



Effects of Copper Amino Acids Complex on Growth Performance and Serum Cu-Zn SOD Activity in Piglets

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ABSTRACT

The objective of this study was to evaluate the influence of different level of copper amino acids complex on growth performance and serum Cu-Zn SOD activity in piglets. A total of 288 (144 castrated males and 144 females) healthy post-weaned at 23-day-old piglets (Duroc × Landrace × Yorkshire) with an average body weight of (8.79±1.15) kg were randomly divided into 6 groups with 8 replicates in each group, and 6 piglets (3 castrated males and 3 females) per replicate. Control group was fed a basal diet, experimental groups were fed diets supplemented with copper amino acids addition level was 25, 50, 100 and 150 mg/kg Cu or 150 mg/kg Cu from CuSO₄ of the control group, respectively. Growth performance, serum biochemical parameters (Cu-Zn SOD activity) and diarrhea incidence were measured in the 2-phase feeding program (1-14 d, 15-42 d). Results showed that: compared with the control diets, 1) Diets supplemented with Av-Cu or CuSO₄ did not affect the average daily feed intake (ADFI) of weaned piglets; supplementing the diet with Av-Cu, the average daily gain (ADG) was linearly increased ($P < 0.05$), and the feed conversion ratio (FCR) was linearly decreased ($P < 0.05$); 90-100 mg/kg Cu from Av-Cu has the same effects on ADG and FCR compared to 150 mg/kg Cu from CuSO₄ according to linear analysis. 2) compared with the control diets, supplementing the diet with Av-Cu can decrease diarrhea incidence of piglets during the whole experimental period ($P < 0.05$). 3) Av-Cu has significant effect on serum Cu-Zn SOD activity on day 14 ($P < 0.05$). This study showed that, oral administration Av-Cu to weaned piglet has the potential to promote growth performance and antioxidant capacity, and reduce diarrhea incidence.

Article Information

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Authors' Contribution

RF designed the experiments. HL, XT, RQ, FS and SY performed the experiments and tested index. HL wrote the paper and analyzed the data. FY and CZ provided the copper mineral.

Key words

Copper amino acids complex, CuSO₄, Cu-Zn SOD, Diarrhea, Growth performance, Piglet.

INTRODUCTION

Copper (Cu) is one of essential elements for animals (NRC, 1998). Previous studies have shown that high dose of copper plays an important role in promoting piglet growth, reducing diarrhea incidence and feed conversion, increasing immunity and anti-stress function, etc. (Barber *et al.*, 1955; Bunch *et al.*, 1961; Hawbarber *et al.*, 1961; McDowell *et al.*, 1992; Cromwell *et al.*, 2001; Dorton *et al.*, 2003; Ma *et al.*, 2015). Organic Cu has a better effect on animal growth performance compared with inorganic Cu due to more safety, had a higher absorb rate, environmentally friendly (Coffey *et al.*, 1994; Veum *et al.*, 2004; Zhao *et al.*, 2014). Because of a low digestibility in the gastrointestinal tract, the Cu was often added several times higher than the recommended amount in dietary, which resulted in an increased Cu excretion of fecal and final leads to environmental pollution (Holden *et al.*, 2002;

McDowell *et al.*, 2003). In addition, it has been demonstrated that high dietary Cu from inorganic sources antagonizes other nutrients utilization, such as Zinc (Zhao *et al.*, 2008) and phosphorus (Banks *et al.*, 2004). Therefore, it's necessary to find out an efficiency Cu source to replace inorganic Cu as feed additives. The present study was designed to evaluate the effects of a copper amino acids complex called Availa® Cu 170 (Av-Cu) on growth performance and serum Cu-Zn SOD activity in piglets, and explore whether lower dose of Av-Cu has the same effects of high level of CuSO₄ on growth performance of piglets.

