Alanyl-Glutamine Alleviates Diquat-Induced Oxidative Damage in Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of alanyl-glutamine (Ala-Gln) in alleviating oxidative damage induced by Diquat in weaned piglets. A two-factor design was adopted in the experiment. Twenty-four healthy 21-day-old weaned piglets with similar parity were selected and randomly divided into 2 groups, with 12 replicates per group and 1 piglet per replicate. The two groups were fed a basal diet and a basal diet supplemented with 0.3% Ala-Gln, respectively. After a 7-day pre-feeding period, based on the previous feeding regimen, the piglets were divided into 4 groups, with 6 replicates per group and 1 piglet per replicate, namely the basal diet group, basal diet + 0.3% Ala-Gln group, basal diet stress group, and basal diet + 0.3% Ala-Gln stress group. Oxidative stress in piglets was simulated by intraperitoneal injection of 8 mg/kg BW Diquat, while the non-stressed groups received an equal volume of sterile physiological saline. The experimental period lasted for 7 days. The results showed that: 1) Under oxidative stress conditions, compared with the basal diet stress group, dietary supplementation with Ala-Gln significantly increased serum glutamine (Gln) and glutathione (GSH) contents, as well as the activities of glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD), and total antioxidant capacity (T-AOC) ($P<0.05$). 2) Under both normal physiological and oxidative stress conditions, compared with the corresponding basal diet group and basal diet stress group, dietary supplementation with Ala-Gln significantly increased jejunal GSH-Px activity and T-AOC ($P<0.05$), and significantly decreased malondialdehyde (MDA) content ($P<0.05$); dietary supplementation with Ala-Gln also significantly increased hepatic GSH-Px activity and T-AOC ($P<0.05$), and significantly decreased MDA content ($P<0.05$). 3) Under oxidative stress conditions, compared with the basal diet stress group, dietary supplementation with Ala-Gln significantly increased the mRNA expression level of glutathione peroxidase 4 (GPx4) in the liver ($P<0.05$), and significantly decreased the mRNA expression level of superoxide dismutase 1 (SOD1) ($P<0.05$). It can be concluded that dietary supplementation with Ala-Gln can enhance the antioxidant

capacity of piglets and decrease MDA content under both normal physiological and oxidative stress conditions, thereby mitigating oxidative stress-induced damage to tissues in weaned piglets; and the effect is more pronounced under oxidative stress conditions.

Full Text

Abstract

This study investigated the effects of alanyl-glutamine (Ala-Gln) on alleviating oxidative damage induced by Diquat in weaned piglets. A 2×2 factorial design was employed with twenty-four 21-day-old weaned piglets in good health and of similar parity. The piglets were initially randomly divided into 2 groups (12 replicates per group, 1 pig per replicate) and fed either a basal diet or a basal diet supplemented with 0.3% Ala-Gln. After a 7-day pre-feeding period, the piglets were further divided into 4 groups (6 replicates per group, 1 pig per replicate): basal diet group, basal diet + 0.3% Ala-Gln group, basal diet + stress group, and basal diet + 0.3% Ala-Gln + stress group. Oxidative stress was induced by intraperitoneal injection of 8 mg/kg BW Diquat, while non-stressed groups received an equivalent volume of sterile saline. The experimental period lasted 7 days. The results showed: 1) Under oxidative stress, dietary Ala-Gln supplementation significantly increased serum glutamine (Gln) and glutathione (GSH) contents, as well as glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD) activities, and total antioxidant capacity (T-AOC) compared with the basal diet stress group ($P<0.05$). 2) Under both normal physiological and oxidative stress conditions, dietary Ala-Gln supplementation significantly increased jejunal and hepatic GSH-Px activity and T-AOC, while significantly decreasing malondialdehyde (MDA) content ($P<0.05$). 3) Under oxidative stress, dietary Ala-Gln supplementation significantly increased hepatic glutathione peroxidase 4 (GPx4) mRNA expression and significantly decreased superoxide dismutase 1 (SOD1) mRNA expression ($P<0.05$). These findings indicate that dietary Ala-Gln supplementation can enhance antioxidant capacity and reduce MDA content in piglets under both normal physiological and oxidative stress conditions, thereby mitigating oxidative stress-induced tissue damage in weaned piglets, with more pronounced effects under oxidative stress conditions.

