Influence of copper amino acid complex on growth performance and serum Cu-ZnSOD activity in piglets

Zhifeng Xiang [1, a], Quansheng Chen [1, b], Taoying Zhou [1]

[1] College of biology and agriculture, Huanggang Polytechnic College Huanggang City, China,

[a] 15908681946@163.com, [b] wanbofanyi@163.com

Abstract. A trial was conducted to evaluate the effects of copper amino acids complex in piglet diet. 288 (Duroc x Landrace x Yorkshire) hybrid piglets weaned at 23 d with body weight 8.79±1.15 kg were selected and allotted to one of the 6 treatments.Results showed that: 1) The addition of AV Cu or CuSO4 will not affect the daily average yield (ADFI) of weaned piglets, but the dietary AV Cu will linearly increase the daily gain (ADG) of weaned piglets and reduce the feed gain (FGR); the 90-100 mg/kg copper of AV Cu is equivalent to the 150 mg/kg copper of CusO4 on the ADG and FCR.; 2) Av-Cu inclusion in diet decreased diarrhea incidence of piglets.Base on this experiments, supplementation of Av-Cu into weaning piglet diet reduced diarrhea incidence, promoted growth and facilitated feed conversion of weaning piglets, as well as altered blood biochemistry indices.

Keywords: Amino acids complex ; piglet; growth performance; Cu-Zn SOD.

1. Introduction

Copper ,as one of essential elements in animal production [1], many studies has shown that high copper can accelerate piglet growth rate, reduce the rate of diarrhea, lower feed conversion, increase immunity and anti-stress, etc. [2-7] . The effects of copper and add form has a certain relationship, most of the domestic and foreign scholars think organic copper has a better effect compared with inorganic copper [8,9]. Because the digestion rate of copper in the body is very low, so when feeding the high content of copper, the copper enrichment in the feces, with droppings, in turn, cause environmental pollution [3,10]. This study is designed to study different doses of avail-Cu used in the production of pigs and piglets to explore the feasibility of adding avail-Cu for safe and efficient development of new swine diets provide a theoretical basis for copper sources.

1.1 Materials and method

Design and Animal Assignment: complete random design with dietary treatment as the only factor was adopted. There were 6 treatments and 8 replicates per treatment, with 6 piglets per replicate.

1.1.1 Design and Animal Assignment

Complete random design with dietary treatment as the only factor was adopted. There were 6 treatments and 8 replicates per treatment, with 6 piglets per replicate. The trial was conducted in Huang gang former pig field, Huazhong Institute of Agricultural Sciences. 288 (Duroc x Landrace x Yorkshire) hybrid piglets weaned at around 23 d with body weight 8.79±1.15 kg were selected and allotted to 6 treatments (Table 1).

Table 1 Experiment treatments

Copper source	Treatmenta					
	Basalb	AvCu25	Av Cu50	Av Cu100	Av Cu150	CuSO4
CuSO4c, mg/kgCud	-	-	-	-	-	150
Availa-Cu170, mg/kgCud	-	25	50	100	150	-

1.1.2 Animal management

Trial animals were raised in 3 houses with same structure next to each other. There are 16 pens in each house, with a drinker and a feeder in each pen. The floor is slatted floor. The animal trial was started right after the animals were allotted without acclimation period. Animals were fed four times per day(8:00, 12:00, 15:00 and 18:00). Animals were able to access water ad libitum.

1.1.3 rial diet

Ingredients with low copper content were selected for basal diet. Prior to basal diet production, all feedstuffs selected were analyzed for Cu content to avoid contamination. And Cu content of basal diet was analyzed . Diet composition and analytical results are shown in Table 2 and 3.

Table 2 Composition and nutrient levels of basal diets for 1stexperiment phase (d 1 - 14) (air -dry basis %)

Items	Content %
Ingredients	
Corn	60.00
Soybean meal	10.50
Extruded soybean	12.16
Fish meal	7.00
Whey powder	4.00
CaHPO4	0.36
Flour	3.00
NaCl	0.30
Limestone	0.70
Soybean oil	0.50
98Lys	0.30
98Met	0.05
98Thr	0.13
Trp	0.21
premix1	1.00
Total	100.00
Calculated nutrients level	
CP2	19.28
Ca	0.75
Total P	0.69
Available P	0.38
NaCl	0.30
Lys	1.42
Thr	0.91
Try	0.24
Met	0.40