MATERIALS AND METHODS

Animals and experimental design

All animal work was approved by the Committee of Laboratory Animal Management and Animal Welfare of Hunan Agricultural University (Changsha, Hunan province, China). A total of 288 piglets (Duroc × Landrace × Yorkshire, with an initial body weight (BW): 8.79±1.15 kg, half castrated and half females) weaned at 23 days of

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age were randomly assigned to one of the 6 treatments: 1) basal diets group (without copper supplement); 2-5) basal diets with 25, 50, 100 and 150 mg/kg Cu from copper amino acids complex [Availa® Cu 170, Av-Cu, Zinpro (Wuxi) Additives Bio-technology Co. Ltd.]; or 6) 150 mg/kg Cu from CuSO₄. All piglets were divided in to 48 pens, and each treatment had 8 replicate pens with 6 piglets per pen (3 castrated males and 3 females).

Animal diets and management

The study was conducted in 2-phase feeding program (1-14 d, 15-42 d). The basal diets (low copper ingredients) of phase 1 and phase 2 were formulated according to the nutrient requirements of NRC (2012) (Table I). Control group was feed basal diets, the others groups were feed basal diets with 25, 50, 100 and 150 mg/kg Cu from Av-Cu, and 150 mg/kg Cu from CuSO₄. The trial was conducted in Xiang Yi Pig Farm, Yiyang Institute of Agricultural Sciences. All pigs were housed in pens equipped with a nipple drinker and a feeder. Piglets were fed four times (8:00, 12:00, 15:00 and 18:00) every day and had free access to water *ad libitum* during the entire experimental period. Breeding management and vaccination programs were conducted according to the normal procedure.

Nutrient composition analysis and Cu analysis

The diets of phase 1 and phase 2 were sampled then were dried at 60°C in a forces-air oven for 96 h, air-equilibrated, weighed to ground to pass through a 1-mm screen, and stored in sealed plastic bags at -4°C refrigerator. Feed sample determined for CP, EE, CF, Ash, Ca and total P according to the AOCS (2009). All experimental diets including basal diets of phase 1 and phase 2, and drinking water were sampled for Cu analysis. Cu content was determined by Jena (Germany) Vario 6 Atom Absorption Spectrophotometer according to the method described in GB/T13885-2003.

Growth performance

Piglets were weighed on days 1, 14 and 42 before feeding. Feed consumption was determined weekly by pen. Average daily weight gain (ADG), Average daily feed gain (ADFI) and feed conversion ratio (FCR) were calculated. Av-Cu intake was also calculated. Piglets were checked daily for the incidence of diarrhea (feces sticky and inconstant and water content >70% was considered diarrhea). The compute formula of Av-Cu intake and diarrhea incidence were as follow:

$$\text{Av-Cu intake (mg)} = \text{Feed intake (g)} \times \text{Av-Cu level (mg/kg)} / 1000$$

$$\text{Diarrhea incidence (\%, pen basis)} = [\text{sum of scouring piglet per day} / (\text{number of piglet} \times \text{day of experiment})] \times 100$$

Table I.- Composition and nutrient levels of basal diets (air -dry basis %).

Items	Phase 1 (1-14d)	Phase 2 (15-42 d)
Ingredients		
Corn	60.00	62.00
Soybean meal	10.50	20.00
Extruded soybean	12.16	5.70
Fish meal	7.00	3.00
Whey powder	4.00	3.00
CaHPO ₄	0.36	0.50
Flour	3.00	3.00
NaCl	0.30	0.35
Limestone	0.70	0.80
Soybean oil	0.50	0.20
98Lys	0.30	0.28
98Met	0.05	0.07
98Thr	0.13	0.10
Trp	0.21	
Premix ¹	1.00	1.00
Total	100.00	100.00
Calculated nutrients level		
CP ²	19.14	18.41
NE(Mcal/kg)	2.49	2.48
Ca	0.80	0.71
Total P	0.66	0.59
Available P	0.40	0.36
NaCl	0.30	0.22
Lys	1.36	1.24
Thr	0.80	0.73
Trp	0.21	0.21
Met	0.39	0.36

¹The premix provided following per kilogram of diet: vitamin A 1,500 IU; vitamin D3 200 IU; vitamin E 85 IU; D-pantothenic acid 35 mg; vitamin B2 12 mg; folic acid 1.5 mg; nicotinic acid 35 mg; vitamin B1 3.5 mg; vitamin B6 2.5 mg; biotin 0.2 mg; vitamin B12 0.05 mg; Fe (as ferrous sulfate) 100 mg; Mn (as manganese sulfate) 20 mg; I (as calcium iodate) 1 mg; Se (as sodium selenite) 0.35 mg; Co (as cobalt sulfate) 0.2mg; Cr (as chromium picolinate) 0.2 mg, 0.5g/kg Colistin Sulfate Premix, and ZnO 2.5 kg. The premix did not contain copper. ²Analytical value.

