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Effects of zinc sources and levels of zinc amino acid complex on growth performance, hematological and biochemical parameters in weanling pigs

Yi Zhang[1], Terry Lynn Ward[2], Fei Ji[3], Chucai Peng[1], Lin Zhu[1], Limin Gong[1], and Bing Dong[1,*]

* **Corresponding Author:** Bing Dong
Tel: +86-131-4619-5659, **Fax:** +86-10-62733688,
E-mail: dongbing@cau.edu.cn

[1] State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing 100193, China
[2] Zinpro Corporation, Eden Prairie, MN 55344, USA
[3] Zinpro (Wuxi) Additives Bio-Technology Co., LTD., Shanghai 201100, China

ORCID
Bing Dong
https://orcid.org/0000-0002-1150-3493

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Objective: The objective of the study was to investigate the effects of zinc amino acid complex (ZnAA) on growth performance, hematological and biochemical parameters in weanling pigs.
Methods: In Exp. 1, a total of 216 Duroc×Landrace×Large White weanling pigs were assigned randomly to 6 dietary treatments. Each treatment had 6 replicates (pens) with 6 pigs each. The diets were corn-soybean meal based with supplementation of 0, 20, 40, 80, 120 mg Zn/kg from ZnAA or 40 mg Zn/kg from feed-grade zinc sulfate. The experiment lasted 42 days. In Exp. 2, a total of 180 weanling pigs were assigned randomly to 3 dietary treatments supplemented with 0, 80, or 800 mg Zn/kg from ZnAA.
Results: In Exp. 1, pigs fed 40 to 80 mg Zn/kg from ZnAA had higher ($p<0.05$) average daily gain (ADG) than the unsupplemented group during d 0 to 14. During d 0 to 42, the pigs fed 20 to 120 mg Zn/kg from ZnAA had increased ($p<0.05$) ADG. Pigs fed 20 to 120 mg/kg Zn from ZnAA had lower feed:gain ($p<0.05$), increased the activity of serum Cu-Zn superoxide dismutase on d 14, and increased serum Zn levels on d 42 ($p<0.05$). In Exp. 2, pigs fed diets with 800 mg Zn/kg had increased average daily feed intake during d 15 to 28 ($p<0.05$) compared to the unsupplemented group. During d 0 to 28, the pigs fed supplemental Zn had increased ADG ($p<0.05$). On d 14 and d 28, pigs fed supplemental Zn had higher the serum alkaline phosphatase activities ($p<0.05$). No significant differences were observed in the hematological parameters and organ indices.
Conclusion: Supplementation with 20 to 80 mg/kg Zn from ZnAA improved the growth performance in weaned pigs. The piglets can tolerate up to 800 mg/kg Zn from ZnAA with limited potential health effects.

Keywords: Zinc Amino Acid Complex; Weanling Pigs; Performance; Hematological Parameters; Biochemical Parameters

INTRODUCTION

Zinc (Zn) is an important trace element for the maintenance of body normal physiological functions [1]. Zn is ubiquitously distributed in animal bones, muscles, liver, kidney and skin. It regulates the activities of multiple biological molecules such as transcription factors, enzymes, growth factors, etc [2]. Zn deficiency can cause retarded growth in pigs [3] and depressed immune responses [4] and parakeratosis [5]. Nowadays, feeding supplemental Zn to pigs is a common application in the pig industry to improve feed intake and immune function and to decrease gastro-intestinal stress after weaning, which in turn effectively enhances the growth performance in pigs [6,7].

On the other hand, widely supplementing Zn in pig feed raises a potential concern regarding environmental pollution. According to the 2012 National Research Council (NRC)

[8] recommendations, the feed addition of Zn is 100 mg/kg for weanling piglets weighing 7 to 11 kg; and is 80 mg/kg for those weighing 11 to 25 kg. In 2014, European Food Safety Authority (EFSA) proposed 150 mg Zn/kg in complete feed for pigs [9]. In 2017, the European Medicines Agency (EMA) recommended the future limit on Zn oxide in pig feeds. They claimed that the environmental risks arising from dietary Zn oxide addition are greater than the gain from the prevention of piglet diarrhea [10]. In China, Ministry of Agriculture (MOA) has reduced the lower limit of supplemental Zn from 70 mg/kg in 2007 to 40 mg/kg in 2009 [11]. Animal scientists are devoted to seek solutions to restrict the supplemental dose of Zn and improve its utilization in animals.

