

Effects of Ferrous Glycine on Growth Performance, Apparent Iron Digestibility, and Serum Iron-Related Parameters in Weaned Piglets (Postprint)

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Abstract

This experiment was conducted to investigate the effects of ferrous glycine on growth performance, apparent iron digestibility, and serum iron-related indices in weaned piglets. Twelve (28 ± 1)-day-old weaned piglets (Duroc \times Landrace \times Large White) were selected and allocated into 2 groups following the principle of balanced distribution based on body weight and health status, with 6 replicates per group and 1 piglet per replicate, individually housed in metabolic cages. The trial consisted of two phases: Phase 1, both groups received an iron-deficient basal diet for 10 days to induce a near-anemic state; Phase 2, the control group was supplemented with 100 mg/kg ferrous sulfate (as iron) in the iron-deficient basal diet, whereas the experimental group received 100 mg/kg ferrous glycine (as iron) supplementation, with an experimental period of 10 days. The results demonstrated that: compared with ferrous sulfate supplementation, dietary ferrous glycine tended to reduce the feed conversion ratio of piglets ($P < 0.10$) by 4.57%, but exhibited no significant differences in average daily gain or average daily feed intake ($P > 0.10$); dietary ferrous glycine significantly decreased iron excretion in feces and total iron excretion ($P < 0.05$) by 23.11% and 22.09%, respectively; dietary ferrous glycine highly significantly increased apparent iron digestibility and apparent metabolic rate ($P < 0.01$) by 13.34% and 22.42%, respectively; additionally, dietary ferrous glycine significantly elevated serum iron saturation and blood iron content ($P < 0.05$). In conclusion, ferrous glycine can enhance the growth performance of weaned piglets, increase blood iron content, improve iron metabolic status, reduce fecal iron excretion, and serves as a green and efficient novel iron supplement.

Full Text

Effects of Ferrous Glycine on Growth Performance, Iron Apparent Digestibility, and Serum Iron-Related Indices in Weaned Piglets

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Abstract: This experiment was conducted to investigate the effects of dietary ferrous glycine on growth performance, iron apparent digestibility, and serum iron-related indices in weaned piglets. Twelve (28 ± 1) day-old *Duroc* × *Landrace* × *Large Yorkshire* weaned piglets were selected and divided into 2 groups according to the principle of balanced distribution based on body weight and health status, with 6 replicates per group and 1 piglet per replicate, individually housed in metabolic cages. The experiment consisted of two stages: in stage 1, both groups were fed an iron-deficient basal diet for 10 days to induce a near-anemic state; in stage 2, the control group received the iron-deficient basal diet supplemented with 100 mg/kg ferrous sulfate (as iron), while the experimental group received 100 mg/kg ferrous glycine (as iron) for 10 days. The results showed that compared with ferrous sulfate, dietary ferrous glycine tended to decrease the feed-to-gain ratio by 4.57% ($P < 0.10$), but had no significant effects on average daily gain or average daily feed intake ($P > 0.10$). Dietary ferrous glycine significantly decreased iron excretion in feces and total iron excretion by 23.11% and 22.09%, respectively ($P < 0.05$), and extremely significantly increased iron apparent digestibility and apparent metabolic rate by 13.34% and 22.42%, respectively ($P < 0.01$). Meanwhile, dietary ferrous glycine significantly increased serum iron saturation and blood iron content ($P < 0.05$). In conclusion, ferrous glycine can improve growth performance, increase blood iron content, improve iron metabolic status, and reduce fecal iron excretion in weaned piglets, making it a green and efficient new iron supplement.

Key words: ferrous sulfate; ferrous glycine; weaned piglets; growth performance; iron apparent digestibility; serum iron indices

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Iron is one of the most important trace elements for growth and development in livestock and poultry, serving as a crucial component and activator of numerous enzymes. It participates in various biochemical reactions and plays vital roles in promoting animal growth, enhancing immunity, and improving antioxidant

capacity [1-2]. Young animals have particularly high iron requirements and are prone to iron deficiency anemia, which can even lead to death [3-5].

Currently, due to low cost, inorganic iron (ferrous sulfate) supplementation is common in sow and piglet diets. However, it has several drawbacks, including crystalline water content, susceptibility to oxidation, low digestibility and utilization rates, and environmental pollution. Amino acid chelated iron, a third-generation iron source additive that has developed rapidly in recent years both domestically and internationally—including compounds such as lysine iron, methionine iron, and ferrous glycine—closely resembles the natural form of iron supplements in animals. These compounds offer high biological efficacy, good absorption efficiency, and environmental friendliness, while also providing dual supplementation of amino acids and trace elements [6-7]. Moreover, they demonstrate clear advantages in absorption, metabolism, and improvement of animal production performance. Research on amino acid chelated iron has promoted the healthy and rapid development of feed iron additives for weaned piglets and represents a current hotspot in domestic research, development, and application [8-9].