Table 3 Composition and nutrient levels of basal diets in 2ndexperiment phase (d 15–42) (air-dry basis%)

Items	Content
Ingredients	
Corn	62.00
Soybean meal	20.00
Extruded soybean	5.99
Fish meal	2.44
Whey powder	3.27

CaHPO4	0.50
Flour	3.00
NaCl	0.35
Limestone	0.80
Soybean oil	0.20
Lys	0.28
Met	0.07
Thr	0.10
Premix1	1.00
Total	100.00
Calculated nutrients level	
CP2	19.30
Ca	0.75
Total P	0.59
Available P	0.36
NaCl	0.22
Lys	1.24
Thr	0.73
Try	0.21
Met	0.36

1.1.4.Sampling and Analysis

Feed and water sampling

All feedstuffs, Copper amino acids complex, CuSO4 and all treatment diets including basal diet were sampled. The samples were stored in 4 ℃ refrigerator. Water sample was collected in a clean-sterile polypropylene bottle and stored in -20℃ refrigerator.

Measurements of performance

On d 1, 14 and 42, individual BW of piglets were weighed after 8 h fasting. Feed consumption were recorded on pen basis. Daily ADG and FCR were calculated.

Blood sample collection

One female and one male piglet in each pen were randomly selected for blood sample collection. On day 14 and 42 prior to feeding, blood sample was collected from precaval vein into a vacuum tube, and then was settled at room temperature for 30 min,.

Lab analysis

A Feed approximate analysis and Cu content: CP, EE, CF, Ash, Ca and total P content were analyzed by conveintional feed approximate analysis method; Cu content was analyzed using the method described in GB/T13885-2003(Analysis of Ca, Cu, Fe, Mg, Mn, K, Na and Zn in animal feed). Sample was ashed at550 ± 15℃, dissolved and diluted into a constant volume with HCL. Sample solution was introduced into air-acetylene flame. Absorption of Cu atom was measured and compared to the standard.

B Serum Cu-ZnSOD, ceruloplasmin activities and Cu content: test kit produced by Nanjing Jiancheng Co. were used. Analysis procedures are provided by the producer.

Calculations

Average daily growth(g/d) = [final BW (g) － initial BW (g)]/day of experiment

Average daily feed intake (g)= [feed placed (kg) － feed remained (kg)]/day of experiment*1000

FCR (pen basis) = feed consumption (kg)/ total pen ADG (kg)

SOD activity (U/ml)= (blank OD-sample OD)/blank OD/50%*diluting times

Statistical analysis

Performance data were subjected to statistical analysis with SPSS 20.0. At first outliers were identified and excluded by Explore function, then normality and homogeneity of variance of data were validated. Serum biochemistry data were then subjected to analysis of variance by GLM with treatment and gender as fixed factors. Analysis results indicated that there were no main effects of gender and no interaction of gender and treatment on serum biochemistry parameters. So performance data and serum biochemistry data for all treatment groups were subjected to one-way ANOVA, and means were compared by Duncan's if significant differences were detected ($p<0.05$). Finally, data from treatment groups other than CuSO4 group , ceruloplasmin activity and Cu-ZnSOD activity to supplemental level of Av-Cu.

2. Results

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

Approximate composition and Cu content of basal diet were shown in Table 4 and 5. Cu content in drinking water (Cu≤1.0 mg/L) met the standard of drinking water (GB5479-2006).

Table 4 Analytical composition of basal diet

Nutrients	1st phase	2nd phase
DM	90.24	89.37
CP	18.75	19.02
EE	7.90	7.22
CF	10.01	15.01
Ash	5.28	5.00

Table 5 Copper content of the experimental diets

Treatment	Cu in 1st phase pellet mg/kg	Cu in 2nd phase pellet mg/kg
Basal (Av-Cu 0 mg/kg)	15.3	18.3
Av-Cu 25 mg/kg	23.9	38.9
Av-Cu 50 mg/kg	66.6	66.9
Av-Cu 100 mg/kg	117.7	106.8
Av-Cu 150 mg/kg	135.3	137.8
CuSO4 150 mg/kg	134.2	146.2

2.2 Performance of piglets

2.2.1 Performance of piglets in 1st phase of experiment

In the first phase of the experiment (d 1-14), there were no differences observed on performance measurements (ADFI, ADG and FCR), neither the linear relationship between these measurements and Av-Cu level in the diets.