Organic Zn is a group of Zn chelates in which the ligands donate free electron pairs to Zn [12]. Reported organic Zn includes Zn chelates with amino acids, protein and carbohydrates. Multiple factors can affect the absorption and utilization of Zn in the body, such as chelation strengths, mineral interaction and diet compositions [13]. Amino acids are frequently used as dietary ligands for synthesis of Zn chelates. Organic Zn sources are absorbed via peptide or amino acid transport systems resulting in improved digestibility and availability of Zn. Organic Zn has been shown to enhance Zn retention by reducing urinary Zn excretion compared with inorganic Zn, whereas there was no differences in apparent Zn absorption [14]. The bioavailability of zinc from Zn-methionine was significantly better than a double dose of zinc from Zn sulfate [15].

Zinc secretion from the supplemented animals have raised concerns on environmental pollution [16]. The reported ranges of organic Zn in feed supplements are mostly over 80 mg/kg Zn [17-19] in weanling pigs. A much lower dose was reported in growing pigs [20]. We hypothesized that a lower dose of organic Zn in weanling pig diets could be used without negatively impacting growth performance. The objectives of this study were to investigate the effects of supplemental levels of zinc amino acid complex (ZnAA) on the growth performance, blood hematological and biochemical parameters in weanling pigs, in order to provide the fundamental data for its efficiency and safety in the application in pig feeds.

MATERIALS AND METHODS

Materials

The ZnAA (containing 17% of zinc) used in the current experiment was a 1:1 complex of zinc lysine and zinc glutamic acid (Zinpro Zn170) which was provided by Zinpro Corporation (Edina, MN, USA).

Animals and experimental design

All animal procedures and animal care were approved by the Institutional Animal Care and Use Committee of China Agricultural University (Beijing, China).

The experiments were conducted in the Pig Research Facility at the Swine Nutrition Research Centre of National Feed Engineering Technology Research Centre (Chengde, Hebei, China). One nursery barn was used in the study. The barn was a closed facility with mechanical ventilation equipment, equipped with 36 pens and 6 pigs (three barrows and three gilts) per pen resulting in 0.45 m^2 per pig (1.8 m×1.5 m/6). The floor was one-half slatted concrete floor. Each pen was equipped with 1 nipple waterer and 1 feeder.

Experiment 1

A total of 216 crossbred pigs (Duroc×Landrace×Yorkshire) with an average initial body weight (BW) of 8.47±1.40 kg were blocked according to gender, ancestry and BW. Pigs were allotted to one of six dietary treatments with six replicate pens in each treatment. The six groups were as follows: i) control (basal diet no Zn supplementation)+40 mg Zn/kg from zinc sulfate. ii) control; iii) control+20 mg Zn/kg from ZnAA; iv) control+40 mg Zn/kg from ZnAA; v) control+80 mg Zn/kg from ZnAA; vi) control+120 mg Zn/kg from ZnAA; The basal diet, comprised of corn, soybean meal, soy protein concentrate and dried whey, was formulated to meet or exceed NRC [8] requirements for weanling piglets except for Zn (Table 1) in a 2-phase feeding program. Analyzed Zn concentrations in diets are listed in Table 2. The experiment lasted 42 days and was divided into an early (d 0 to 14) and late phase (d 15 to 42). Pigs had *ad libitum* to access to feed and water.

Each piglet was weighed on d 0, 14 and 42. Feed consumption was recorded daily to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F:G). ADG, ADF, and F/G were calculated as: ADG = total weight gain/days of experiment; ADFI = total feed consumption/days of experiment; F/G = ADFI/ADG. Pig fecal consistency was observed and scored from d 0 following the standards of diarrhea scoring. The feces were scored twice a day at 8:00 am and 3:00 pm. The highest score of the pig on each day was used to calculate the diarrhea index. The calculation formula was: Diarrhea rate = [total number of diarrhea pigs/(total number of pigs×days of experiment)]×100%; Diarrhea index = sum of all fecal scores from all piglets during the experiment/(days of experiment ×numbers of pigs). Fecal score falling into 2 and 3 on the same day was deemed as diarrhea on that day.

