Effects of Iron Glycine Chelate on Growth, Tissue Mineral Concentrations, Fecal Mineral Excretion, and Liver Antioxidant Enzyme Activities in Broilers

W. Q. Ma · H. Sun · Y. Zhou · J. Wu · J. Feng

Abstract The study was conducted to determine the effects of iron glycine chelate (Fe-Gly) on growth, tissue mineral concentrations, fecal mineral excretion, and liver antioxidant enzyme activities in broilers. A total of 360 1-day-old commercial broilers (Ross × Ross) were randomly allotted to six dietary treatments with six replications of ten chicks per replicate. Broilers were fed a control diet with no Fe supplementation, while five other treatments consisted of 40, 80, 120, and 160 mg Fe/kg diets from Fe-Gly, and 160 mg Fe/kg from ferrous sulfate, respectively. After a 42-day feeding trial, the results showed that 120 and 160 mg Fe/kg as Fe-Gly improved the average daily gain ($P<0.05$) and average daily feed intake ($P<0.05$) of broilers (4–6 weeks). Addition with 120 and 160 mg Fe/kg from Fe-Gly and 160 mg Fe/kg from $FeSO_4$ increased Fe concentration in serum ($P<0.05$), liver ($P<0.05$), breast muscle ($P<0.05$), tibia ($P<0.05$), and feces ($P<0.01$) at 21 and 42 days. There were linear responses to the addition of Fe-Gly from 0 to 160 mg/kg Fe on Fe concentration in serum (21 days, $P=0.005$; 42 days, $P=0.001$), liver ($P=0.001$), breast muscle ($P=0.001$), tibia ($P=0.001$), and feces (21 days, $P=0.011$; 42 days, $P=0.032$). Liver Cu/Zn superoxide dismutase activities of chicks were increased by the addition of 80, 120, and 160 mg Fe/kg as Fe-Gly to diets at 42 days. There were no differences in liver catalase activities of chicks among the treatments ($P>0.05$). This study indicates that addition with Fe-Gly could improve growth performance and iron tissue storage and improves the antioxidant status of broiler chickens.

Keywords Iron glycine chelate · Growth · Mineral content · Antioxidant enzyme · Broiler

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Introduction

Iron (Fe) is an essential trace element for broiler growth, which functions in numerous energy metabolism, neurotransmitter synthesis, and phagocyte antimicrobial activity, as well as the synthesis of DNA, collagen, and bile acids [1]. A level of 80 mg Fe/kg dry matter has been recommended in the diet for broiler chickens [2]. Traditionally, Fe is supplemented in the form of inorganic salts, such as sulfates, oxides, and carbonates, to allow the bird to reach its genetic growth potential [3].

In recent decades, studies have shown that chelated or proteinated sources of Fe have higher relative availability compared with inorganic Fe [4–8]. The body weights of newborn and weanling piglets increased significantly when the sows were maintained on feed supplemented with iron proteinate [9, 10]. Yu et al. [11] reported that Fe from amino acid complex increased plasma Fe and total Fe binding capacity in the blood, as well as hemosiderin and ferritin Fe in the liver and spleen of weanling pigs. Feng et al. [7] found that 90 mg iron glycine chelate (Fe-Gly) per kilogram has beneficial effects on growth and hematological and immunological functions of weanling pigs compared with those of ferrous sulfate ($FeSO_4$).

Table 1 Ingredient and composition (as-fed basis) of the basal diet (in grams per kilogram)

Ingredients	0–21 days	22–42 days
Corn	569.0	609.0
Soybean meal	230.0	210.0
Extruded soybean	100.0	90.0
Fish meal	40.0	30.0
Soybean oil	20.0	20.0
Calcium hydrogen phosphate	15.0	15.0
Vitamin mineral premix[a]	10.0	10.0
Limestone	8.0	8.0
Salt	3.0	3.0
Chloride choline	1.0	1.0
Lysine	2.0	2.0
Methionine	2.0	2.0
Nutrient composition		
ME (MJ/kg)[b]	13.04	13.14
CP	207	192
Ca	10.2	9.7
P	7.9	76
Met+Cys	9.4	8.9
Lys	13.1	12.0
Thr	7.6	7.0
Fe	85.81	72.52