Blood sample collection and Cu-Zn SOD activity analysis

Two pigs from each pen (one female and one male) were chosen and blood samples were collected from precaval vein on days 14 and 42 before morning feeding. Blood samples were settled at room temperature for 30 min, and then centrifuged at 3000 r/min for 10 min at 4°C. The resulting serum samples were collected into a 1.5 mL sterile tube and stored at -20°C until further analyzed. Serum samples were used to evaluate the Cu-Zn SOD activity according to the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China).

Table II.- Nutrition composition of basal diets.

Nutrients	Phase 1	Phase 2
DM	88.40	88.52
CP	19.14	18.41
EE	7.75	6.78
CF	9.90	13.59
Ash	5.21	4.91

DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber

Statistical analysis

The data were subjected to one-way ANOVA with SPSS 20.0, significant differences amongst treatments were determined using Duncan's multiple range tests, $P < 0.05$ was considered statistically significant. Av-cu treatment groups (including basal diets group) were subjected to linear regression of ADG, and FCR to supplemental level of Av-Cu, and Av-Cu intake, respectively, and were subjected to quadratic regression analysis of serum Cu-Zn SOD activity to supplemental level of Av-Cu.

RESULTS

Approximate composition of diets and Cu content in diet and drinking water

Approximate composition and Cu content of basal diets were shown in Tables II and III. Cu content in drinking water ($\text{Cu} \leq 1.0 \text{ mg/L}$) meet the standard of drinking water (GB5479-2006).

Table III.- Copper content in the experimental diets.

Item	Treatment	Phase 1 (mg/kg)	Phase 2 (mg/kg)
A	Basal diet (Av-Cu 0 mg/kg)	14.2	17.2
B	Av-Cu 25 mg/kg	24.5	37.5
C	Av-Cu 50 mg/kg	65.1	65.9
D	Av-Cu 100 mg/kg	112.6	107.2
E	Av-Cu 150 mg/kg	134.2	136.0
F	CuSO_4 150 mg/kg	133.0	143.0