Keywords: oxidative stress; Ala-Gln; piglets; antioxidant capacity

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Introduction

Under normal physiological conditions, oxidation and reduction exist in dynamic equilibrium within animal bodies, with free radicals being continuously generated and promptly eliminated. When this homeostasis is disrupted, free radicals accumulate due to inadequate decomposition or conversion. When free radicals

exceed the capacity of the antioxidant defense system, oxidative damage occurs, impairing digestive and immune systems and reducing production performance [1]. In swine production, oxidative stress is a common physiological phenomenon in weaned piglets and a major factor causing economic losses.

Glutamine (Gln) is the most abundant free amino acid in mammalian blood, reaching 1.93 mmol/L in sow milk at 21 days of lactation [2]. As an important immune enhancer, Gln reduces oxidative damage during oxidative stress. Studies have shown that dietary Gln supplementation increases free Gln and GSH contents in piglet plasma and jejunal tissue. Gln alleviates the decrease in intestinal Gln content and the increase in oxidized glutathione (GSSG)/reduced GSH ratio induced by weaning stress [3], and reduces apoptosis during disease or stress states, primarily by enhancing antioxidant enzyme defense, heat shock protein expression, and inducing autophagy [4]. As the main energy source for intestinal cells, Gln also effectively promotes proliferation and differentiation of small intestinal epithelial cells and lymphocytes, and repairs intestinal mucosa [5]. However, due to its low water solubility, thermal instability, and easy decomposition into toxic pyroglutamic acid and ammonia, monomeric Gln is limited in livestock feed applications. Dipeptide forms of Gln (such as alanyl-glutamine, Ala-Gln) overcome these disadvantages, showing greater stability in aqueous and thermal environments (remaining stable for 2 years at room temperature) with approximately 4 times higher water solubility than monomeric Gln [6]. Intestinal mucosa can absorb Gln dipeptides, which rapidly decompose into Gln in tissues and cells for utilization. The absorption of Gln dipeptides by the small intestine is non-saturable and non-competitive, offering greater absorption advantages compared to monomeric Gln. Consequently, Gln dipeptides are widely used in clinical total parenteral nutrition as Gln substitutes. However, few studies have reported the effects of Gln dipeptides on antioxidant capacity in weaned piglets. Therefore, this study used Ala-Gln to investigate its effects on serum, jejunal, and hepatic antioxidant indices in Diquat-induced oxidative stress piglets, aiming to provide a scientific basis for Ala-Gln application in piglet diets.

Materials and Methods

1.1 Experimental Material

The Ala-Gln used in this experiment was purchased from Shanghai Chaoqiang Chemical Co., Ltd., with purity 99%.

1.2 Experimental Animals and Design

A 2×2 factorial design was employed. Twenty-four 21-day-old weaned castrated male piglets in good health and of similar parity were randomly divided into 2 groups (12 replicates per group, 1 pig per replicate). The two groups were

fed either a basal diet or a basal diet supplemented with 0.3% Ala-Gln. After a 7-day pre-feeding period, the piglets were further divided into 4 groups (6 replicates per group, 1 pig per replicate): basal diet group, basal diet + 0.3% Ala-Gln group, basal diet + stress group, and basal diet + 0.3% Ala-Gln + stress group. Oxidative stress was induced by intraperitoneal injection of 8 mg/kg BW Diquat, while non-stressed groups received an equivalent volume of sterile saline. The Diquat dosage was determined according to Xu et al. [7]. The experimental period lasted 7 days.

1.3 Experimental Diets

The basal diet was a corn-soybean meal-based diet. The experimental diet was prepared by adding 0.3% Ala-Gln to the basal diet. The diet formulation followed NRC (2012) guidelines and was formulated based on true ileal digestible amino acids. The composition and nutrient levels of the basal diet are shown in Table 1 .

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

The premix provided the following per kilogram of diet: Fe 120 mg, Cu 7 mg, Mn 25 mg, Zn 130 mg, I 0.2 mg, Se 0.3 mg, Co 1.5 mg, VA 4,800 IU, VD 480 IU, VE 40 IU, VK 1.5 mg, VB 3 mg, VB 8 mg, VB 3.5 mg, VB 0.04 mg, pantothenic acid 25 mg, niacin 35 mg, biotin 0.15 mg, folic acid 1 mg.

1.4 Management

The experiment was conducted at the Animal Experimental Center of the Key Laboratory of Animal Nutrition in Jiangxi Province, Jiangxi Agricultural University. The pig house temperature was maintained at 23–26°C with relative humidity of 55–65%. Pigs were fed powdered feed ad libitum with free access to water. Deworming, castration, and vaccination were performed according to routine farm procedures.

