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The effect of iron gl cine chelate on tissue mineral levels, *fecal* mineral concentration, and liver antio idant en me activit in weanling pigs

J. Feng[a,*], W.Q. Ma[a], Z.R. Xu[a], J.X. He[b], Y.Z. Wang[a], J.X. Liu[a]

[a] *College of Animal Science, ZheJiang University (Hua Jia Chi Campus), The Key Laboratory of Molecular Animal Nutrition, Ministry of Education, HangZhou 310029, PR China*

[b] *College of Environmental and Resource Sciences, ZheJiang University (Hua Jia Chi Campus), HangZhou 310029, PR China*

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ABSTRACT

Twent four weaning pigs were used to evaluate the effects of iron gl cine chelate (Fe Gl) on tissue mineral levels, fecal mineral concentration and liver antio idant en me activities of weanling pigs. Pigs were allotted to si treatments based on live weight and litter origin. Treatments consisted of: (1) control (no Fe supplementa tionl); (2) 30 mg Fe/kg diet from Fe Gl ; (3) 60 mg Fe/kg diet from Fe Gl ; (4) 90 mg Fe/kg diet from Fe Gl ; (5) 120 mg Fe/kg diet from Fe Gl ; (6) positive control, 120 mg Fe/kg diet from ferrous sulphate ($FeSO_4$). Feeding the diets containing Fe Gl for 40 da s resulted in an increased Fe concentration in heart ($P<0.05$), liver ($P<0.05$), kid ne ($P<0.05$), spleen ($P<0.05$) and feces ($P<0.01$). There were linear responses to the addition of Fe Gl from 0 to 120 mg Fe/kg Fe on concentration in the liver and kidne . $FeSO_4$ also enhanced heart, liver, spleen and fecal Fe concentration ($P<0.05$ or $P<0.01$) compared with the control. Spleen Fe concentration was enhanced ($P=0.01$) and fecal Fe concentration was little reduced ($P=0.09$) when pigs were fed with 120 mg Fe as Fe Gl /kg compared with 120 mg Fe as $FeSO_4$/kg. Linear responses to the addition of Fe Gl were observed on catalase and succinate deh drogenase (SDH) activities. 90 mg Fe as Fe Gl /kg increased SOD ($P=0.02$) and SDH ($P=0.03$) activit compared with the negative control. However, there were no sig nificant differences in pancreas mineral concentration, fecal Cu, Zn and Mn concentration and liver anthine o idase activities among the treatments ($P>0.05$).

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* Corresponding author. Tel.: +86 571 86986127; fa : +86 571 86994963.
E-mail address: fengj@ ju.edu.cn (J. Feng).

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1. Introduction

Addition of Fe from iron chelated with amino acids or protein to the diet has can prevent and treat Fe deficienc in animals or humans (Veum et al., 1995; Spears et al., 1999; Bovell Benjamin et al., 2000; Kegle et al., 2002; Feng et al., 2007). *A study showed that* chelated or proteinated source of Fe had 125 185% relative availabilit compared with ferrous sulphate (Henr and Miller, 1995). Research on pigs indicated that iron methionine *had a higher bioavailability* than ferrous sulphate in nursing pigs (Spears et al., 1992). Mortalit , birth and weanling bod weight of *piglets* were improved significantl when sows were fed with iron proteinate (Close, 1998, 1999). Yu et al. (2000) found iron from an amino acid comple increased plasma iron and total iron binding capacit in the blood, hemosiderin and ferritin iron in the liver and spleen of weanling pigs.

In the last decade, studies have shown that iron chelated with gl cine could be absorbed and utili ed easil , and maintain high iron bioavailabilit in rats or humans, despite the presence of iron absorption inhibitor factors such as ph tic acid (Allen et al., 1998; Iost et al., 1998; La risse et al., 2000; Oscar and Ashmead, 2001). Iron gl cine chelate (Fe Gl) is currentl used as an efficient iron fortificant in human food, especiall in infant food (Fo et al., 1998; Giorgini et al., 2001). In a previous stud it was found that, at an appropriate dosage, Fe Gl improved performance, hematological and immunological characteristics in weanling pigs (Feng et al., 2007). The main objectives of the current trial were to investigate the effects of dietar Fe Gl on tissue mineral status, fecal mineral concentration, and liver antio idant en me activit in weanling pigs.

