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Effect of replacing dietary $FeSO_4$ with equal Fe-levelled iron glycine chelate on broiler chickens

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ABSTRACT: Iron (Fe) is an essential mineral for animal development and function. The present study was carried out to evaluate the effect of replacing $FeSO_4$ with iron glycine chelate (Fe-Gly) in the equal Fe level in diets on broiler chickens. The broilers were randomly allotted to 6 dietary treatments with 5 replicate pens and 10 birds per pen. The treatments consisted of: Control group (100 mg Fe from $FeSO_4$/kg diet), Experimental group 1 (80 mg Fe from $FeSO_4$ + 20 mg Fe from Fe-Gly/kg diet), Experimental group 2 (60 mg Fe from $FeSO_4$ + 40 mg Fe from Fe-Gly/kg diet), Experimental group 3 (40 mg Fe from $FeSO_4$ + 60 mg Fe from Fe-Gly/kg diet), Experimental group 4 (20 mg Fe from $FeSO_4$ + 80 mg Fe from Fe-Gly/kg diet), and Experimental group 5 (100 mg Fe from Fe-Gly/kg diet). The results showed that replacing $FeSO_4$ with Fe-Gly in the diets did not significantly improve broiler growth performance ($P > 0.05$). But it significantly ($P < 0.05$) improved the blood biochemical parameters. Xanthione oxidase activity in blood serum showed no significant difference between all treatments at day 21 except for Experimental group 5 ($P > 0.05$). In addition, catalase activity in blood serum and Cu/Zn superoxide dismutase activity in liver were increased with the increasing replacement level of Fe-Gly ($P < 0.05$). But for all of the above indicators, the observed values of Experimental groups 3, 4, and 5 did not significantly differ ($P > 0.05$). This study indicates that replacing $FeSO_4$ with Fe-Gly in the equal Fe level in the diets cannot improve the growth performance of broilers. But it can effectively improve the blood biochemical parameters and antioxidative enzyme activity. The least substitution ratio for low feeding cost and beneficial effect on the broilers was 60%.

Keywords: broiler; amino acid; chelated iron; growth performance; blood biochemical parameters; antioxidant enzyme activity

INTRODUCTION

Iron (Fe) is an essential trace element for broiler growth, which has many functions, e.g. in energy metabolism, neurotransmitter synthesis, and phagocyte antimicrobial activity, as well as in the synthesis of DNA, collagen, and bile acids (Brock 1994). According to NRC (1994), a level of 80 mg Fe/kg dry matter has been recommended in the diet for broiler chickens. To allow the bird to reach its genetic growth potential, it is a common way to add Fe in diet, and Fe is traditionally supplied in the form of sulfates.

During the past several decades, studies have shown that chelated or proteinated sources of Fe have higher relative availability compared with inorganic Fe (Bovell-Benjamin et al. 2000; Kegley et al. 2002; Ji et al. 2007, 2009). Recent research on organic minerals proved that chelate minerals can be more effectively absorbed in the intestines than inorganic oxide and sulfate (Wedekind et al. 1992; Aoyagi and Baker 1993). Accordingly, chelated Fe, especially Fe-Gly, has been used more frequently as the Fe fortification in animal nutrition. Ma et al. (2012) reported that addition of Fe-Gly im-

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proved the growth performance, Fe tissue storage, and antioxidant status of broiler chickens. Some previous studies revealed that Fe-Gly at an appropriate dosage improved growth performance, hematological and immunological characteristics, Fe tissue storage, and antioxidant enzyme activity in weanling pigs (Ji et al. 2007, 2009).

However, some studies showed that dietary Fe-Gly had no significant effects on feed intake, feed/gain ratio, blood immunoglobulins concentration, and B lymphocyte proliferation in weanling piglets (Ji et al. 2007). And Bao et al. (2007) showed that there was no significant difference in body weight gain between animals supplemented with organic trace minerals and the positive control (inorganic form). Nollet et al. (2007) reported that replacing inorganic minerals with organic sources had no effect on the performance parameters of broiler. The discrepancies among the reports on the effect of organic Fe might be explained by differences in the animal species, experimental method, and quality of organic Fe used in these studies. The common point of these results was that the researchers fed the broilers a lower level of Fe from Fe-Gly than from $FeSO_4$. In our experiment $FeSO_4$ was replaced with Fe-Gly in the equal Fe level in broiler diets. Additionally, Arbor Acres chosen for the present experiment is a commonly bred broiler species in China, and until now less data on its feeding Fe-Gly has been at disposal. The main objectives were to investigate the effects of replacing $FeSO_4$ with Fe-Gly on the broiler chickens performance and to provide reference for the application of Fe-Gly in broiler production.