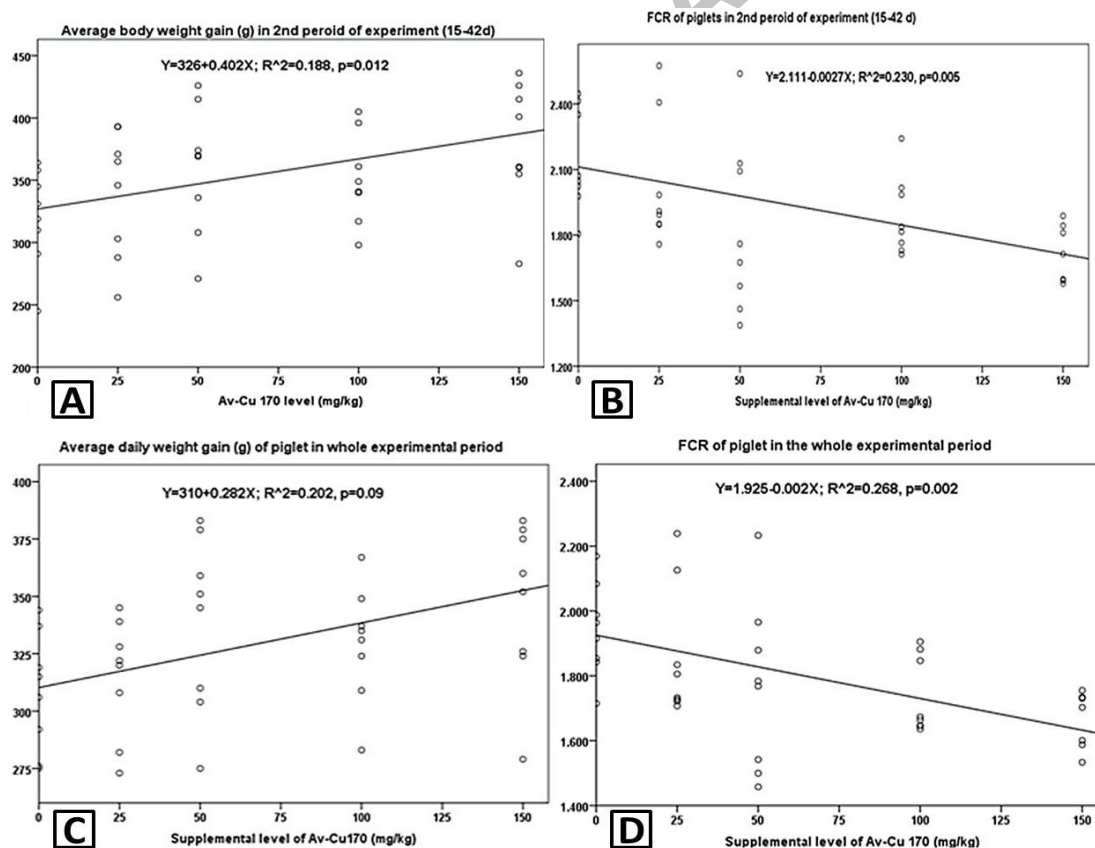


Fig. 1. Linear regression of ADG, FCR to supplemental level of Av-Cu. A, linear regression of ADG (g) to supplemental level of Av-Cu (mg/ kg) in phase 2; B, linear regression of FCR to supplemental level of Av-Cu (mg/ kg) in phase 2; C, linear regression of ADG (g) to supplemental level of Av-Cu (mg/ kg) in whole experimental period; D, linear regression of FCR to supplemental level of Av-Cu (mg/ kg) in whole experimental period.

Table IV.- Growth performance of piglets in phase 1, phase 2, and entire experimental period.

	A ¹	B	C	D	E	F	SEM	p ²	linear ³
1-14 d									
ADFI (g/d)	429	391	492	395	446	416	6	0.46	0.303
ADG (g/d)	283	265	304	286	282	282	7	0.19	0.012
FCR	1.53	1.46	1.58	1.37	1.57	1.54	0.04	0.03	0.005
15-42 d									
ADFI (g/d)	679	692	656	658	671	665	6	0.46	0.300
ADG (g/d)	320	339	359	351	380	365	7	0.19	0.012
FCR	2.14	2.03	1.83	1.89	1.72	1.84	0.04	0.03	0.005
0-42 d									
ADFI (g/d)	596	581	600	571	596	586	4	0.38	0.230
ADG (g/d)	308	315	338	329	347	338	5	0.12	0.009
FCR	1.94	1.86	1.77	1.74	1.66	1.74	0.03	0.04	0.002

¹A, basal diets group; B-E, basal diets with 25, 50, 100 and 150 mg/kg Cu from copper amino acids complex, respectively; F, basal diets with 150 mg/kg Cu from CuSO₄. ²Data for all treatments were analyzed for variance. ³Data for basal group and Av-Cu groups were used for linear regression.

Growth performance of piglets

The results of growth performance of phase 1, phase 2, and entire experimental period of piglets is shown in Table IV. There are no significant differences about ADG, ADFI, in phase 1 (1-14 d) and phase 2(15-42 d). Compared to basal diets group, the groups supplemented with 50, 100, 150 mg/kg Cu from Av-Cu, and 150 mg/kg Cu from CuSO₄ had a lower FCR in phase 1 (1-14 d) and phase 2(15-42 d) ($P < 0.05$). There was a significant linear relationship between ADG to supplemental level of Av-Cu [$Y=326+0.402X$ ($R^2=0.188$, $P = 0.012$)] (Fig. 1A), and a significant linear relationship between FCR to supplemental level of Av-Cu [$Y=2.111-0.0027X$ ($R^2=0.230$, $P=0.005$)] (Fig. 1B). According to the linear regression, 97 mg/kg Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO₄ on ADG, and 100 mg/kg Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO₄ on FCR.