Amino acid chelated iron refers to complexes with cyclic structures formed by the binding of iron to amino acids through coordination and ionic bonds. Glycine is highly water-soluble and has the smallest molecular weight among amino acids. Theoretically, ferrous glycine chelate synthesized from glycine and ferrous iron can more easily cross intestinal epithelial cells and be absorbed intact. However, few studies have reported on the application of ferrous glycine in piglets. Therefore, this experiment investigated the effects of ferrous glycine on growth performance, iron apparent digestibility, and serum iron-related indices in piglets to provide a theoretical reference for its application in piglet diets.

1.1 Experimental Materials

The ferrous sulfate used contained 31% iron and was provided by Guangxi Xingtengke Feed Company; the ferrous glycine contained 17% iron and was provided by Shandong Heshi Company. Both were feed-grade.

1.2 Experimental Design and Management

Twelve (28 \pm 1)day-old *Duroc \times *Landrace \times Large Yorkshire crossbred male piglets weighing approximately 8.0 kg were randomly divided into 2 groups with 6 replicates per group and 1 piglet per replicate. Each piglet was individually housed in stainless steel metabolic cages. Both groups were fed an iron-deficient basal diet for 10 days to induce a near-anemic state. Subsequently, the control group received the iron-deficient basal diet supplemented with 100 mg/kg ferrous sulfate (as iron), while the experimental group received 100 mg/kg ferrous glycine (as iron). After 5 days of feeding to determine individual feed intake, piglets were limit-fed at 80% of normal intake, followed by a 5-day total collection of feces and urine. The total experimental period was 20 days. The experi-**

ment was conducted at the experimental farm of the College of Animal Science, South China Agricultural University, which had complete facilities meeting the experimental requirements. Experimental pigs were housed in metabolic cages with free access to deionized water throughout the trial. Daily management and disease prevention followed routine procedures.

1.3 Experimental Diets

The experimental diet was a corn-soybean meal basal diet, with all nutrient levels meeting the nutritional requirements of weaned piglets (NRC, 2012). The composition and nutrient levels are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Items	Ingredients	Content
	Corn	
	Soybean meal	
	Fish meal	
	Whey	
	Soybean oil	
	CaHPO ₄	
	Lys	
	Thr	
	NaCl	
	Limestone	
	Premix ¹	
	Total	
Nutrient levels ²	DE/(MJ/kg)	
	CP	
	Ca	
	TP	
	AP	
	Lys	
	Met+Cys	
	Thr	
	Trp	
	Fe/(mg/kg)	

¹The premix provided the following per kg of the diet: VA 12,000 IU, VD₃ 3,500 IU, VE 22.5 mg, VK₃ 3 mg, VB₁ 3 mg, VB₂ 3.7 mg, VB₆ 5 mg, nicotinic acid 30.3 mg, pantothenic acid 13.8 mg, folic acid 1.5 mg, Se 0.3 mg, Cu 50 mg, Zn 100 mg, Mn 40 mg, I 0.3 mg.

²Nutrient levels were all calculated values.

1.4.1 Blood Samples

At the end of the experiment, blood was collected from the anterior vena cava of each piglet. Two samples were collected per piglet: one (5 mL) was placed in a centrifuge tube containing sodium heparin anticoagulant to prepare whole blood; the other (10 mL) was placed in a tube without anticoagulant, allowed to stand for 30 minutes, then centrifuged for 10 minutes at 3,000 r/min to prepare serum. The serum was aliquoted into 1 mL EP tubes and stored at -20 °C for later analysis.

1.4.2 Fecal and Urine Samples

During the total collection period, feces and urine from each piglet were collected daily. Fecal samples were thoroughly mixed, and 25% of the total fecal weight per piglet was sampled using the quartering method. For every 100 g of feces, 5.0 mL of 10% tartaric acid was added, along with a small amount of toluene as preservative, and samples were stored at -20 °C for later analysis. Urine samples were collected and total volume was recorded; 10% of the volume was sampled into brown bottles, and 10% sulfuric acid was added (ratio of 2:1,000), plus a small amount of toluene as preservative, and stored at -20 °C.

1.4.3 Slaughter Sampling

After the experiment, piglets were slaughtered. The liver, spleen, heart, kidney, and longissimus dorsi muscle were collected, weighed, and recorded. Surface moisture was absorbed with filter paper, and samples were placed in pre-labeled sealed bags, rapidly frozen in liquid nitrogen, and then transferred to -80 °C storage.