2. Materials and methods

2.1. Animals and experimental design

One hundred and eight 35 da old piglets (Duroc × Landrace × Yorkshire) weighing 7.8 ± 0.72 kg were blocked based on weight, se and ancestr and randoml allotted to si dietar treatments, each of which was replicated three times with 10 pigs per replicate. Treatments consisted of: (1) control (no Fe supplementationl); (2) 30 mg Fe from Fe Gl /kg diet; (3) 60 mg Fe from Fe Gl /kg diet; (4) 90 mg Fe from Fe Gl /kg diet; (5) 120 mg Fe from Fe Gl /kg diet; (6) positive control, 120 mg Fe from ferrous sulphate ($FeSO_4$)/kg diet.

Pigs were housed in concrete floored indoor pens (10 pigs per pen) and fed a mai e so bean meal based diet formulated to meet National Research Council (NRC, 1998) nutrient requirement estimates (Table 1). In the 40 da stud , all pigs were given *ad libitum* access to feed and water.

Table 1
Composition of basal diet (as fed basis)

Ingredient	g/kg	Composition[a]	
Mai e	543.5	DE (MJ/kg)	14.38
So bean meal	170	Crude protein (g/kg)	207.2
E truded so bean	100	*Ether extract (g/kg)*	*4.3*
Whe	80	Calcium (g/kg)	10.5
Fish meal	60	Phosphorus (g/kg)	7.6
Wheat middling	10	L sine (g/kg)	13.5
Calcium h drogen phosphate	10	Fe (mg/kg)	*79*
Limestone	8	Cu (mg/kg)	*58*
So bean oil	5	Zn (mg/kg)	*146*
Vitamin mineral premi [b]	10	Mn (mg/kg)	*74*
Salt	2		
L sine	1.5		

[a] DE based on calculated values, others were anal ed values.

[b] Supplied the following per kilogram of diet: Vitamin A 15000 IU; Vitamin D2 3000 IU; Vitamin E 30 IU; *Vitamin B2 3.5 mg*; Vitamin B1 3.0 mg; Vitamin B12 0.025 mg; biotin 0.06 mg; pantothenic acid 20 mg; nicotinic acid 15 mg; Cu 50 mg; Zn 120 mg; Mn 60 mg; Se 0.67 mg; Co 1 mg.

2.2. Blood, tissues, and feces collection

At the end of the feeding trial, 24 pigs (four piglets of each treatment) were selected and humanel slaughtered. Heart, liver, kidne , spleen and pancreas samples were e cised and immediatel stored at −70 °C until anal sis for antio idant en me activities and mineral concentrations. Fecal samples were free e dried and fro en at −20 °C until mineral anal sis.

2.3. Determination of mineral concentration in tissues and feces

Fecal samples were prepared for mineral anal sis using a method described b Armstrong et al. (2004). Uniform samples were cut from tissues, wet digested using nitric perchloric acid and then diluted with deioni ed distilled water for anal ses of minerals (Hill et al., 1983). Contents of Fe, Cu, Zn, and Mn were anal ed with flame atomic absorption spectrophotometr (AA 6300, Shimad u Corp., Tok o, Japan).

2.4. Mesurement of SOD, CAT, SDH and XOD activities

Liver samples were homogeni ed in 0.1 M Tris HCl buffer at 4 °C, pH 7.4, to make a 10% (w/v) homo genate, using a pol tron *homogenizer* for 5 min and a sonic homogeni er for 3 min. The homogenates were centrifuged at 3000 × g for 5 min at 4 °C and then the supernatants were collected and stored at −20 °C for en me anal sis. Liver Cu/Zn SOD activities were determined with the methods of Shaw et al. (2002). Assa for catalase (CAT) activit was performed b following the reduction in H_2O_2 absorbance at 240 nm as reported b Venturino et al. (2001). Succinate deh drogenase (SDH) activit was determined b the method of Tune et al. (2006). Xanthine o idase (XOD) activit was measured according to the method described b Hashimoto (1974). Protein was estimated b the method of Lowr et al. (1951). Units of SOD, CAT, SDH and XOD activities were e pressed as per milligram of protein.

2.5. Statistical analysis

Data were anal ed b ANOVA as a randomi ed complete block design using the GLM procedures of SAS (1988). Individual pigs were the e perimental unit for all indices. The planned single df tests included the linear and quadratic effects of Fe Gl , the control *versus* $FeSO_4$ (120 mg Fe/kg), $FeSO_4$ *versus* Fe Gl (120 mg Fe/kg) treatments. Differences between two treatment means were compared using the Student *t* test (Steel and Torrie, 1960). An alpha level of 0.05 was used for determination of statistical significance of differences among treatments.