Table 6 Performance of piglets in different treatmentsin first 14 d of experiment（Mean±SD）

Treatments	ADFI(g)	ADG(g)	FCR
Basal (Av-Cu 0 mg/kg)	429±73	283±56	1.526±0.115
Av-Cu 25 mg/kg	391±103	265±49	1.457±0.170
Av-Cu 50 mg/kg	492±83	304±15	1.585±0.146
Av-Cu 100 mg/kg	395±89	286±25	1.375±0.224
Av-Cu 150 mg/kg	446±91	282±25	1.570±0.239
CuSO4 150 mg/kg	416±17	282±25	1.543±0.057
SEM	12	5	0.026

P value*	0.164	0.543	0.168
P value for linear**	0.672	0.764	0.246

2.2.2 Performance of piglets in 2nd phase of experiment

In the 2nd phase of experiment (15-42d), significant difference was observed on FCR of piglet, but not on ADFI and ADG. FCR for basal group (without supplemental Cu) was significantly higher than other groups except for 25 mg/kg Av-Cu, while FCR for 150 mg/kg Av-Cu was significantly lower than for basal group and 25 mg/kg Av-Cu group. FCR for 150 mg/kg CuSO4 group was lower than for basal group, but not for any of the Av-Cu groups. Av-Cu in piglet diet linear increased ADG and decreased FCR. Regression of ADG to supplemental level of Av-Cu was showed in Fig 1 and regression of FCR to supplemental level of Av-Cu was revealed in Fig 2.

Table 7 Performance of piglets in different treatment in 15-42 d of experiment（Mean±SD）

Treatments	ADFI (g)	ADG(g)	FCR
Basal(Av-Cu 0 mg/kg)	679±43	320±39	2.142±0.233c
Av-Cu25 mg/kg	692±29	339±51	2.028±0.296bc
Av-Cu50 mg/kg	656±31	359±52	1.826±0.395ab
Av-Cu100 mg/kg	658±47	351±36	1.888±0.181abc
Av-Cu150mg/kg	671±10	380±50	1.717±0.131a
CuSO4150mg/kg	665±43	365±49	1.841±0.194ab
SEM	6	7	0.041
P value*	0.458	0.190	0.031
P value for linear**	0.303	0.012	0.005

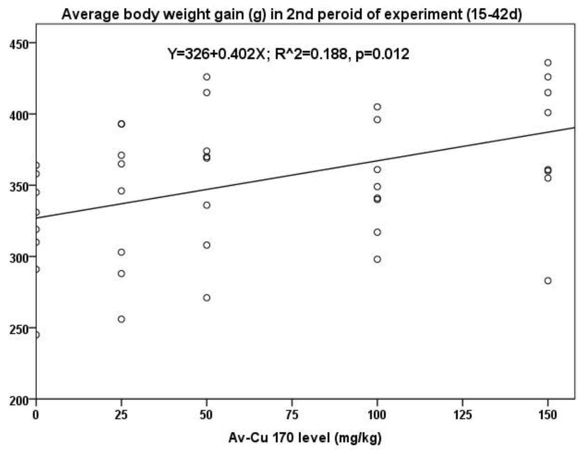

Fig 1. Linear regression of ADG(g)in the 2nd period of experiment (d 15-42) on supplemental Copper amino acids complex level (mg/kg).

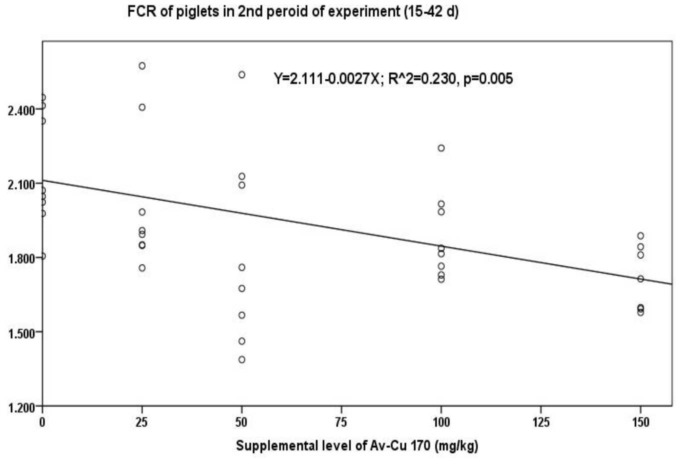

Fig 2. Linear regression of FCR in the 2nd period of experiment (d 15-42) on supplemental Copper amino acids complex level (mg/kg).

