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## The effect of iron glycinate chelate on tissue mineral levels, fecal mineral concentration, and liver antioxidant enzyme activity in weanling pigs

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### ABSTRACT

Twenty-four weanling pigs were used to evaluate the effects of iron glycinate chelate (Fe-Gly) on tissue mineral levels, fecal mineral concentration and liver antioxidant enzyme activities of weanling pigs. Pigs were allotted to six treatments based on live weight and litter origin. Treatments consisted of: (1) control (no Fe supplementation); (2) 30 mg Fe/kg diet from Fe-Gly; (3) 60 mg Fe/kg diet from Fe-Gly; (4) 90 mg Fe/kg diet from Fe-Gly; (5) 120 mg Fe/kg diet from Fe-Gly; (6) positive control, 120 mg Fe/kg diet from ferrous sulphate (FeSO<sub>4</sub>). Feeding the diets containing Fe-Gly for 40 days resulted in an increased Fe concentration in heart ( $P < 0.05$ ), liver ( $P < 0.05$ ), kidney ( $P < 0.05$ ), spleen ( $P < 0.05$ ) and feces ( $P < 0.01$ ). There were linear responses to the addition of Fe-Gly from 0 to 120 mg Fe/kg Fe on concentration in the liver and kidney. FeSO<sub>4</sub> 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-Gly/kg compared with 120 mg Fe as FeSO<sub>4</sub>/kg. Linear responses to the addition of Fe-Gly were observed on catalase and succinate dehydrogenase (SDH) activities. 90 mg Fe as Fe-Gly/kg increased SOD ( $P = 0.02$ ) and SDH ( $P = 0.03$ ) activity compared with the negative control. However, there were no significant differences in pancreas mineral concentration, fecal Cu, Zn and Mn concentration and liver anthinone oxidase activities among the treatments ( $P > 0.05$ ).

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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 deficiency in animals or humans (Veum et al., 1995; Spears et al., 1999; Bovell Benjamin et al., 2000; Kegel et al., 2002; Feng et al., 2007). A study showed that chelated or proteinated source of Fe had 125–185% relative availability compared with ferrous sulphate (Henry and Miller, 1995). Research on pigs indicated that iron methionine had a higher bioavailability than ferrous sulphate in nursing pigs (Spears et al., 1992). Mortality, birth and weaning body weight of piglets were improved significantly when sows were fed with iron proteinate (Close, 1998, 1999). Yu et al. (2000) found iron from an amino acid complex increased plasma iron and total iron binding capacity 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 glycine could be absorbed and utilized easily, and maintain high iron bioavailability in rats or humans, despite the presence of iron absorption inhibitory factors such as phytic acid (Allen et al., 1998; Iost et al., 1998; Lührs et al., 2000; Oscar and Ashmead, 2001). Iron glycine chelate (Fe-Gly) is currently used as an efficient iron fortificant in human food, especially in infant food (Follet et al., 1998; Giorgini et al., 2001). In a previous study it was found that, at an appropriate dosage, Fe-Gly 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 dietary Fe-Gly on tissue mineral status, fecal mineral concentration, and liver antioxidant enzyme activity in weanling pigs.

## 2. Materials and methods

### 2.1. Animals and experimental design

One hundred and eighty-three 35-day-old piglets (Duroc × Landrace × Yorkshire) weighing  $7.8 \pm 0.72$  kg were blocked based on weight, sex and ancestry and randomly allotted to six dietary treatments, each of which was replicated three times with 10 pigs per replicate. Treatments consisted of: (1) control (no Fe supplementation); (2) 30 mg Fe from Fe-Gly/kg diet; (3) 60 mg Fe from Fe-Gly/kg diet; (4) 90 mg Fe from Fe-Gly/kg diet; (5) 120 mg Fe from Fe-Gly/kg diet; (6) positive control, 120 mg Fe from ferrous sulphate ( $\text{FeSO}_4$ )/kg diet.

