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Effects of Dietary Pharmacological Zinc on Growth, Liver Metallothionein, Cu, Zn-SOD Concentration and Serum Parameters in Piglets

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Abstract: The purpose of the present study was to investigate the effects of pharmacological level of zinc from Zinc oxide (ZnO) on growth, liver Metallothionein (MT), Cu, Zn-SOD activity and serum parameters in piglets. A total of 96 crossbred piglets with an initial body weight of 22.95±1.05 kg were blocked by body weight and randomly assigned to two treatments with three replications. Each group was fed the diet supplemented with 100 or 3000 mg kg^{-1} zinc from ZnO for 30 days. During the feeding trial, all pigs were given free access to feed and water. At the end of the feeding trial, 10 pigs from each treatment were randomly selected and slaughtered to collect liver samples after being bled via anterior vena cava puncture. The addition of 3000 mg kg^{-1} ZnO improved average daily gain ($p<0.05$), average feed intake ($p<0.05$) and feed gain ratio ($p<0.05$), respectively. The supplementation of 3000 mg kg^{-1} ZnO increased concentration of MT ($p<0.05$) and activity of Cu, Zn-SOD ($p<0.05$) in liver. And serum level of Ca ($p<0.05$), P ($p<0.05$) was decreased with Alkaline Phosphatase (ALP) activity ($p<0.05$) increased. Serum Urine Nitrogen (SUN) was decreased ($p<0.05$). Supplementation of dietary pharmacological zinc also increased Insulin-like Growth Factor I (IGF-I, $p<0.05$) and insulin ($p<0.05$) level. In summary, high dietary zinc exerts its beneficial effects on growth performance in piglets through increasing serum IGF-I, liver MT and Cu, Zn-SOD levels.

Key words: Zinc, pigs, metallothionein, Cu, Zn-SOD, hormone, China

INTRODUCTION

Nursery pig diets are commonly supplemented with 2000-3000 mg kg^{-1} of Zn as ZnO to improve growth and reduce scours (Carlson *et al*., 1999; Hill *et al*., 2000). However, the high levels of Zn excreted by supplemented pigs have raised concerns about its potential environmental pollution (Meyer *et al*., 2002). Additionally, the physiological mechanism behind the growth-promoting effect of additional intake of ZnO is still a matter of controversy. Elucidating such a mechanism is expected to optimize the growth-promoting efficacy of ZnO while minimizing the amount of Zn supplemented to the piglet's diet. Some studies suggest that ZnO exerts its effect through inhibiting the growth of pathogenic *E. coli* (Poulsen, 1989). However, there is evidence showing that dietary ZnO supplementation had no effect on the killing or number of *E. coli in vivo* or *in vitro* (Jensen-Waern *et al*., 1998; Roselli *et al*., 2003). Zn is needed for various physiological processes and has been found to be present on >200 metalloenzymes (Prasad *et al*., 1971; Hossain *et al*., 2001; Padmakar *et al*., 2005; Bashandy *et al*., 2006; Kasim, 2006; Pourfallah *et al*., 2011; Hosseini *et al*., 2011). Thus, some researchers suggested that zinc enhanced the growth of piglets through a systemic effect (via the blood) rather than a direct influence on the gastrointestinal tract.

The current study was conducted to investigate the effects of high dietary concentration zinc from ZnO on growth performance, liver Metallothionein (MT), Cu, Zn-SOD level, serum parameters and hormones indexes in piglets.

MATERIALS AND METHODS

Animals and experimental design: The protocol of this study was approved by the Institution Animal Care and Use Committee at Zhejiang University and was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals. The feeding trial was carried out in Anji Zhengxin Husbandry Company, HuZhou. A total of 96 crossbred

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Table 1: Ingredient inclusion and chemical composition of basal diet as fed basis

Ingredients (g kg^{-1})	Values
Corn	620.0
Soybean meal	240.0
Fish meal	20.0
Wheat middling	50.0
Rice bran	35.0
Limestone	8.0
Calcium phosphate	18.0
Salt	3.0
Mineral premix[a]	3.0
Vitamin premix[b]	3.0
Chemical composition (analyzed, g kg^{-1})[c]	
Digestible energy (MJ kg^{-1})	13.4
Crude protein	183.4
Calcium	9.7
Phosphorus	6.2
Lysine	12.5
Methionine	6.5