2.2.3 Performance of piglets in the whole period of experiment

Significant difference on FCR for the whole experimental period was observed, but not on ADFI and ADG. 100 and 150 mg/kg Av-Cu, 150 mg/kg $CuSO_4$ group had lower FCR than basal group.

Table 8 Growth performance of the piglet in whole experimental period (Mean±SD)

Treatments	ADFI (g)	ADG(g)	FCR
Basal(Av-Cu 0 mg/kg)	596±36	308±26	1.941±0.144c
Av-Cu 25 mg/kg	581±20	315±26	1.862±0.205bc
Av-Cu 50 mg/kg	600±22	338±38	1.766±0.264abc
Av-Cu 100 mg/kg	571±38	329±25	1.737±0.118ab
Av-Cu 150 mg/kg	596±26	347±36	1.663±0.087a
CuSO4 150 mg/kg	586±31	338±34	1.745±0.132ab
SEM	4	5	0.027
P value*	0.381	0.121	0.039
P value for linear**	0.231	0.009	0.002

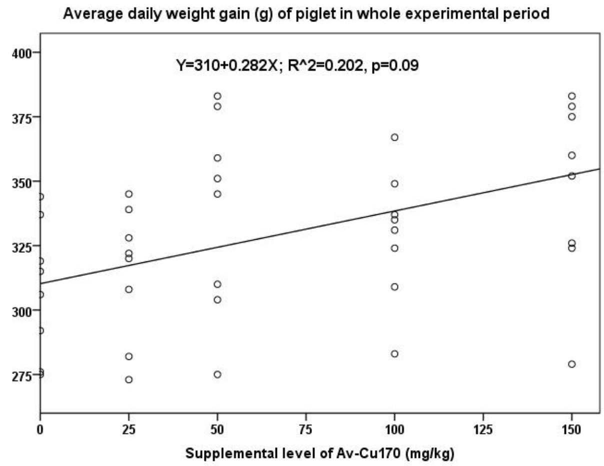

Fig 3. Linear regression of ADG (g) in the whole experimental period on supplemental Copper amino acids complex level (mg/kg).

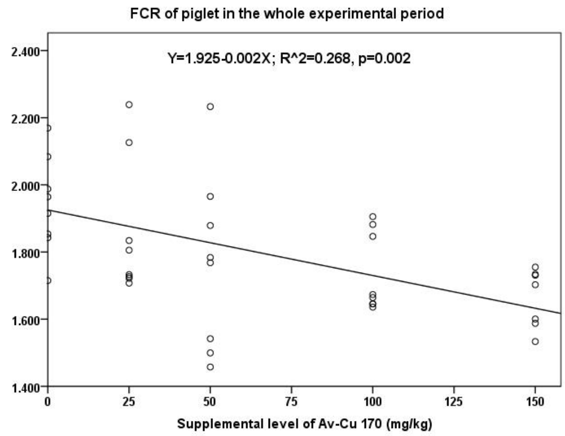

Fig 4. Linear regression of FCR in the whole experimental period on supplemental Copper amino acids complex level (mg/kg).

2.2.4 Regression of performance to Av-Cu 170 intake

Regression analysis revealed in Fig 5-8.

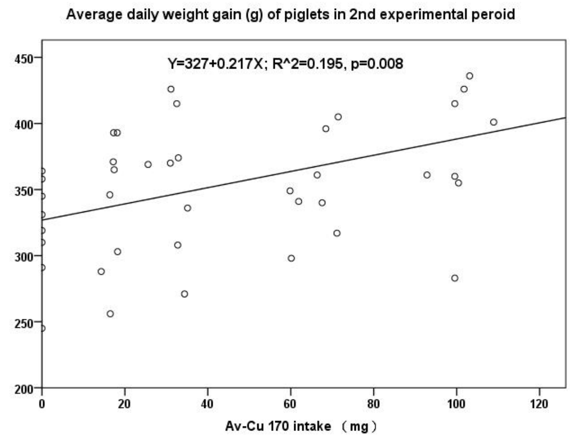

Fig 5. Linear regression of ADG (g) in the 2nd period of experiment (d 15-42) on Copper amino acids complex intake (mg).