3. Results

3.1. Tissue mineral concentrations

The effects of different levels of Fe Gl on tissue mineral concentrations of weanling pigs are pre sented in Table 2. Increasing dietar Fe Gl levels increased the Fe content of the heart (P=0.02), the liver (P=0.003), the kidne s (P=0.005) and the spleen (P=0.001), the highest organ concentrations occurring in the animals receiving the highest amount of Fe Gl . Moreover, there were linear responses to the addition of Fe Gl from 0 to 120 mg Fe/kg on Zn concentration in liver and kidne . Compared to the negative control, 120 mg $FeSO_4$/kg also enhanced the Fe concentration of the heart (P=0.04), the liver (P=0.02) and the spleen (P=0.001). In addition, spleen Fe storage was improved when pigs were fed 120 mg/kg Fe as Fe Gl compared with 120 mg/kg Fe as $FeSO_4$ (P=0.005). However, there were no significant differences in pancreas mineral contents when pigs were offered different levels of iron as Fe Gl and $FeSO_4$ compared with control (P>0.05).

Table 2
Effect of amount and chemical composition of iron on tissue trace element levels in weanling pigs[a]

Item[b]	Fe Gl [c]					$FeSO_4$[c]	S.E.M.[d]	P value			
	0[e]	30[e]	60[e]	90[e]	120[e]	120[e]		Control *vs.* $FeSO_4$	*$FeSO_4$ vs. Fe-Gl*	Fe Gl	
										Linear	Quad
Heart (mg/kg)											
Fe	28.8	30.8	29.5	33.8	34.7	31.7	0.82	0.04	*0.54*	0.02	0.92
Cu	4.37	4.55	4.96	4.20	4.42	4.34	0.12	0.35	*0.86*	0.80	0.35
Zn	18.2	18.5	18.4	20.2	18.5	18.2	0.31	0.98	*0.20*	0.41	0.48
Mn	0.61	0.65	0.69	0.64	0.68	0.63	0.02	0.82	*0.28*	0.48	0.60
Liver (mg/kg)											
Fe	102	106	117	120	129	117	2.73	0.02	*0.005*	0.003	0.88
Cu	16.9	18.4	19.2	17.8	17.8	19.2	0.57	0.46	*0.55*	0.75	0.20
Zn	49.8	49.6	52.2	54.6	55.2	55.5	0.89	0.13	*0.17*	0.03	0.86
Mn	2.06	2.01	2.06	2.02	2.03	2.09	0.03	0.81	*0.60*	0.83	0.87
Kidne (mg/kg)											
Fe	49.2	49.8	52.7	51.9	53.1	50.8	0.36	0.27	*0.048*	0.005	0.47
Cu	4.74	4.83	4.44	4.53	4.57	4.93	0.08	0.29	*0.68*	0.25	0.50
Zn	18.4	18.3	18.8	18.8	19.6	18.7	0.15	0.24	*0.24*	0.02	0.27
Mn	1.46	1.46	1.52	1.44	1.40	1.49	0.02	0.47	*0.34*	0.34	0.22
Pancreas (mg/kg)											
Fe	24.4	23.1	25.9	25.8	27.5	25.9	0.74	0.60	*0.083*	0.17	0.69
Cu	3.71	3.81	3.34	3.54	3.38	3.40	0.15	0.41	*0.069*	0.51	0.93
Zn	25.7	26.2	28.1	28.3	26.7	27.1	0.51	0.59	*0.81*	0.16	0.11
Mn	1.49	1.60	1.49	1.48	1.53	1.60	0.02	0.11	*0.48*	0.87	0.90
Spleen (mg/kg)											
Fe	64.4	65.3	72.4	71.7	78.01	73.4	1.13	0.001	*0.01*	0.001	0.59
Cu	2.32	2.46	2.06	2.01	2.13	2.20	0.11	0.77	*0.83*	0.40	0.78
Zn	20.7	21.2	21.4	20.6	20.7	20.3	0.12	0.13	*0.10*	0.60	0.11
Mn	1.34	1.37	1.40	1.37	1.39	1.39	0.01	0.08	*0.22*	0.08	0.18

[a] Non orthogonal comparisons between the control *vs.* $FeSO_4$ (120 mg/kg), and *the $FeSO_4$ (120 mg/kg) vs. Fe-Gly (120 mg/kg) treatments*. Linear and quadratic effects of increasing Fe concentrations in Fe Gl form (0 to 120 mg/kg).

[b] Tissue levels are e pressed per kg wet weight.

[c] Fe source.

[d] S.E.M. stands for standard error of the mean.

[e] Fe addition (mg/kg).

3.2. *Fecal mineral concentrations*

Anal ed values of Fe, Cu, Zn, and Mn for fecal samples are presented in Table 3.