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

**Table 1**

Composition of basal diet (as fed basis)

Ingredient	g/kg	Composition <sup>a</sup>	
Maize	543.5	DE (MJ/kg)	14.38
Soybean meal	170	Crude protein (g/kg)	207.2
Extruded soybean	100	Ether extract (g/kg)	4.3
Wheat	80	Calcium (g/kg)	10.5
Fish meal	60	Phosphorus (g/kg)	7.6
Wheat middling	10	Lysine (g/kg)	13.5
Calcium hydroxide phosphate	10	Fe (mg/kg)	79
Limestone	8	Cu (mg/kg)	58
Soybean oil	5	Zn (mg/kg)	146
Vitamin mineral premix <sup>b</sup>	10	Mn (mg/kg)	74
Salt	2		
Lysine	1.5		

<sup>a</sup> DE based on calculated values, others were analyzed values.

<sup>b</sup> 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 humanely slaughtered. Heart, liver, kidney, spleen and pancreas samples were excised and immediately stored at  $-70^{\circ}\text{C}$  until analysis for antioxidant enzyme activities and mineral concentrations. Fecal samples were freeze-dried and frozen at  $-20^{\circ}\text{C}$  until mineral analysis.

## 2.3. Determination of mineral concentration in tissues and feces

Fecal samples were prepared for mineral analysis using a method described by Armstrong et al. (2004). Uniform samples were cut from tissues, wet digested using nitric perchloric acid and then diluted with deionized distilled water for analyses of minerals (Hill et al., 1983). Contents of Fe, Cu, Zn, and Mn were analyzed with flame atomic absorption spectrophotometry (AA 6300, Shimadzu Corp., Tokyo, Japan).

## 2.4. Measurement of SOD, CAT, SDH and XOD activities

Liver samples were homogenized in 0.1 M Tris-HCl buffer at  $4^{\circ}\text{C}$ , pH 7.4, to make a 10% (w/v) homogenate, using a polytron homogenizer for 5 min and a sonic homogenizer for 3 min. The homogenates were centrifuged at  $3000 \times g$  for 5 min at  $4^{\circ}\text{C}$  and then the supernatants were collected and stored at  $-20^{\circ}\text{C}$  for enzyme analysis. Liver Cu/Zn SOD activities were determined with the methods of Shaw et al. (2002). Assay for catalase (CAT) activity was performed by following the reduction in  $\text{H}_2\text{O}_2$  absorbance at 240 nm as reported by Venturini et al. (2001). Succinate dehydrogenase (SDH) activity was determined by the method of Tune et al. (2006). Xanthine oxidase (XOD) activity was measured according to the method described by Hashimoto (1974). Protein was estimated by the method of Lowry et al. (1951). Units of SOD, CAT, SDH and XOD activities were expressed as per milligram of protein.

## 2.5. Statistical analysis

Data were analyzed by ANOVA as a randomized complete block design using the GLM procedures of SAS (1988). Individual pigs were the experimental unit for all indices. The planned single df tests included the linear and quadratic effects of Fe $\text{G}_2$ , the control versus  $\text{FeSO}_4$  (120 mg Fe/kg),  $\text{FeSO}_4$  versus Fe $\text{G}_2$  (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 $\text{G}_2$  on tissue mineral concentrations of weanling pigs are presented in Table 2. Increasing dietary Fe $\text{G}_2$  levels increased the Fe content of the heart ( $P=0.02$ ), the liver ( $P=0.003$ ), the kidney ( $P=0.005$ ) and the spleen ( $P=0.001$ ), the highest organ concentrations occurring in the animals receiving the highest amount of Fe $\text{G}_2$ . Moreover, there were linear responses to the addition of Fe $\text{G}_2$  from 0 to 120 mg Fe/kg on Zn concentration in liver and kidney. Compared to the negative control, 120 mg  $\text{FeSO}_4$ /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 $\text{G}_2$  compared with 120 mg/kg Fe as  $\text{FeSO}_4$  ( $P=0.005$ ). However, there were no significant differences in pancreas mineral contents when pigs were offered different levels of iron as Fe $\text{G}_2$  and  $\text{FeSO}_4$  compared with control ( $P>0.05$ ).

