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Effects of Chelated Copper and Zinc Supplementation on Growth Performance, Nutrient Digestibility, Blood Profiles, and Fecal Noxious Gas Emission in Weanling Pigs

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ABSTRACT

This study was conducted to evaluate the effects of chelated Cu and Zn on growth performance, nutrient digestibility, blood profiles, and fecal noxious gas emission in weanling pigs. A total of 90 weanling pigs with an initial body weight (BW) of 5.27 ± 0.04 kg were randomly allotted to two dietary treatments for 42 d. Pigs were then fed a control diet (CON) or a Zinpro diet (CON + 0.1% chelate copper and zinc). There were nine replicate-pens with five pigs in each pen. During d 0 to 14 and d 14 to 28, the ADFI decreased ($p<0.05$) and the G/F increased ($p<0.05$) in pigs fed the Zinpro diet compared with those that received the CON diet. During d 28 to 42, the ADFI increased ($p<0.05$) in pigs fed the Zinpro diet relative to those fed the CON diet. Additionally, the apparent total tract digestibility of DM, N, and energy increased ($p<0.05$) in the Zinpro group when compared to the CON group on d 14 and 28. The lymphocyte percentage was also greater ($p<0.05$) in the Zinpro group than in the CON group. Overall, dietary supplementation with 0.1% chelate copper and zinc improved the growth performance and nutrient digestibility in weanling pigs.

(**Key words :** Blood profiles, Growth performance, Nutrient digestibility, Weanling pig, Zinpro)

INTRODUCTION

Owing to the biological function of Cu and Zn in animal nutrition, diets are usually supplemented with these materials in amounts sufficient to ensure good performance and to maintain good health of pigs (Stansbury et al., 1990; Underwood and Suttle, 1999). Feeding high concentrations of Cu and Zn as Cu sulfate (125 to 250 ppm) and ZnO (2,500 to 3,000 ppm) has been shown to improve performance (Cromwell, 1989; Hill et al., 2001; Williams et al., 2005; Shelton et al., 2011), increase stool firmness (Hill et al., 2000) and modulate microbial balance (Roselli et al., 2005; Broom et al., 2006) of weanling pigs. However, the excretion of Cu and Zn from animal manure poses serious pollution issues (Jondreville et al., 2003).

Some studies reported that chelated formsof Zn and Cu are more bioavailable than their inorganic forms (Fouad, 1976; Spears, 1996). It has also been reported that chelated forms of minerals have higher absorbability than traditional forms, which leads to better absorbance through amino acid transport systems (Mazzoni et al., 2010). Indeed, Mazzoni et al. (2010) found that 250 or 2,500 ppm amino acid chelated Zn improved intestinal morphology in weaned pigs. Therefore, we hypothesized that 1,000 ppm Zn would have beneficial effects on performance and nutrient digestibility in weanling pigs.

This study was conducted to evaluate the effects of chelated Cu and Zn on growth performance nutrient digestibility, blood profiles, and fecal noxious gas emission in weanling pigs.

MATERIALS AND METHODS

1. Experimental design, animals, housing and diets

A total of 90 [(Landrace × Yorkshire) × Duroc, 21 d of age] weanling pigs with an initial body weight (BW) of 5.27 ± 0.40 kg were randomly allotted into two dietary treatments according to their BW (nine replicate-pens with five pigs per pen). The diets were fed in three phases during the experiment, phase 1 (d 0 to 14), phase 2 (d 14 to 28), and

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phase 3 (d 28 to 42). Pigs were fed a control diet (CON) or a Zinpro die (CON + 0.1% chelate copper and zinc). All diets were formulated to meet or exceed the NRC (1998) recommendations for weanling pigs (Table 1).

Pigs were housed in an environmentally controlled nursery facility with slatted plastic flooring. The temperature of the room was maintained at 30 ± 1°C for the first week, after which it was gradually reduced by 1°C per week. Each pen was provided with a stainless steel feeder and one nipple waterer that allowed ad libitum access to feed and water throughout the experiment. The animal care and use protocol was approved by the Animal Care and Use Committee of Dankook University.