MATERIAL AND METHODS

Fe sources. In our experiment, reagent-grade Fe sulfate ($FeSO_4 \cdot 7H_2O$; Tianjin Tianli Chemical Industry Co., Ltd., Tianjin, China) with Fe concentration of more than 20.14%, and iron glycine chelate (Fe-Gly; Guangzhou Tanke Technology Co., Ltd., Guangdong, China) with Fe concentration of more than 14% were used. In our experiment, the Fe content was indicated the amount of Fe element.

Animals and experimental design. A total of 300 1-day-old Arbor Acres commercial broilers with a body weight of 45 ± 1 g were used in a 21-day experiment. The broilers were randomly allotted to 6 dietary treatments with 5 replicate pens and 10 birds per pen. The basal maize-soybean meal diet (Table 1) was formulated to meet NRC (1994) requirements for starting chickens, except for Fe. Six treatments were kept at the same Fe level of 100 mg/kg diet, consisting of: Control group (100 mg Fe from $FeSO_4$/kg diet), Experimental group 1 (80 mg Fe from $FeSO_4$ + 20 mg Fe from Fe-Gly/kg diet), Experimental group 2 (60 mg Fe from $FeSO_4$ + 40 mg Fe from Fe-Gly/kg diet), Experimental group 3 (40 mg Fe from $FeSO_4$ + 60 mg Fe from Fe-Gly/kg diet), Experimental group 4 (20 mg Fe from $FeSO_4$ + 80 mg Fe from Fe-Gly/kg

Table 1. Ingredient and nutrient composition of basal diets

Ingredient	g/kg	Composition[a]	%
Maize	547.6	ME (MJ/kg)	12.62
Soybean meal	348.6	crude protein	21.53
Fish meal	35	lysine	1.23
Soybean oil	36	calcium	0.33
Dicalcium phosphate	12	nonphytate phosphorus	0.46
Limestone	13	methionine + cysteine	0.87
D,L-Methionine	1.6		
Salt	3		
Choline	1		
Mineral mixtures	2		
Vitamin premix	0.2		

[a]provided per kg of diet: vitamin A 13 500 IU, vitamin D3 3600 IU, vitamin E 33 IU, vitamin K3 6 mg, vitamin B1 4.5 mg, vitamin B2 10.5 mg, vitamin B6 6 mg, vitamin B12 0.03 mg, calcium pantothenate 18 mg, niacin 60 mg, folic acid 1.8 mg, biotin 0.165 mg, choline 1000 mg, Fe 100 mg, Cu 8 mg, Mn 120 mg, Se 0.3 mg, I 0.7 mg; metabolizable energy (ME) based on calculated values, other results based on analysis

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Table 2. Experimental treatments

Supplemental Fe concentration		mg/kg
Control group	$FeSO_4$	100
	Fe-Gly	0
Experimental group 1	$FeSO_4$	80
	Fe-Gly	20
Experimental group 2	$FeSO_4$	60
	Fe-Gly	40
Experimental group 3	$FeSO_4$	40
	Fe-Gly	60
Experimental group 4	$FeSO_4$	20
	Fe-Gly	80
Experimental group 5	$FeSO_4$	0
	Fe-Gly	100

diet), and Experimental group 5 (100 mg Fe from Fe-Gly/kg diet) (Table 2).

The broilers were reared according to standard routine practices. The birds were housed in stainless steel battery cages on concrete floor covered with clean rice bran and hulls. The experimental house was kept at 33°C during the first 3 days and then the temperature was reduced by 3°C per week until it reached 24°C. Light of 24 h/day was provided via fluorescent lights. The broilers were given *ad libitum* access to feed and water. On day 1, the broilers were vaccinated against infectious bronchitis, and Newcastle disease vaccination was performed on day 8.