Fecal Fe concentration increased linearl with the increasing dietar Fe Gl levels (P=0.002), and reached the highest level in 120 mg Fe as Fe Gl /kg. Moreover, 120 mg Fe as $FeSO_4$/kg enhanced Fe concentration in feces compared with the control (P=0.01). Fecal Fe concentration had a decrease trend when pigs fed diet supplemental 120 mg Fe as Fe Gl /kg compared with diet in addition with 120 mg Fe as $FeSO_4$/kg (P=0.09). Mineral contents of Cu, Zn and Mn in feces did not differ in pigs among all the treatments.

3.3. *Liver antioxidant enzyme activities*

Fig. 1 shows the effect of different levels of iron as Fe Gl on liver SOD, CAT, XOD, and SDH activit in weanling pigs. There were linear responses to the addition of Fe Gl on CAT and SDH activities (P=0.41 and P=0.001, respectivel). As shown in the figure, 90 mg Fe as Fe Gl /kg increased SOD (P=0.02) and SDH (P=0.03) activit . No significant response to XOD could be found among the Fe Gl , $FeSO_4$ treatments and the control.

Table 3
Effect of amount and chemical composition of iron on fecal mineral concentrations in weanling pigs[a]

Item[b]	Fe Gl [c]					$FeSO_4$[c]	S.E.M.[d]	*P* value			
	0[e]	30[e]	60[e]	90[e]	120[e]	120[e]		Control *vs.* $FeSO_4$	*$FeSO_4$ vs. Fe-Gly*	Fe Gl	
										Linear	Quad
Fe	252	269	282	307	314	331	7.0	0.01	*0.09*	0.001	0.83
Cu	31	32	30	31	29	30.51	0.5	0.77	*0.21*	0.08	0.15
Zn	174	170	173	176	177	186	3.0	0.54	*0.19*	0.66	0.72
Mn	2.2	2.1	2.2	2.2	2.2	2.3	0.05	0.18	*0.26*	0.81	0.72

[a] Non orthogonal comparisons between the control *vs.* $FeSO_4$ (120 mg/kg), and *the $FeSO_4$ (120 mg/kg) vs. Fe-Gly (120 mg/kg) treatments*. Linear and quadratic effects of increasing Fe concentrations in Fe Gl form (0 to 120 mg/kg).

[b] The trace elements are e pressed as mg/kg dr matter.

[c] Fe source.

[d] S.E.M. stands for the standard error of the mean.

[e] Fe addition (mg/kg).