2. Chemical analysis

Samples of diets were analyzed using standard methods (AOAC, 2000) for DM (method 934.01), nitrogen (N; method 968.06), and ether extract (method 920.39). Calcium (method 984.01) and phosphorus (method 965.17) contents were determined according to the AOAC (1995). Lactose was analyzed by gas-liquid chromatography via trimethylsilyl-O-methyl-oxime derivatives (Mawhinney et al., 1980). The individual amino acid composition was measured using an amino acid analyzer (Beckman 6300, Beckman Coulter, Inc., Fullerton, California, US) after 24-h of 6 N-HCl hydrolysis at 110°C (AOAC, 2000). To determine the cysteine and methionine levels, the samples were oxidized with performic acid overnight at 0°C. Performic acid is an oxidizing reagent that quantitatively converts cysteine to cysteic acid and methionine to methionine sulfone (Moore, 1963). Nitrogen was determined using a Kjectec 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden). The gross energy was determined by measuring the heat of combustion in the

Table 1. Feed compositions of control diet (as-fed basis)

Items	d 0 to 14	d 14 to 28	d 28 to 42
Ingredient, %			
Extruded corn	29.18	44.49	61.97
Soybean meal, 48% CP	16.94	21.20	27.80
Fish meal, 66% CP	5.00	3.50	–
Plasma powder	6.00	3.00	–
Soy oil	3.65	2.55	1.05
Lactose	15.30	8.30	–
Whey	15.00	10.00	5.00
Monocalcium phosphate	1.45	–	–
Dicalcium phosphate	–	1.50	1.50
Sugar	5.00	3.00	–
L-Lysine-HCl, 78%	0.29	0.39	0.46
DL-Methionine, 50%	0.32	0.30	0.24
L-Threonine, 89%	0.13	0.19	0.20
Choline chloride, 25%	0.20	0.10	0.10
Vitamin premix[1)]	0.10	0.10	0.10
Trace mineral premix[2)]	0.20	0.20	0.20
Limestone	1.24	0.98	1.13
Salt	–	0.20	0.25
Calculated composition			
ME, kcal/kg	3,640	3,540	3,410
Analyzed composition, %			
Crude protein	21.12	19.87	19.07
Lysine	1.65	1.53	1.32
Methionine	0.61	0.60	0.55
Calcium	0.97	0.91	0.87
Phosphorus	0.82	0.73	0.67
Crude fiber	1.39	1.79	2.49

[1)] Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 µg.
[2)] Provided per kg of complete diet: Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

samples using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, US).

3. Experimental procedures, sampling and assay

During the experiment, pigs were weighed individually at the beginning of the experiment (d of weanling = d 0), and at d 14, 28 and 42 and the number of dead pigs was recorded. Feed consumption was determined on a pen basis during the experiment. This information was then used to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain/feed ratio (G/F). From d 0 to 7, d 21 to 28 and d 35 to 42, 0.2% chromium oxide was added to the diet as an indigestible marker for determination of the apparent total tract digestibility (ATTD) of DM, N and energy (Ball and Aherne, 1987). Fecal samples were collected from all pigs via rectal massage at d 12, 13, 14, d 26, 27, 28, and d 40, 41, 42. Fecal samples were freeze dried, after which they were finely ground to be able to pass through a 1-mm screen and then analyzed using the aforementioned methods (AOAC, 2000). Chromium levels were determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) according to Williams et al. (1962). Nutrient digestibility was then calculated using the chromium technique (Sauer et al., 2000).

For the blood profile, two pigs from each pen (n = 18 per treatment) were randomly selected and blood samples were collected into vacuum tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) to obtain whole blood via anterior vena cava puncture on d 0, 14, 28, and 42. The white blood cell (WBC) and red blood cell (RBC) concentrations and lymphocytes percentage were determined using an Automatic Blood Analyzer (ADVIA 120, Bayer, NY). Samples for serum analysis were then centrifuged at 3,000 g for 15 min, after which the serum was removed and analyzed for immunoglobulin (IgG) using a nephelometer (Dade Behring, Marburg, Germany).

Fecal samples were collected directly via massaging the rectum of two pigs in each pen (18 pigs per treatment) at the end of the experiments. Samples from each pen were pooled and placed on ice for transportation to the lab. One gram of composite fecal sample from each pen was then diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and homogenized. Next,

Table 2. Effects of dietary supplementation of chelate copper and zinc on growth performance in weanling pigs[1)]

Items	CON	Zinpro	SE[2)]
BW, kg			
d 0	5.28	5.25	0.04
d 14	8.35	8.31	0.26
d 28	14.61	14.78	0.05
d 42	22.39	22.62	0.63
d 0 to 14			
ADG, g	219	218	18
ADFI, g	343[a]	317[b]	7
G/F	0.638[b]	0.687[a]	0.033
d 14 to 28			
ADG, g	447	462	26
ADFI, g	678[a]	648[b]	2
G/F	0.660[b]	0.713[a]	0.029
d 28 to 42			
ADG, g	556	560	25
ADFI, g	920[b]	945[a]	9
G/F	0.605	0.593	0.015
d 0 to 42			
ADG, g	407	414	15
ADFI, g	647	637	8
G/F	0.629	0.650	0.023

1) CON, control diet; Zinpro, CON + 0.1% chelate copper and zinc.
2) Standard error. Nine replicate pens of 5 pigs/pen per treatment.
a,b Means in the same row with different superscripts differ (p<0.05).

