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Supplemental dietary iron glycine modifies growth, immune function, and antioxidant enzyme activities in broiler chickens

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ABSTRACT

The objective of this study was to evaluate the effects of iron glycine (Fe-Gly) on growth, immune function, and serum antioxidant enzyme activities in broiler chickens. A total of 480 1-d-old broiler chickens [average body weight (BW), 45.9 ± 0.5 g] were randomly allotted to 8 dietary treatments with 6 replicate pens and 10 broiler chickens per replicate pen. The control treatment contained 160 mg Fe/kg diet from $FeSO_4$, while 7 other treatments consisted of 40, 60, 80, 100, 120, 140, and 160 mg Fe/kg diet from Fe-Gly. After a 21-d-feeding period, there were increasing responses to the addition of 40–160 mg Fe/kg from Fe-Gly, with the greatest ADG (quadratic, $P < 0.05$) and ADFI (linear and quadratic, $P < 0.05$) observed in broiler chickens fed with 100 mg Fe/kg. The weight of thymus gland increased (linear and quadratic, $P < 0.05$) with the increasing levels of Fe as Fe-Gly, and it was greater with 160 mg Fe/kg from Fe-Gly compared with the control ($P < 0.05$). Serum catalase (CAT), xanthine oxidase (XOD), and superoxide dismutase (SOD) increased as addition of Fe from Fe-Gly increased from 40 to 160 mg /kg (linear, $P < 0.05$), and the SOD activity was greater in broiler chickens fed the diet containing 160 mg of Fe as Fe-Gly compared with those fed the control diet ($P < 0.05$). The divalent metal transporter 1 (DMT1) mRNA expression was decreased with the increase of Fe as Fe-Gly in diets (linear and quadratic, $P < 0.05$), and it was lower in broiler chicken fed the diet with 160 mg/kg Fe as Fe-Gly compared with those fed the control diet with $FeSO_4$ ($P < 0.05$). This study indicated that addition of Fe-Gly could obviously modify antioxidant status of broiler chickens, and moreover, improve growth performance and immune function partially.

1. Introduction

Iron is an essential trace element that plays a vital role in various physiologic processes. NRC (1994) has recommended 80 mg Fe/kg diet for broiler chickens. A majority of broiler chicken diets comprising maize and soybean meal have more than 80 mg Fe/kg; however, this may not be sufficient for rapidly growing broiler chickens to reach their genetic growth potential (Vahl and van'T Klooster, 1987). At present, the Fe supplement commonly used in chicken diets under practical conditions is the inorganic form, such as sulfates, oxides, and carbonates.

In recent decades, it has been reported that a trace mineral chelated with amino acid or protein has high bioavailability in animals. A study showed that chelated or proteinated source of Fe had 125–185% relative availability compared to $FeSO_4$ (Henry and Miller, 1995), and dietary addition of Fe chelated with amino acids or protein can

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prevent and cure Fe deficiency in animals or humans (Bovell-Benjamin et al., 2000; Feng et al., 2007; Kegley et al., 2002; Spears et al., 1999; Veum et al., 1995). A research with broiler chickens indicated that iron methionine (Fe-Met) had a greater bioavailability than iron sulfate (Shinde et al., 2011). Seo et al. (2008a, b) reported that Fe-Met to be more efficient in enriching the Fe concentration in liver, spleen, and muscle of broiler chickens than $FeSO_4$. It was established that iron glycine (Fe-Gly) could be absorbed and utilized easily in rat and human, which could have much more beneficial effects on the prevention and treatment of Fe deficiency than $FeSO_4$ (Iost et al., 1998; Oscar and Ashmead, 2001).

Iron fortification is crucial for broiler chickens because of increasing demand for Fe because of the rapid increases in red blood cell volume and body mass. At present, data on the utilization of dietary Fe-Gly in broiler chicken production are limited, especially in the Arbor Acre chicken, which is commonly used in China. Therefore, the aim of our study was to evaluate the efficacy of different levels of dietary supplemental Fe-Gly on the growth performance, immune function, and antioxidant property in Arbor Acre broiler chickens.

2. Materials and methods

2.1. Fe sources

In our experiment, reagent-grade iron sulfate ($FeSO_4.7H_2O$; Tianjin Tianli Chemical Industry Co., Ltd., Tianjin, China) was used with Fe concentration of 20.1%; Fe^{2+}-glycine [Fe-Gly (II); Guangzhou Tanke Technology Co., Ltd., Guangzhou, China] was also used with Fe concentration of 14.0% and Gly concentration of 38.0%.