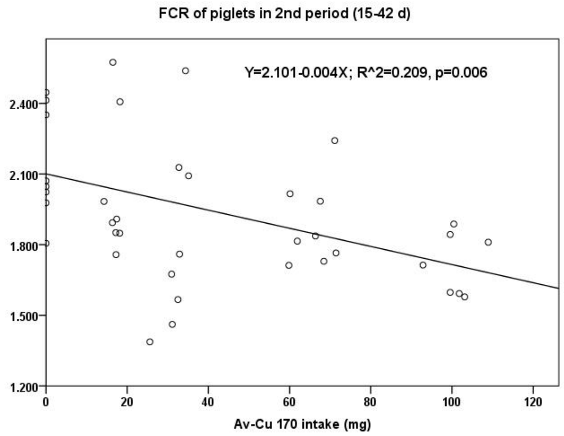

Fig 6. Linear regression of FCR in the 2nd period of experiment (d 15-42) on Copper amino acids complex intake (mg).

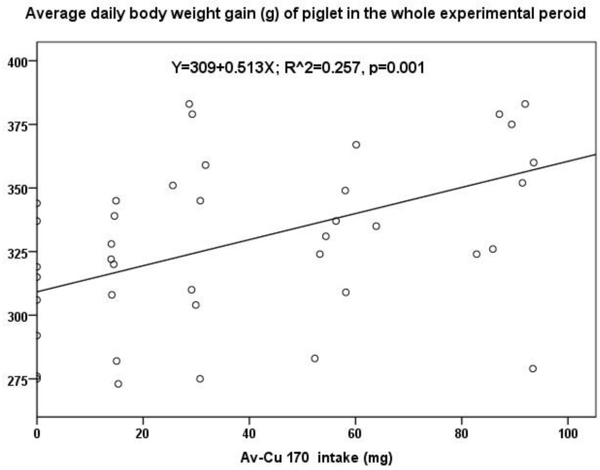

Fig 7. Linear regression of ADG (g) in the whole experimental period on Copper amino acids complex intake (mg/kg).

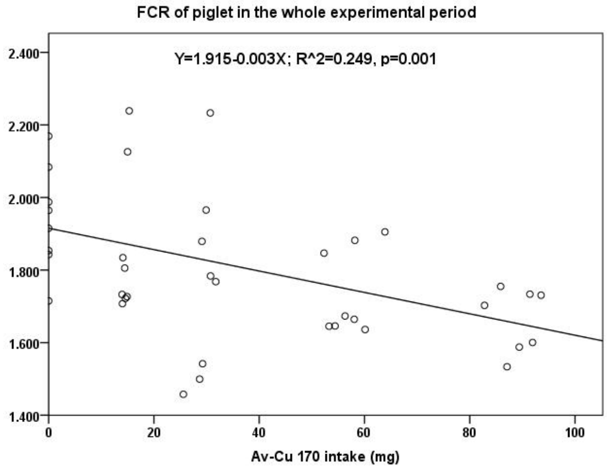

Fig 8. Linear regression of FCR in the whole experimental period on Copper amino acids complex intake (mg).

2.2.5 Diarrhea incidence

In the 1st phase, 25, 50 and 100 mg/kg Av-Cu groups had lower diarrhea incidence than basal group, and 25 mg/kg Av-Cu group had lower diarrhea incidence than 150 mg/kg CuSO4 group. No differences were observed between 150 mg/kg CuSO4 group and basal group.

Table 9 Diarrhea rateof piglet in different treatments(mean±SD)

Treatment	1st period	2ndperiod	Whole peroid
Basal(Av-Cu 0 mg/kg)	6.85 ± 2.68^{C}	1.04 ± 0.76^{c}	2.98 ± 1.27^{B}
Av-Cu 25 mg/kg	2.21 ± 0.82^{A}	0.67 ± 0.67^{abc}	1.29 ± 0.59^{A}
Av-Cu 50 mg/kg	3.13 ± 1.79^{AB}	0.74 ± 0.83^{bc}	1.54 ± 0.69^{A}
Av-Cu 100 mg/kg	3.27 ± 1.53^{AB}	0.00 ± 0.00^{a}	1.14 ± 0.49^{A}
Av-Cu 150 mg/kg	5.44 ± 1.35^{BC}	0.09 ± 0.22^{ab}	1.74 ± 0.92^{A}
$CuSO_4$ 150 mg/kg	5.06 ± 1.23^{BC}	0.52 ± 0.59^{abc}	2.03 ± 0.49^{AB}
SEM	0.33	0.10	0.14
P value	0.000	0.015	0.000

