

Effects of zinc on growth performance and biochemical parameters of piglets

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Abstract: The effects of zinc on growth performance, activities of enzymes, and concentrations of hormones were investigated in this study. Crossbred (Duroc × Landrace × Large White) piglets (initial body weight: 26.66 ± 2.45 kg) were randomly assigned into 4 groups receiving a basal diet and were supplemented with 60, 300, 1000, or 3000 mg/kg of zinc (as ZnSO₄·7H₂O). The results showed that supplementation of zinc at 3000 mg/kg increased ($P < 0.05$) the average daily feed intake, weight gain, gain/feed, and relative organ weight, and promoted the activities of alkaline phosphatase and Cu-Zn superoxide ($P < 0.05$) by 84.17% and 49.86%, respectively, when compared with the control group. It also led to higher hepatic metallothionein content and tissue zinc concentration ($P < 0.05$) than in the control group. Furthermore, the concentrations of insulin and neuropeptide Y (NPY) in the 3000 mg/kg group were increased ($P < 0.05$) by 55.19% and 26.53%, respectively, compared to the control group. The present study suggests that pharmacological dietary supplementation of zinc increased growth performance, activities of enzymes, tissue zinc concentrations, and plasma insulin and NPY concentrations.

Key words: Zinc, growth performance, tissue zinc concentration, enzyme activity, hormone concentration, piglet

Introduction

Zinc is an essential trace element for humans and animals that has many physiological functions, such as immune, antioxidant, growth, and reproduction functions (1). Pharmacological doses of Zn (approximately 300 to 3000 mg/kg) are often used in the diets of nursery pigs immediately following weaning in the swine industry and have been reported to enhance growth performance (2). There have been a number of studies on the effects of Zn supplementation on growth performance, reproduction, antioxidant, and immune functions for animals. Pharmacological doses of ZnO, one of

the most widely used Zn sources, have been proven to promote postweaning piglet growth and improve antibacterial function, reducing the incidence of diarrhea (3). Additional experiments have demonstrated that ZnO aided the wound healing process (4). However, an overdose of Zn in pig diets can cause environmental pollution; thus, we should consider the environmental impact of its application (5).

It is well known that different chemical forms of Zn differ in their bioavailability and efficacy on various animals. When comparisons were made of different Zn sources, serum Zn concentrations suggested that

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the bioavailability of ZnO ranged from 69% to 73% compared with ZnSO₄ (6). However, Edwards and Baker (7) reported that analytical grade ZnO was as efficacious (89% relative Zn bioavailability) as the standard analytical grade ZnSO₄·7H₂O. Although many studies have focused on the effects of ZnO and the organic complexes of Zn in weaning pigs, few studies have been carried out to study the effects of ZnSO₄ on the physiological performance of growing piglets.

The objective of this study was to investigate the effects of different doses of ZnSO₄·7H₂O on growth performance, organ weight, enzyme activities, tissue Zn concentrations, and hormone levels in growing piglets.

Materials and methods

Animals and treatment

The care, use, and sacrifice of the animals were in accordance with the institutional guidelines for the care and use of laboratory animals of the Internal Animal Care and Use Committee. A total of 120 crossbred (Duroc × Landrace × Large White) piglets,

with initial body weight of 26.66 ± 2.45 kg, were randomly assigned into 4 different groups with 3 replications in each group (10 piglets per replication, half male and half female per pen). The piglets were housed in half-opened pigsties with concrete floors and had free access to diets and water throughout the trials. The piglets received corn-soybean basal diets (Table 1) and were supplemented with 60 (as the control group), 300, 1000, or 3000 mg/kg of Zn from ZnSO₄·7H₂O. The basal diet was formulated to meet the nutrient requirements of the National Research Council (8) for piglets of 20 to 50 kg. The piglets were fed a common diet for a 7-day adaptation period. Food intakes were recorded daily per pen and the experiments lasted 16 days. The piglets were weighed at the start and end of the feeding trial.

Parameter analysis

At the end of the trial, 3 male and 3 female piglets per group, after a 24-h fast, were slaughtered by exsanguinating of the carotid. Blood samples were drawn into tubes containing aprotinin and EDTA and were centrifuged at 1000 × g for 5 min at 4 °C to obtain plasma. The plasma was stored at -70 °C until use. The tissues were immediately removed, weighed,

Table 1. Composition of the basal diet (as-fed basis).