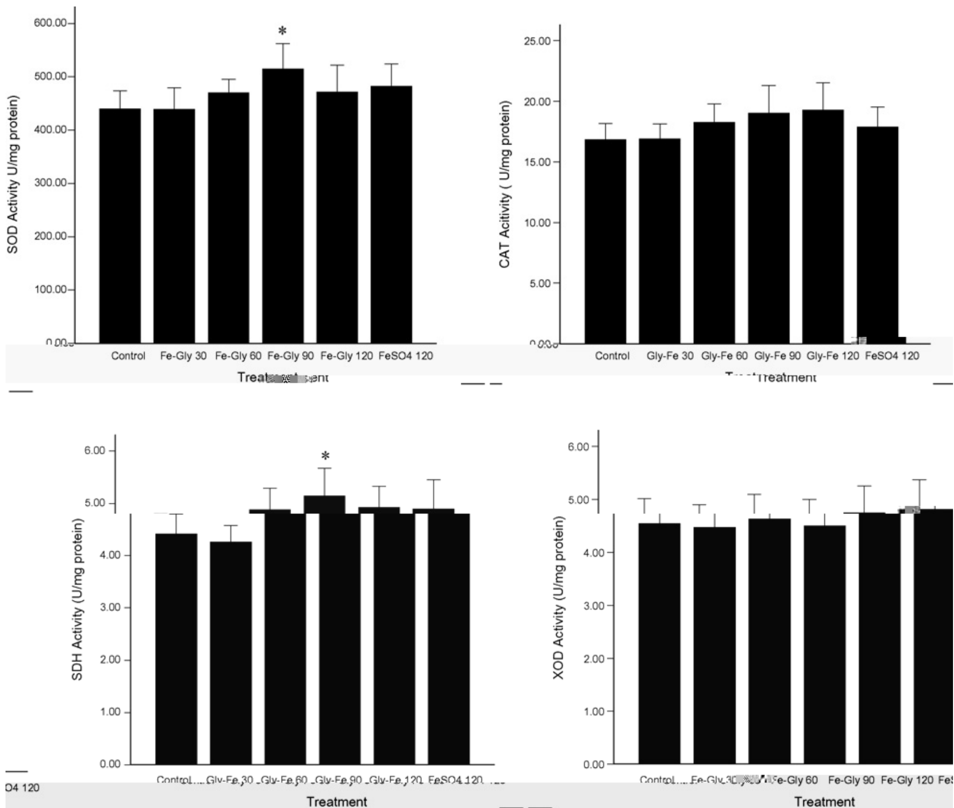

Fig. 1. The effects of iron gl cine and ferrous sulfate on liver SOD, CAT, SDH and XOD activities in weanling pig. Values were means for 4 piglets. Control (no Fe supplementation), Fe Gl groups supplement 30 120 mg Fe as Fe gl cine chelate/kg diet as, $FeSO_4$ 120 group (positive control) supplements 120 mg Fe/kg diet from ferrous sulphate. *The mean difference is significant at the 0.05 level compared with the control.

4. Discussion

Tissue mineral concentration data are usuall used to evaluate mineral status of animals and humans. The present stud showed that Fe concentration in heart, liver, kidne and spleen, but not in the pancreas increased with the increasing levels of Fe as Fe Gl in weanling pigs. Spra and Widdow son (1950) compared nursing pigs receiving a dail dose of supplemental Fe (11 mg/kg BW) during the first 3 week of life with pigs receiving no supplemental Fe and noted that supplemental Fe greatl increased the amount of Fe in the bod . Furugouri (1972) also reported a linear decrease in liver ferritin, nonheme and total Fe when dietar Fe decreased. When nurser pigs were fed diets supplemented with 0, 25, 50, 100, 150 ppm iron in the diet (as fed basis) from ferrous sulphate, whole bod iron stores increased linearl due to increasing dietar iron concentrations (Rincker et al., 2004, 2005). Yu et al. (2000) reported that total iron in the liver, spleen, and muscle significantl increased as the level of Fe amino acid chelate supplement was increased ($P<0.05$). These results are in agreement with the increase in tissue Fe concentration due to increases in dietar Fe concentration reported in the current stud . The present results also showed that there were linear responses to the addition of Fe Gl from 0 to 120 mg/kg Fe on Zn concentration in liver, kidne and pancreas. Hill and Matrone (1970) reported that the trace minerals Cu, Fe, and Zn are transition metals, which have similar chem ical and ph sical properties (*i.e.*, similar electronic structure). Thus, an imbalance in one mineral can have an antagonistic effect on the concentration of another mineral. Rincker et al. (2005) also found the increasing dietar Fe concentration resulted in a linear increase in dietar Fe ($P=0.001$), dietar Zn ($P=0.003$), fecal Fe ($P=0.001$) e cretion and fecal Zn ($P=0.020$) e cretion. Iron gl cinate has been proved to have high iron bioavailabilit in animal or human. It has been suggested that the higher bioavailabilit of iron gl cine is probabl due to the chemical structure of this compound, which par tiall prevents iron ph tate interactions (Bovell Benjamin et al., 2000; La risse et al., 2000). Galdi et al. (1988) reported higher absorption in anemic rats fed iron gl cine compared with ferrous sulphate. Bovell Benjamin et al. (2000) conducted a comparative stud of the absorption of iron from ferrous gl cinate and iron sulphate in a whole mai e meal and found a significantl greater geometric mean percentage of iron was absorbed from ferrous gl cinate (6.8%) than from $FeSO_4$ (1%). La risse et al. (2000) showed that twice as much iron was absorbed as from foods fortified with ferrous gl cinate than from $FeSO_4$ fortified foods. In the present stud , spleen Fe retention was improved when pigs were fed iron as Fe Gl compared with 120 mg/kg Fe as $FeSO_4$ treatment. This ma be related with the good absorption of iron gl cine implicating that the bioavailabilit of iron from Fe Gl is higher than that of iron from ferrous sulphate.

Anal sis of fecal mineral concentration indicated that fecal Fe concentrations were enhanced ($P<0.05$) as the dietar concentration of Fe as Fe Gl increased, and 120 mg/kg Fe as $FeSO_4$ also enhanced Fe concentration in feces compared with the control ($P=0.01$). This is in accordance with the results of others studies with other forms of iron compounds. Fecal Fe was decreased when Fe was reduced in the pig diet regardless of source (sulphate *versus* combination of sulphate and chelate) (Creech et al., 2004). Increasing the dietar Fe (0 150 mg/kg) as iron sulphate resulted in a linear increase in fecal Fe e cretion ($P<0.01$) (Rincker et al., 2005). There also e isted a trend for a decrease when pigs fed diet supplemental 120 mg/kg Fe as Fe Gl compared with diet in addition with 120 mg Fe as $FeSO_4$/kg ($P=0.09$). This finding combined with the results of tissue Fe storage supports the view that iron chelated with gl cine is better absorbed and utili ed than iron sulphate.