2.2.6 Effects of Different Levels of Copper in Serum Cu-ZnSOD Weaned Indicators

Significant differences on Cu-ZnSOD activities on day 14 of experiment were observed in that basal group ,150 mg/kg Av-Cu groups and 150 mg/kg CuSO4 were lower than 25 mg/kg Av-Cu groups(Table 10). Quadratic regression analysis showed significant quadratic relationship between serum Cu-ZnSOD activities and the amount of Av-Cu, Quadratic regression of Cu-ZnSOD to dietary Av-Cu level was showed in Fig 9 Serum Cu-ZnSOD will be highest activities after add about 70 mg/kg Av-Cu. There was no significant difference in serum test on day 42 piglets in each group Cu-ZnSOD activity.

Table 10 Weaned serum Cu-ZnSOD activity (U/mL)

Treatment	D 14	D42
Basal (Av-Cu 0 mg/kg)	143.21a	162.05
Av-Cu 25 mg/kg	186.46b	155.79
Av-Cu 50 mg/kg	163.28ab	162.95
Av-Cu 100 mg/kg	180.21ab	160.29
Av-Cu 150 mg/kg	140.10a	163.73
CuSO4 150 mg/kg	138.02a	151.63
SEM	5.84	4.12
P value*	0.045	0.957
P value for linear**	0.652	0.730
P value for quadratic**	0.047	0.943

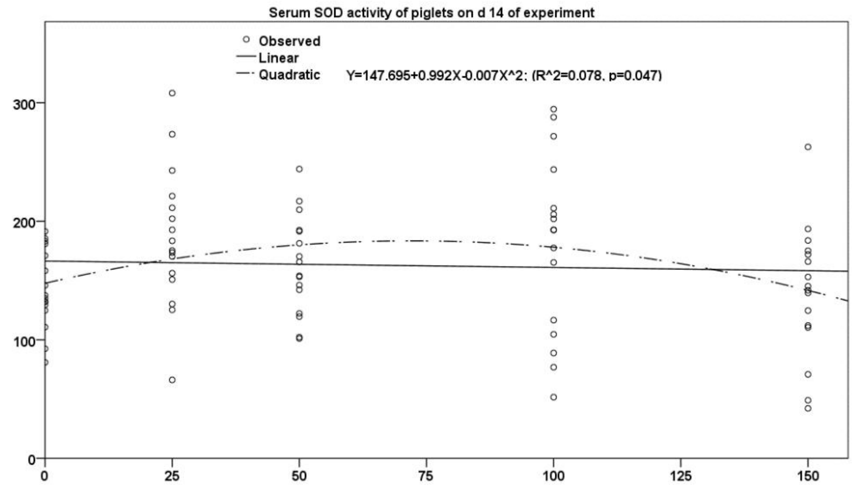

Fig 9. Regression of serum Cu-ZnSOD activity in the 1st period of experiment (d 1-14) on supplemental Copper amino acids complex level (mg/kg).

3. Discussion

From the current results, copper is about 100~250mg/kg, which has a significant impact on the growth performance of pigs [6,7,11]. In this study, the added copper improved the growth rate and feed conversion efficiency of pigs. Adding more than 90-100 mg/kg of Av copper was the same as adding 150 mg/kg CusO4, which improved the performance. Most of the copper was not absorbed after eating fixed copper, followed by undigested copper, These copper will be discharged from the face, causing environmental pollution. Compared with the wrapping rate of CuSO4, the addition of Av Cu should have economic and environmental advantages.