Ingredient	g/kg	Component ¹	
Corn	620	Digestible energy (MJ/kg)	13.86
Soybean meal	210	Crude protein (g/kg)	185.3
Wheat middling and red dog	80	Calcium (g/kg)	8.0
Extruded soybean	20	Phosphorus (g/kg)	5.9
Imported fish meal	30	Lysine (g/kg)	11.5
Premix ²	40	Methionine + cysteine (g/kg)	6.6
		Fe (mg/kg)	58.5
		Cu (mg/kg)	48.5
		Zn (mg/kg)	21.1

¹ All of the data were analyzed values except digestible energy, which was calculated using swine National Research Council values (8).

² Supplied the following per kilogram of diet of vitamin premixes (contained in the premix): 1400 IU of vitamin A, 160 IU of vitamin D₃, 13 IU of vitamin E, 0.6 IU of vitamin K, 12 µg of vitamin B₁₂, 1 mg of vitamin B₁, 1 mg of vitamin B₆, 55 µg of biotin, 0.3 g of choline, 0.3 mg of folic acid, 8 mg of pantothenic acid, 2.6 mg of riboflavin, 4 mg of copper, 0.14 mg of iodine, 60 mg of iron, 2 mg of manganese, and 0.15 mg of selenium.

washed using ice-cold 0.9% NaCl, frozen in liquid nitrogen, and stored at -70°C . The activities of alkaline phosphatase (ALP) in the plasma and Cu-Zn superoxide dismutase (Cu-Zn SOD) in the liver were measured using commercial kits (Jiancheng Biochemical Reagent Co., Nanjing, China). The hepatic metallothionein (MT) concentration was determined using the Cd/hemoglobin affinity assay, based on the method of Eaton and Toal (9). Zn contents in the serum, liver, kidney, and femur were measured using the dry ashing method (10) through flame atomic absorption spectroscopy (AA-6500; Shimadzu Corporation, Kyoto, Japan). Plasma neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), cholecystokinin (CCK), and insulin and glucagon concentrations were measured using radioimmunoassay kits (Beijing North Institute of Biological Technology).

Statistical analysis

The data were analyzed using the ANOVA procedure of SPSS 16.0, and comparisons were made using the least significant difference test. $P < 0.05$ was considered significant.

Results

Growth performance

The greatest increases in the average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G/F) were observed in the 3000 mg/kg group, being increased ($P < 0.05$) by 37.50%, 24.24%,

and 10.86%, respectively, compared to those in the control group. No significant differences ($P > 0.05$) in ADG, ADFI, or G/F were observed between the 300 and 1000 mg/kg groups, but both groups had higher ($P < 0.05$) ADG and ADFI values than the control group (Table 2).

Organ weight

The relative organ weights were calculated as the ratio of organ weight to body weight. The relative liver weights in the 3000 mg/kg group were increased by 22.27% ($P < 0.05$) compared with that of the control, whereas there was no significant difference among the other groups. The relative spleen weights of the control group were 11.24%, 9.47%, and 9.47% lower ($P < 0.05$) than those of the 300, 1000, and 3000 mg/kg groups, respectively. The relative femur weights in the 300, 1000, and 3000 mg/kg Zn groups were increased ($P < 0.05$) by 12.99%, 18.64%, and 29.38% compared with that of the control group, but no significant difference was observed between the 300 and 1000 mg/kg groups. The relative kidney weights were increased ($P < 0.05$) by 23.50%, 24.07%, and 29.23% in the 300, 1000, and 3000 mg/kg groups compared with that of the control, respectively, whereas there was no significant difference between the 300 and 1000 mg/kg groups or between the 1000 and 3000 mg/kg groups (Table 3).

Tissue Zn concentration

Compared with the control, Zn contents in the serum, liver, femur, and kidneys for piglets in the 1000 mg/kg group were increased ($P < 0.05$) by

Table 2. Effects of the different levels of Zn¹ on the growth performance of the piglets².

Examined parameter	60 ³	300 ³	1000 ³	3000 ³	SEM ⁴
Initial weight (kg)	25.73	26.64	26.97	27.28	0.37
Final weight (kg)	35.07 ^B	36.78 ^{AB}	38.07 ^A	40.13 ^A	0.65
ADG (kg)	0.584 ^B	0.634 ^B	0.694 ^B	0.803 ^A	0.047
ADFI (kg)	1.32 ^C	1.46 ^B	1.54 ^{AB}	1.64 ^A	0.04
G/F	0.442 ^B	0.434 ^B	0.451 ^B	0.490 ^A	0.09

¹Zn source was ZnSO₄·7H₂O.

²Means based on 3 replications per treatment group; values with different letters within a row indicate significant differences ($P < 0.05$).

³Zn concentration (mg/kg).

⁴Standard error of the mean.