Sample collection. On days 1 and 21 of the feeding trial, the weight of broilers and feed consumption were measured to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G).

At the end of the experiment, the broilers were fasted overnight, and 25 chickens (5 per pen) were randomly selected from each treatment and blood samples were then collected from the wing vein using a sterilized syringe. After collection, 2 ml of the blood sample/chicken were transferred into an EDTA-K_2 tube (Jiangsu Yu Li Medical Device Co., Ltd., Jiangsu, China) immediately to analyze hemoglobin (HGB), red blood cell (RBC), and hematocrit (HCT). And 10-ml blood samples/chicken were kept in constant temperature water bath at 37°C for 30 min, and then centrifuged at 3000 rpm for 15 min to separate the serum, which was transferred to 5 ml Eppendorf tubes and frozen at –20°C until analysis. Then the broilers were euthanized using sodium pentobarbital. Liver samples were removed from the carcasses and immediately stored at –80°C until analysis for antioxidant enzyme activity.

Sample measurements

Blood biochemical parameters. Hemoglobin (HGB; g/l), red blood cells (RBC; $\times 10^{12}$/l), and hematocrit (HCT; %) were analyzed using a Harbin Electric Power Hospital (Harbin Electric Company Ltd., Harbin, China). Total Fe binding capacity (TIBC; mg/l) in serum was determined by assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to manufacturer's instructions. Absorbance of the sample was measured by UVmini-1240 spectrophotometer (Shimadzu Corp., Tokyo, Japan).

Antioxidant enzyme activity. Liver samples were prepared for analysis according to the method of Ji et al. (2009). Liver Cu/Zn superoxide dismutase (SOD) activity was determined by the method of Shaw et al. (2002). Protein was measured by the method of Lowry et al. (1951). A unit of SOD activity was expressed as the activity of an enzyme per mg protein.

The activities of catalase (CAT) and xanthione oxidase (XOD) in serum were determined by assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), following the procedure of the manufacturer's instructions. Absorbance of the sample was recorded by spectrophotometer UVmini-1240 (Shimadzu Corp.). Units of CAT and XOD activity were expressed as the activity of an enzyme per ml serum. At least duplicate determination was carried out for each serum sample in all enzyme assays.

Statistical analysis. The analyses were carried out using the SPSS software (Version 17.0, 2008). Data for all the treatments were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test. And $P < 0.05$ as a criterion of statistical significance was declared.

RESULTS AND DISCUSSION

Growth performance. The effects of the treatments on broiler chickens' growth performance are summarized in Table 3. From the values measured on day 21 of the experiment it was concluded that replacing $FeSO_4$ with Fe-Gly in the equal Fe level in the diets did not significantly influence the broilers growth performance ($P > 0.05$).

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Table 3. Effects of replacing $FeSO_4$ with Fe-Gly in the equal Fe (mg/kg) level in the diets on growth performance

Treatment	$FeSO_4$	Fe-Gly	ADG (g)	ADFI (g)	F/G
Control group	100	0	30.54	48.20	1.578
Experimental group 1	80	20	30.52	48.13	1.577
Experimental group 2	60	40	30.55	48.22	1.579
Experimental group 3	40	60	30.56	48.24	1.578
Experimental group 4	20	80	30.51	48.03	1.576
Experimental group 5	0	100	30.51	48.01	1.574
SEM			0.479	0.196	0.008
P-value			0.374	0.265	0.882

ADG = average daily gain, ADFI = average daily feed intake, F/G = feed : gain ratio, SEM = standard error of the mean

Fe deficiency is one of the most common potential mineral deficiencies in broilers. Most of the dietary Fe sources for broilers come from feed. According to NRC (1994), the minimum dietary Fe requirement of broilers is 80 mg/kg. Our study showed that 100 mg/kg Fe from $FeSO_4$ (Control group) was sufficient for maximum growth. However, replacing $FeSO_4$ with Fe-Gly in the equal level in the diet did not improve the broilers growth performance. This was supported by the studies of Nollet et al. (2007) and Wang et al. (2008) stating that birds fed lower levels of chelated minerals displayed no significant difference in body weight gain compared to those fed higher levels of inorganic minerals. It was concluded that the form of Fe source is not important for broiler growth on condition of sufficient doses of Fe intake.