1.5 Sample Collection and Processing

On day 7 of the experimental period, blood samples were collected from the anterior vena cava after overnight fasting (by replicate). After clotting, serum was separated by centrifugation at 3,000 r/min for 15 min and stored at -20°C for analysis. Piglets were anesthetized by intravenous injection of 5% sodium pentobarbital, then euthanized by exsanguination. The abdomen was opened, and the liver and jejunum were rapidly separated. After removing fat and visible connective tissue, samples were rinsed with pre-cooled (4°C) saline. Liver samples were immediately frozen in liquid nitrogen and stored at -70°C.

The small intestine and mesentery were pushed to the lower left to expose the duodenal peritoneal fixation segment. The jejunal head position was located and ligated with suture. After cutting the mesentery, approximately 10 cm of the middle jejunum was taken, gently rinsed with pre-cooled saline, and a 2 cm

segment was stored in a 1.5 mL cryovial, snap-frozen in liquid nitrogen, and stored at -70°C.

1.6 Assays

1.6.1 Serum Antioxidant Indices Using kits from Nanjing Jiancheng Bioengineering Institute, serum Gln, GSH, and MDA contents, as well as GSH-Px, CAT, T-SOD activities, and T-AOC were determined according to kit instructions.

1.6.2 Jejunal and Hepatic Antioxidant Indices Using kits from Nanjing Jiancheng Bioengineering Institute, GSH and MDA contents, GSH-Px, CAT, T-SOD activities, and T-AOC in jejunum and liver were determined according to kit instructions.

1.6.3 Hepatic GPx4 and SOD1 mRNA Expression 1.6.3.1 Total RNA Extraction and Reverse Transcription

Total RNA was extracted from liver using Trizol method according to kit instructions. RNA concentration and purity were measured (ideal OD 260/280 ratio: 1.8–2.2). First-strand cDNA was synthesized using TaKaRa RR047A reverse transcription kit and stored at -20°C.

1.6.3.2 GPx4 and SOD1 mRNA Expression Detection

GPx4 and SOD1 mRNA primers and probes were designed using Primer Express 2.0 software and synthesized by a commercial company. Primer and probe sequences are shown in Table 2 . Reactions were performed on an FTC2000 real-time PCR system (Canada) under the following conditions: 50 µL reaction volume containing 25 µL 2×Hotstart Fluo-PCR mix, 1 µL each of forward/reverse primer, 0.5 µL Probe (25 pmol/µL), 1 µL cDNA template, and 21.5 µL dH O. Thermal cycling: 94°C for 4 min; 40 cycles of 94°C for 20 s, 60°C for 30 s. -actin served as internal control. GPx4 and SOD1 mRNA expression was calculated using the 2^(-ΔCt) method.

Table 2 Primer and probe sequences of GPx4, SOD1 and -actin mRNA

Target gene	Accession No.	Sequence
Glutathione peroxidase 4 (GPx4)	NM_214407.1	5’ - CCAGTTTGGGAGGCAGGAG- 3’ 5’ - GGACTTTCATCCACTTCCACAG- 3’ 5’ - TCCCCATTCACACAGATCTTGCTGAAC- 3’

Target gene	Accession No.	Sequence
Superoxide dismutase 1 (SOD1)	XM_005657141.1	5’ - TGGAGACCTGGGCAATGTG- 3’ 5’ - CCACCTCTGCCCAAGTCATC- 3’ 5’ - CATCGAAGATTCTGTGATCGCCCTC- 3’
-actin	AF054837.1	5’ - GGGTATGGGTCAGAAAGATTCC- 3’ 5’ - TCTCCATGTCGTCCCAGTTG- 3’ 5’ - CTCAGAGCAAGAGAGGTATCCTGACCCTC- 3’

1.7 Statistical Analysis

Data were analyzed using SPSS 17.0 software. Two-way ANOVA was performed to analyze main effects (Ala-Gln and Diquat) and their interaction. Duncan’ s multiple range test was used for post-hoc comparisons. Results are expressed as “mean ± SE” . mRNA expression data calculated by 2^(-ΔCt) are also expressed as “mean ± SE” . $P<0.05$ was considered statistically significant.