[a] Supplied the following per kilogram of diet: vitamin A, 25,000 IU; vitamin D_2, 5,000 IU; vitamin E, 12.5 IU; vitamin K, 2.5 mg; vitamin B_1, 1.0 mg; vitamin B_2, 8.0 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 0.015 mg; calcium pantothenate, 20 mg; nicotinic acid, 17.5 mg; Cu, 10 mg; Zn, 40 mg; Mn, 80 mg; Se, 0.15 mg; I, 0.35 mg

[b] ME based on calculated values; others were analyzed values

It was demonstrated in studies with rats and humans that iron chelated with glycine was well absorbed and utilized [12–15]. Accordingly, the Fe-Gly is now used as an iron fortificant in human nutrition and especially in infant food [16]. Our previous study revealed that Fe-Gly at an appropriate dosage improves growth performance, hematological and immunological characteristics, iron tissue storage, and antioxidant enzyme activities in weanling pigs [7, 8]. But few studies have been conducted on Fe-Gly in broilers. The main objective of the present research was to investigate the effects of iron glycine chelate on growth performance, tissue mineral concentrations, fecal mineral excretion, and liver antioxidant enzyme activities in broilers.

Materials and Methods

Animals and Experimental Design

Three hundred and sixty 1-day-old commercial broiler chicks (Ross×Ross) were randomly allotted to six dietary treatments, each of which was replicated six times with ten birds per replicate. Dietary treatments were as follows: (1) control (no Fe supplementation), (2) control+40 mg/kg of Fe as Fe-Gly, (3) control+80 mg/kg of Fe as Fe-Gly, (4) control+120 mg/kg of Fe as Fe-Gly, (5) control+160 mg/kg of Fe as Fe-Gly, and (6) control+160 mg/kg of Fe from $FeSO_4$ (positive control).

Chicks were reared in an environmentally controlled room and fed a corn–soybean meal-based diet formulated to meet National Research Council [2] nutrient requirement estimates (Table 1) for 42 days. The initial room temperature

Table 2 Effects of different levels of iron glycine on the growth performance of broiler chickens

Fe source	Fe-Gly					$FeSO_4$	SEM	P value		Fe-Gly	
Fe addition (mg/kg)	0	40	80	120	160	160		Control vs. $FeSO_4$	$FeSO_4$ vs. Fe-Gly	Linear	Quad
Item											
0–21 days											
Initial weight (g)	41.67	41.33	41.00	41.17	41.17	41.17	0.175	0.360	1.000	0.448	0.521
21-day weight (g)	630.7	608.7	643.5	651.7	630.3	641.7	8.398	0.579	0.557	0.562	0.769
ADG (g)	27.78	28.05	28.24	28.26	28.50	28.13	0.321	0.607	0.500	0.613	0.951
ADFI (g)	42.93	45.81	47.16	48.30	48.27	44.14	0.531	0.297	0.023	0.001	0.007
F/G	1.55	1.64	1.67	1.72	1.69	1.57	0.026	0.564	0.069	0.099	0.383
22–42 days											
42-day weight (g)	2,182	2,219	2,256	2,323	2,305	2,252	119.4	0.047	0.442	0.104	0.339
ADG (g)	73.94	76.73	76.75	79.61	79.78	76.70	0.760	0.014	0.024	0.034	0.739
ADFI (g)	108.34	117.34	119.36	120.62	116.69	117.54	1.090	0.043	0.570	0.003	0.001
F/G	1.47	1.53	1.55	1.52	1.46	1.53	0.015	0.209	0.044	0.912	0.074

Nonorthogonal comparisons between the control vs. $FeSO_4$ (160 mg/kg) and the $FeSO_4$ (160 mg/kg) vs. Fe-Gly (160 mg/kg) treatments. Linear and quadratic effects of increasing Fe concentrations in Fe-Gly form (0 to 160 mg/kg)

