

## Effects of Iron Dipeptide Chelate on Growth Performance, Serum Iron, and Antioxidant Indices in Piglets: Postprint

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### Abstract

This experiment aimed to investigate the effects of dipeptide-chelated iron on growth performance, serum iron, and antioxidant indices in piglets. Eighty healthy 40-day-old three-way crossbred piglets were selected and randomly divided into two groups: a control group and an experimental group, with 4 replicates per group and 10 piglets per replicate. The iron supplementation level in the diets of both the control and experimental groups was 180 mg/kg (as iron), with ferrous sulfate being the sole iron source in the control group and dipeptide-chelated iron being the sole iron source in the experimental group. The experimental period lasted 21 days. The results showed that compared with the control group, the average daily gain (ADG) of the experimental group increased by 10.37% ( $P > 0.05$ ), and the feed-to-gain ratio (F/G) decreased by 5.59% ( $P < 0.05$ ); the serum iron content in the experimental group was significantly higher than that in the control group ( $P < 0.05$ ), while serum ferritin and hemoglobin contents were higher than those in the control group ( $P > 0.05$ ); serum superoxide dismutase (SOD) activity in the experimental group was significantly higher than that in the control group ( $P < 0.05$ ), and serum malondialdehyde (MDA) content was significantly lower than that in the control group ( $P < 0.05$ ). These results indicate that dipeptide-chelated iron can significantly promote growth, improve iron metabolism status, and enhance antioxidant capacity in piglets.

## Full Text

# Effects of Ferrous N-carbamylglycinate Chelate on Growth Performance, Serum Iron and Antioxidant Indices of Piglets

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## Abstract

This study investigated the effects of ferrous N-carbamylglycinate chelate (Fe-CGly) on growth performance, serum iron status, and antioxidant indices in piglets. Eighty healthy 40-day-old Duroc×Landrace×Large Yorkshire crossbred piglets were randomly allocated to two groups with four replicates per group and ten piglets per replicate. Both groups received diets supplemented with 180 mg/kg iron, with the control group receiving ferrous sulfate and the experimental group receiving Fe-CGly. The 21-day trial revealed that compared with the control group, the experimental group exhibited a 10.37% increase in average daily gain (ADG) ( $P > 0.05$ ) and a 5.59% decrease in feed-to-gain ratio (F/G) ( $P < 0.05$ ). Serum iron content was significantly higher in the experimental group ( $P < 0.05$ ), while serum ferritin and hemoglobin levels were elevated but not significantly ( $P > 0.05$ ). Additionally, serum superoxide dismutase (SOD) activity was significantly higher ( $P < 0.05$ ) and serum malondialdehyde (MDA) content was significantly lower ( $P < 0.05$ ) in the experimental group. These results demonstrate that Fe-CGly can effectively promote piglet growth, improve iron metabolism, and enhance antioxidant capacity.

**Keywords:** ferrous N-carbamylglycinate chelate; hemoglobin; antioxidant; piglets

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## Introduction

Iron is an essential trace element for animal growth and development, serving as a critical component of hemoglobin and myoglobin and playing vital roles in numerous biochemical reactions. Iron deficiency in piglets can lead to anemia, reduced growth rates, and compromised disease resistance. Amino acid chelated iron represents the third generation of iron feed additives that have developed rapidly in recent years. These supplements closely resemble the natural form

of iron in animals and offer advantages including high bioavailability, efficient absorption, chemical stability, and environmental friendliness, making them a focal point of current research and development in China. Studies have shown that peptide and free amino acid absorption operate through independent, non-interfering pathways. Peptides are transported via peptide transport systems that are electrogenic and pH-gradient driven, offering benefits such as low energy consumption, rapid transport, and carriers that are not easily saturated. In contrast, amino acids are absorbed through sodium-dependent active transport. Although both utilize transport mechanisms, peptides have relatively lower osmotic pressure and their carriers demonstrate superior quantity and transport capacity compared to amino acid carriers. Consequently, dipeptide chelated iron may achieve higher absorption rates and efficiency than single amino acid chelated iron. However, research on dipeptide chelated iron remains scarce both domestically and internationally. Therefore, this study examined the effects of dipeptide chelated iron on growth performance, serum iron status, and antioxidant indices in piglets to provide a theoretical basis for its application in piglet diets.