On d 0 and 42, one barrow and one gilt were selected randomly from each pen for blood sampling. Blood was collected from the anterior vena cava using anticoagulant-free tubes followed by centrifugation at 1,500 g for 10 min at 4°C. The sera were stored at –20°C for further analysis.

Experiment 2

In China, the Ministry of Agriculture has a mandatory require-

Table 1. Composition and chemical composition of the basal diets (as-fed basis)

Items	Phase 1	Phase 2
Ingredient (%)		
Maize	58.47	59.51
Soybean meal	16.50	20.00
Soybean oil	3.00	2.72
Soybean protein concentrate	9.07	3.47
Fish meal	2.00	2.30
Dried whey	6.99	8.50
Dicalcium phosphate	1.32	1.04
Limestone	0.75	0.61
Salt	0.20	0.25
L-lysine-HCl	0.52	0.49
L-threonine	0.17	0.15
L-tryptophan	0.02	0.02
Methionine hydroxy analogue	0.29	0.24
Choline chloride	0.20	0.20
Vitamin-mineral premix[1)]	0.50	0.50
Total	100.00	100.00
Nutrient levels		
Digestible energy (MJ/kg)	14.82	14.61
Crude protein	20.56	18.87
Lysine	1.53	1.40
Methionine	0.57	0.50
Methionine+cysteine	0.87	0.79
Threonine	0.95	0.87
Calcium	0.80	0.70
Total phosphorus	0.65	0.60

[1)] Premix provided per kg of diet: early phase: Vitamin A, 12 KIU; Vitamin D_3, 2 KIU; Vitamin E, 30.0 IU; Vitamin K_3, 2.50 mg; Vitamin B_1, 2.50 mg; Vitamin B_2, 4.00 mg; Vitamin B_6, 7.0 mg; Vitamin B_{12}, 20.0 μg; Nicotinic acid, 40.0 mg; Pantothenic acid, 12.5 mg; Folic acid, 0.7 mg; Biotin, 80 μg; Fe, 100 mg; Cu, 90 mg; Mn, 30 mg; I, 0.25 mg; Se, 0.3 mg. Zinc was not be supplied in the premix. Late phase: Vitamin A, 6 KIU; Vitamin D_3, 2 KIU; Vitamin E, 25.0 IU; Vitamin K_3, 2.0 mg; Vitamin B_1, 2.0 mg; Vitamin B_2, 4.0 mg; Vitamin B_6, 7.0 mg; Vitamin B_{12}, 20.0 μg; Nicotinic acid, 40.0 mg; Pantothenic acid, 12.5 mg; Folic acid, 0.7 mg; Biotin, 80 μg; Fe, 100 mg; Cu, 90 mg; Mn, 20 mg; I, 0.25 mg; Se, 0.3 mg. Zinc was not supplied in the premix.

ment for inclusion of hematological evaluation for all new feedstuffs or feed additives [21]. It is believed this requirement is in place because hematological parameters are closely associated with systemic metabolic status. We thus performed hematological test of pig plasma to demonstrate the healthy status of the pigs. According to the Guideline [21], ten times the effective dose should be selected to evaluate the additive's safety in animals. We had found three doses (20, 40, and 80 mg/kg ZnAA) effective in ADG and F:G in Phase I and the entire phase in Exp 1, compared to the unsupplemented group. Therefore the high dose of 80 mg/kg and its ten-time group 800 mg/kg were selected for Exp 2. A total of 108 Duroc×Landrace×Yorkshire crossbred weanling pigs (9.16±1.04 kg) were blocked into three groups based on gender, ancestry and BW with six replicates in each treatment and six pigs (three barrows and three gilts) per pen. Pigs had *ad libitum* access to feed and water. Pigs were fed with a basal diet as in Exp 1 supplemented with 0, 80, or 800 mg Zn/kg from ZnAA for 28 days. A 2-phase feeding program was applied: d 0-14 and d 15-28 postweaning. On d 0, 14, and 28, one pig from each pen (total three barrows and three gilts for each treatment) was selected for blood sampling from the anterior vena cava using ethylenediaminetetraacetic acid dipotassium salt-containing and anticoagulant-free tubes for preparation of plasma and serum, respectively. Hematological parameters were assayed within 1 h after sampling. The sera were stored at –20°C for further analysis.