During the entire experimental period there was a significant effect on FCR ($P < 0.05$), but no effect on ADFI and ADG. Supplemented with 100 and 150 mg/kg Cu from Av-Cu, and 150 mg/kg Cu from CuSO₄ had lower FCR than basal diets group ($P < 0.05$). They also had a significant linear relationship between ADG to supplemental level of Av-Cu [$Y=310+0.282X$ ($R^2=0.202$, $P=0.009$)] (Fig. 1C), and FCR to supplemental level of Av-Cu [$Y=1.925-0.0020X$ ($R^2=0.268$, $P=0.002$)] (Fig. 1D). According to the linear regression, 100 mg/kg Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO₄ on ADG, and FCR. Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO₄ on FCR.

Linear regression analysis revealed that ADG and FCR of piglets in phase 2 and in whole period of experiment were linearly affected by Av-Cu intake. Av-

Cu intake linearly increased ADG and decreased FCR of piglets in phase 2 (Fig. 2A, B) and whole experimental period (Fig. 2C, D).

Diarrhea incidence

Diarrhea occurred primarily in the phase 1 of experiment, and sporadically in phase 2 (Table V). In phase 1, diets supplemented with 25, 50 and 100 mg/kg Cu from Av-Cu had lower diarrhea incidence than basal diets group ($P < 0.05$), and supplemented with 25 mg/kg Cu from Av-Cu had lower diarrhea incidence than 150 mg/kg Cu from CuSO₄ ($P < 0.05$). In phase 2, supplemented with 100 and 150 mg/kg Cu from Av-Cu had lower diarrhea incidence than basal diets group ($P < 0.05$). For the whole experimental period, diets supplemented with 25, 50, 100, and 150 mg/kg Cu from Av-Cu significantly reduced diarrhea incidence of piglets compared to basal diet group ($P < 0.05$). No differences between 150 mg/kg CuSO₄ and any other groups.

Table V.- Diarrhea incidence of piglets in different treatments %.

Treatment	Phase 1	Phase 2	Whole peroid
A ¹	6.85 ^C	1.04 ^C	2.98 ^B
B	2.21 ^A	0.67 ^{ABC}	1.29 ^A
C	3.13 ^{AB}	0.74 ^{BC}	1.54 ^A
D	3.27 ^{AB}	0.00 ^A	1.14 ^A
E	5.44 ^{BC}	0.09 ^{AB}	1.74 ^A
F	5.06 ^{BC}	0.52 ^{ABC}	2.03 ^{AB}
SEM	0.33	0.10	0.14
p value	<0.001	0.015	<0.001

For detail of treatments, see Table IV.

Table VI.- Effects of Av-Cu on serum Cu-Zn SOD activity (U/mL).

Treatment	d 14	d 42
A ¹	143.21 ^a	162.05
B	186.46 ^b	155.79
C	163.28 ^b	162.95
D	180.21 ^b	160.29
E	140.10 ^a	163.73
F	138.02 ^a	151.63
SEM	5.84	4.12
P value ²	0.045	0.957
P value for linear ³	0.652	0.730
P value for quadratic ³	0.047	0.943

^{a,b}Means in a column without common superscript differ significant ($p < 0.05$). For detail of treatments, see Table IV.

Effects of different levels of Cu on serum Cu-Zn SOD

The effects of different levels of Cu on serum Cu-Zn SOD are listed in Table VI. Compared to basal diets group, supplemented with 25, 50, and 100 mg/kg Cu from Av-Cu had higher Cu-Zn SOD activities on day 14 ($P < 0.05$). Diets supplemented with 150 mg/kg Cu from Av-Cu and 150 mg/kg Cu from CuSO_4 had no difference compared with basal diets group. There was a significant quadratic relationship between serum Cu-Zn SOD activities to supplemental level of Av-Cu [$Y=147.695+0.992X-0.007X^2$ ($R^2=0.078$, $P=0.047$)] (Fig. 3). According to the quadratic regression analysis, diets supplemented with about 70 mg/kg Cu from Av-Cu would have the highest Cu-Zn SOD activities.