1.5.1 Growth Performance of Piglets

Body weight was measured on an empty stomach on days 10 and 21 of the experiment to calculate average daily gain. Feed intake was recorded to calculate average daily feed intake, and the feed-to-gain ratio was calculated for the experimental period.

1.5.2 Serum Iron Related Indices

Assay kits for serum iron content, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), serum iron saturation, and blood iron content were purchased from Nanjing Jiancheng Bioengineering Institute, and operations were performed according to the manufacturer's instructions.

1.5.3 Determination of Iron Content in Diets, Tissues, and Feces

Iron content in diets, tissues, and feces was determined according to the national standard GB/T 13885–2003.

1.6 Data Processing and Analysis

Experimental data were analyzed using SPSS 17.0 statistical software with independent samples t-test. Results are expressed as mean \pm standard error. $P < 0.01$ indicated extremely significant difference, $P < 0.05$ indicated significant difference, and $P < 0.10$ indicated a trend toward significant difference.

2.1 Effects of Ferrous Glycine on Growth Performance of Weaned Piglets

As shown in Table 2, compared with the ferrous sulfate group, the ferrous glycine group showed a trend toward decreased feed-to-gain ratio ($P < 0.10$), with a reduction of 4.57%. No significant differences were observed in average daily gain or average daily feed intake between the two groups ($P > 0.05$).

Table 2 Effects of ferrous glycine on growth performance of weaned piglets

Items	FeSO ₄ group	Fe-Gly group
IW/kg	9.50 ^a ±0.73	9.76±0.32
FW/kg	13.78±0.66	14.05±0.65
ADG/g	214.16±12.11	214.08±9.17

In the same row, values with different capital letter superscripts indicate extremely significant difference ($P < 0.01$), different small letter superscripts indicate significant difference ($P < 0.05$), and same small letters or no letters indicate no significant difference ($P > 0.05$). The same applies to the following tables.

2.2 Effects of Ferrous Glycine on Iron Apparent Digestibility and Metabolic Rate of Weaned Piglets

As shown in Table 3, compared with the ferrous sulfate group, the ferrous glycine group showed significantly decreased iron excretion in feces and total iron excretion ($P < 0.05$), with reductions of 23.11% and 22.09%, respectively. Iron apparent digestibility and apparent metabolic rate were extremely significantly increased ($P < 0.01$), with improvements of 13.34% and 22.42%, respectively.

Table 3 Effects of ferrous glycine on iron apparent digestibility and metabolic rate of weaned piglets

Items	FeSO ₄ group	Fe-Gly group
Iron intake/(mg/kg)	190.00±12.78	180.00±15.25

2.3 Effects of Ferrous Glycine on Tissue Iron Content of Weaned Piglets

As shown in Table 4 , ferrous glycine had no significant effect on iron content in tissues and organs of weaned piglets (P>0.05).

Table 4 Effects of ferrous glycine on tissue iron content of weaned piglets (g/g)

Tissues	FeSO ₄ group	Fe-Gly group
Heart	22.73±0.57	23.18±1.08

2.4 Effects of Ferrous Glycine on Serum Iron Related Indices of Weaned Piglets

As shown in Table 5 , compared with the ferrous sulfate group, the ferrous glycine group showed significantly increased serum iron saturation (P<0.05), with an increase of 15.57%. Blood iron content was also significantly increased (P<0.05), with an increase of 37.05%. Serum total iron binding capacity decreased by 4.65%, but the difference was not significant (P>0.05).

Table 5 Effects of ferrous glycine on serum iron related indices of weaned piglets

Items	FeSO ₄ group	Fe-Gly group
Serum iron content/(mol/L)	24.91±1.27	27.38±1.94

Trace elements are essential nutrients for animals, directly participating in almost all physiological and biochemical functions. They serve as important components and activators of various enzymes and play extremely important roles in life activities [1]. Iron deficiency in animals can easily lead to anemia, impaired immune function, metabolic disorders, and consequently slow growth, pale skin, and rough hair coat.

The development of iron supplements has undergone three stages: the first generation consists of inorganic iron salts, including ferrous sulfate, ferrous carbonate, ferrous phosphate, ferrous chloride, etc. [10], with ferrous sulfate being commonly used in production. However, these supplements have low digestion and absorption rates, are susceptible to feed pH, crude fiber, and phytic

acid content, resulting in waste of mineral resources and environmental pollution. The second generation comprises organic iron salt additives, which are chelates of iron with proteins (such as casein) or organic acids (such as citric acid, fumaric acid, lactic acid, etc.). However, these have problems such as insignificant improvement effects and high economic costs. Amino acid chelated iron is known as the third-generation iron feed additive [11], consisting of metal elements bound to amino acids through coordination and ionic bonds to form cyclic chelate structures.