Table 2. Analyzed Zn concentration in diets for pigs

	Zn source	Added Zn (mg/kg)	Dietary Zn[1)] (mg/kg)	
			d 0 to d 14	d 15 to d 42
Exp. 1	Zn sulfate	40	86.7	83.7
	ZnAA	0	44.3	38.2
		20	68.0	57.1
		40	86.6	82.8
		80	125.3	113.1
		120	177.3	160.3
			d 0 to d 14	d 15 to d 28
Exp. 2	ZnAA	0	46.0	42.5
		80	128.3	126.4
		800	812.4	830.9

ZnAA, zinc amino acid complex.
[1)] Values based on chemical analysis of triplicate samples of diets. Values for Zn concentrations are reported on an as-fed basis.

Chemical analysis

The activities of serum alkaline phosphatase (ALP) and Cu-Zn Superoxide Dismutase (CuZn-SOD) were measured using commercially available kits according to manusfacturers instructions (ALP, BioSino Biotechnology and Science Company, Beijing, China) and an automatic biochemical analyzer (Hitachi 7160, Hitachi Group, Tokyo, Japan). Superoxide dismutase (SOD) activity was measured using the SOD assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), based on the xanthine⁄xanthine oxidase system. After total SOD (CuZn-SOD and Mn SOD) was determined, samples were again analyzed in the presence of 500 M KCN to inhibit CuZn SOD and to obtain Mn SOD activity. CuZn-SOD activity was obtained by subtracting Mn SOD activity. Feed and serum Zn levels were determined with ICP-MS (Agilent 7500, Agilent Technologies, Inc., USA).

Analysis of hematological and serum biochemical parameters

Hematological parameters, including white blood cells, red blood cells, hemoglobin, hematocrit, and platelet count were determined using a Sysmex Microcell Counter CL-180 (Tokyo, Japan). Serum biochemical parameters were determined us-

ing corresponding commercially available kits (BioSino Biotechnology and Science Company, Beijing, China), which included glucose, ALP, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, triglyceride, creatinine, and urea nitrogen. Analyses were conducted on an automatic biochemical analyzer (Hitachi 7160, Hitachi Group, Japan).

Statistical analysis

Data were analyzed using one-way analysis of variance in accordance with the general linear model procedures of SAS 9.2 (SAS Institute Inc., Cary, NC, USA) as a randomized complete block design including the terms for treatments and block (weight). Each pen was deemed as one experimental unit for growth performance, while an individual pig was considered as the experimental unit for other indices. Interactive matrix algebra procedure (IML) of SAS was adopted to generate the coefficients of unequally spaced contrasts. Subsequently, the linear and quadratic responses of Zn level of ZnAA were assessed by the orthogonal polynomial contrast. Significance level was set at $p<0.05$.

RESULTS

Experiment 1

Growth performance: The growth performance of the weanling pigs is presented in Table 3. In the early phase (d 0 to 14), ADG was higher ($p<0.05$) for pigs fed diets supplemented with 20, 40, and 80 mg Zn/kg from ZnAA compared to pigs fed the unsupplemented diet. Further, there was a quadratic response ($p<0.05$) in ADG and F:G to increasing ZnAA. During d 15 to d 42, the pigs fed supplemental Zn from ZnAA had lower F:G (linear and quadratic, $p<0.05$). The overall ADG was significantly lower in weanling pigs fed the control diet, as compared to the supplemented groups ($p<0.05$). The F:G was lower ($p<0.05$) for pigs fed diets supplemented with 20, 40, 80, and 120 mg Zn/kg from ZnAA compared to pigs fed the unsupplemented diet. There were linear and quadratic responses ($p<0.05$) in F:G of ZnAA supplemented pigs to increasing ZnAA.

The diarrhea rate and diarrhea index: The effects of graded levels of ZnAA on the diarrhea rate and diarrhea index of weanling pigs were not different among the pigs fed 0 to 120 mg Zn/kg from ZnAA or the zinc sulfate group in the early phase (d 0 to d 14), late phase (d 15 to d 42) and the entire phase (d 0 to d 42) ($p>0.05$) (data not shown).