Table 3. Effects of the different levels of Zn¹ on the relative organs weights of the piglets (%)².

Examined parameter	60 ³	300 ³	1000 ³	3000 ³	SEM ⁴
Liver	2.11 ^B	2.29 ^B	2.30 ^B	2.58 ^A	0.02
Spleen	1.69 ^B	1.88 ^A	1.85 ^A	1.85 ^A	0.02
Kidney	0.349 ^C	0.431 ^B	0.433 ^{AB}	0.451 ^A	0.009
Femur	0.354 ^C	0.400 ^B	0.420 ^B	0.458 ^A	0.007

¹Zn source was ZnSO₄·7H₂O.

²Means based on 3 replications per treatment group; values with different letters within a row indicate significant differences (P < 0.05).

³Zn concentration (mg/kg).

⁴Standard error of the mean.

59.75%, 440.67%, 63.57%, and 183.91%, respectively. In the 3000 mg/kg group, they were increased (P < 0.05) by 83.56%, 911.47%, 121.35%, and 484.28%, respectively. However, no difference (P > 0.05) was observed between the control group and the 300 mg/kg Zn group (Table 4).

Biochemical parameters

Compared with the control group, the piglets fed 300, 1000, and 3000 mg/kg of Zn showed significantly elevated activities of Cu-Zn SOD (12.39%, 34.17%, and 49.87%; P < 0.05) in the liver as well as in the plasma ALP (27.05%, 58.60%, and 84.19%; P < 0.05). The MT content of the piglets fed 300, 1000, and 3000 mg/kg of Zn was increased (P < 0.05) by 208.70%, 605.80%, and 1156.38% compared with that of the control group, respectively (Table 5).

Hormone concentration

Plasma NPY and insulin concentrations in the 3000 mg/kg group were significantly higher than those of the other groups, being increased (P < 0.05) by 26.53% and 55.19% compared with that of the control group, respectively. However, there was no significant difference among the control, 300, and 1000 mg/kg groups. Plasma CGRP, CCK, and glucagon concentrations showed no significant difference among all of the treatments (Table 6).

Discussion

This study was designed to determine the effects of different Zn doses from ZnSO₄·7H₂O on growth performance and various biochemical parameters in piglets of 20 to 50 kg. Zn is an essential trace

Table 4. Effects of the different levels of Zn¹ on the liver, kidney, and femur Zn contents of the piglets².

Examined parameter	60 ³	300 ³	1000 ³	3000 ³	SEM ⁴
Serum (µmol/L)	40.45 ^B	43.78 ^B	64.62 ^A	74.25 ^A	3.47
Liver (mg/kg)	80.64 ^C	86.54 ^C	436.56 ^B	815.65 ^A	63.78
Kidney (mg/kg)	32.63 ^C	32.88 ^C	92.64 ^B	190.65 ^A	13.71
Femur (mg/kg)	70.46 ^C	85.83 ^C	115.25 ^B	155.96 ^A	7.07

¹Zn source was ZnSO₄·7H₂O.

²Means based on 3 replications per treatment group; values with different letters within a row indicate significant differences (P < 0.05).

³Zn concentration (mg/kg).

⁴Standard error of the mean.

Table 5. Effects of the different levels of Zn¹ on the biochemical parameters in the plasma and liver of the piglets².

Examined parameter	60 ³	300 ³	1000 ³	3000 ³	SEM ⁴
Plasma					
ALP activity (U/mL)	62.56 ^D	79.48 ^C	99.22 ^B	115.23 ^A	4.53
Liver					
Cu-Zn SOD (U)	115.65 ^D	129.98 ^C	155.17 ^B	173.78 ^A	5.08
MT contents (μmol/mg)	13.82 ^D	42.55 ^C	97.37 ^B	173.8 ^A	12.74

¹ Zn source was ZnSO₄·7H₂O.

² Means based on 3 replications per treatment group; values with different letters within a row indicate significant differences (P < 0.05).

³ Zn concentration (mg/kg).

⁴ Standard error of the mean.

Table 6. Effects of the different contents of Zn¹ on the neuropeptides and hormones of the piglets².

Examined parameter	60 ³	300 ³	1000 ³	3000 ³	SEM ⁴
Insulin (uIU/mL)	4.24 ^B	4.58 ^B	4.97 ^B	6.58 ^A	1.10
Glucagon (pg/mL)	237.48	236.28	252.36	248.89	38.70
CGRP (pg/mL)	136.99	134.71	125.86	132.98	20.01
NPY (pg/mL)	273.22 ^B	275.20 ^B	294.99 ^B	345.70 ^A	30.65
CCK (pmol/L)	26.95	27.30	26.67	27.50	8.51

¹ Zn source was ZnSO₄·7H₂O.