[a]All data were analyzed values except digestible energy which was calculated using swine NRC (1998) values; [b]Contained per kg of diet: Cu, 25 mg from $CuSO_4.5H_2O$; Fe, 140 mg from $FeSO_4.H_2O$; Mn, 40 mg from $MnSO_4.5H_2O$; Se, 0.1 mg from $Na_2SeO_3.5H_2O$; I, 0.3 mg from KI; [c]Contained per kg of diet: Vitamin A, 6600 IU; Vitamin D_3, 700 IU; vitamin E, 88 IU; Vitamin K, 4.4 mg; riboflavin, 8.8 mg; D-pantothenic acid 24.2 mg; niacin, 33 mg; choline chloride 330 mg; vitamin B_{12}, 22 μg; D-biotin, 300 μg; folic acid, 2.5 mg

pigs (Duroc x Landrace x Yorkshire) with an average body weight of 22.95±1.05 kg were blocked by initial weight and equalized for sex and ancestry, randomly allotted to two dietary treatments: basal diet supplemented with 100 or 3000 mg kg^{-1} zinc from zinc oxide with three replicate pens per treatment and 16 pigs per pen. All experimental treatments used the same corn-soybean meal basal diet formulated met or exceeded NRC (1998) recommendations for nutrients except digestible energy (Table 1).

The pigs were penned in 3.25×5.25 m with a nipple drinker and feeder to allow pigs *ad libitum* access to feed and water. The duration of the feeding trial was 30 days. Preceding the study, pigs were allowed a 7 days adaptation period during which they were offered basal diet for *ad libitum* consumption. Body weight and feed intake were recorded at the end of trial. Body weight gain was calculated by the difference between initial body weight and final body weight. Feed gain ratio was calculated by dividing the amount of feed consumed with the corresponded body weight gain.

Chemical analysis of basal diet: Samples of the mixed basal diet were analyzed for dry metter after oven drying to a constant weight for crude protein by a N analyzer (N×6.25) for a crude fat based on ether extraction and for ash and crude fiber; all methods were based on standard procedures (AOAC, 1995). Calcium was analyzed with atomic absorption spectrophotometry after wet-ashing and P was determined by a colorimetric procedure (AOAC, 1995). Amino acids were analyzed with ionexchange chromatography after acid hydrolysis as 6 mol L^{-1} HCl and 0.1% phenol under vacuum for 24 h at 110±2°C. Methionine was oxidized to methionine sulfone by treatment with performic acid before hydrolysis (Schram *et al*., 1954).

Blood and liver sampling: At the end of the feeding trial, ten pigs from each treatment were randomly selected (3 or 4 pigs per pen, 10 pigs per treatment in total) and blood samples were taken by anterior vena cava puncture after 12 h fasting. The samples were then centrifuged at 2,500×g at 4°C for 15 min. Serum collected from each sample was collected and stored at -20°C until needed for analysis. After blood sampling, the selected 20 pigs were transported to Huzhou meat factory and slaughtered by exsanguination after electrical stunning. Liver samples were collected from the 1st lobe and kept in -70°C freezer after soaked in liquid nitrogen.

Determination of concentration of MT and Cu, Zn-SOD activity in liver: Liver samples were rinsed with deionized water after thawing and one inch square samples were cut from the center portion of the thawed tissues. MT concentration was determined following the method of (Eaton and Toa, 1982). Cu, Zn-SOD was extracted according to the method of Lee *et al.* (1988) and the activity was measured using a commercially available ^{125}I RIA kit (Beijing North Institute of Biological Technology, Beijing, China). The minimum detectable concentration of Cu, Zn-SOD was 0.1 ng g^{-1} and the intraassay CV was 10%.

Analysis of blood samples: The concentrations of serum Total Protein (TP), Triglyceride (TG), Urea Nitrogen (SUN), Cholesterol (CHL), Ca and P were determined by corresponding commercial kits (Cicheng Biochemical Reagent Co., Ningbo, China) with the recommended procedures. The enzymatic activities of Alkaline Phosphatase (ALP), Glutamic-Oxaloacetic Transaminase (GOT) and Glutamic-Pyruvic Transaminase (GPT) were also determined by commercial kits (Jiancheng Biochemical Reagent Co., Nanjing, China). The concentration of Insulin (INS) was analyzed using a commercially available ^{125}I RIA kit (Beijing North Institute of Biological Technology, Beijing, China). The assay used human INS and antibodies against human INS as the standard. Minimum detectability of INS was 0.1 ng mL^{-1} and the intraassay CV was 10%. Serum Growth Hormone (GH) was determined using a ^{125}I RIA kit provided by National Hormone and Peptide Program HARBDE-UCLA Medical Center, USA. The minimum detectable concentration of GH was 0.1 ng mL^{-1} and the intraassay CV was 10%. Serum Insulin-Like Growth Factor I (IGF-I) was analyzed using a commercially available ^{125}I RIA kit

