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Short communication

Iron sources on iron status and gene expression of iron related transporters in iron-deficient piglets

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ABSTRACT

The study was conducted to compare the effects of ferrous sulfate ($FeSO_4$) and iron glycine chelate (Fe-Gly) on iron status and gene expression of iron related transporters in iron-deficient piglets. Eighteen piglets (Duroc × Landrace × Yorkshire; 6.50 ± 0.52 kg) were selected to conduct the study. In period 1, all pigs were fed an iron-deficient diet (basal diet; 20.23 mg Fe/kg of diet) for 19 days until iron-deficient anemia was induced. In period 2, the eighteen piglets were randomly allotted to 3 dietary treatments for another 10 days. Dietary treatments were as follows: (1) control group (basal diet); (2) $FeSO_4$ group (basal diet + 120 mg/kg of iron as $FeSO_4$); (3) Fe-Gly group (basal diet + 120 mg/kg of iron as Fe-Gly). After 10-d feeding trial, piglets fed Fe-Gly or $FeSO_4$ had higher ($P < 0.05$) iron content in heart, liver and lung, whereas serum total iron binding capacity (TIBC) was lower ($P < 0.05$) than iron-deficient piglets. Piglets fed Fe-Gly had higher ($P < 0.05$) peptide transporter 1 (PepT1) mRNA level, lower ($P < 0.05$) ferroportin1 mRNA level both in duodenum and jejunum when compared with pigs fed $FeSO_4$. However, growth performance of piglets did not differ among all treatments ($P > 0.05$). In summary, current study showed that Fe-Gly was more effective in improving iron status of iron-deficient piglets than $FeSO_4$. Moreover, gene expression of iron related transporters differ between $FeSO_4$ and Fe-Gly group. Results also suggest that PepT1 may play a crucial role in absorption of Fe-Gly.

1. Introduction

Fe is one of the most important trace elements for animal growth and health. However, iron deficiency is a prevalent nutritional problem for humans and animals. Iron supplementation is appropriate to prevent and treat iron deficiency anemia (Hurrell, 1997). It was reported that metal chelated with amino acid has good bioavailability in animals (Feng et al., 2010). Yu et al. (2000) reported that Fe from amino acid complex elevated plasma iron concentration as well as hemosiderin and ferritin Fe in the liver and spleen of weaned pigs. Feng et al. (2007) revealed that 90 mg Fe-Gly/kg of diet had positive effects on growth performance, hematological and immunological functions of weanling pigs compared with $FeSO_4$. The higher efficiency of Fe-Gly may result from the fact that it could be easily absorbed and maintain high bioavailability in humans in spite of the presence of iron absorption inhibitory factors such as phytic acid (Oscar and Ashmead, 2001). Therefore, Fe-Gly is currently used as an efficient iron reinforcing agent in infant dairy and food (Giorgini et al., 2001).

Abbreviations: ADFI, average feed intake; ADG, average daily gain; BW, body weight; DMT1, divalent metal transporter 1; Fe-Gly, iron glycine chelate; $FeSO_4$, ferrous sulfate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PepT1, peptide transporter 1; RBC, red blood cell; TIBC, total iron binding capacity.

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Table 1
Composition of the basal diet (as-fed basis).

Ingredient	g/kg	Component	g/kg
Maize	589	DE[a] (MJ/kg)	14.3
Casein	148	Crude protein	185
Lactose	80	Calcium	8.3
Sucrose	50	Total phosphorus	6.5
Glucose	100	Lysine	12.3
Cellulose	10	Methionine	4.9
Choline chloride	1	Fe (mg/kg)	20.2
Salt	3		
Calcium carbonate	6		
Calcium hydrogen phosphate	10		
Vitamin mineral premix[b]	3		

[a] DE (digestible energy) values for maize, lactose, sucrose, glucose and cellulose were provided by Feed Database in China (2011).
[b] Supplied the following per kilogram of diet: vitamin A 2200 IU; vitamin D_3 220 IU; vitamin E 16 IU; vitamin K_3 0.5 mg; vitamin B_1 1 mg; vitamin B_{12} 17.5 mg; vitamin B_2 3.5 mg; vitamin B_6 1.5 mg; pantothenic acid 10 mg; nicotinic acid 15 mg; folic acid 0.3 mg; biotin 0.05 mg; Cu 6 mg; Zn 100 mg; Mn 4 mg; I 0.14 mg; Se 0.3 mg.