SEM standard error of the mean

Table 3 Effects of different levels of iron glycine on mineral contents of serum and liver in broiler chickens

Fe source	Fe-Gly					FeSO$_4$	SEM	P value			
Fe addition (mg/kg)	0	40	80	120	160	160		Control vs. FeSO$_4$	FeSO$_4$ vs. Fe-Gly	Fe-Gly	
Item										Linear	Quad
21 days											
Serum (mg/L)											
Fe	1.574	1.688	1.753	1.863	1.931	1.913	0.039	0.001	0.806	0.005	0.884
Cu	0.173	0.174	0.169	0.173	0.174	0.175	0.002	0.799	0.891	0.963	0.754
Zn	1.528	1.530	1.530	1.533	1.539	1.536	0.011	0.799	0.936	0.808	0.931
Mn	0.012	0.012	0.011	0.011	0.012	0.012	0.000	0.722	0.855	0.776	0.533
Liver (mg/kg)											
Fe	121.13	123.70	129.31	139.32	145.82	140.29	2.451	0.044	0.936	0.001	0.462
Cu	3.72	3.73	3.70	3.73	3.55	3.74	0.040	0.905	0.817	0.314	0.378
Zn	64.45	64.23	64.87	64.47	64.54	64.05	0.761	0.881	0.989	0.951	0.955
Mn	2.81	2.79	2.81	2.79	2.80	2.84	0.025	0.584	0.760	0.896	0.956
42 days											
Serum (mg/L)											
Fe	1.558	1.560	1.552	1.575	1.560	1.560	0.000	0.001	0.736	0.001	0.127
Cu	0.012	0.012	0.012	0.011	0.012	0.012	0.050	0.883	0.870	0.836	0.130
Zn	1.578	1.879	1.996	2.069	2.141	2.097	0.010	0.964	1.000	0.841	0.974
Mn	0.174	0.174	0.175	0.174	0.176	0.176	0.003	0.870	0.992	0.855	0.980
Liver (mg/kg)											
Fe	124.67	128.85	133.68	140.18	148.34	141.00	2.143	0.034	0.987	0.001	0.493
Cu	3.74	3.73	3.72	3.71	3.73	3.76	0.048	0.936	0.757	0.940	0.898
Zn	64.68	64.31	65.07	64.61	64.69	64.23	0.498	0.832	0.858	0.942	0.949
Mn	2.83	2.81	2.84	2.81	2.78	2.87	0.024	0.507	0.448	0.657	0.764

Nonorthogonal comparisons between the control vs. FeSO$_4$ (160 mg/kg) and the FeSO$_4$ (160 mg/kg) vs. Fe-Gly (160 mg/kg) treatments. Linear and quadratic effects of increasing Fe concentrations in Fe-Gly form (0 to 160 mg/kg)

SEM standard error of the mean

for chicks was set at approximately 32°C and reduced by 2 to 3°C weekly until reaching 22°C at the 4th week, which was maintained for the remaining 2 weeks. In the starter (0 to 21 days) and grower (21 to 42 days) phases, each pen was offered the respective diet, and all chicks were given ad libitum access to feed and water.

Sample Collection and Analytical Methods

On days 21 and 42 of the feeding trial, all chicks were held without feed for 12 h, and the weight and feed consumption were measured to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G). Meanwhile, 72 chicks (two chicks per pen) were humanely killed by cervical dislocation at days 21 and 42. Plasma samples were isolated from blood by centrifuging at 3,000×g for 10 min and stored at −70°C until analysis. Liver and left breast muscle samples were removed from the carcasses and immediately stored at −70°C until analysis for

mineral concentrations or antioxidant enzyme activities. Fat was removed from the left tibia by a 36-h Soxhlet extraction in ethyl alcohol followed by a 36-h extraction with diethyl ether and then stored at −70°C until analysis. Fecal samples were freeze-dried and frozen at −20°C until mineral analysis. The experiment was conducted in accordance with the Chinese guidelines for animal welfare and approved by the animal welfare committee of Animal Science College, Zhejiang University.