(INCSTAR Co., Stillwater, MN, USA). In the assay, recombinant human IGF-I and mouse antiIGF-I monoclonal antibody were used as the standard. Recovery rate ranged from 92.3-110.0%. The intraassay CV was 10% and the minimum detectable concentration of IGF-I was 0.1 nmol L^{-1}.

Statistical analysis: Data were analyzed for all variables using SAS (1989) Software. Data were subjected to t-test procedure to establish differences between means. For all data, the model included treatment as main effect. A probability of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Growth performance: The ADFI and ADG were increased by 14.55% ($p<0.05$) and 21.43% ($p<0.05$), respectively and the feed gain ratio was decreased by 4.02% ($p<0.05$), respectively due to addition of pharmacological level of zinc in the diet (Table 2).

Liver MT concentration and Cu, Zn-SOD: As shown in Table 3, supplemental pharmacological level of zinc in diet significantly increased the concentration of MT in liver, it was 18 times higher than that of the control group. The activity of Cu, Zn-SOD in liver was also increased by 48.36% ($p<0.05$) with the supplementation of zinc.

Serum biochemical indicator: As shown in Table 4, the concentration of SUN was decreased by 22.75% ($p<0.05$) with the supplementation of pharmacological level of zinc.

Table 2: Performance of piglets fed diet supplemented with or without pharmacological level of zinc[a]

	Zinc (mg kg^{-1})		
Diets	100	3000	SEM[c]
Initial weight (kg)	22.59	23.32	3.36
Final weight (kg)	35.26	38.62	5.62
ADG (kg)[b]	0.42	0.51	0.10
ADFI (kg)[b]	1.10	1.26	0.07
Feed:Gain[b]	2.59	2.49	0.07

[a]Values are presented as means; n = 3 per treatment with 16 pigs per pen contributing to a pen mean; [b]Means differ $p<0.05$; [c]Standard error of the mean

Table 3: The effects of supplemental pharmacological zinc on liver concentrations of MT and Cu, Zn-SOD measured after 30 days in piglets[a]

	Zinc (mg kg^{-1})		
Concentrations	100	3000	SEM[c]
MT (umol g^{-1})[b]	0.01	0.18	0.018
Cu, Zn-SOD (ng g^{-1})[b]	393.35	583.56	52.020

[a]Values are presented as means; n = 3 per treatment with 3 or 4 pigs per pen contributing to a pen mean (10 pigs per treantment in total); [b]Means differ $p<0.05$; [c]Standard error of the mean

Serum Ca and P in were decreased by 35.35% ($p<0.05$) and 12.37% ($p<0.05$), respectively compared to that of the control group while ALP activity was increased by 81.75% ($p<0.05$).

Serum INS, GH and IGF-I: As shown in Table 5, supplemental pharmacological level of zinc increased serum INS and IGF-1 by 24.76% ($p<0.05$) and 35.10% ($p<0.05$), respectively. There was no significant difference in serum GH level.

The data supported previous findings that excess dietary Zn increased ADG, ADFI and feed gain ratio (Hahn and Baker, 1993; Hill *et al*., 2000). The improvement of body weight gain resulting from the addition of ZnO was primarily a result of increased feed intake. Hahn and Baker (1993) reported that pigs fed 3000 mg kg^{-1} Zn from ZnO had increased gain and feed intake but no increase in feed gain ration however, other researchers have reported that excess Zn also improves feed efficiency (Carlson *et al*., 1999; Willliams *et al*., 2005).