SOD can promote excessive oxidation, eliminate free radicals in students' bodies, reduce the damage of free radical chain reaction to the body, etc., which has a great impact on the body. SOD can clear many enzymes in the body system, produce free oxygen free radicals, reduce the important role of unsaturated fatty acids on the membrane in the process of lipid oxidation, and thus reduce the unsaturated fatty acids in cells. It protects against damage. Therefore, the activity of sod can reflect the degree of anti lipid oxidation promotion in the body, representing the degree of damage to the body. As one of the key enzymes of free radical toxicity, Cu znsod is widely used in animal tissues [12]. The research shows that, within a certain range, the accurate increase of copper content in the feed is effective in improving the activity of Cu ZnSOD in animal tissues, but the activity of Cu ZnSSOD will not increase linearly with the increase of copper content in the feed [13]. The square regression analysis in this study showed that there was a significant square relationship between the serum Cu ZnSD activity and the amount of Av Cu on the 14th day. There was no significant difference in serum Cu ZnSD activity among 42 day old piglets in each group.

4. Conclusion

In present study, we observed that: 1). Inclusion of Av-Cu or CuSO4 did not affect FI of weaning piglets, but Av-Cu in diet linearly increased ADG and decreased FCR of weaning piglets in 2nd phase and whole period of the study. 90-100 mg/kg Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO4 on the effects on ADG and FCR; 2). Av-Cu inclusion in diet decreased diarrhea incidence of piglets, especially in the 1st phase of study; 3). Av-Cu inclusion in diet Significant differences on Cu-ZnSOD activities on day 14 of experiment were observed in that basal group ,150 mg/kg Av-Cu groups and 150 mg/kg CuSO4 were lower than 25 mg/kg Av-Cu groups .Base on this experiments, supplementation of Av-Cu into weaning piglet diet reduced diarrhea incidence, promoted growth and facilitated feed conversion of weaning piglets, as well as altered blood biochemistry indices. 90-110 mg/kg Cu from Av-Cu was equivalent to 150 mg/kg Cu from CuSO4.

Acknowledgment

This work was financially supported by Huanggang Polytechnic College doctoral start-up fund project.

References

[1] NRC. Nutrient requirements of swine. 10th ed. Washington, DC: National Academy Press; 1998.

[2] Cromwell, G.L.,. Antimicrobial and promicrobial agent. In: Lewis, A.J., Southern, L.L., et al. (Eds.), Swine Nutrition. CRC Press, Boca Raton, FL, 2001, 401–406.

[3] McDowell, L.R.,. Mineral in Animal and Human Nutrition. Academic Press. Inc, New York, 1992.176–200

[4] Dorton, K.L., Engle, T.E., Hamar, D.W., Siciliano, P.D., Yemm, R.S.. Effects of copper source and concentration on copper status and immune function in growing and finishing steers. Anim. Feed Sci. Technol. 2003(110), 31–44.

[5] Barber R S, R Braude and K.G.Mitchell.antibiotics and copper supplements for fattening pigs.Br.J.Nutr. 1955,9:378-381

[6] Bunch. R.J.,V.C Speer, V. W. Hays, J. H. Hawbaker and D. V. Catron..Effects of copper sulfate ,copper oxide and chlortetracycline on baby pig performance.J.Anim.Sci. 1961,20:723-726.

[7] Hawbaber J.H.,V.C. Speer , V. W. Hays, and D.V.Catron..Effect of copper sulfate and other chemotherapeutics in growing swine rations.J.Anim. 1961,Sci.20:163-167.

[8] Coffey,R．D．，Cromwell，G L．and Monegue，H．J．Efficacy ofa copper-lysine complex as a

[9] growth promotant for weanling pigs[J]．J Anim Sci，1994．72(11):2880—2886

[10] Veum，T．L．Copper proteinate in weanling pig diets for enhancing growth performance and

[11] reducing fecal copper excretion compared with copper sulfate[J]一 Anim Sci，2004,82(4):1062-1070．

[12] Holden P., Carr J., Honeyman M., Kliebenstein, J., McKean J. Harmon J., Mabry J., Hoyer S. 2002 .Minimizing the use of antibiotics in pig production. AS-lllowa State University Extension. Ames,lowa,50011.IPIC 8,11.

[13] barber R.S. R Braude and K.G.Mitchell. Antibiotics and copper supplements for fattening pigs. British J Nutrition.1995(9):378-381.

[14] CHEN J R，WENG C N，HO T Y，et a1．Identification of the copper—zinc superoxide dismutase activity in

[15] Mycoplasma hyopneumoniae[J]．Veterinary Microbiology，2000，73(4)：301—310．

[16] Ma Deying, Shan Anshan. Effects of copper free radicals to animals defense enzyme activity and gene expression of the system[J]. China Animal Husbandry and Veterinary medicice. 2003,05:27-29