Table 3. Effects of dietary supplementation of chelate copper and zinc on apparent total tract nutrient digestibility in weanling pigs[1)]

Items, %	CON	Zinpro	SE[2)]
d 14			
Dry matter	81.16[b]	86.41[a]	0.38
Nitrogen	82.08[b]	87.34[a]	0.69
Energy	84.03[b]	88.89[a]	0.08
d 28			
Dry matter	86.28[b]	91.81[a]	0.86
Nitrogen	87.66[b]	93.21[a]	1.06
Energy	89.31[b]	93.64[a]	0.56
d 42			
Dry matter	80.09	80.39	0.32
Nitrogen	82.40	82.07	0.62
Energy	83.32	83.60	0.19

1) CON, control diet; Zinpro, CON + 0.1% chelate copper and zinc.
2) Standard error. Nine replicate pens of 2 pigs/pen per treatment.
a,b Means in the same row with different superscripts differ (p<0.05).

counts of viable bacteria in the fecal samples were conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 37°C under anaerobic conditions, while the MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after samples were removed from the incubator.

Fecal samples were collected directly by massaging the rectum of two pigs in each pen on d 40, 41, and 42 and then used to determine the fecal pH value and fecal noxious gas emissions. The fecal pH value was determined immediately using a glass-electrode pH meter (77P, Istek, Korea). Additionally, 300 g fresh feces samples from each pen were fermented in 2.6 L plastic boxes for 48 h at 32°C. After the fermentation period, a Gastec (model GV-100) gas sampling pump was utilized for gas detection (Gastec detector tube No. 3La for NH_3 No. 4LK for H_2S; No. 70 and 70L for total mercaptans No. 81L for acetic acid, Gastec Corp., Kanagawa, Japan).

4. Statistical analysis

Data were analyzed by ANOVA using the General Linear Models procedure of the SAS software (SAS Institute, 2006). The pen was defined as the experimental unit during the experimental period. Differences among treatments were separated by a t-test. Variability in the data is expressed as the standard error means (SEM) and a probability level of $p<0.05$ was considered to be statistically significant.

RESULTS

1. Growth performance

During d 0 to 14 and d 14 to 28, the ADFI decreased ($p<0.05$) and the G/F increased ($p<0.05$) in pigs fed the Zinpro diet when compared with those fed the CON diet. During d 28 to 42, the ADFI increased ($p<0.05$) in pigs that received the Zinpro treatment relative to those in the CON group. The BW and ADG were not affected ($p>0.05$) by dietary treatment throughout the experiment.

Table 4. Effects of dietary supplementation of chelate copper and zinc on blood profiles in weanling pigs[1)]

Items	CON	Zinpro	SE[2)]
RBC ($\times 10^6/\mu l$)			
d 0	6.17	6.03	0.19
d 14	6.35	6.24	0.41
d 28	5.78	5.61	0.31
d 42	6.44	6.45	0.36
WBC ($\times 10^3/\mu l$)			
d 0	8.99	8.70	0.78
d 14	15.02	13.29	0.88
d 28	14.77	14.35	2.26
d 42	15.56	15.65	0.75
Lymphocyte (%)			
d 0	54.20	54.48	2.58
d 14	46.43	52.55	2.86
d 28	48.20[b]	60.53[a]	2.19
d 42	53.05	50.45	2.94
IgG (mg/dL)			
d 0	283.00	273.00	26.23
d 14	239.75	224.25	26.34
d 28	232.25	234.00	23.88
d 42	299.00	284.00	20.65

[1)] CON, control diet; Zinpro, CON + 0.1% chelate copper and zinc.
[2)] Standard error. Nine replicate pens of 2 pigs/pen per treatment.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

2. Nutrient digestibility

The ATTD of DM, N, and energy were higher ($p<0.05$) in the Zinpro group than the CON group on d 14 and 28. There was no difference ($p>0.05$) in the ATTD of DM, N, and energy among dietary treatments.