**Table 2**Effect of amount and chemical composition of iron on tissue trace element levels in weanling pigs<sup>a</sup>

Item <sup>b</sup>	Fe Gly <sup>c</sup>					FeSO <sub>4</sub> <sup>c</sup>	S.E.M. <sup>d</sup>	P value			
	0 <sup>e</sup>	30 <sup>e</sup>	60 <sup>e</sup>	90 <sup>e</sup>	120 <sup>e</sup>	120 <sup>e</sup>		Control vs. FeSO <sub>4</sub>	FeSO <sub>4</sub> vs. Fe-Gly	Fe Gly	
										Linear	Quad
<b>Heart (mg/kg)</b>											
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
<b>Liver (mg/kg)</b>											
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
<b>Kidney (mg/kg)</b>											
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
<b>Pancreas (mg/kg)</b>											
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
<b>Spleen (mg/kg)</b>											
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

<sup>a</sup> Non orthogonal comparisons between the control vs. FeSO<sub>4</sub> (120 mg/kg), and the FeSO<sub>4</sub> (120 mg/kg) vs. Fe-Gly (120 mg/kg) treatments. Linear and quadratic effects of increasing Fe concentrations in Fe Gly form (0 to 120 mg/kg).

<sup>b</sup> Tissue levels are expressed per kg wet weight.

<sup>c</sup> Fe source.

<sup>d</sup> S.E.M. stands for standard error of the mean.

<sup>e</sup> Fe addition (mg/kg).

### 3.2. Fecal mineral concentrations

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

Fecal Fe concentration increased linearly with the increasing dietary Fe Gly levels (P=0.002), and reached the highest level in 120 mg Fe as Fe Gly/kg. Moreover, 120 mg Fe as FeSO<sub>4</sub>/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 Gly/kg compared with diet in addition with 120 mg Fe as FeSO<sub>4</sub>/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 Gly on liver SOD, CAT, XOD, and SDH activity in weanling pigs. There were linear responses to the addition of Fe Gly on CAT and SDH activities (P=0.41 and P=0.001, respectively). As shown in the figure, 90 mg Fe as Fe Gly/kg increased SOD (P=0.02) and SDH (P=0.03) activity. No significant response to XOD could be found among the Fe Gly, FeSO<sub>4</sub> treatments and the control.

**Table 3**

Effect of amount and chemical composition of iron on fecal mineral concentrations in weanling pigs<sup>a</sup>

Item <sup>b</sup>	Fe-Gly <sup>c</sup>					FeSO <sub>4</sub> <sup>c</sup>	S.E.M. <sup>d</sup>	P value			
	0 <sup>e</sup>	30 <sup>e</sup>	60 <sup>e</sup>	90 <sup>e</sup>	120 <sup>e</sup>			Control vs. FeSO <sub>4</sub>		FeSO <sub>4</sub> vs. Fe-Gly	
							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