However, the data as to biological effects and absorption characteristics of Fe-Gly is limited. Therefore, the main objectives of present research were to compare the effects of $FeSO_4$ and Fe-Gly on iron status and gene expression of iron related transporters in iron-deficient piglets.

2. Materials and methods

2.1. Animals and experimental design

Eighteen 25-day-old piglets (Duroc × Landrace × Yorkshire; 6.50 ± 0.52 kg) were selected to conduct the study. A basal diet (iron-deficient diet; Table 1) was formulated to reach nutrient requirements for piglets (NRC, 1998). Diets were determined for crude protein (984.13), calcium (935.13) and phosphorus content (964.06) of as described by AOAC (1995). Lysine and methionine content of diets were analyzed by an automatic amino acid analyzer L-8800 (Hitachi, Tokyo, Japan). The Fe content in diets was analyzed by atomic absorption spectrophotometer (model AA6501, Shimadzu Ltd., Kyoto, Japan). All pigs were fed the basal diet for 19 days (period 1). Thereafter, the eighteen piglets were randomly allotted to 3 dietary treatments (6 replicates per treatment and 1 pig per replicate) for another 10 days (period 2). Dietary treatments were as follows: (1) control group (basal diet; Table 1); (2) $FeSO_4$ group (basal diet + 120 mg/kg of iron as $FeSO_4$); (3) Fe-Gly group (basal diet + 120 mg/kg of iron as Fe-Gly). Pigs were given *ad libitum* access to feed and water. Piglets were weighed on d 19 and d 29. Feed consumption was recorded.

2.2. Sample collection

On d 1, d 7, d 14, d 19 and d 29, EDTA-treated whole blood for hemoglobin and RBC measurements were obtained from jugular vein. Serum was separated by centrifugation at 3, 000 × *g* for 15 min at 4 °C and stored at −20 °C until analysis. On d 29, all piglets were killed by exsanguination. Heart, liver, lung and kidney were quickly removed, washed briefly in cold phosphate buffer, cut into small portions, snap-frozen in liquid nitrogen until analysis. Duodenum and jejunum was removed, washed with 0.1% diethylpyrocarbonate water, packed with sterile and RNase-free silver paper, and rapid frozen in liquid nitrogen until analyses of divalent metal transporter 1 (DMT1), ferroportin1 and PepT1.

2.3. Determination of biochemical parameters in blood and tissues

Concentrations of hemoglobin and RBC (red blood cell) in blood were assayed using an automated hematology analyzer (Sysmex K-1000D, Sysmex Inc., Kobe, Japan). Heart, liver, lung and kidney nonheme iron was determined colorimetrically after acid digestion of tissues (Torrance and Bothwell, 1968). Concentrations of serum iron and TIBC were measured using the method of Fielding (1980).

2.4. Real-time PCR analysis

Primers for DMT1, Ferroportin1, PepT1 and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were designed with Primer 5.0 and GenBank (Table 2). Total RNA was extracted by Trizol Reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Real-time PCR was conducted using the iQTW5 real-time multiplexing system (Bio-Rad Inc., Hercules, California, USA). Moloney murine leukemia virus (MMLV) reverse transcriptase reagent kits and Oligo primer transcriptase were used to reverse RNA to cDNA. The flourescence quantitative-PCR mixtures contained 2.0 mL cDNA, 10.0 mL SYBR green PCR master mix, 7.0 mL RNase-free deionised water, 0.5 mL upstream primers and 0.5 mL downstream primers. The standard