Specific indicators: We measured the serum specific indicators including the activities of CuZn-SOD, ALP, and Zn levels (Table 4). On d 14, ALP was lower in the unsupplemented group compared to 80 and 120 ZnAA. Serum Zn was lower in the unsupplemented group compared to 20, 40, 80, and 120 mg/kg ZnAA as well as $ZnSO_4$. serum CuZn-SOD activity was greater ($p<0.05$) in pigs fed Zn from ZnAA compared to pigs fed the unsupplemented diet. On d 42, the activity of serum ALP and Zn levels showed differences between treatments. There was a linear increase in serum ALP and Zn with increasing ZnAA ($p<0.05$) and a quadratic increase in serum CuZn-SOD with increasing ZnAA ($p<0.05$).

Experiment 2

Table 3. Effects of zinc sulfate and zinc amino acid complex (ZnAA) on growth performance in weanling pigs[1] (Zn, mg/kg)

Items	$ZnSO_4$	ZnAA					SEM	p-value		
	40	0	20	40	80	120		ANOVA	Linear[2]	Quadratic[2]
Early phase, d 0 to 14										
ADG (g)	231[ab]	201[a]	237[b]	249[b]	255[b]	227[ab]	12	0.049	0.221	0.007
ADFI (g)	404	395	408	396	390	403	22	0.993	0.976	0.822
F:G	1.75	1.97	1.73	1.62	1.58	1.78	0.11	0.223	0.308	0.028
Mortality rate (%)	2.78	2.78	2.78	0.00	2.78	2.78	2.54	0.960	0.898	0.631
Late phase, d 15 to 42										
ADG (g)	542	518	557	546	552	548	13	0.420	0.309	0.223
ADFI (g)	772	852	825	770	766	778	33	0.338	0.085	0.165
F:G	1.43	1.65	1.48	1.43	1.39	1.43	0.06	0.090	0.021	0.037
Mortality rate (%)	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-	-
Entire experiment, d 0 to 42										
ADG (g)	438[ab]	413[a]	450[b]	447[b]	453[b]	441[b]	9	0.046	0.128	0.019
ADFI (g)	649	700	686	645	641	653	24	0.417	0.104	0.170
F:G	1.49[ab]	1.70[a]	1.52[b]	1.45[b]	1.42[b]	1.48[b]	0.06	0.026	0.011	0.006
Mortality rate (%)	2.78	2.78	2.78	0.00	2.78	2.78	2.54	0.960	0.898	0.631

SEM, standard error of the mean; ANOVA, analysis of variance; ADG, average daily gain; ADFI, average daily feed intake; F:G, feed to gain ratio.
[1] Data are the means of six replicates of six pigs (three barrows and three gilts) per pen.
[2] Linear and quadratic analysis of increasing ZnAA (0 to 120 mg Zn from ZnAA).
[abc] Means within the same column lacking a common superscript letter differ ($p<0.05$).

Table 4. Effects of zinc sulfate and zinc amino acid complex (ZnAA) on serum Zn levels, activities of ALP and CuZn-SOD in weanling pigs[1] (Zn, mg/kg)

Items	$ZnSO_4$	ZnAA					SEM	p-value		
	40	0	20	40	80	120		ANOVA	Linear[2]	Quadratic
0 d										
ALP (U/L)	317.45	369.29	346.71	333.37	297.51	330.20	36.48	0.807	0.342	0.372
Zn (μmol/L)	16.01	17.58	16.08	16.27	15.75	15.03	1.62	0.928	0.325	0.827
CuZn-SOD (U/mL)	41.35	43.89	44.46	44.79	41.35	40.26	4.05	0.948	0.396	0.806
14 d										
ALP (U/L)	209.94	185.66	196.73	263.83	260.82	210.06	29.61	0.314	0.440	0.072
Zn (μmol/L)	11.78	10.91	9.76	12.66	14.08	12.96	1.28	0.237	0.058	0.335
CuZn-SOD (U/mL)	54.00^{ab}	45.01^{a}	52.81^{b}	57.70^{b}	62.85^{b}	58.54^{b}	3.39	0.021	0.008	0.027
42 d										
ALP (U/L)	185.90^{ab}	143.83^{a}	173.14^{ab}	183.38^{ab}	206.37^{b}	209.16^{b}	15.07	0.050	0.003	0.207
Zn (μmol/L)	11.19^{b}	8.31^{a}	9.63^{a}	11.05^{b}	12.32^{b}	12.13^{b}	0.86	0.022	0.001	0.088
CuZn-SOD (U/mL)	59.86	54.98	60.30	68.31	67.54	60.89	4.47	0.301	0.291	0.025