Results

2.1 Effects of Ala-Gln on Serum Antioxidant Indices of Oxidative Stress Piglets

The effects of Ala-Gln on serum antioxidant indices are shown in Table 3 . Diquat significantly affected serum GSH-Px and T-SOD activities, T-AOC, and MDA content ($P<0.05$), but not serum Gln, GSH contents, or CAT activity ($P>0.05$). Ala-Gln significantly affected serum Gln and GSH contents, GSH-Px and T-SOD activities, and T-AOC ($P<0.05$), but not serum MDA content or CAT activity ($P>0.05$). The interaction between Diquat and Ala-Gln significantly affected serum T-SOD activity and T-AOC ($P<0.01$), but not serum Gln, GSH, MDA contents, or GSH-Px, CAT activities ($P>0.05$).

Multiple comparisons revealed that Diquat-induced oxidative stress significantly decreased serum GSH content and T-SOD activity ($P<0.05$). Under

normal physiological conditions, dietary Ala-Gln supplementation significantly decreased serum T-SOD activity (P<0.05), significantly increased serum Gln content and T-AOC (P<0.05), but had no significant effect on serum GSH content or GSH-Px and CAT activities (P>0.05). Under oxidative stress conditions, dietary Ala-Gln supplementation significantly increased serum Gln, GSH, and MDA contents, as well as GSH-Px and T-SOD activities (P<0.05).

Table 3 Effects of Ala-Gln on serum antioxidant indices of weaned piglets challenged with oxidative stress

(-) indicates no stress; (+) indicates stress. Ala-Gln: alanyl-glutamine. In the same row, values with different small letter superscripts differ significantly (P<0.05), while values with the same or no superscripts do not differ significantly (P>0.05). The same applies below.

2.2 Effects of Ala-Gln on Jejunal Antioxidant Indices of Oxidative Stress Piglets

The effects of Ala-Gln on jejunal antioxidant indices are shown in Table 4 . Diquat significantly affected jejunal GSH-Px, CAT, T-SOD activities, MDA content, and T-AOC (P<0.05). Ala-Gln significantly affected jejunal GSH-Px, T-SOD activities, MDA content, and T-AOC (P<0.05), but not CAT activity (P>0.05). The interaction between Diquat and Ala-Gln significantly affected jejunal MDA content, GSH-Px activity, and T-AOC (P<0.05), but not CAT or T-SOD activities (P>0.05).

Multiple comparisons revealed that Diquat-induced oxidative stress significantly decreased jejunal GSH-Px, T-SOD activities, and T-AOC (P<0.05), and significantly increased jejunal MDA content (P<0.05). Under normal physiological conditions, dietary Ala-Gln supplementation significantly increased jejunal GSH-Px activity and T-AOC (P<0.05), significantly decreased jejunal MDA content (P<0.05), but had no significant effect on CAT or T-SOD activities (P>0.05). Under oxidative stress conditions, dietary Ala-Gln supplementation significantly increased jejunal GSH-Px, T-SOD activities, and T-AOC (P<0.05), and significantly decreased jejunal MDA content (P<0.05).

Table 4 Effects of Ala-Gln on jejunal antioxidant indices of weaned piglets challenged with oxidative stress

2.3 Effects of Ala-Gln on Hepatic Antioxidant Indices of Oxidative Stress Piglets

The effects of Ala-Gln on hepatic antioxidant indices are shown in Table 5 . Diquat significantly affected hepatic GSH-Px, CAT, T-SOD activities, MDA content, and T-AOC (P<0.05). Ala-Gln significantly affected hepatic T-SOD, GSH-Px activities, MDA content, and T-AOC (P<0.05), but not CAT activity (P>0.05). The interaction between Diquat and Ala-Gln significantly affected

hepatic MDA content, GSH-Px activity, and T-AOC ($P<0.05$), but not CAT or T-SOD activities ($P>0.05$).

Multiple comparisons revealed that Diquat-induced oxidative stress significantly decreased hepatic GSH-Px, T-SOD activities, and T-AOC ($P<0.05$), and significantly increased hepatic MDA content ($P<0.05$). Under normal physiological conditions, dietary Ala-Gln supplementation significantly increased hepatic GSH-Px activity and T-AOC ($P<0.05$), significantly decreased MDA content ($P<0.05$), but had no significant effect on CAT or T-SOD activities ($P>0.05$). Under oxidative stress conditions, dietary Ala-Gln supplementation significantly increased hepatic GSH-Px, T-SOD activities, and T-AOC ($P<0.05$), and significantly decreased hepatic MDA content ($P<0.05$).