Determination of Mineral Concentrations in Tissues and Feces

The mineral levels in samples (liver, tibia, breast muscle, and feces) were conducted using a method described by Shelton and Southern [17]. Samples were dried at 100°C for 24 h and ashed for 10 h (liver, breast muscle, and feces) or 36 h (tibia) at 550°C. The ashed samples were dissolved in nitric acid–perchloric acid mixture (1:1) and diluted with deionized water for analysis of minerals [18]. Contents of Fe, Cu, Zn, and Mn

Table 4 Effects of different levels of iron glycine on mineral contents of breast muscle and tibia in broiler chickens

Fe source	Fe-Gly					FeSO$_4$	SEM	P value			
Fe addition (mg/kg)	0	40	80	120	160	160		Control vs. FeSO$_4$	FeSO$_4$ vs. Fe-Gly	Fe-Gly	
Item										Linear	Quad
21 days											
Breast muscle(mg/kg)											
Fe	8.54	9.03	9.52	10.54	11.31	11.05	0.251	0.007	0.737	0.001	0.336
Cu	1.54	1.55	1.60	1.56	1.55	1.56	0.042	0.612	0.850	0.748	0.863
Zn	0.54	0.54	0.55	0.56	0.54	0.55	0.009	0.683	0.848	0.727	0.688
Mn	0.59	0.59	0.60	0.61	0.58	0.58	0.013	0.881	0.959	0.936	0.685
Tibia (mg/kg)											
Fe	18.15	19.69	21.07	24.00	24.33	24.19	0.586	0.007	0.421	0.001	0.717
Cu	49.30	49.36	49.39	49.51	49.36	49.56	0.272	0.792	0.295	0.916	0.913
Zn	43.14	43.29	43.04	43.68	43.45	43.48	0.514	0.906	0.886	0.811	0.981
Mn	28.59	28.88	29.04	28.93	28.92	29.22	0.318	0.559	0.473	0.806	0.803
42 days											
Breast muscle (mg/kg)											
Fe	8.59	9.05	9.74	10.83	11.54	11.08	0.266	0.005	0.506	0.001	0.487
Cu	1.55	1.56	1.56	1.57	1.56	1.58	0.010	0.442	0.408	0.774	0.877
Zn	0.54	0.56	0.55	0.56	0.54	0.55	0.009	0.675	0.818	0.847	0.419
Mn	0.59	0.60	0.62	0.61	0.59	0.59	0.014	0.897	0.952	0.985	0.596
Tibia (mg/kg)											
Fe	18.40	20.21	21.43	24.88	24.70	24.67	0.599	0.002	0.188	0.001	0.528
Cu	51.71	52.48	51.59	51.87	51.63	51.95	0.326	0.839	0.922	0.792	0.810
Zn	52.60	51.53	51.72	52.18	52.18	52.53	0.470	0.956	0.795	0.964	0.637
Mn	34.31	33.67	34.49	34.55	34.48	35.34	0.353	0.136	0.072	0.707	0.923

Nonorthogonal comparisons between the control vs. FeSO$_4$ (160 mg/kg) and the FeSO$_4$ (160 mg/kg) vs. Fe-Gly (160 mg/kg) treatments. Linear and quadratic effects of increasing Fe concentrations in Fe-Gly form (0 to 160 mg/kg)

SEM standard error of the mean

were measured with flame atomic absorption spectrophotometry (AA-6300, Shimadzu Corp., Tokyo, Japan).

Measurement of Superoxide Dismutase and Catalase Activities

Liver samples were prepared for analysis according to the method of Feng et al. [8]. Liver Cu/Zn superoxide dismutase (SOD) activities were determined by the methods of Shaw et al. [19]. An assay for catalase (CAT) activity was performed by following the reduction in H$_2$O$_2$ absorbance at 240 nm as reported by Venturino et al. [20]. Protein was measured by the method of Lowry et al. [21]. Units of SOD and CAT activities were expressed as the activity of an enzyme per milligram of protein.

Statistical Analysis

Data were analyzed by ANOVA as a randomized complete block design using the GLM procedures of SAS [22].