² Means based on 3 replications per treatment group; values with different letters within a row indicate significant differences (P < 0.05).

³ Zn concentration (mg/kg).

⁴ Standard error of the mean.

element that plays an important role in metabolism, as a component of numerous metalloenzymes and transcription factors. In the present study, our data showed that pharmacological doses of Zn (300 to 3000 mg/kg) increased the ADG, ADFI, G/F, and relative organ weights. However, Shinde et al. (11) did not observe any changes in the growth rate in guinea pigs supplemented with Zn, either from organic or inorganic sources. Schell and Kornegay (6) reported that the Zn concentration in the tissues of weanling pigs fed pharmacological doses of Zn (3000 mg/kg) increased, but the growth performance did not. A likely explanation for the inconsistent results

in growth performance may lie in the dosage and timing of the supplementation of Zn, the nutrition levels, and environmental factors.

Several factors have been reported to influence both the actual and relative organ weights of slaughtered pigs (12). In the present study, the relative organ weights of the spleen, kidney, and femur of piglets fed 300, 1000, and 3000 mg/kg of Zn were increased significantly and the relative liver weights of the piglets fed 3000 mg/kg were increased significantly compared to those of the control group, respectively. The results were similar to those of a previous study (13), which indicated that pigs from

sows fed 5000 mg/kg of additional Zn from ZnO had heavier liver, heart, thyroid, and adrenal weights relative to their body weights than those of pigs from sows in the other treatments.

Briefly, the tissue Zn levels of piglets fed 1000 and 3000 mg/kg of Zn increased significantly compared with those of the control group. The results were consistent with previous findings (3,6) that serum and soft tissue Zn concentrations were clearly affected by Zn status. Schell and Kornegay (6) reported that the liver Zn concentrations of weanling pigs (28 days) fed diets with 3000 mg/kg of Zn from feed-grade ZnO, feed-grade ZnSO₄, and reagent-grade ZnSO₄ were 1564, 1354, 1592 mg/kg, respectively, based on fresh matter. Nevertheless, the liver Zn concentration in this study was only 815.65 mg/kg based on fresh matter, possibly due to the difference in piglet age. The older the piglets, the stronger homeostatic mechanisms they have. A strong homeostatic mechanism is able to reduce the high hepatic Zn concentration. Several experiments support our hypothesis (13). The true reason for this is not known and requires further investigation. Zn concentration in the kidney was increased in piglets with enhanced dietary Zn levels, and the changes in the Zn concentration in the kidney were similar to those of the liver. Schell and Kornegay (6) reported that kidneys seem to be less sensitive indicators of Zn status than the liver when Zn is supplemented at pharmacological levels.

MT not only exerts a protective effect against stress by acting as an antioxidant, but also functions as a source of Zn storage and transfers protein (14). The hepatic MT concentration, which was believed to be Zn-dependent, was decreased in Zn-deficient animals and increased in animals with Zn supplementation. Physiologically, the functions of MT appear to be principally involved in the detoxification of heavy metals and the metabolism of several essential trace elements (15). Zn can induce the synthesis of MT, and MT is involved in regulating and maintaining the metabolism and balance of Zn. In our study, the MT content in the liver was increased dramatically when dietary Zn levels were enhanced, which is consistent with the findings of a study by Carlson (16). This is important for Zn homeostasis regulated by MT. Our results indicated that there was a dose-

effect relationship between MT and Zn (Figure). Sun et al. (17) reported that the MT content in the liver of rats was increased by both Zn deficiency and Zn overload, which might be explained by the fact that Zn deficiency could induce the mobilization of MT and then diminish or replenish the deficiency of Zn. MT concentrations are also increased after different types of stress, such as the restriction of food intake and infection, whereas stress reduces plasma and liver Zn levels by substantial amounts (18). In our study, since it is known that the diets contained sufficient nutritive materials meeting or exceeding the requirements of growth for piglets, and the same results were not observed, none of the piglets in our experiments were in a state of stress.

Zn is an integral part of about 200 metalloenzymes, such as ALP and carbonic anhydrase (19). Our study demonstrated that ALP activity in the plasma was increased by high dietary Zn, which was identical to the findings of other studies (17,20). ALP is an enzyme involved in calcium absorption, and its activity is very important for the growth and development of piglets. ALP was the first Zn enzyme to be discovered in which there are 3 closely spaced metal ions (2 Zn²⁺ and 1 Mg²⁺) at the active center. Zn²⁺ at all 3 sites also produces a maximally active enzyme (21). Our results demonstrated the importance of Zn in ALP activity. High levels of Zn might indirectly promote the growth of piglets by enhancing ALP activity.