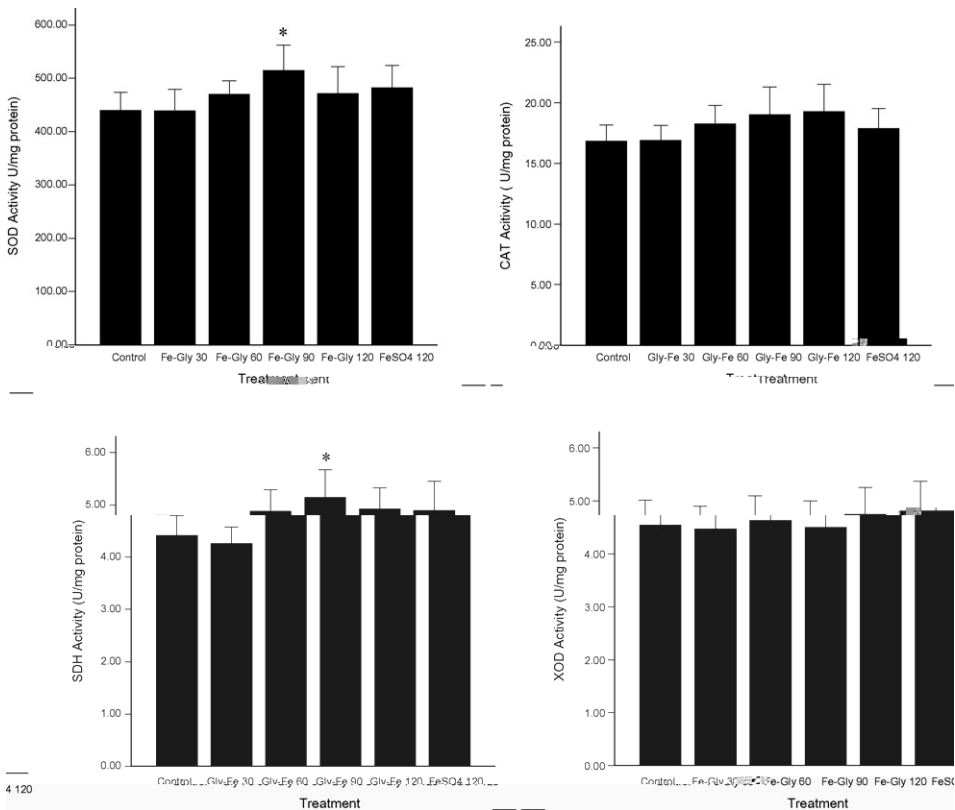
<sup>a</sup> Non orthogonal comparisons between the control vs. FeSO<sub>4</sub> (120 mg/kg), and the FeSO<sub>4</sub> (120 mg/kg) vs. Fe-Gly (120 mg/kg) treatments. Linear and quadratic effects of increasing Fe concentrations in Fe-Gly form (0 to 120 mg/kg).

<sup>b</sup> The trace elements are expressed as mg/kg dry matter.

<sup>c</sup> Fe source.

<sup>d</sup> S.E.M. stands for the standard error of the mean.

<sup>e</sup> Fe addition (mg/kg).



**Fig. 1.** The effects of iron glycine 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-Gly groups supplement 30–120 mg Fe as Fe-glycine chelate/kg diet as, FeSO<sub>4</sub> 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 usually used to evaluate mineral status of animals and humans. The present study showed that Fe concentration in heart, liver, kidney and spleen, but not in the pancreas increased with the increasing levels of Fe as Fe G<sub>2</sub> in weanling pigs. Spray and Widdowson (1950) compared nursing pigs receiving a daily 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 greatly increased the amount of Fe in the body. Furugouri (1972) also reported a linear decrease in liver ferritin, nonheme and total Fe when dietary Fe decreased. When nursing pigs were fed diets supplemented with 0, 25, 50, 100, 150 ppm iron in the diet (as fed basis) from ferrous sulphate, whole body iron stores increased linearly due to increasing dietary iron concentrations (Rincker et al., 2004, 2005). Yu et al. (2000) reported that total iron in the liver, spleen, and muscle significantly 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 dietary Fe concentration reported in the current study. The present results also showed that there were linear responses to the addition of Fe G<sub>2</sub> from 0 to 120 mg/kg Fe on Zn concentration in liver, kidney and pancreas. Hill and Matrone (1970) reported that the trace minerals Cu, Fe, and Zn are transition metals, which have similar chemical and physical 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 dietary Fe concentration resulted in a linear increase in dietary Fe ( $P = 0.001$ ), dietary Zn ( $P = 0.003$ ), fecal Fe ( $P = 0.001$ ) excretion and fecal Zn ( $P = 0.020$ ) excretion. Iron glycinate has been proved to have high iron bioavailability in animal or human. It has been suggested that the higher bioavailability of iron glycinate is probably due to the chemical structure of this compound, which partially prevents iron phosphate interactions (Bovell Benjamin et al., 2000; Larisse et al., 2000). Galdi et al. (1988) reported higher absorption in anemic rats fed iron glycinate compared with ferrous sulphate. Bovell Benjamin et al. (2000) conducted a comparative study of the absorption of iron from ferrous glycinate and iron sulphate in a whole maize meal and found a significantly greater geometric mean percentage of iron was absorbed from ferrous glycinate (6.8%) than from FeSO<sub>4</sub> (1%). Larisse et al. (2000) showed that twice as much iron was absorbed as from foods fortified with ferrous glycinate than from FeSO<sub>4</sub> fortified foods. In the present study, spleen Fe retention was improved when pigs were fed iron as Fe G<sub>2</sub> compared with 120 mg/kg Fe as FeSO<sub>4</sub> treatment. This may be related with the good absorption of iron glycinate implicating that the bioavailability of iron from Fe G<sub>2</sub> is higher than that of iron from ferrous sulphate.