2.2. Animals and experimental design

Four hundred and eighty 1-d-old Arbor Acres commercial male broiler chickens [average body weight (BW), 45.9 ± 0.5 g] were weighed and allocated randomly to 8 treatments with 6 replicates of 10 broiler chickens per replicate. The treatments consisted of: the control with 160 mg Fe from $FeSO_4$/kg diet and the diets supplemented with 40, 60, 80, 100, 120, 140, and 160 mg Fe from Fe-Gly/kg. Experimental duration was 21 d.

Nutrient levels of the basal diet were based on NRC (1994) recommended nutrient requirements of broiler chickens (Table 1). Broiler chickens were randomly placed in elevated pens in the house, and it was kept at 33 °C during the first 3 d and then reduced by 3 °C every week until a temperature of 24 °C was reached. A light was provided 24 h/d via fluorescent lights. Broiler chickens were given ad libitum access to feed and water. On d 1, the broiler chickens were vaccinated against infectious bronchitis, and newcastle disease vaccination was performed on d 8.

2.3. Sample collection

On d 1 and 21 of the experiment, BW and feed consumption of all birds were measured by pens to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed/gain ratio (*F*/*G*). At 21 d, broiler chickens were deprived of feed for 12 h. After that, 12 broilers per treatment group (2 birds per replicate) were selected based on mean BW and bled via cervical vein for serum sample, and then slaughtered and dissected by a trained team to collect spleen, thymus, bursa of fabricius, and 5 cm duodenum (about 10 cm distal to the pylorus). Serum was obtained by centrifuging blood sample at 3000*g* for 15 min at room temperature, and then stored at −20 °C until analysis of serum enzyme activities.

The spleen, thymus and bursa of fabricius were washed and weighted in tap water, and stored at −20 °C until measure of immune organ weight. Duodenum was collected and snap-frozen in liquid nitrogen and stored at −80 °C until divalent metal transporter 1 (DMT1) mRNA expression analysis.

Table 1
Ingredient and composition of basal diet[a].

Item	Content
Ingredient (g/kg)	
Maize	547.6
Soybean meal (43% CP)	348.6
Fish meal (anchovy, 55% CP)	35.0
Soybean oil	36.0
Dicalcium phosphate (23% Ca and 18% P)	12.0
Limestone	13.0
DL-Met (99%)	1.6
Salt	3.0
Choline chloride (50%)	1.0
Vitamin–mineral premix [b]	2.0
Composition [c]	
ME (MJ/kg)	12.62
CP (%)	21.53
Lys (%)	1.23
Ca (%)	0.33
P (%)	0.46
Met+Cys (%)	0.87
Fe (mg/kg)	90.31

[a] ME=metabolizable energy, and CP=crude protein.
[b] Provided per kilogram of diet: vitamin A, 13,500 IU; vitamin D_3, 3600 IU; vitamin E, 33 IU; vitamin K_3, 6 mg; vitamin B_1, 4.5 mg; vitamin B_2, 10.5 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.03 mg; pantothenate acid, 18 mg (calcium pantothenate); niacin, 60 mg; folicacid, 1.8 mg; and biotin, 0.165 mg; Zn, 60 mg (zinc sulphate); Cu, 8 mg (copper sulphate); Mn, 120 mg (manganese sulphate); Se, 0.3 mg (sodium selenite); and I, 0.7 mg (potassium iodide).
[c] ME and P were calculated values; others were analyzed values.

2.4. Immune organ weight and serum antioxidant enzyme activities

The weight of the spleen, thymus, or bursa of fabricius was expressed in terms of the body weight. An assay for catalase (CAT) activity was performed by following the reduction in H_2O_2 absorbance at 240 nm as reported by Venturino et al. (2001). Serum Cu/Zn superoxide dismutase (SOD) activities were determined by the methods of Shaw et al. (2002). Xanthine oxidase (XOD) activity was measured according to the method described by Hashimoto (1974). The samples of above serum enzymes were determined using colorimetric methods with a spectrophotometer (Biomate 5; Thermo Electron Corporation, Rochester, NY, USA). The intra-CV of all measurements is about 3% and inter-assay CV is about 5%.

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