ALP, alkaline phosphatase; CuZn-SOD, Cu-Zn superoxide dismutase; SEM, standard error of the mean; ANOVA, analysis of variance; ALP, alkaline phosphatase.
[1] Data are the means of six replicates of one pig per pen.
[2] Linear analysis of increasing ZnAA (0 to 120 mg Zn from ZnAA).
[abc] Means within the same column lacking a common superscript letter differ ($p < 0.05$).

Growth performance: In Exp. 2, we selected two doses of Zn from ZnAA, 80 and 800 mg Zn/kg to evaluate the safety of ZnAA in weaned pigs (Table 5). For the growth performance, in the later phase (d 15 to d 28), pigs fed ZnAA had higher ADFI ($p<0.05$) compared to the unsupplemented group. In the entire phase (d 0 to d 28), ADG was higher for pigs fed 80 or 800 mg Zn/kg from ZnAA than the unsupplemented group ($p<0.05$). The supplemented groups tended to show lower F:G compared to the unsupplemented control ($p = 0.05$).

Hematological parameters: The effects of different dietary supplementation levels of Zn from ZnAA on the hematological parameters of weaned piglets are presented in Table 6. No significant differences were found in the hematological parameters among the pigs fed 80 or 800 mgZn/kg as ZnAA and the pigs fed the unsupplemented diet ($p>0.05$).

Table 5. Effects of zinc amino acid complex (ZnAA) on growth performance in weaned pigs[1] (Zn, mg/kg)

Items	ZnAA			SEM	p-value
	0	80	800		ANOVA
Phase I, 0 to 14 d					
ADG (g)	259	282	268	14	0.501
ADFI (g)	427	416	420	16	0.875
F:G	1.68	1.47	1.59	0.10	0.386
Mortality rate (%)	0.00	0.00	2.78	1.60	0.391
Phase II, 15 to 42 d					
ADG (g)	329	393	400	22	0.091
ADFI (g)	594^{a}	632^{b}	665^{b}	15	0.021
F:G	1.82	1.65	1.69	0.11	0.516
Mortality rate (%)	0.00	0.00	0.00	-	-
Entire experiment, 0 to 42 d					
ADG (g)	294^{a}	338^{b}	334^{b}	11	0.040
ADFI (g)	511	524	543	11	0.149
F:G	1.74	1.56	1.64	0.05	0.079
Mortality rate (%)	0.00	0.00	2.78	1.60	0.391

SEM, standard error of the mean; ANOVA, analysis of variance; ADG, average daily gain; ADFI, average daily feed intake; F:G, feed to gain ratio.
[1] Data are the means of six replicates of six pigs (three barrows and three gilts) per pen.
[abc] Means within the same column lacking a common superscript letter differ ($p < 0.05$).

Table 6. Effects of zinc amino acid complex (ZnAA) on hematological parameters in weaned pigs[1] (Zn, mg/kg)

Items	ZnAA			SEM	p-value
	0	80	800		ANOVA
0 d					
WBC (10^9/L)	17.25	16.67	16.32	1.43	0.899
RBC (10^{12}/L)	5.74	6.03	5.66	0.24	0.533
HGB (g/L)	100.00	100.17	94.83	3.91	0.566
HCT (%)	0.32	0.32	0.29	0.01	0.150
PLT (10^9/L)	379.33	449.83	406.33	34.66	0.385
14 d					
WBC (10^9/L)	19.22	18.33	20.27	1.42	0.640
RBC (10^{12}/L)	6.28	6.71	6.81	0.28	0.416
HGB (g/L)	106.83	105.17	102.17	4.34	0.750
HCT (%)	0.39	0.39	0.38	0.02	0.940
PLT (10^9/L)	315.83	303.17	307.33	35.41	0.967
28 d					
WBC (10^9/L)	19.32	21.82	21.07	1.40	0.461
RBC (10^{12}/L)	6.41	6.61	6.82	0.21	0.406
HGB (g/L)	113.17	108.83	112.00	3.00	0.588
HCT (%)	0.35	0.34	0.35	0.01	0.911
PLT (10^9/L)	358.00	359.83	407.17	37.18	0.587

SEM, standard error of the mean; ANOVA, analysis of variance; WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; PLT, platelet count.
[1] Data are the means of six replicates of one pig per pen.