Table 5 Effects of Ala-Gln on liver antioxidant indices of weaned piglets challenged with oxidative stress

2.4 Effects of Ala-Gln on Hepatic GPx4 and SOD1 mRNA Expression

The effects of Ala-Gln on hepatic GPx4 and SOD1 mRNA expression are shown in Table 6 . Diquat, Ala-Gln, and their interaction significantly affected hepatic GPx4 and SOD1 mRNA expression ($P<0.05$).

Under normal physiological conditions, dietary Ala-Gln supplementation increased hepatic GPx4 mRNA expression by 95.8% ($P<0.05$). Under oxidative stress conditions, dietary Ala-Gln supplementation increased hepatic GPx4 mRNA expression by 166.7% ($P<0.05$).

Diquat-induced oxidative stress significantly increased hepatic SOD1 mRNA expression ($P<0.05$). Under normal physiological conditions, dietary Ala-Gln supplementation decreased hepatic SOD1 mRNA expression by 59.4% ($P>0.05$). Under oxidative stress conditions, dietary Ala-Gln supplementation decreased hepatic SOD1 mRNA expression by 40.0% ($P<0.05$).

Table 6 Effects of Ala-Gln on liver GPx4 and SOD1 mRNA expressions of weaned piglets challenged with oxidative stress

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Discussion

3.1 Effects of Ala-Gln on Serum Antioxidant Indices of Oxidative Stress Piglets

The transition from sow milk to solid feed after weaning causes a sharp drop in feed intake that cannot meet the piglet' s Gln requirement. As an important antioxidant substance, exogenous Gln supplementation can effectively improve piglet growth performance, protect intestinal morphology, and alleviate weaning stress [3,8-12]. As a Gln substitute, Ala-Gln has similar functions. During stress, the body' s demand for Gln increases and endogenous Gln cannot meet

requirements. Since weaned piglets cannot obtain Gln from sow milk, dietary Gln or Ala-Gln supplementation is particularly important [13]. The antioxidant system in animals mainly consists of GSH-Px, superoxide dismutase (SOD), CAT, and low molecular weight compounds (vitamin C, vitamin E, GSH, etc.). Serum antioxidant enzyme activity reflects the redox status in vivo. Dietary Gln supplementation can also prevent GSH synthesis 障碍 caused by insufficient precursors [14].

Our previous study found that dietary supplementation with 0.3% Ala-Gln significantly improved growth performance in 21-28 day-old piglets [15]. The current study found that under normal physiological conditions, dietary Ala-Gln supplementation significantly increased serum Gln content, supplementing the body' s Gln, with a trend toward increased serum GSH content and GSH-Px and CAT activities. This is similar to findings by Dai et al. [16]. The study also found that serum T-SOD activity was significantly decreased, possibly because the body in redox balance does not require as many antioxidant enzymes to maintain homeostasis. Similarly, Zhang et al. [17] found that dietary Gln supplementation increased serum GSH-Px activity and decreased serum SOD activity in 35-day-old piglets. Xi et al. [18] found that Gln dipeptide supplementation increased serum SOD activity in weaned piglets, possibly due to differences in supplement specifications and levels. Diquat injection to simulate oxidative stress significantly decreased serum GSH-Px and T-SOD activities and T-AOC, while significantly increasing serum MDA content. This confirms that during persistent oxidative stress, serum GSH-Px and T-SOD activities decrease to regulate oxidative-reductive balance. Studies show that decreased antioxidant enzyme activity in animals results from feedback regulation by hydrogen peroxide (H O) end products or inactivation of antioxidant enzymes by superoxide anion (O). Xu et al. [7] also found that Diquat injection significantly decreased serum GSH-Px and T-SOD activities and hydroxyl radical scavenging capacity, and significantly increased serum MDA content on days 7, 14, 21, and 28, with a trend toward decreased serum CAT activity and increased H O content.

This study found that under oxidative stress, dietary supplementation with 0.3% Ala-Gln significantly increased serum Gln and GSH contents, as well as GSH-Px, SOD activities, and T-AOC, with a trend toward decreased serum MDA content. During oxidative stress, the body requires enhanced antioxidant capacity to maintain redox homeostasis. Exogenous Ala-Gln supplementation increases serum Gln content, promotes GSH-Px synthesis, maintains SOD function, and significantly improves T-AOC, alleviating oxidative stress intensity and protecting piglets from stress damage.