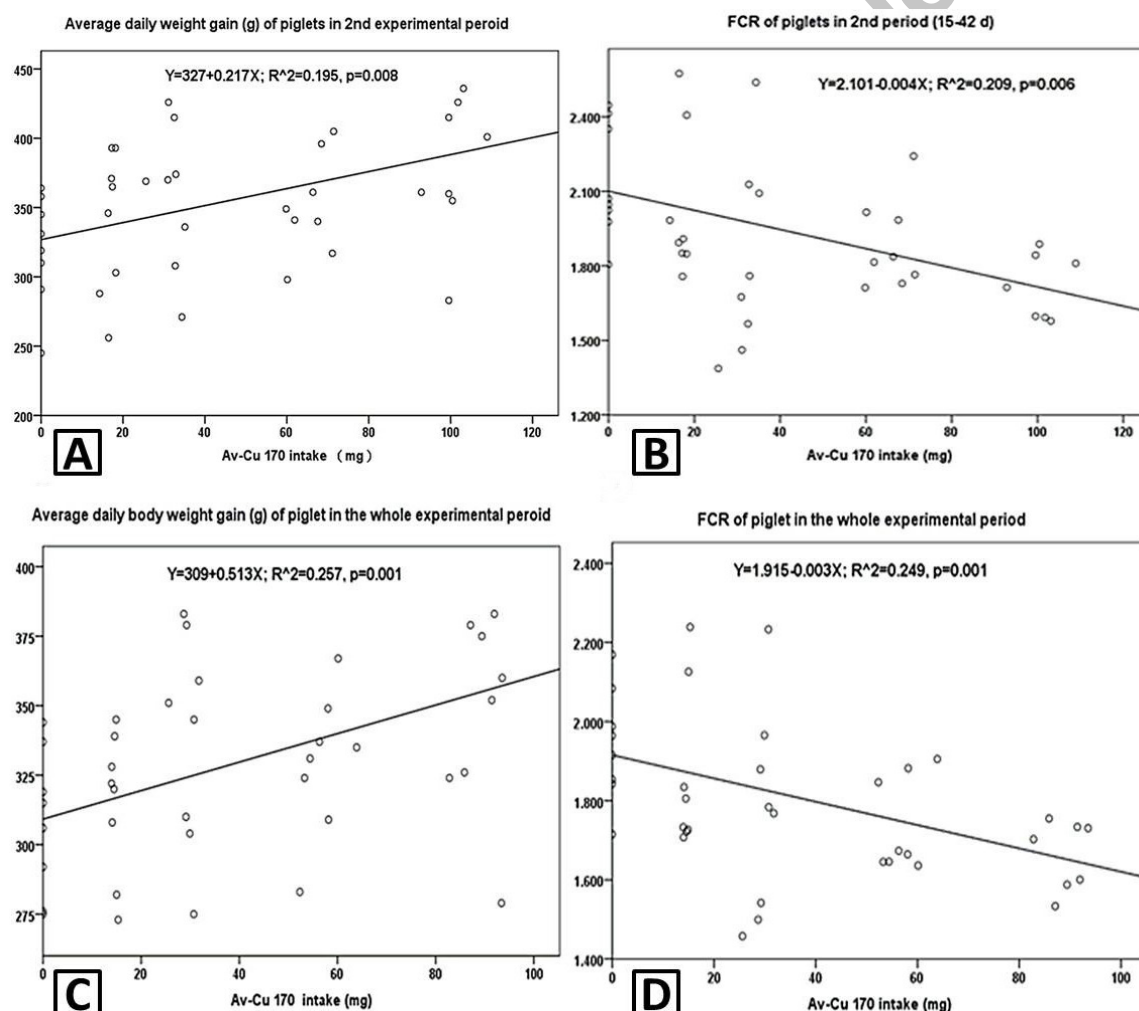


Fig. 2. Linear regression of ADG, FCR to intake level of Av-Cu. **A**, linear regression of ADG (g) to intake level of Av-Cu (mg/kg) in phase 2; **B**, linear regression of FCR to intake level of Av-Cu (mg/kg) in phase 2; **C**, linear regression of ADG (g) to intake level of Av-Cu (mg/kg) in whole experimental period; **D**, linear regression of FCR to intake level of Av-Cu (mg/kg) in whole experimental period.