Serum biochemical parameters: The effects of different dietary supplementation levels of Zn from ZnAA on the serum biochemical parameters of weaned piglets are presented in Table 7. The activities of serum ALP in the pigs fed 80 and 800 mg Zn/kg from ZnAA were higher than that of the pigs fed the unsupplemented diet at d 14 and d 28 ($p<0.05$).

DISCUSSION

Absorption of minerals is a major factor limiting their use for biological funcitons within the body. A trace mineral can be absorbed but is not necessarily utilized. Inorganic sourced mineral ions are easily combined with other components in digesta to form insoluble complexes, which reduce mineral absorption. Whereas, an organic trace mineral or chelated trace mineral, is in a chemically inert form via ligand binding and as such, is protected from negative interaction with the digesta components, such as phytate. Organic trace minerals use either amino acid or peptide uptake mechanisms for their absorption, thus they are expected to be absorbed and circulated to target tissues very efficiently. The effects of organic Zn on growth performance are conflicting [22-24]. Our study found that dietary supplementation of 20, 40, and 80 mg Zn/kg as ZnAA increased the ADG in weaned piglets compared to pigs fed an unsupplemented diet from d 0 to d 14 and d 0 to d 42. This response was similar to that of Zinc sulfate at 40 mg Zn/kg.

Table 7. Effects of zinc amino acid complex (ZnAA) on serum biochemical parameters in weaned pigs[1] (Zn, mg/kg)

Items	ZnAA			SEM	p value
	0	80	800		ANOVA
0 d					
TBILI (μmol/L)	2.60	2.05	2.55	00.51	0.707
CRE (μmol/L)	95.17	94.55	81.58	06.49	0.292
UN (mmol/L)	2.64	2.82	2.92	00.28	0.785
ALP (U/L)	338.40	351.13	346.23	24.35	0.933
ALT (U/L)	33.00	32.87	33.90	03.94	0.980
AST (U/L)	59.92	61.20	57.25	06.57	0.911
TP (g/L)	38.67	43.72	40.55	04.07	0.684
ALB (g/L)	29.00	30.27	27.98	02.01	0.730
GLU (mmol/L)	2.77	2.80	2.70	00.45	0.986
14 d					
TBILI (μmol/L)	3.12	3.42	3.20	00.39	0.855
CRE (μmol/L)	105.33	100.92	107.90	06.93	0.776
UN (mmol/L)	3.69	3.26	3.08	00.21	0.168
ALP (U/L)	214.43[a]	279.37[b]	270.80[b]	16.41	0.038
ALT (U/L)	36.02	39.12	40.97	03.37	0.593
AST (U/L)	55.10	55.95	58.22	04.01	0.853
TP (g/L)	47.88	46.65	46.92	03.41	0.965
ALB (g/L)	31.52	29.28	28.27	02.18	0.577
GLU (mmol/L)	4.29	3.96	4.31	00.54	0.875
28 d					
TBILI (μmol/L)	4.17	4.25	3.97	00.34	0.837
CRE (μmol/L)	112.57	103.07	104.43	06.91	0.592
UN (mmol/L)	3.67	3.10	3.25	00.30	0.411
ALP (U/L)	184.10[a]	230.53[b]	237.72[b]	11.55	0.017
ALT (U/L)	41.72	48.78	48.68	02.33	0.093
AST (U/L)	47.97	51.42	47.75	05.40	0.867
TP (g/L)	44.42	43.08	37.73	03.58	0.410
ALB (g/L)	27.48	24.00	22.55	01.70	0.160
GLU (mmol/L)	4.39	3.95	4.59	00.30	0.340

SEM, standard error of the mean; ANOVA, analysis of variance; TBILI, total bilirubin; CRE, creatinine; UN, urea nitrogen; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; ALB, albumin; GLU, glucose.