Individual chicks were the experimental unit for all indices. The planned single degree of freedom tests included the linear and quadratic effects of Fe-Gly, the control versus FeSO$_4$ (160 mg/kg Fe), and FeSO$_4$ versus Fe-Gly (160 mg/kg Fe) treatments. Differences of treatment means were compared using Student's t test. An alpha level of 0.05 was used for determination of differences among treatments.

Results

Growth Performance

The effects on growth performance are shown in Table 2. From the observed weights at day 21, it may be concluded that the level of Fe in the diet did not significantly influence broiler growth performance. High Fe supplementation (120 and 160 mg Fe/kg as Fe-Gly) improved ADG (P<0.05) and ADFI (P<0.05) of broilers over the birds fed basal diet at

day 42. However, no effect on F/G was observed among all treatments ($P>0.05$) throughout the experimental period.

Tissue Mineral Concentrations

The concentration of serum Fe was increased ($P<0.05$) in chicks fed diets supplemented with 120 and 160 mg/kg Fe as Fe-Gly or 160 mg/kg Fe as $FeSO_4$ at day 21 (Table 3). Similar effects in the liver of chicks were also observed ($P<0.05$). Moreover, the Fe concentrations in serum (21 days, $P=0.005$; 42 days, $P=0.001$) and liver (21 and 42 days, $P=0.001$) were elevated with the increasing dietary Fe-Gly levels. No significant differences on the contents of Cu, Zn, and Mn in serum and liver could be observed between the treatments.

Table 4 shows the effects of Fe-Gly on mineral contents of breast muscle and tibia in broilers. Compared with the control group, the addition of 120 and 160 mg/kg Fe as Fe-Gly or 160 mg/kg Fe as $FeSO_4$ enhanced the Fe concentration of the breast muscle ($P<0.05$). Tibia Fe concentration was improved ($P<0.05$) in chicks fed 80, 120, and 160 mg/kg Fe as Fe-Gly or 160 mg/kg Fe as $FeSO_4$. Moreover, there were linear responses to the addition of Fe-Gly from 0 to 160 mg/kg Fe on Fe concentrations in breast muscle ($P=0.001$) and tibia ($P=0.001$). However, there were no differences in breast muscle and tibia Cu, Zn, and Mn contents when broilers were offered different levels of iron as Fe-Gly and $FeSO_4$ compared with the control ($P>0.05$).

Fecal Mineral Excretion

Analyzed values of Fe, Cu, Zn, and Mn for fecal samples are presented in Table 5. Fecal Fe concentration elevated linearly with the increasing dietary Fe-Gly levels (21 days, $P=0.011$; 42 days, $P=0.021$). Moreover, supplementation of 160 mg/kg Fe-Gly or 160 mg/kg Fe as $FeSO_4$ enhanced ($P<0.05$) Fe concentration in feces compared with the control. Mineral contents of Cu, Zn, and Mn in feces of chicks did not differ among all the treatments ($P>0.05$).

Liver Antioxidant Enzyme Activities

As shown in Fig. 1, SOD activity did not differ among all treatments at 21 days ($P>0.05$). However, it was enhanced ($P<0.05$) when the diet was supplemented with 80, 120, or 160 mg/kg Fe as Fe-Gly at day 42. In addition, CAT activity at 21 days was slightly higher for chicks fed the basal diet supplemented with iron than in the chicks fed the basal diet, but no great changes were found among all the treatments ($P>0.05$).