### 1.1 Experimental Materials

Ferrous N-carbamylglycinate chelate was synthesized by the Institute of Subtropical Agriculture, Chinese Academy of Sciences (patent publication number: CN102093467A), with an iron content of 14.9% and chelation rate of 84%. Ferrous sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , analytical grade) was purchased from China National Pharmaceutical Group Shanghai Chemical Reagent Company.

### 1.2 Experimental Design

Eighty healthy 40-day-old Duroc×Landrace×Large Yorkshire crossbred piglets were selected and adapted for 4 days in nursery pens before fasting and weighing. The piglets were randomly divided into two groups based on body weight: a control group receiving ferrous sulfate and an experimental group receiving Fe-CGly. Each group comprised four replicates with ten piglets per replicate. The control diet was supplemented with 488 mg/kg ferrous sulfate (providing 180 mg/kg iron), while the experimental diet contained 932 mg/kg Fe-CGly (also providing 180 mg/kg iron). The trial lasted 21 days. The basal diet was formulated according to NRC (2012) standards, with composition and nutrient levels shown in .

### 1.3 Feeding Management

The experiment was conducted at the breeding base of Xinjiang Tiankang Animal Husbandry Biotechnology Co., Ltd. Piglets were group-housed in nursery pens and managed according to standard protocols for disinfection and immunization. Prior to the trial, piglets were fed creep feed for 4 days before transitioning to experimental diets. During the trial, feed was provided at 07:00,

12:00, and 18:00 daily. Piglets had ad libitum access to feed (with slight residual feed remaining after satiation) and water. Daily feed intake was recorded. Severe diarrhea or illness cases were treated or removed from the study.

#### 1.4 Sample Collection and Processing

On day 21, piglets were weighed and two piglets per pen were randomly selected for blood collection via anterior vena cava puncture. Two blood samples were collected from each piglet: one in an anticoagulant-containing tube and another in a 10 mL tube without anticoagulant. The latter was left overnight at 4 °C, centrifuged to prepare serum, aliquoted into EP tubes, and stored at -20 °C for subsequent analysis.

#### 1.5 Measurement Indicators and Methods

Growth performance parameters including initial and final body weight (on days 1 and 21) were recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G). Serum iron content and total iron-binding capacity were measured using a Beckman CX4 automatic biochemical analyzer. Serum ferritin was determined by enzyme-linked immunosorbent assay (ELISA). Hemoglobin content was measured using an automatic hematology analyzer. Superoxide dismutase (SOD) activity, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

#### 1.6 Data Processing and Analysis

Data are expressed as mean  $\pm$  standard deviation. Statistical analysis was performed using SPSS 16.0 software, with independent samples t-test used for inter-group comparisons. Differences were considered significant at  $P < 0.05$ .

#### 2.1 Effects of Ferrous N-carbamylglycinate Chelate on Growth Performance of Piglets

As shown in , dietary supplementation with Fe-CGly improved piglet growth performance. Compared with the control group, ADG increased by 10.37% ( $P > 0.05$ ) and F/G decreased by 5.59% ( $P < 0.05$ ) in the experimental group.

#### 2.2 Effects of Ferrous N-carbamylglycinate Chelate on Serum Iron Indices of Piglets

demonstrates that compared with the control group, serum iron content increased by 11.26% ( $P < 0.05$ ) in the experimental group. Ferritin and hemoglobin levels increased by 9.02% and 1.45%, respectively, while total iron-binding capacity decreased by 2.06%, though these differences were not statistically significant ( $P > 0.05$ ).

### 2.3 Effects of Ferrous N-carbamylglycinate Chelate on Serum Antioxidant Indices of Piglets

As presented in , serum MDA content decreased by 26.55% ( $P < 0.05$ ) and SOD activity increased by 21.40% ( $P < 0.05$ ) in the experimental group compared with the control group, while T-AOC showed no significant difference ( $P > 0.05$ ).