[1] Data are the means of six replicates of one pig per pen.

[abc] Means within the same column lacking a common superscript letter differ ($p<0.05$).

Zinc sulfate and Zn oxide are commonly used as inorganic Zn controls in evaluation of organic Zn products. Pharmacological Zn oxide (3,000 to 5,000 mg Zn/kg) is effective in promoting growth and reducing diarrhea; however, due to the high Zn excretion and risk for environmental pollution, China MOA currently is considering reducing the allowable dose of Zn oxide in feeds [25]. Comparatively, Zinc sulfate is more efficiently utilized. Part of the reason is that sulfate, of several ligands tested, has been demonstarted to be the most effective in enhancing Zn uptake [26]. Lee reported that 120 mg Zn/kg from Zn methionine functioned similar to the same dose of Zinc sulfate [27]. Presently, most reports on the effective dose of organic Zn are over 80 mg Zn/kg [17-19]. In this study, we reported a lower effective dose of ZnAA from 20 to 80 mg Zn/kg for promoting growth performance. This indicates that a higher bioavailability can be achieved and explored for use of organic Zn which offers improved environmental protection.

Post weaning diarrhea is a serious common problem for pig production in the world. A large number of studies have been carried out on the dietary addition of high doses of Zn oxide to decrease post-weaning diarrhea [22,28]. In this study, the dose of 40 mg/kg Zn as ZnAA did not show significantly lower mortality rates in the early phase (d 0 to d 14) and the entire phase (d 0 to d 42) ($p = 0.96$) compared with other dietary treatments. Mortality rate was very low with limited development of severe diarrhea in any treatment group which may reflect a low level of weaning stress. Unfortunately, this limits the opportunity to infer beneficial impact of ZnAA on improving diarrhea incidence.

Alkaline phosphatase is an enzyme that catalyzes the release of inorganic phosphorus from phosphate monoester hydrolysis. Zinc is a necessary element for the synthesis of ALP, and body Zn level is positively correlated with the activity of this enzyme. A rat experiment showed that Zn level in the diet affected ALP activity [29]. In our study, serum ALP activity of weaned piglets tended to increase with increasing ZnAA dosage ($p = 0.055$), which demonstrates that serum ALP ac-

tivity was positively correlated with systemic Zn levels. Our study also determined that systemic Zn levels were closely correlated with the dietary Zn levels, which is in agree with previous reports [30,31]. CuZn-SOD is a Zn-dependent enzyme which plays an important role in the antioxidant defense system to eliminate free radicals produced by metabolism. Our study identified that ZnAA beneficially enhanced CuZn-SOD activity in weaned piglets.

Safety evaluation is crucial for Zn additives. Prolonged addition of high-dose Zn oxide has been reported to be toxic to the liver in pigs [32]. Therefore, it is necessary to investigate the safety of ZnAA in piglets. Hematological parameters are a set of basic blood test indicators and are closely associated with systemic metabolic status; thus can be effective in assessing body health conditions [33]. Serum biochemical parameters, serum ALP, AST, and ALT activities, can reflect the metabolic status of liver and kidney, closely associating with the growth performance of pigs [34]. In experiment 2, the serum ALP activity in pigs fed 80 or 800 mg/kg Zn as ZnAA were significantly higher than the pigs fed the unsupplemented diet at d 14 and d 28. The serum ALP activity in pigs fed diets containing 800 mg Zn/kg as ZnAA was similar to the serum ALP of pigs fed 80 mg/kg Zn as ZnAA. This indicates that a high dose of ZnAA may be well tolerated by weaned pigs considering the lack of difference in growth performance, hematological parameters and serum biochemical parameters between 80 and 800 mg/kg supplemental Zn as ZnAA.

CONCLUSION

Our results demonstrated that ZnAA (40 to 80 mg/kg) is an efficient Zn supplement to promote the growth performance in weanling pigs. The piglets can tolerate up to 800 mg/kg Zn from ZnAA with limited potential health effects.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript. Ward TL is an employee of Zinpro Corporation, and Ji F is an employee of Zinpro (Wuxi) Additives Bio-Technology Co., LTD.

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