3. Blood characteristics

The concentrations of RBC, WBC, IgG, and the lymphocyte percentage were not affected ($p>0.05$) by dietary treatment throughout the experiment, except that the lymphocyte percentage was higher ($p<0.05$) in pigs fed the Zinpro diet than those fed the CON diet on d 28.

4. Fecal microbial shedding, fecal pH, fecal noxious gas emission

There were no differences ($p>0.05$) between treatments in

Table 5. Effects of dietary supplementation of chelate copper and zinc on fecal microbial shedding in weanling pigs[1)]

Items, Log_{10} cfu/g	CON	Zinpro	SE[2)]
Escherichia coli	7.55	5.81	0.87
Lactic acid bacteria	6.84	4.10	0.88

[1)] CON, control diet; Zinpro, CON + 0.1% chelate copper and zinc.
[2)] Standard error. Nine replicate-pens of 2 pigs/pen per treatment.

Table 6. Effects of dietary supplementation of chelate copper and zinc on fecal pH and fecal noxious gas emission in weanling pigs[1)]

Items	CON	Zinpro	SE[2)]
pH	7.77	8.00	0.08
NH_3 (ppm)	150.00	133.33	21.17
Total mercaptan (ppm)	53.33	2.00	38.44
H_2S (ppm)	–[3)]	–[3)]	–
Acetic acid (ppm)	–[3)]	–[3)]	–

[1)] CON, control diet; Zinpro, CON + 0.1% chelate copper and zinc.
[2)] Standard error. Nine replicate-pens of 2 pigs/pen per treatment.
[3)] Not detected.

fecal *E. coli*, lactic acid bacteria counts, fecal pH, fecal NH_3, total mercaptans, H_2S, or acetic acid contents.

DISCUSSION

Pharmacological concentrations of Zn and Cu are commonly added to weanling pig diets as growth promoters (Shelton et al., 2011). However, no studies have found additive effects of Zn and Cu in weanling pigs (Smith et al., 1997; Hill et al., 2000). Nevertheless, there has been increased interest in the use of organic chelated mineral complexes as mineral sources for weanling pigs because of their higher potential bioavailability when compared to minerals from inorganic sources. In the current study, pigs fed the Zinpro diet had lower G/F than those fed the control diet during d 0 to 14 and d 14 to 28, which was likely because the ADFI decreased in response to administration of Zinpro in the diet. In agreement with our results, Castillo et al. (2008) reported that dietary supplementation with 80 mg/kg organic Zn improved the G/F during the first two weeks after weanling. Mullan et al. (2002, 2004) also demonstrated improvements when feeding weanling pigs organic Zn. However, Carlson et al. (2004) did not find positive effects on growth performance in weanling pigs fed an organic Zn diet. In addition, Stansbury et al. (1990) failed to demonstrate improvements in growth performance when pigs were fed diets containing 31.25 to 250 ppm chelated Cu. In the present study, Zinpro application decreased ADFI during phase 1 and phase 2, but increased ADFI during phase 3. Mazzoni et al. (2010) reported that 200 mg/kg chelated zinc improved the villous height of the duodenum when compared with pigs fed the control diet after 7 d of weanling, suggesting that dietary inclusion of chelated zinc improved the health of the gastro-intestine. Similarly, increased nutrient digestibility may also explain the improved gastro-intestinal health observed in the present study, and thus the increased G/F.

In our study, the lymphocyte percentage was increased in pigs fed the Zinpro diet when compared with those fed the CON diet, which was similar to the results reported by Van Heugten (2003), who found that lymphocyte proliferation was greater in pigs fed 80 mg/kg of Zn from Zn lysine or Zn methione. Hall et al. (1993) also reported increased proliferation of lymphocytes in pigs receiving 40 mg/kg of Zn compared with pigs fed diet without supplemental Zn.

Hojberg et al. (2005) reported that lactic acid bacteria were reduced and coliforms were increased inpiglets fed high doses of ZnO. Broom et al. (2006) demonstrated that lactic acid bacteria were reduced, but that ZnO had no effect on *E. coli.* In the present study, no differences were observed in the microbial shedding of *E. coli* and lactic acid bacteria. Similarly, Castillo et al. (2008) reported that lactobacillus counts in jejunum digesta did not differ between pigs fed the control and the chelated Zn diets. Taken together, these findings indicate that ZnO would affect gut microbiota, whereas chelated Zn would be absorbed and contribute to enterocyte and whole body metabolism.

CONCLUSIONS

In conclusion, dietary supplementation with 0.1% chelate copper and zinc improved growth performance and nutrient digestibility in weanling pigs.

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