysmex Corporation, Japan). Serum samples were pretreated using a microwave digestion system MARS 6 (CEM Corporation, USA), and zinc content was determined by inductively coupled plasma mass spectrometry (Agilent 7900, USA).

1.3.3 Tissue and Organ Zinc Deposition Analysis

At the conclusion of the experiment, three healthy pigs were randomly selected from each replicate and euthanized by electrical stunning. Liver, spleen, and pancreas samples were collected. All samples were pretreated using a microwave digestion system MARS 6 (CEM Corporation, USA), and zinc deposition was measured by inductively coupled plasma mass spectrometry (Agilent 7900, USA).

For bone zinc analysis, the right front hoof was heat-pressed at 120°C for 20 min. After removing muscle and connective tissue, the fourth metacarpal bone was isolated, pulverized, and dried at 75°C to constant weight. Following 24 h of moisture equilibration at room temperature, samples were analyzed for zinc deposition.

Approximately 5 g of hair was collected using ceramic scissors on the final day for hair zinc determination. Longissimus dorsi, biceps brachii, Boston butt, and rib meat samples were dried at 65°C to constant weight, moisture-equilibrated at room temperature for 24 h, and ground with a mortar for muscle zinc analysis.

1.4 Statistical 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

Different zinc sources and levels had no significant effects on the growth performance of growing-finishing pigs ($P > 0.05$). The average initial body weight was (33.70 ± 0.50) kg, and the average final body weight was (88.09 ± 2.12) kg. During the 30-60 kg phase, ADFI was (1,724.94 ± 26.84) g/d, ADG was (716.84 ± 29.15) g/d, and F/G was 2.42 ± 0.14. During the 61-90 kg phase, ADFI was (2,365.13 ± 58.46) g/d, ADG was (643.62 ± 41.41) g/d, and F/G was 3.70 ± 0.25.

2.2 Effects of Different Zinc Sources and Levels on Serum Enzyme Activities

The effects of different zinc sources and levels on serum enzyme activities are presented in Table 2 . Compared with the control group, dietary supplementation with 40 mg/kg Zn-Met significantly reduced serum ALT, AST, and LDH activities ($P < 0.05$) while significantly increasing ALP activity ($P < 0.05$). Supplementation with 40 mg/kg ZnSO significantly decreased serum ALT and LDH activities ($P < 0.05$). The 80 mg/kg ZnSO group showed significantly reduced serum ALT activity ($P < 0.05$) and significantly increased ALP activity ($P < 0.05$). Across all treatment groups, serum ALT, AST, and LDH activities were lowest in the 40 mg/kg Zn-Met group, which also exhibited the highest ALP activity.

Table 2 Effects of different zinc sources and levels on serum enzyme activities of growing-finishing pigs

Trial period	Items	Control	40 mg/kg Zn-Met	40 mg/kg ZnSO	80 mg/kg ZnSO	P-value
30-60 kg	ALT	55.33 ± 5.13	42.83 ± 2.40	46.33 ± 1.21	46.50 ± 4.54	<0.001
	AST	42.17 ± 3.54	33.83 ± 4.06	36.50 ± 4.42	40.25 ± 2.92	<0.001
	ALP	121.00 ± 10.05	154.00 ± 10.05	124.88 ± 15.03	145.71 ± 5.84	<0.001
	LDH	585.43 ± 11.57	482.57 ± 24.30	532.38 ± 24.61	538.25 ± 29.83	<0.001

Trial period	Items	Control	40 mg/kg Zn-Met	40 mg/kg ZnSO	80 mg/kg ZnSO	P-value
61–90 kg	ALT	77.33 ± 6.82	58.33 ± 3.48	64.00 ± 4.80	67.17 ± 5.23	<0.001
	AST	34.40 ± 0.49	30.20 ± 1.72	32.00 ± 3.16	35.00 ± 3.38	<0.001
	ALP	93.88 ± 3.71	122.71 ± 8.61	122.50 ± 5.51	116.38 ± 20.94	<0.001
	LDH	299.33 ± 4.16	252.17 ± 17.26	269.50 ± 23.45	293.83 ± 23.38	<0.001

In the same row, values with different superscript letters indicate significant differences ($P < 0.05$), while values with the same or no superscript letters indicate no significant difference ($P > 0.05$). The same notation applies to subsequent tables.