3.2 Effects of Ala-Gln on Jejunal and Hepatic Antioxidant Indices of Oxidative Stress Piglets

The small intestine and liver are the two most active sites in animals, providing continuous nutrients and immune factors, while also being most vulnerable to

damage during stress. Both tissues have well-developed antioxidant systems with sophisticated mechanisms for antioxidant enzyme expression and synthesis.

Under normal physiological conditions, dietary Ala-Gln supplementation significantly increased jejunal GSH-Px activity and T-AOC, significantly decreased jejunal MDA content, and showed a trend toward increased jejunal T-SOD activity, possibly because the piglet intestine encounters various stressors requiring stronger antioxidant capacity to maintain tissue homeostasis. During oxidative stress, jejunal GSH-Px, T-SOD, and CAT activities and T-AOC significantly decreased, while MDA content significantly increased. However, Ala-Gln supplementation significantly increased jejunal GSH-Px and T-SOD activities and T-AOC, and significantly decreased jejunal MDA content. This indicates that Ala-Gln can enhance jejunal antioxidant enzyme activity, improve antioxidant capacity, and reduce oxidative stress damage. Additionally, dietary Ala-Gln supplementation significantly increased hepatic GSH-Px activity and T-AOC, and significantly decreased hepatic MDA content. Oxidative stress significantly decreased hepatic GSH-Px, T-SOD, and CAT activities and T-AOC, while significantly increasing hepatic MDA content, causing hepatic stress injury. Numerous studies have also found that oxidative stress decreases hepatic SOD activity and increases MDA content in piglets [19-21]. Dietary Ala-Gln supplementation significantly increased hepatic GSH-Px, CAT, and T-SOD activities under oxidative stress, possibly because increased serum Gln content enhanced GSH-Px synthesis, protecting tissues from oxidative damage, while CAT and T-SOD maintained enzyme activity to exert normal antioxidant functions. This demonstrates that dietary Ala-Gln supplementation can improve hepatic antioxidant capacity in piglets.

3.3 Effects of Ala-Gln on Hepatic GPx4 and SOD1 mRNA Expression

GSH-Px and SOD are two important antioxidant enzymes that promptly clear accumulated free radicals and maintain redox homeostasis during persistent oxidative stress. GPx4 is an important member of the GSH-Px family, playing crucial antioxidant functions across different tissues [22]. GPx4 is the only enzyme in mammalian cells that can directly reduce phospholipid hydroperoxides on biological membranes, thereby protecting membranes from oxidative stress damage [23]. Studies have shown that hepatic GPx4 mRNA expression is significantly higher than in other tissues, with relatively high SOD mRNA expression as well [24].

This study found that to maintain redox homeostasis, oxidative stress increased hepatic GPx4 and SOD1 mRNA expression, enhancing hepatic antioxidant enzyme activity. With exogenous Ala-Gln supplementation, GPx4 mRNA expression significantly increased under both normal and stress conditions, while SOD1 mRNA expression significantly decreased. This is consistent with Hiraishi et al. [25], indicating that the entire antioxidant defense system in animals operates through enzymatic mechanisms, maintaining relatively constant antioxidant capacity across normal, stress, and pathological states. Hiraishi et al. [25]

also demonstrated that SOD mRNA expression is induced by superoxide anion radicals during oxidative stress. When Gln content increases, GSH-Px mRNA expression rises, promptly clearing tissue free radicals and reducing the need for SOD. Fluorescence quantitative PCR results further confirm that Ala-Gln supplementation can reduce free radicals generated by oxidative stress, enhancing antioxidant capacity through increased GSH-Px mRNA expression.

Conclusion

1. Under normal physiological conditions, dietary Ala-Gln supplementation significantly increased serum Gln content and T-AOC, significantly increased jejunal and hepatic GSH-Px activity and T-AOC, and significantly decreased jejunal and hepatic MDA content.
2. Under oxidative stress conditions, dietary Ala-Gln supplementation significantly increased some antioxidant indices in serum, jejunum, and liver, decreased MDA content, significantly increased hepatic GPx4 mRNA expression, and significantly decreased SOD1 mRNA expression.
3. Dietary Ala-Gln supplementation can alleviate oxidative stress-induced tissue damage in weaned piglets, with more significant effects under oxidative stress conditions.

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Note: Figure translations are in progress. See original paper for figures.