## Discussion

Ferrous N-carbamylglycinate chelate is a novel chelate product developed from amino acid chelated iron, with limited research reported worldwide. Li et al. used peptide chelated iron as an iron supplement in pregnant and lactating sows and piglets, finding it more effectively transferred into breast milk and increased milk iron content compared with ferrous sulfate. Zhang et al. demonstrated that dipeptide chelated iron significantly improved iron metabolism in an iron-deficient rat model. The present study is the first to investigate the effects of completely replacing dietary ferrous sulfate with Fe-CGly on weaned piglet growth performance. The results showed that Fe-CGly improved growth performance, reduced feed-to-gain ratio, and promoted growth in weaned piglets, consistent with previous findings on amino acid chelated iron supplementation.

Iron is a component of hemoglobin, myoglobin, cytochrome oxidase, and peroxidase, and is closely related to erythropoiesis and antioxidant status. Hemoglobin and serum ferritin are specific indicators reflecting iron storage and primary metrics for evaluating iron status in piglets, showing significant correlation with liver iron content. Hemoglobin concentrations above 100 g/L are considered appropriate in piglets, with values below 80 g/L indicating anemia. Previous studies reported that dietary amino acid chelated iron significantly improved iron status and increased serum iron and hemoglobin levels. In this study, Fe-CGly supplementation significantly elevated serum iron content, enhanced iron reserves, increased serum ferritin and hemoglobin levels, and decreased total iron-binding capacity, indicating improved iron metabolism. However, except for serum iron, no significant differences were observed in ferritin, hemoglobin, or total iron-binding capacity between groups. This may be attributed to the strict iron absorption regulation mechanism in animals. When dietary iron meets physiological requirements, the hepcidin-ferroportin1 iron regulatory axis and iron-responsive element/iron regulatory protein (IRE/IRP) system act on iron absorption proteins in the upper small intestine, downregulating iron absorption. This reduces ferroportin-mediated iron transport across the basolateral membrane of enterocytes and iron export from macrophages, thereby decreasing serum iron and transferrin saturation to maintain iron homeostasis. In this study, hemoglobin levels in both groups approached 100 g/L, within the normal appropriate range, which may explain the lack of significant differences in serum iron, total iron-binding capacity, and hemoglobin between Fe-CGly and ferrous sulfate groups.

Iron is a major participant in abnormal oxygen free radical generation under pathological conditions. Iron proteins are vulnerable to attack by reactive oxygen species ( $O_2 \cdot$ ), releasing ferric iron which is reduced to ferrous iron, thereby liberating iron from ferritin and generating hydroxyl radicals ( $OH \cdot$ ) through Fenton reactions. Hydroxyl radicals can react with virtually any biomolecule at extremely rapid rates, damaging biological macromolecules. Excess iron can also induce massive production of reactive oxygen species (ROS), and when ROS production exceeds antioxidant defense capacity, oxidative stress occurs, leading to DNA damage, lipid peroxidation, protein modification, and other destructive effects. However, toxicological evaluations of Fe-CGly have demonstrated its safety and non-toxicity, showing no teratogenic or mutagenic effects and no tissue damage in rat liver or kidney even at high doses, though its effects on the antioxidant system remain unreported. The body generates oxygen free radicals through enzymatic and non-enzymatic systems that attack polyunsaturated fatty acids in biological membranes, initiating lipid peroxidation and forming lipid peroxides such as aldehydes, ketones, and hydroxyl compounds. Therefore, this study evaluated the effects of Fe-CGly on oxidative-antioxidant status by measuring serum SOD activity, T-AOC, and MDA content. Compared with the control group, dietary Fe-CGly supplementation significantly reduced serum MDA content and increased SOD activity, indicating that Fe-CGly can enhance antioxidant enzyme system activity and free radical scavenging capacity, thereby reducing lipid peroxides and maintaining homeostasis.

In conclusion, dietary supplementation with Fe-CGly at 180 mg/kg iron can significantly promote piglet growth, increase serum iron content, improve iron metabolism, reduce serum MDA content, enhance serum SOD activity, and strengthen antioxidant capacity in piglets.