Discussion

Fe deficiency is one of the most common potential mineral deficiencies in animals, which produces microcytic hypochromic anemia in chickens. For this reason, supplementation of Fe with a large safety margin to meet the dietary

Table 5 The effects of different levels of iron glycine on feces mineral concentrations in broiler chickens

Fe source	Fe-Gly					$FeSO_4$	SEM	P value		Fe-Gly	
Fe addition (mg/kg) Item	0	40	80	120	160	160		Control vs. $FeSO_4$	$FeSO_4$ vs. Fe-Gly	Linear	Quad
21days											
Feces (mg/kg)											
Fe	294.04	312.63	335.10	343.16	355.18	401.11	18.97	0.021	0.579	0.011	0.645
Cu	10.83	10.73	10.80	10.86	10.83	11.19	0.198	0.701	0.681	0.948	0.956
Zn	35.08	34.98	35.43	34.34	35.32	35.36	0.826	0.929	0.990	0.984	0.950
Mn	85.54	83.02	86.28	87.79	86.84	88.80	1.058	0.349	0.332	0.460	0.905
42 days											
Feces (mg/kg)											
Fe	293.00	316.55	338.20	345.78	356.94	380.95	9.590	0.019	0.457	0.032	0.615
Cu	10.85	10.83	10.93	10.85	10.87	11.25	0.212	0.687	0.683	0.973	0.697
Zn	35.25	35.48	35.50	35.66	35.59	35.39	0.790	0.958	0.949	0.908	0.960
Mn	88.44	85.18	86.22	88.41	88.60	89.02	0.901	0.795	0.808	0.679	0.438

Nonorthogonal comparisons between the control vs. $FeSO_4$ (160 mg/kg) and the $FeSO_4$ (160 mg/kg) vs. Fe-Gly (160 mg/kg) treatments. Linear and quadratic effects of increasing Fe concentrations in Fe-Gly form (0 to 160 mg/kg)

SEM standard error of the mean

Fig. 1 Effects of iron glycine on SOD and CAT activities. Control (no Fe supplemental), 40, 80, 120, and 160 (Fe-Gly) group supplements with 40, 80, 120, and 160 mg Fe/kg diet from Fe-Gly, 160 p group (positive control) supplements 160 mg Fe/kg diet from ferrous sulfate. *significant difference compared with the control
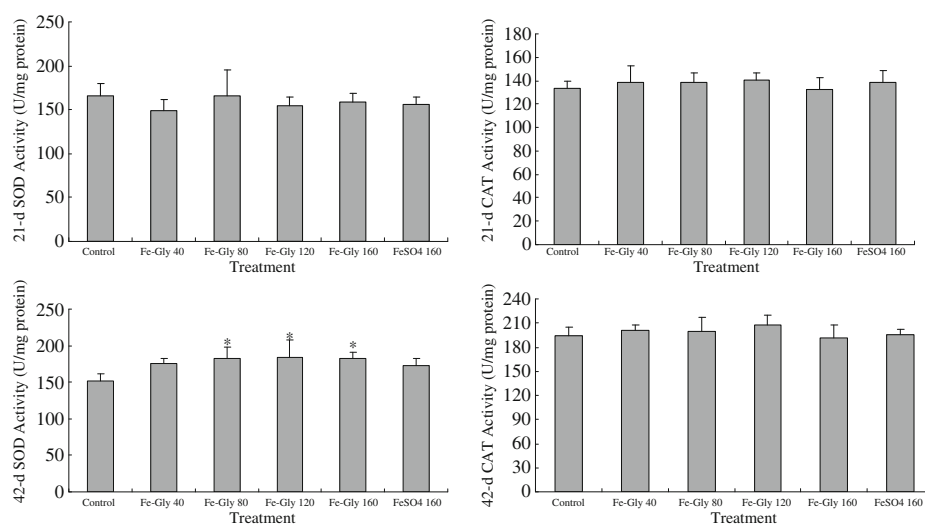
needs of broilers is used. It has been reported that iron from ferrous glycinate was better absorbed than that from $FeSO_4$ [12, 23]. And, ferrous glycinate has better effect on precaution and treatment of iron deficiency anemia in humans, especially in infants or young children [13, 15]. Langini et al. [23] reported that the absorption of Fe was 30.9 % in weanling rats given infant formula labeled with [^{59}Fe]glycine compared with 15.8 % with [^{59}Fe]sulfate. Layrisse et al. [14] showed that the absorption of Fe in Fe-Gly is twice that of $FeSO_4$ in a breakfast meal based on maize flour. Glycine has the lowest molecular weight of all the amino acids, which favors 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 [24]. Additionally, Fe from Fe-Gly has lower prooxidant properties since diets supplemented with additional Fe had positive effects on feed conversion ratio (FCR) in broilers [3]. Therefore, higher absorption and lower prooxidant properties may be both attributed to the positive effect of Fe-Gly on performance in the current study. However, our result is in conflict with Kulkarni et al. [24], who reported that significantly improved BWG and FCR were observed in boilers fed 120 mg/kg $FeSO_4$ compared to those fed ferrous aminoate [25], which needs further investigation.