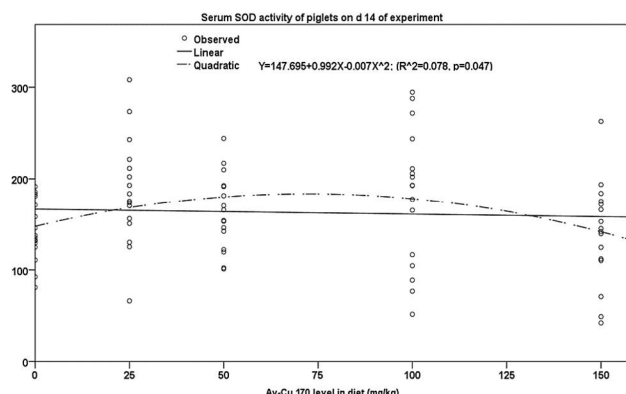


Fig. 3. The quadratic regression analysis of serum Cu-Zn SOD activity to supplemental level of Av-Cu (mg/kg).

DISCUSSION

Previous studies have shown that diet supplemented with 100 to 250 mg/kg Cu had a significant promoting effect on growth performance of pigs (Bunch *et al.*, 1961; Hawbaber *et al.*, 1961; Baber *et al.*, 1955; Ma *et al.*, 2015). In this study, diets supplemented with Cu from Av-Cu and CuSO_4 had a tendency to increase the ADG and ADFI, and reduce FCR of pigs. Cu has a low digestibility in the gastrointestinal tract, hence was often added several times higher than the recommended amount according to NRC (2012) in the diet to meet the fast growing requirement. This resulted in an increased fecal copper excretion leading to environmental pollution (Holden *et al.*, 2002; McDowell *et al.*, 2003). It seems that organic copper has a better digestibility compared with inorganic copper (Coffey *et al.*, 1994; Veum *et al.*, 2004; Zhao *et al.*, 2014). This study also showed that adding about 90-100 mg/kg Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO_4 on growth performance. Moreover, supplemented Av-Cu is likely to have both economic and environment benefits compared to CuSO_4 .

Piglet has a negative growth performance when weaned early in swine industry (Rhouma *et al.*, 2017). Furthermore, many key stress factors related with the weaning period, such as separation from the sow, feed changes, adapting to a new environment, mixing of pigs from different group and histological changes in the small intestine, may negatively affect the response of immune system and lead to an intestinal gut dysfunction (Gresse *et al.*, 2017; Li *et al.*, 2018; Wang *et al.*, 2014). Previous study showed better liver function with organic trace mineral supplementation which enhanced gluconeogenesis partly through upregulating PCK1 (Batistel *et al.*, 2016). Mei *et al.* (2010) reported that high dietary copper on microflora

and their activities and metabolic products might contribute to the intestinal health and result in increased growth performance. Wang *et al.* (2012) showed that copper decreased the diarrhea rate. In our study, we also found that copper decreased the diarrhea rate in weaned piglets and Av-Cu is better than CuSO_4 due to high antibacterial activity from Av-Cu.

SOD is a key enzyme in regulating oxidative stress by dismutation of superoxide radicals to oxygen and hydrogen peroxide (Wang *et al.*, 2010; Sujiwattanasarat *et al.*, 2015). SOD can remove many enzymes in body system to produce oxygen free radicals, and reduce the membrane unsaturated fatty acids which play an important role in the process of lipid per-oxidation and hence, protect cells from damage. So the activity of SOD can reflect the degree of the anti-lipid per-oxidation *in vivo*, which represents the body of the damage to the body. As one of the key enzymes of free-radical toxicity, Cu-Zn SOD is present in animal tissues (Chen *et al.*, 2000). Research shows that within a certain range properly raised level of fodder copper can effectively improve Cu-Zn SOD activity in animal body tissue, but Cu-Zn SOD activity is not linearly increased with the increase of fodder copper levels (Ma 2003). In this study, Quadratic regression analysis showed significant quadratic relationship between serum Cu-Zn SOD activities and the amount of Av-Cu on d 14. There is no significant difference in serum Cu-Zn SOD activity on day 42 piglets in each group.

CONCLUSION

In conclusion, diet supplemented with Av-Cu had a tendency to increased ADG, and reduced FCR. Adding about 90-100 mg/kg Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO_4 on growth performance. Av-Cu has significant effect on serum Cu-Zn SOD activity on day 14. Under our experiment conditions, we suggest supplementing diet with 90-100 mg/kg Cu from Av-Cu for piglets.

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Statement of conflict of interest

The authors declare no conflict of interest.

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