Blood biochemical parameters. The results of blood biochemical analyses are presented in Table 4. From the parameters measured on day 21 of the experiment it was concluded that replacing $FeSO_4$ with Fe-Gly in the equal Fe level in the diets significantly ($P < 0.05$) affected blood biochemical parameters. Compared with the Control, Experimental groups 1–5 improved HGB by 2.91, 1.59, 5.56, 7.14, and 6.35%, RBC by 1.27, 2.11, 3.38, 4.52, and 2.53%, and HCT by 5.86, 8.78, 11.96, 9.68, and 12.68%, respectively. However, the TIBC for all the groups except Experimental group 1 decreased significantly ($P < 0.05$) as compared to the Control. A significant negative correlation ($P < 0.05$) was found between the Fe-Gly replacement level and TIBC. But for all of the above indicators except RBC, the observed values in Experimental groups 3–5 were not significantly different ($P > 0.05$).

In our experiment, the values of HGB, RBC, HCT, and TIBC were within physiological limits (Feldman et al. 2000). Fe is an important constituent element of HGB, RBC, and HCT; furthermore the Fe content directly decides on their generation. TIBC is the blood capacity to bind Fe with

Table 4. Effects of replacing $FeSO_4$ with Fe-Gly in the equal Fe (mg/kg) level in the diets on blood biochemical parameters

Treatment	$FeSO_4$	Fe-Gly	HGB (g/l)	RBC (10^{12}/l)	HCT (%)	TIBC (mg/l)
Control group	100	0	75.60^{c}	2.37^{d}	29.02^{c}	2.49^{a}
Experimental group 1	80	20	77.80^{bc}	2.40^{c}	30.72^{b}	2.47^{a}
Experimental group 2	60	40	76.80^{c}	2.42^{bc}	31.57^{ab}	2.38^{b}
Experimental group 3	40	60	79.80^{ab}	2.45^{ab}	32.49^{a}	2.34^{bc}
Experimental group 4	20	80	81.00^{a}	2.47^{a}	31.83^{ab}	2.29^{c}
Experimental group 5	0	100	80.40^{ab}	2.43^{bc}	32.70^{a}	2.26^{c}
SEM			0.50	0.01	0.29	0.02
P-value			0.001	0.013	0.007	0.000

HGB = hemoglobin, RBC = red blood cell, HCT = hematocrit, TIBC = total Fe binding capacity, SEM = standard error of the mean

$^{a–d}$values within a row with different superscripts differ ($P < 0.05$)

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transferrin or the amount of Fe needed for 100% saturation of transferrin. TIBC often decreases with the increasing serum Fe concentration. Our study showed that replacing $FeSO_4$ with Fe-Gly in the equal Fe level in the diets significantly influenced the blood biochemical parameters ($P < 0.05$).

Fe deficiency is one of the most common potential mineral deficiencies in animals, which produces microcytic hypochromic anemia in chickens. To avoid this, in practice Fe is overdosed to safely cover the broilers' dietary need. Langini et al. (1988) reported that ferrous glycinate absorption was better than that of $FeSO_4$. And, ferrous glycinate was of great benefit to precaution and treatment of Fe deficiency anemia in humans, especially in infants or young children (Iost et al. 1998; Pineda and Ashmead 2001). Glycine has the lowest molecular weight of all the amino acids, which favours the stability of the chelate compounds, preventing the ferrous ion from undesirable chemical reactions in the stomach and intestines that limit the absorption of Fe (Kulkarni et al. 2011). Layrisse et al. (2000) reported that the absorption of Fe in Fe-Gly was twice that of $FeSO_4$ in a breakfast meal based on maize flour. In an isotope ^{59}Fe experiment, Fe absorption rate from Fe-Gly was 30.9% in weanling rats; however, that from sulfate was only 15.8% (Langini et al. 1988). Therefore, in the current study, the positive effect of Fe-Gly on blood biochemical parameters may be attributed to its higher absorption.