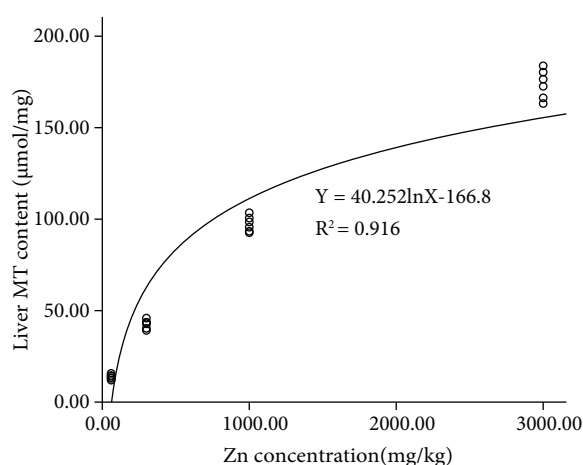


Figure. Relationships between dietary Zn levels and liver MT content of piglets.

Note: Each value represents 1 piglet per treatment.

The enzyme Cu-Zn SOD, the first line of defense against oxygen-derived free radicals, promotes the conversion of superoxide free-radical anions into hydrogen peroxide (22). Cu-Zn SOD has been shown to have a protective effect and might be an antioxidant enzyme in lipid peroxidation. This well-known mechanism prevents the formation of highly cytotoxic oxygen-derived free radicals (23). In our study, the activity of Cu-Zn SOD in piglets showed a significant increase at enhanced dietary Zn levels. Zn is a component of Cu-Zn SOD and it directly affects the stability of the enzyme. The increase of Cu-Zn SOD activity may be related to the improvement of the stability or slowdown of degradation by itself.

It is well accepted that NPY is one of the most potent appetite-regulating neuropeptides and it stimulates food intake behavior when injected into the paraventricular nucleus (24). In our study, the NPY concentration in piglets fed with 3000 mg/kg Zn was markedly higher than that of the other groups; however, there was no significant difference among the control, 300, and 1000 mg/kg groups. Scientists have paid more attention to this peptide due to its function in regulating food intake in Zn deficiency. Huntington et al. (25) reported that Zn deficiency resulted in significantly higher levels of NPY and NPY mRNA and lower food intake. The authors recognized that increased NPY concentration is a compensation mechanism of decreased food intake. However, in our study, the Zn concentrations of the diets met or exceeded the Zn needed for piglets, and so NPY did not play the role of a compensation mechanism but increased food intake at a high Zn status.

CGRP, produced in the central and peripheral nervous systems, stomach, pancreatic gland, and gut, is one type of brain-gut peptide, and it decreases food intake through the central and peripheral pathways. Lutz et al. (26) reported that CGRP suppressed the food intake of rats through the central and peripheral pathways. CCK, which is one of the first discovered gastrointestinal hormones, is one of the most

abundant neurotransmitters and can strongly inhibit food intake (27). The present study suggested that the high levels of Zn in the diets had no effect on plasma CGRP and CCK concentrations. This prompted us to study the relationship between Zn and CGRP at the molecular and cellular level.

Insulin and glucagon are major hormones regulating blood sugar. Insulin regulates blood sugar through stimulating blood glucose absorption and suppressing the synthesis of glucose; the glucagon function is opposite to insulin. In the present study, the plasma insulin concentration of piglets fed with 3000 mg/kg Zn was dramatically increased by 55.19% ($P < 0.05$) compared with that of the control group, whereas no significant difference in the glucagon concentration was observed among any of the groups. There are 2 possible reasons for the increased insulin concentration: high Zn concentration not only enhanced carboxypeptidase activity, which accelerated the conversion of proinsulin to insulin, but also improved the stability of the insulin. Insulin can promote the absorption of amino acid and glucose and increase the synthesis of protein, fat, and glycogen. An increased insulin concentration provided a strong theoretical basis for growth development.

The present study indicated that pharmacologic levels (300-3000 mg/kg) of Zn as $ZnSO_4 \cdot 7H_2O$ increased growth performance, organ weight, and tissue Zn concentrations, and improved biochemical parameters such as ALP and Cu-Zn SOD activities and MT content and endocrine parameters such as NPY, CGRP, CCK, insulin, and glucagon concentrations. Furthermore, the greatest effects were observed in the group fed with 3000 mg/kg Zn.

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