Tissue mineral concentrations are commonly used to evaluate the mineral status of animals and humans. The present study showed that the Fe concentrations in serum (21 days, $P=0.005$; 42 days, $P=0.001$), liver ($P=0.001$), breast muscle ($P=0.001$), and tibia ($P=0.001$) increase with the increasing levels of Fe as Fe-Gly in chickens. The results are similar to those reported by Cao et al. [26], who also observed that the tissue iron concentrations increase with increasing levels of dietary Fe. Yu et al. [11] reported that total Fe in the liver, spleen, and muscle significantly increased as the supplement level of Fe amino acid chelate was increased in pigs. Rincker et al. [27, 28] also found pigs

maintained on diets supplemented with 0, 25, 50, 100, and 150 mg/kg Fe as $FeSO_4$ in the feed resulted in a linear increase in whole body iron stores ($P=0.001$). Consistent with our results, the Fe-Gly was also better absorbed and more bioavailable in rats or humans [5, 14] Olivares et al. [29] demonstrated that Fe from glycinate was 2 to 2.5 times more bioavailable than that from $FeSO_4$ when added to the milk. Furthermore, Bovell-Benjamin et al. [5] also found that the Fe was absorbed from Fe-Gly (6.8 %) to a greater extent than from FeSO4 (1 %). Those results may explain the increased tissue Fe concentrations and suggest that a good absorption of Fe-Gly could improve the bioavailability of Fe from Fe-Gly.

Dietary Fe (160 mg/kg Fe as Fe-Gly or $FeSO_4$) enhanced Fe concentration in feces compared with the control. We also found linear responses to concentrations of fecal Fe with the increasing levels of Fe-Gly. This is in accordance with the results of Creech et al. [30], who observed a decreased level of fecal Fe in piglets fed with reduced levels of Fe in the diet, whereas increased dietary Fe level (0 to 150 mg/kg) as $FeSO_4$ resulted in a linear increase of fecal Fe excretion.

The continuous presence of an excessive intake of Fe could lead to free radical formation [31, 32]. SOD and CAT are antioxidant enzymes considered as the indicators for oxidative stress [33]. SOD functions to convert the active oxygen groups into H_2O_2, and CAT is responsible for the destruction of excess H_2O_2 [34]. In the present study, the Fe-Gly diet did dramatically stress the antioxidant system of chicks, as shown by a major increase in SOD at 42 days. This agrees with other studies performed in rats. Davis and Feng [35] found that dietary Fe (140 mg Fe/kg diet) caused a significantly increased SOD activity in rat liver. However, other researchers reported that a moderate dietary Fe excess (≤400 mg Fe/kg diet) did not affect the SOD activity in rat liver [36, 37], except for rats fed Fe-deficient diets [33].

Brandsch et al. [38] also suggested that the increase of CAT activity in rat liver may be attributed to the increased Fe concentrations in the liver itself rather than the induction of oxidative stress. Therefore, in the present experiment, the Fe concentration of the basal diet (21 days, 191.60 mg/kg; 42 days, 192.57 mg/kg) probably caused the activity of SOD to be at an abnormally low level, which was restored to normal by the supplementation of Fe. This may indicate that the moderately high Fe intake does not pose a major risk for oxidative stress in chicks, although the point warrants further research.

Conclusion

In conclusion, the results of this study indicate that additions of Fe-Gly improve the growth performance, iron tissue storage, and the antioxidant status of broiler chickens. Additionally, Fe-Gly reduces the fecal Fe concentrations compared to $FeSO_4$.

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