## References

- [1] WU X, YIN Y L, XING F F, et al. Application research progress of trace element amino acid chelates at home and abroad[J]. Swine Industry Science, 2008(3): 68-71.
- [2] TORRES-FUENTES C, ALAIZ M, VIOQUE J. Iron-chelating activity of chickpea protein hydrolysate peptides[J]. Food Chemistry, 2012, 134(3): 1585-1588.
- [3] SUN J, LIU D S, SHI R B. Supplemental dietary iron glycine modifies growth, immune function, and antioxidant activities in broiler chickens[J]. Livestock Science, 2015, 176: 129-134.
- [4] ZHANG Y Z, SUN X M, XIE C Y, et al. Effects of ferrous carbamoyl glycine on iron state and absorption in an iron-deficient rat model[J]. Genes & Nutrition, 2015, 10: 54.
- [5] WANG M Z, LIU M Z. Effects of amino acid chelated iron on production performance of early-weaned piglets[J]. China Animal Husbandry & Veterinary

Medicine, 2007, 34(10): 14-15.

[6] TIAN P. Effects of iron methionine chelate on production performance of weaned piglets[J]. Journal of Domestic Animal Ecology, 2005, 26(2): 33-35.

[7] HU P, CHENG M J, JIANG T, et al. Effects of ferrous glycinate on growth performance of weaned piglets[J]. Feed Industry, 2011, 32(13): 29-32.

[8] FINAZZI D, AROSIO P. Biology of ferritin in mammals: an update on iron storage, oxidative damage and neurodegeneration[J]. Archives of Toxicology, 2014, 88(10): 1787-1802.

[9] NRC. Nutrient requirements of swine[M]. 11th rev. ed. Washington, DC: National Academies Press, 2012.

[10] SPEARS J W, SCHOENHERR W D. Efficacy of iron methionine as a source of iron for nursing pigs[J]. Journal of Animal Science, 1992, 70(Suppl. 1): 243.

[11] SUN X M, WU X, YIN Y L, et al. Iron absorption and its regulation in young animals[J]. Chinese Journal of Animal Nutrition, 2012, 24(6): 1001-1006.

[12] PRÁ D, FRANKE S I R, HENRIQUES J A P, et al. Iron and genome stability: an update[J]. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 2012, 733(1/2): 92-99.

[13] PEREIRA M C, OLIVEIRA L C A, MURAD E. Iron oxide catalysts: Fenton and Fenton-like reactions—a review[J]. Clay Minerals, 2012, 47(3): 285-302.

[14] HALLIWELL B. Antioxidant characterization: methodology and mechanism[J]. Biochemical Pharmacology, 1995, 49(10): 1341-1348.

[15] DONG A L, HU Y L, QI Y B, et al. Research progress on iron metabolism disorders and other pathogenic factors mediated hepatic oxidative damage and antioxidant protection strategies[J]. Acta Biophysica Sinica, 2012, 28(4): 307-316.

[16] DIXON S J, STOCKWELL B R. The role of iron and reactive oxygen species in cell death[J]. Nature Chemical Biology, 2014, 10(1): 9-17.

[17] YIN J, REN W K, WU X S, et al. Oxidative stress-mediated signaling pathways: a review[J]. Journal of Food, Agriculture & Environment, 2013, 11(2): 132-139.

[18] CHEN K, YOU J, TANG Y, et al. Supplementation of superfine powder prepared from *Chaenomeles speciosa* fruit increases endurance capacity in rats via antioxidant and Nrf2/ARE signaling pathway[J]. Evidence-Based Complementary and Alternative Medicine, 2014, 2014: 976438.

[19] WAN D, ZHOU X H, XIE C Y, et al. Toxicological evaluation of ferrous N-carbamylglycinate chelate: acute, sub-acute toxicity and mutagenicity[J]. Regulatory Toxicology and Pharmacology, 2015, 73(2): 644-651.

[20] COBBINA S J, CHEN Y, ZHOU Z X, et al. Toxicity assessment due to sub-chronic exposure to individual and mixtures of toxic heavy metals[J]. Journal of Hazardous Materials, 2015, 294: 109-120.

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