Iron is an essential micronutrient, but e cess intake and storage of iron induces increased produc tion of reactive o gen species (ROS) and is thought to cause various diseases (To okuni, 1996; Fiers et al., 1999; Nicholls and Budd, 2000; Zodl et al., 2003). CAT and SOD are considered the primar antio idant en mes because the are involved in the direct elimination of ROS (Beckman et al., 1988; Rao and Jagadeesan, 1996). The present stud indicated that CAT activit increased with the addition of Fe Gl . This agrees with the studies performed with rats. Lee et al. (1981) observed reduced CAT activit in the RBC and liver of Fe deficient rats. Brandsch et al. (2002) found that catalase activit in rat liver increased b feeding high iron diets, and *they postulated* it was because of increased iron concentrations in the liver rather than to induction b o idative stress. Moderate dietar iron e cess ($\leq$400 mg iron/kg diet) did not affect the SOD activit in rat liver (Bristow Craig et al., 1994; Ibrahim et al., 1997), but SOD activit decreased when rat fed with iron deficienc diet (Rao and Jagadeesan,

1996). Increased SOD activit was observed when pigs fed with 90 mg Fe as Fe Gl /kg in the present stud . Rincker et al. (2004) thought even though Fe contributed b feed ingredients provided basal dietar Fe concentrations in e cess of the NRC (1998) postweaning requirement (80 mg/kg), the dietar Fe was not adequate to sustain Fe stores in pigs fed lower supplemental Fe concentrations, and the supplementation of 100 mg of Fe/kg of diet was required in addition to the Fe provided b dietar Fe ingredients to alleviate severe decreases in Fe stores. In the present e periment, basal diet contained 79 mg Fe/kg which just met the requirement for postweaning pigs. The marginal Fe concentration of the basal diet probabl caused SOD to be abnormall low, and the added Fe restored SOD levels to normal. SDH and XOD are related to the reduction and generation of free radicals. Ishii et al. (2005) reported that a reduction in SDH activit resulted in an increased production of ROS. Zhang et al. (2006) noted that increasing the dietar concentration of Fe as $FeSO_4$ (0 120 mg of Fe of per kg diet) resulted in an increase in SDH activit in the blood of Re rabbits. This is in accordance with current stud . It has been shown that adding e ogenous XOD to generate free radicals can damage muscle function in animal (Barcla and Hansel, 1991). XOD activit was not affected b Fe Gl or $FeSO_4$ addition in diet of piglet in present stud . Feeding rats a wide range of dietar Fe up to 10 times the estimated require ment, did not induce overt o idative stress (Roughead et al., 1999). This indicates that the moderatel high intake of Fe in the present stud probabl does not pose a major risk in increasing o idative stress in weanling pigs, although this point warrants further research.

In conclusion, the results obtained from the current stud indicate that supplementation with Fe Gl could improve iron tissue storage and antio idant en me activities, and also could increase Zinc retention in liver and kidne in weanling pig. Additionall , a reduction in fecal Fe concentrations could be found when pigs were fed diets containing Fe as Fe Gl compared to $FeSO_4$.

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References

Allen, L.H., Bovell Benjamin, A.C., Viteri, F., 1998. Ferrous bis and ferric tris gl cinates as iron fortificants for whole mai e: bioavailabilit and regulation b iron status. FASEB J. 12, A821.

Armstrong, T.A., Cook, D.R., Ward, M.M., Williams, C.M., Spears, J.W., 2004. Effect of dietar copper source (cupric citrate and cupric sulfate) and concentration on growth performance and fecal copper e cretion in weanling pigs. J. Anim. Sci. 82, 1234 1240.

Barcla , J.K., Hansel, M., 1991. Free radicals ma contribute to o idative skeletal muscle fatigue. Can. J. Ph siol. Pharmacol. 69, 279 284.

Beckman, J.S., Minor, R.L., White, C.W., Repine, J.E., Rosen, G.M., Freeman, B.A., 1988. Supero ide dismutase and catalase conju gated to pol eth lene gl col increases endothelial en me activit and o idant resistance. J. Biol. Chem. 263, 6884 6892.

Bovell Benjamin, A.C., Viteri, F.E., Allen, L.H., 2000. Iron absorption from ferrous bisgl cinate and ferric trisgl cinate in whole mai e is regulated b iron status. Am. J. Clin. Nutr. 71, 1563 1569.

Brandsch, C., Ringseis, R., Eder, K., 2002. High dietar iron concentrations enhance the formation of cholesterol o idation products in the liver of adult rats fed salmon oil with minimal effects on antio idant status. J. Nutr. 132, 2263 2269.