2.3 Effects of Different Zinc Sources and Levels on Serum Zinc Content

The effects on serum zinc content are shown in Table 3 . Dietary supplementation with 40 mg/kg Zn-Met significantly increased serum zinc content compared with the control group ($P < 0.05$), with the 40 mg/kg Zn-Met group achieving the highest serum zinc concentration. This suggests that organic zinc may enhance zinc absorption rate through amino acid and small peptide transport pathways.

Table 3 Effects of different zinc sources and levels on serum zinc content of growing-finishing pigs (mg/L)

Trial period	Control	40 mg/kg Zn-Met	40 mg/kg ZnSO	80 mg/kg ZnSO	P-value
30–60 kg	1.35 ± 0.09	1.75 ± 0.12	1.40 ± 0.01	1.45 ± 0.02	<0.001
60–90 kg	0.89 ± 0.01	1.14 ± 0.11	0.91 ± 0.05	1.11 ± 0.11	<0.001

2.4 Effects of Different Zinc Sources and Levels on Zinc Accumulation in Organs, Bone, and Hair

The effects on zinc accumulation in organs, bone, and hair are presented in Table 4 . Compared with the control group, supplementation with 40 mg/kg

Zn-Met or 40 and 80 mg/kg ZnSO had no significant effects on zinc deposition in the spleen, pancreas, or hair (P > 0.05), but significantly increased zinc accumulation in the liver and bone (P < 0.05). However, bone zinc deposition did not differ significantly among the treatment groups (P > 0.05).

Table 4 Effects of different zinc sources and levels on zinc accumulations in organ, bone, and hair of growing-finishing pigs (mg/kg)

Items	Control	40 mg/kg Zn-Met	40 mg/kg ZnSO	80 mg/kg ZnSO	P-value
Liver	106.03 ± 6.56	137.73 ± 9.61	125.61 ± 8.54	147.03 ± 10.83	<0.001
Spleen	3.57 ± 0.24	3.65 ± 0.22	3.52 ± 0.15	3.57 ± 0.17	>0.05
Pancreas	1.56 ± 0.10	1.58 ± 0.08	1.57 ± 0.15	1.54 ± 0.01	>0.05
Bone	112.55 ± 3.18	134.43 ± 2.52	127.29 ± 12.97	136.58 ± 7.69	<0.001
Hair	38.99 ± 2.71	38.22 ± 2.63	38.17 ± 3.32	38.65 ± 2.58	>0.05

2.5 Effects of Different Zinc Sources and Levels on Muscle Zinc Deposition

The effects on muscle zinc deposition are shown in Table 5 . Dietary supplementation with 40 mg/kg Zn-Met or 40 and 80 mg/kg ZnSO increased zinc deposition in the longissimus dorsi, biceps brachii, Boston butt, and rib meat compared with the control group, but these differences were not statistically significant (P > 0.05). The 40 mg/kg Zn-Met group showed the highest muscle zinc deposition among all treatments.

Table 5 Effects of different zinc sources and levels on muscle zinc accumulations of growing-finishing pigs (mg/kg)

Items	Control	40 mg/kg Zn-Met	40 mg/kg ZnSO	80 mg/kg ZnSO	P-value
Longissimus dorsi	37.66 ± 2.38	38.75 ± 0.43	37.67 ± 1.37	38.65 ± 0.22	>0.05
Biceps brachii	50.97 ± 2.57	53.44 ± 4.58	52.71 ± 1.81	53.04 ± 2.88	>0.05
Boston butt	63.48 ± 3.73	65.14 ± 3.97	64.19 ± 1.15	64.26 ± 3.01	>0.05
Rib meat	42.66 ± 2.06	44.87 ± 3.47	41.65 ± 1.39	43.11 ± 2.63	>0.05

Discussion

3.1 Effects of Different Zinc Sources and Levels on Serum Enzyme Activities and Zinc Content

The basal diet zinc content in this experiment was consistent with values reported in previous studies [14-16]. The zinc present in basal diet ingredients has low utilization efficiency and exists in undefined forms, thus exerting minimal influence on serum enzyme activities, serum zinc content, and tissue zinc deposition.