Analysis of fecal mineral concentration indicated that fecal Fe concentrations were enhanced ( $P < 0.05$ ) as the dietary concentration of Fe as Fe G<sub>2</sub> increased, and 120 mg/kg Fe as FeSO<sub>4</sub> 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) (Creach et al., 2004). Increasing the dietary Fe (0–150 mg/kg) as iron sulphate resulted in a linear increase in fecal Fe excretion ( $P < 0.01$ ) (Rincker et al., 2005). There also existed a trend for a decrease when pigs fed diet supplemental 120 mg/kg Fe as Fe G<sub>2</sub> compared with diet in addition with 120 mg Fe as FeSO<sub>4</sub>/kg ( $P = 0.09$ ). This finding combined with the results of tissue Fe storage supports the view that iron chelated with glycinate is better absorbed and utilized than iron sulphate.

Iron is an essential micronutrient, but excess intake and storage of iron induces increased production of reactive oxygen species (ROS) and is thought to cause various diseases (Tokuni, 1996; Fiers et al., 1999; Nicholls and Budd, 2000; Zodl et al., 2003). CAT and SOD are considered the primary antioxidant enzymes because they are involved in the direct elimination of ROS (Beckman et al., 1988; Rao and Jagadeesan, 1996). The present study indicated that CAT activity increased with the addition of Fe G<sub>2</sub>. This agrees with the studies performed with rats. Lee et al. (1981) observed reduced CAT activity in the RBC and liver of Fe deficient rats. Brandsch et al. (2002) found that catalase activity in rat liver increased by feeding high iron diets, and they postulated it was because of increased iron concentrations in the liver rather than to induction by oxidative stress. Moderate dietary iron excess ( $\leq 400$  mg iron/kg diet) did not affect the SOD activity in rat liver (Bristow Craig et al., 1994; Ibrahim et al., 1997), but SOD activity decreased when rat fed with iron deficient diet (Rao and Jagadeesan,

1996). Increased SOD activity was observed when pigs fed with 90 mg Fe as Fe G<sub>2</sub>/kg in the present study. Rincker et al. (2004) thought even though Fe contributed by feed ingredients provided basal dietary Fe concentrations in excess of the NRC (1998) postweaning requirement (80 mg/kg), the dietary 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 by dietary Fe ingredients to alleviate severe decreases in Fe stores. In the present experiment, basal diet contained 79 mg Fe/kg which just met the requirement for postweaning pigs. The marginal Fe concentration of the basal diet probably caused SOD to be abnormally 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 activity resulted in an increased production of ROS. Zhang et al. (2006) noted that increasing the dietary concentration of Fe as FeSO<sub>4</sub> (0–120 mg of Fe of per kg diet) resulted in an increase in SDH activity in the blood of Red rabbits. This is in accordance with current study. It has been shown that adding exogenous XOD to generate free radicals can damage muscle function in animal (Barclay and Hansel, 1991). XOD activity was not affected by Fe G<sub>2</sub> or FeSO<sub>4</sub> addition in diet of piglet in present study. Feeding rats a wide range of dietary Fe up to 10 times the estimated requirement, did not induce overt oxidative stress (Roughhead et al., 1999). This indicates that the moderate high intake of Fe in the present study probably does not pose a major risk in increasing oxidative stress in weanling pigs, although this point warrants further research.

In conclusion, the results obtained from the current study indicate that supplementation with Fe G<sub>2</sub> could improve iron tissue storage and antioxidant enzyme activities, and also could increase Zinc retention in liver and kidney in weanling pig. Additionally, a reduction in fecal Fe concentrations could be found when pigs were fed diets containing Fe as Fe G<sub>2</sub> compared to FeSO<sub>4</sub>.

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