Bristow Craig, H.E., Strain, J.J., Welch, R.W., 1994. Iron status, blood lipids and endogenous antio idants in response to dietar iron levels in male and female rats. Int. J. Vitam. Nutr. Res. 64, 324 329.

Close, W.H., 1998. The role of trace mineral proteinates in pig nutrition. In: L ons, T.P., Jacques, K.A. (Eds.), Biotechnolog in the Feed Industr . Nottingham Universit Press, Nottingham, UK, pp. 469 483.

Close, W.H., 1999. Organic minerals for pigs: an update. In: L ons, T.P., Jacques, K.A. (Eds.), Biotechnolog in the Feed Industr . Nottingham Universit Press, Nottingham, UK, pp. 51 60.

Creech, B.L., Spears, J.W., Flowers, W.L., Hill, G.M., Llo d, K.E., Armstrong, T.A., Engle, T.E., 2004. Effects of dietar trace mineral concentration and source (inorganic vs. chelated) on performance, mineral status, and fecal mineral e cretion in pigs from weaning through finishing. J. Anim. Sci. 82, 2140 2147.

Feng, J., Ma, W.Q., Xu, Z.R., Wang, Y.Z., Liu, J.X., 2007. Effects of iron gl cine chelate on growth, haematological and immunological characteristics in weaning pigs. Anim. Feed Sci. Technol. 134, 261 272.

Fiers, W., Be aert, R., Declercq, W., Vandenabeele, P., 1999. More than one wa to die: apoptosis, necrosis and reactive o gen damage. Oncogene 18, 7719 7730.

Fo , T.E., Eagles, J., Fairweather Tait, S.J., 1998. Bioavailabilit of iron gl cine as a fortificant in infant foods. Am. J. Clin. Nutr. 67, 664 668.