Metallothionein (MT) is a low molecular weight, cysteine rich protein present in many living organism (Dunn *et al*., 1987). This protein has been considered to be an intracellular marker of excess zinc inside cells based on the increased induction of MT when dietary Zn intakes are well above normal (Tran *et al*., 1999). Transcriptional regulation of MT by dietary Zn was demonstrated in rats and cultured cells treated with Actinomycin D (Tran *et al*., 1999). Liver MT was

Table 4: The effects of supplemental pharmacological zinc on serum metabolites measured after 30 days in piglets[a]

	Zinc (mg kg^{-1})		
Serum metabolites	100	3000	SEM[c]
Ca (mg dL^{-1})[b]	9.9	6.4	0.30
P (mg dL^{-1})[b]	9.7	8.5	0.49
TP (mg dL^{-1})	71.6	71.3	0.92
ALB (mg dL^{-1})	37.5	36.3	3.43
SUN (mg dL^{-1})[b]	21.1	16.3	2.21
CHL (mg dL^{-1})	108.7	111.3	9.63
TG_3 (mg dL^{-1})	48.8	50.5	10.18
GPT (IU L^{-1})	53.2	58.5	5.21
GOT (IU L^{-1})	43.3	45.7	3.21
ALP (IU L^{-1})[b]	142.5	259.0	22.20

Table 5: The effects of supplemental pharmacological zinc on selected serum endocrine parameters measured after 30 days in piglets[a]

	Zinc (mg kg^{-1})		
Serum	100	3000	SEM[c]
GH (ng mL^{-1})	13.4	13.9	0.84
INS (ng mL^{-1})[b]	10.5	13.1	0.80
IGF-I (nmol L^{-1})[b]	9.4	12.7	0.89

[a]Values are presented as means; n = 3 per treatment with 3 or 4 pigs per pen contributing to a pen mean (10 pigs per treatment in total), [b]Means differ $p<0.05$; [c]Standard error of the mean

significantly increased with the addition of dietary Zn in the study which is consistent with the findings of Carlson *et al.* (1999) and Marrinez *et al.* (2004). Zinc is the composition of Cu, Zn-SOD that has indirect impact on stability of the enzyme. It is speculated that the activity of Cu, Zn-SOD increased which may be related to increase stability and decrease degradation rate of the enzyme in body.

MT has multi-biological functions and can combine with the free radicals, thereby protects cells from oxidative reaction of the superoxide free radical damage. Cu, Zn-SOD is one of the main scavengers on superoxide free radical in body. The concentration of MT and activity of Cu, Zn-SOD were significantly enhanced that played an important role on maintaining the integrity of biofilm improving the immunity of body and promoting growth and health of animals.

Many studies have demonstrated that pharmacological level of zinc increased activity of ALP (Fryer *et al.*, 1992; Hill and Miller, 1983). Zinc is a necessary metal ion to synthesis ALP and has a strong positive correlation to the activity of ALP. The increased ALP could improve activity of osteoblasts and deposition of calcium and phosphorus bone tissues. This result was consistent with concentration of Ca and P decreased in serum.

In the study, serum insulin was significantly increased with the supplementation of excess zinc which was similar to the other studies (Carroll *et al.*, 1998; Carlson *et al.*, 2004). Zinc is a necessary microelement for insulin not only a composition of insulin but also can stablize its molecular structure. When proinsulin is degradated to insulin, trypsin and carboxypeptidase are needed. Carboxypeptidase which need Zn^{2+} to activate was significantly decreased by in zinc-deficient rats (Dorup *et al.*, 1991; Roth and Kirchgessner, 1994).

In most studies of mechanism concerning animal growth block due to zinc-deficient, it was found that the level of IGF-I in serum was obviously decreased. McNall *et al.* (1995) found that 7.5 kb mRNA of IGF-I in liver cells was significantly decreased in zinc-deficent rats. INS and IGF-I are synthetic metabolism hormone that can promote cells to uptake amino acid and glucose and increase synthesis protein, fat and glycogen. The level of INS and IGF-1 were elevated in the study which provided a strong theoretical basis on growth-promoting effect of pharmacological level of zinc.

And it may be an overall effect rather than only in digestive tract level. The elevated IGF-I level measured in pigs offered the supplemented diets may have played a role in the protein and energy metabolism changes indicated by the results since, IGF-I mediates the effects of GH on protein metabolism and the uptake of glucose by muscle tissue. The lack of any significant effect of high zinc on serum GH however, suggests that the effect on IGF-I may have been independent of GH though a single blood sample is not a sensitive enough technique to assess treatment effects on GH given the pulsatile nature of GH release in most mammals (Barb *et al.*, 2002).

CONCLUSION

These results suggested high dietary zinc exerts its beneficial effects on growth performance in piglets through increasing serum IGF-I, liver MT and Cu, Zn-SOD levels.

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