Compared with inorganic iron salts and simple organic acid iron salts, amino acid chelated iron has good stability and high solubility in the animal gastrointestinal tract. It not only alleviates antagonism among minerals and the chelation of iron with anti-nutritional factors in feed, but is also easily digested and absorbed with high utilization rates, while providing dual supplementation of amino acids and iron [12-13].

Weaned piglets have high iron requirements but low absorption rates [14], making organic iron, with its good oral absorption and high biological value, an excellent choice. Many studies have shown that compared with ferrous sulfate, ferrous glycine is more easily transported and absorbed [15], with biological utilization rates 125%~185% that of ferrous sulfate at the same level [16].

Feng et al. [17] reported that compared with adding 120 mg/kg ferrous sulfate, adding an equivalent amount of ferrous glycine chelate to weaned piglet diets reduced fecal iron content, a finding consistent with Creech et al. [18]. Muniz et al. [19] compared organic and inorganic mineral elements in piglets aged 24-57 days and found that organic trace elements improved piglet growth performance, but had no significant differential effects on mineral deposition in the liver, heart, spleen, and kidneys. Liu et al. [20] added 0.05% ferrous glycine to the diet of weaned piglets in the experimental group and found that compared with the control group (inorganic iron), diarrhea rate decreased by 56.3%, daily gain increased by 9.1% (significant difference), and feed-to-gain ratio decreased by 6.2%. Cocato et al. [21] studied 44 weaned piglets at 21 days of age and found that dietary iron methionine improved feed conversion more effectively than ferrous sulfate. Feng et al. [22] showed that compared with ferrous sulfate, dietary ferrous glycine significantly improved growth performance in broilers, significantly increased serum immunoglobulin G (IgG) and immunoglobulin M (IgM) contents, and enhanced serum superoxide dismutase (SOD) and catalase (CAT) activities.

In this experiment, the results were generally consistent with previous studies. Compared with ferrous sulfate, ferrous glycine showed a trend toward decreasing feed-to-gain ratio by 4.57% and had no significant effects on other growth performance metrics. It had no significant effect on iron deposition in tissues and organs, but significantly reduced fecal iron excretion, which has positive implications for environmental protection.

Yang [23] demonstrated that ferrous glycine significantly increased catalase

(CAT) activity, decreased serum malondialdehyde content, and significantly increased IgM content. Lysine iron, methionine iron, and ferrous fumarate can increase the expression of divalent metal transporter 1 (DMT1) in the duodenum, promoting iron absorption. Li [24] showed that at the same iron supplementation level, the IgM content in the organic iron group was higher than in the inorganic group. Chen et al. [25] added ferrous sulfate, ferrous fumarate, and ferrous glycine to the diets of 25-day-old weaned piglets and found that serum IgG content in piglets fed ferrous glycine was 12.2% and 10.6% higher than in the ferrous sulfate and ferrous fumarate groups, respectively. Zhang et al. [26] reported that compared with the same dose of ferrous sulfate, dipeptide chelated iron significantly increased average daily gain, decreased feed-to-gain ratio, and significantly increased serum iron content in piglets.

The primary sites of iron absorption are the duodenum and proximal jejunum, mediated by mature enterocytes on the villi. Currently, two distinct mechanisms are believed to exist for amino acid chelated iron absorption in the small intestine: 1) Amino acid chelated trace elements are dissociated in the intestine and absorbed as ions. For example, iron ions are transported into enterocytes via DMT1 located on the brush border membrane of the small intestine, then transferred into the blood through ferroportin 1 (FP1) on the basolateral membrane, while the dissociated amino acids are also utilized by the body through intestinal epithelial cells [27-29]. 2) Amino acid chelates enter body tissues as small peptides through specific or non-specific transporters [such as peptide transporter 1 (PepT1)] and are absorbed intact by the small intestine [30].

In this experiment, compared with ferrous sulfate, ferrous glycine extremely significantly increased iron apparent digestibility and apparent metabolic rate and increased blood iron content, consistent with previous research findings.

Compared with inorganic iron (ferrous sulfate), dietary organic iron (ferrous glycine) can improve growth performance, increase blood iron content, extremely significantly improve iron metabolic status, and significantly reduce fecal iron excretion in weaned piglets, making it a green and efficient new iron supplement.

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