Furugouri, K., 1972. Effect of elevated dietar levels of iron on iron store in liver, some blood constituents and phosphorus deficienc in oung swine. J. Anim. Sci. 34, 573 577.
Galdi, M., Bassi, A., Barrio Rendo, M.E., Valencia, M.E., 1988. Ferric gl cinate iron bioavailabilit as determined b haemoglobin regeneration. Nutr. Rep. Int. 38, 591 598.
Giorgini, E., Fisberg, M., Paula de, R.A., Ferreira, A.M., Valle, J., Braga, J.A., 2001. The use of sweet rolls fortified with iron bis gl cinate chelate in the prevention of iron deficienc anemia in preschool children. Arch. Latinoam. Nutr. 51, 48 53.
Hashimoto, S., 1974. A new spectrophotometric assa method of anthine o idase in crude tissue homogenate. Anal. Biochem. 62, 426 435.
Henr , P.R., Miller, E.R., 1995. Iron availabilit . In: Ammerman, C.B., Baker, D.H., Lewis, A.S. (Eds.), Bioavailabilit of Nutrients for Animals. Academic Press, San Diego, pp. 169 199.
Hill, C.H., Matrone, G., 1970. Chemical parameters in the stud of in vivo and in vitro interactions of transition elements. Fed. Proc. 29, 1474 1481.
Hill, G.M., Miller, E.R., Whetter, P.A., Ullre , D.E., 1983. Concentration of minerals in tissues of pigs from dams fed different levels of dietar inc. J. Anim. Sci. 57, 130 138.
Ibrahim, W., Lee, U.S., Yeh, C.C., S abo, J., Bruckner, G., Chow, C.K., 1997. O idative stress and antio idant status in mouse liver: effects of dietar lipid, vitamin e and iron. J. Nutr. 127, 1401 1406.
Iost, C., Name, J.J., Jeppsen, R.B., Ashmead, H.D., 1998. Repleating hemoglobinin iron deficienc anemiain oung children through liquid milk fortification with bioavailable iron amino acid chelate. J. Am. Coll. Nutr. 17, 187 194.
Ishii, T., Yasuda, K., Akatsuka, A., Hino, O., Hartman, P.S., Ishii, N., 2005. A mutation in the *SDHC* gene of comple II increases o idative stress, resulting in apoptosis and tumorigenesis. Cancer Res. 65, 203 209.
Kegle , E.B., Spears, J.W., Flowers, W.L., Schoenherr, W.D., 2002. Iron methionine as a source of iron for the neonatal pig. Nutr. Res. 22, 1209 1217.
La risse, M., Gart a Casal, M.N., Solano, L., Baron, M.A., Arguello, F., Llovera, D., Ramire , J., Leets, I., Tropper, E., 2000. Iron bioavailabilit in humans from breakfasts enriched with iron bis gl cine chelate, ph tates and pol phenols. J. Nutr. 130, 2195 2199.
Lee, Y.H., La man, D.K., Bell, R.R., 1981. Glutathione pero idase activit in iron deficient rats. J. Nutr. 111, 194 200.
Lowr , O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265 270.
Nicholls, D.G., Budd, S.L., 2000. Mitochondria and neuronal survival. Ph siol. Rev. 80, 315 360.
National Research Council, 1998. Nutrient Requirements of Swine. In: 10th Revised ed. National Academ Press, Washington, DC.
Oscar, P., Ashmead, H.D., 2001. Effectiveness of treatment of iron deficienc anemia in infants and oung children with ferrous bis gl cinate chelate. Nutrition 17, 381 384.
Rao, J., Jagadeesan, V., 1996. Lipid pero idation and activities of antio idant en mes in iron deficienc and effect of carcinogen feeding. Free Radic. Med. 21, 103 108.
Rincker, M.J., Hill, G.M., Link, J.E., Rowntree, J.E., 2004. Effects of dietar iron supplementation on growth performance, hema tological status, and whole bod mineral concentrations of nurser pigs. J. Anim. Sci. 82, 3189 3197.
Rincker, M.J., Hill, G.M., Link, J.E., Me er, A.M., Rowntree, J.E., 2005. Effects of dietar inc and iron supplementation on mineral e cretion, bod composition, and mineral status of nurser pigs. J. Anim. Sci. 83, 2762 2774.
Roughead, Z.K., Johnson, L.K., Hunt, J.R., 1999. Dietar copper primaril affects antio idant capacit and dietar iron mainl affects iron status in a surface response stud of female rats fed var ing concentrations of iron, inc and copper. J. Nutr. 129, 1368 1376.
SAS Institute, 1988. SAS/STAT User s Guide. SAS Institute Inc., Car , NC.
Shaw, D.T., Ro eboom, D.W., Hill, G.M., Booren, A.M., Link, J.E., 2002. Impact of vitamin and mineral supplement withdrawal and wheat middling inclusion on finishing pig growth performance, fecal mineral concentration, carcass characteristics, and the nutrient content and o idative stabilit of pork. J. Anim. Sci. 80, 2920 2930.
Spears, J.W., Schoenherr, W.S., Kegle , E.B., Flowers, W.L., Alhusen, H.D., 1992. Efficienc of iron methionine as a source of iron for nursing pigs. J. Anim. Sci. 70 (Suppl. 1), 243.
Spears, J.W., Creech, B.A., Flowers, W.L., 1999. Reducing copper and inc in swine waste through dietar manipulation. In: Procedings of North Carolina Waste Managements S mposium, North Carolina State Universit , Raleigh, p. 179.
Spra , C.M., Widdowson, E.M., 1950. The effect of growth and development on the composition of mammals. Br. J. Nutr. 4, 332 353.
Steel, R.G.D., Torrie, J.H., 1960. Principles and Procedures of Statistics. McGraw Hill, New York.
To okuni, S., 1996. Iron induced carcinogenesis: the role of redo regulation. Free Radic. Biol. Med. 20, 553 566.
Tune , I., Montilla, P., Muno , M.C., Medina, F.J., Drucker Colin, R., 2006. Effects of transcranial magnetic stimulation on o idative stress induced b 3 nitropropionic acid in cortical s naptosomes. J. Neurosci. Res. 56, 91 95.
Venturino, A., Anguianl, O.L., Gauna, L., Cocca, C., Bergoc, R.M., Pevhen, A.M., 2001. Thiols and pol amines in the potentiation of malathion to icit in larval stages of toad *Bufo arenarum*. Comp. Biochem. Ph siol. C130, 191 198.
Veum, T.L., Bollinger, D.W., Ellersieck, M., 1995. Proteinated trace minerals and condensed fish protein digest in weanling pig diets. J. Anim. Sci. 73 (Suppl. 1), 308.
Yu, B., Huang, W.J., Chiou, P.W., 2000. Bioavailabilit of iron from amino acid comple in weaning pigs. Anim. Feed Sci. Technol. 86, 39 52.
Zhang, Z.Q., Ren, W.Z., Zhang, J.B., 2006. Effect of Different Iron Levels in Diet of jirongIRONG II Re Rabbit on its Blood En me Activit , vol. 28. Journal of Jilin Agricultural Universit (pp. 433 435, in Chinese).
Zodl, B., Zeiner, M., Marktl, W., Steffan, I., Ekmekcioglu, C., 2003. Pharmacological levels of copper e ert to ic effects in Caco 2 cells. Biol. Trace Elem. Res. 96, 143 152.