Antioxidant enzyme activity. As shown in Table 5, XOD activity in blood serum of Experimental group 5 was the lowest, not differing from that of Experimental group 4 ($P > 0.05$). However, no differences in the other items were observed between the groups; SOD value in Experimental group 4 was higher than that in Control group ($P < 0.05$); the values of CAT in groups 3 an 4 were higher than those in the Control group ($P < 0.05$), but there was no significant difference between groups 3 and 4.

Fe is an essential component of haemoglobin and myoglobin. It is also present in various enzymes such as cytochrome, catalase, and peroxidases. Under a continuous excessive Fe intake, free radicals could be produced (Rao and Jagadeesan 1996; Nicholls and Budd 2000). It is known that reactive oxygen species (ROS) are highly toxic, and they can produce a variety of pathological changes through lipid peroxidation and DNA damage. McArdle and Jackson (2000) have documented that a small increase in ROS could induce the expression of antioxidants. SOD, glutathione peroxidase (GSH-Px), and CAT are main antioxidant enzymes in the body, capable of capturing excessive oxidants (Ko et al. 2004). The function of SOD is to convert the active oxygen groups into H_2O_2, and CAT is responsible for the destruction of excessive H_2O_2 (Kohen and Nyska 2002). Accordingly, the activities of SOD and CAT are commonly combined in the investigation of the extent of damage to the whole body. Our study showed that replacing $FeSO_4$ with Fe-Gly increased the activity of SOD and CAT. This agrees with another study performed in rats by Davis and Yi (1999) showing that dietary Fe (140 mg Fe/kg diet) causes a significantly increased SOD activity in rat liver.

XOD is also an enzyme related to the production of free radicals when tissues lack oxygen. Barclay and Hansel (1991) reported that adding exogenous XOD can damage muscle function in

Table 5. Effects of replacing $FeSO_4$ with Fe-Gly in the equal Fe (mg/kg) level in the diets on antioxidant enzyme activity

Treatment	$FeSO_4$	Fe-Gly	SOD	XOD	CAT
Control group	100	0	152.2^{b}	2.40^{a}	14.56^{c}
Experimental group 1	80	20	158.2^{ab}	2.38^{a}	14.75^{bc}
Experimental group 2	60	40	156.4^{ab}	2.42^{a}	15.10^{ab}
Experimental group 3	40	60	162.2^{ab}	2.34^{a}	15.26^{a}
Experimental group 4	20	80	164.8^{a}	2.29^{ab}	15.37^{a}
Experimental group 5	0	100	163.2^{ab}	2.18^{b}	15.23^{a}
SEM			1.628	0.022	0.075
P-value			0.206	0.008	0.002

SOD = liver Cu/Zn superoxide dismutase (U/mg protein), XOD = xanthione oxidase (U/ml), CAT = catalase (U/ml), SEM = standard error of the mean

$^{a–c}$values within a row with different superscripts differ ($P < 0.05$)

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animal because of generating free radicals. In our study, the XOD activity was decreasing with the increasing rate of Fe-Gly substitution except Experimental group 3. But no marked differences were observed between the treatments except Experimental group 5 ($P > 0.05$). In agreement with Davis and Yi (1999), XOD activity was not affected by Fe-Gly or $FeSO_4$ addition in piglets diet.

The results show that the diets where $FeSO_4$ is replaced with Fe-Gly are more effective for broilers. And Fe-Gly can improve the antioxidant status of broilers by elevating the activity of the antioxidant enzyme. This observation is important for Fe-Gly use and supports the idea that Fe is required for the activities of SOD and CAT.

Upon all of the above indicators (blood biochemical parameters and antioxidant enzyme activity), no significant difference was observed between the measured values of Experimental groups 3–5. So we can conclude that the least substitute ratio of replacing $FeSO_4$ with Fe-Gly in the diets for low feeding cost and beneficial effect on the broiler was 60%.

CONCLUSION

Under the experimental conditions, it could be concluded that replacing $FeSO_4$ with Fe-Gly in the equal Fe level in the diets cannot improve the growth performance of broilers. But it is of great benefit to the blood biochemical parameters and antioxidant enzyme activity of broilers. The least substitute proportion for low feeding cost and beneficial effect on the broiler is 60%.

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