Serum zinc content can partially reflect zinc absorption and utilization efficiency since dietary zinc enters the bloodstream following consumption. Hill et al. [17] found that dietary supplementation with 500 mg/kg zinc oxide (ZnO) did not significantly alter serum zinc content. Hahn et al. [18] also reported that animals maintain zinc homeostasis within a certain threshold, with serum zinc content increasing only when dietary zinc exceeds 1,000 mg/kg. Reports on the effects of dietary zinc levels on serum zinc content in pigs have been inconsistent, with many studies showing significant increases only at high supplementation levels [19-21], while others demonstrate significant effects at lower doses [22-23]. Our results indicate that low-level supplementation (40 mg/kg) of Zn-Met significantly increased serum zinc content in growing-finishing pigs, with the 40 mg/kg Zn-Met group achieving the highest concentration. These findings align with studies by Ge et al. [24] and Yu et al. [25] reporting higher serum zinc content with organic versus inorganic zinc sources, likely because organic trace minerals can utilize amino acid and small peptide transport pathways in the small intestine [26], thereby accelerating Zn-Met absorption.

Zinc serves as both a constituent and specific or non-specific activator of multiple enzymes. ALP is an enzyme with high activity under alkaline conditions that is widely distributed throughout various tissues and blood. Since zinc is an essential metal ion for ALP synthesis and positively correlates with its activity, serum ALP activity can be used to assess zinc absorption and utilization, with elevated activity indicating higher zinc utilization efficiency. Numerous studies have demonstrated a positive correlation between serum ALP activity and dietary zinc level, with organic zinc producing higher ALP activity than inorganic sources [27-29]. Our results are consistent with these findings [27-29] and correlate closely with serum zinc content, as increased serum zinc indicates enhanced zinc metabolism and elevated zinc-containing enzyme activities.

ALT and AST are crucial aminotransferases in amino acid metabolism. ALT catalyzes the transamination reaction between -ketoglutarate and aspartate to produce glutamate and oxaloacetate, while AST catalyzes the reaction between -ketoglutarate and alanine to generate glutamate and pyruvate [30]. Both enzymes are primarily located in myocardial and hepatic cells, with large quantities released into the bloodstream when cellular damage occurs. LDH is normally present in the cytoplasm and enters the blood only when tissue pathology increases cell membrane permeability [31-32]. While LDH and AST activities are high in myocardial cells, ALT is predominantly distributed in hepatic cells, resulting in low serum activities under normal physiological conditions. Our study showed that dietary zinc supplementation, particularly organic zinc, reduced serum ALT, AST, and LDH activities in growing-finishing pigs, with the 40 mg/kg Zn-Met group showing the lowest values. This indicates that zinc supplementation does not adversely affect cardiac and hepatic function in pigs.

3.2 Effects of Different Zinc Sources and Levels on Tissue Zinc Deposition

Animals absorb zinc through the intestine, which is then transported via blood to various tissues. Zinc initially deposits in the liver, kidneys, pancreas, and skin before being largely transferred to bone, with only minimal deposition in muscle, brain, and other tissues.

3.2.1 Zinc Deposition in Different Tissues and Organs The liver is the primary organ for zinc metabolism, while the tibia serves as a zinc storage site. Liver trace element concentrations are high and stable, reflecting the body' s trace element status, and bone represents a rapid exchange storage site that adequately reflects zinc deposition status. Our results demonstrate that the spleen, pancreas, and hair are insensitive to dietary zinc levels, whereas the liver and bone are sensitive, with these two organs showing the highest zinc deposition compared to other tissues. These findings are consistent with studies in rats [33,19], chickens [34,35], and weaned rabbits [36], indicating that liver and bone can effectively reflect zinc deposition status.

3.2.2 Zinc Deposition in Different Parts of the Same Tissue Our results revealed that different parts of the same tissue type showed variations in zinc deposition, though these differences were not significant. Such variations may be related to differences in physiological function and metabolic activity [37-38]. Numerous studies have reported that dietary zinc supplementation does not significantly affect muscle zinc deposition [19,22,39-40], which aligns with our findings and suggests that muscle is insensitive to dietary zinc levels. The 40 mg/kg Zn-Met group showed the highest muscle zinc deposition, likely attributable to the higher biological availability of organic zinc, which may deposit more readily in muscle than inorganic sources.

Conclusion

Dietary supplementation with 40 mg/kg Zn-Met or 40 and 80 mg/kg ZnSO can improve serum enzyme activities, serum zinc content, and zinc deposition in the liver and bone of growing-finishing pigs. Under the conditions of this experiment, 40 mg/kg Zn-Met is recommended as the appropriate supplementation level for growing-finishing pigs.

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