Effects of Glutamine on Lipopolysaccharide-Induced Oxidative Stress in Weaned Piglets: Postprint

Authors: Li Huan, yellow cattle, He Liuqin, Tian Junquan, Wu Xiaosong, Yao Kang

Date: 2017-10-23T00:00:00+00:00

Abstract

This experiment established an oxidative stress model using Escherichia coli lipopolysaccharide (LPS) to investigate the effects of glutamine (GLN) on oxidative stress in weaned piglets. Twenty-four healthy 28-day-old three-way crossbred (Duroc × Landrace × Large White) weaned piglets were selected and randomly divided into 3 groups with 8 replicates per group and 1 pig per replicate. The control and stress groups were fed a basal diet, while the GLN group was fed the basal diet supplemented with 1% GLN for a 30-day experimental period. On days 22, 25, 28, and 30 of the experiment, piglets in the stress and GLN groups were intraperitoneally injected with 100 g LPS per kilogram of body weight, while piglets in the control group were intraperitoneally injected with the same dose of sterile physiological saline. On day 30, blood samples were collected from the anterior vena cava and intestinal samples were collected after slaughter to measure oxidative stress-related indicators. The results showed: 1) Before LPS challenge, there were no significant differences in serum antioxidant capacity indices among groups ($P>0.05$). After LPS challenge, serum malondialdehyde (MDA) content in the stress group was significantly higher than that in the control group ($P<0.05$); serum MDA content and superoxide dismutase (SOD) activity in the GLN group were significantly lower than those in both the stress and control groups ($P<0.05$). 2) After LPS challenge, in duodenal mucosa, the relative expression levels of catalase (CAT) and copper-zinc superoxide dismutase (CuZnSOD) genes in the GLN group were significantly higher than those in the stress group ($P<0.05$). In jejunal mucosa, the relative expression levels of CAT, manganese superoxide dismutase (MnSOD), glutathione peroxidase 1 (GPX1), and glutathione peroxidase 4 (GPX4) genes in the GLN group were significantly higher than those in both the control and stress groups ($P<0.05$), while the relative expression level of GPX4 gene in the control group was significantly higher than that in the stress group ($P<0.05$). In ileal mucosa, the

relative expression levels of CAT gene in both the GLN and stress groups were significantly lower than that in the control group (P<0.05), while the relative expression level of GPX4 gene was significantly higher than that in the control group (P<0.05); the relative expression level of MnSOD gene in the GLN group was significantly higher than those in both the control and stress groups (P<0.05), and the relative expression level of CuZnSOD gene was significantly higher than that in the control group (P<0.05). These results indicate that GLN can alleviate LPS-induced oxidative stress in weaned piglets to a certain extent, providing a theoretical basis for reducing oxidative stress in practical production.

Full Text

Effects of Glutamine on Lipopolysaccharide-Induced Oxidative Stress in Weaned Piglets

LI Huan[1], HUANG Niu[1], HE Liuqin[2,3], TIAN Junquan[2,3], WU Xiaosong[1], *YAO Kang[1,2]* [1]College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China
[2]Key Laboratory of Agro-Ecological Processes in Subtropical Region, Hunan Provincial Engineering Research Center for Healthy Breeding of Livestock and Poultry, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South Central, Ministry of Agriculture, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
[3]University of Chinese Academy of Sciences, Beijing 100049, China

Abstract

This study evaluated the effects of glutamine (GLN) on oxidative stress in weaned piglets using an Escherichia coli lipopolysaccharide (LPS) challenge model. Twenty-four healthy 28-day-old crossbred (Duroc × Landrace × Large White) weaned piglets were randomly assigned to three groups with eight replicates per group and one piglet per replicate. Piglets in the control and stress groups were fed basal diets, while those in the GLN group received basal diets supplemented with 1% GLN. The experiment lasted 30 days. On days 22, 25, 28, and 30, piglets in the stress and GLN groups were injected intraperitoneally with 100 g LPS per kg body weight, while control piglets received sterile saline at the same volume. On day 30, blood samples were collected via precaval venipuncture, and intestinal samples were collected post-slaughter to measure oxidative stress-related indices.

The results showed: (1) Serum antioxidant capacity indices did not differ significantly among groups before LPS challenge (P > 0.05). After LPS challenge, serum malondialdehyde (MDA) content in the stress group was significantly higher than in the control group (P < 0.05), while serum MDA content and superoxide dismutase (SOD) activity in the GLN group were significantly lower

than in both the stress and control groups ($P < 0.05$). (2) After LPS challenge, the relative expression levels of catalase (CAT) and copper-zinc superoxide dismutase (CuZnSOD) genes in duodenal mucosa were significantly higher in the GLN group than in the stress group ($P < 0.05$). In jejunal mucosa, the relative expression levels of CAT, manganese superoxide dismutase (MnSOD), glutathione peroxidase 1 (GPX1), and glutathione peroxidase 4 (GPX4) genes in the GLN group were significantly higher than in both the control and stress groups ($P < 0.05$), while GPX4 gene expression in the control group was significantly higher than in the stress group ($P < 0.05$). In ileal mucosa, CAT gene expression in the GLN and stress groups was significantly lower than in the control group ($P < 0.05$), whereas GPX4 gene expression was significantly higher than in the control group ($P < 0.05$). MnSOD gene expression in the GLN group was significantly higher than in both the control and stress groups ($P < 0.05$), and CuZnSOD gene expression was significantly higher than in the control group ($P < 0.05$). These results indicate that GLN can alleviate LPS-induced oxidative stress in weaned piglets, providing a theoretical basis for reducing oxidative stress in practical production.

Keywords: glutamine; weaned piglets; lipopolysaccharide; oxidative stress; antioxidant capacity

Received: 2016-10-13

Funding: National 973 Program Project (2013CB127301); CAS "Hundred Talents Program" Project; National Natural Science Foundation of China (31472107)

Author Introduction: LI Huan (1992–), male, from Nanyang, Henan, master' s student, majoring in animal nutrition and feed science. E-mail: 977141476@qq.com

Corresponding Authors: YAO Kang, professor, doctoral supervisor, Email: yaokang@isa.ac.cn; WU Xiaosong, associate professor, E-mail: wuxiaosong529@126.com

---

During piglet production, various factors including management practices, environmental conditions, and bacterial or viral infections can induce stress responses that not only affect normal growth and development but also compromise immune function and post-slaughter meat quality [1]. During oxidative stress, animals exhibit increased energy demands and generate excessive free radicals, which cause oxidation of nucleic acids and proteins and damage biological membranes through lipid peroxidation reactions, thereby destroying intestinal mucosal integrity and function and reducing growth performance and immune capacity [2-4]. Glutamine (GLN), the most abundant free amino acid in serum of humans and other mammals, plays a crucial role in metabolism and exhibits anti-stress, anti-infection, antioxidant, and immune-enhancing functions [5]. Research indicates that GLN can maintain and increase intracellular glu-

tathione (GSH) content to scavenge various free radicals and peroxide damage, thereby maintaining homeostasis [6]. Studies also show that dietary GLN supplementation significantly increases glutathione peroxidase (GSH-Px) activity and reduces malondialdehyde (MDA) content in blood of yellow-feathered broilers under high-temperature conditions, thereby improving antioxidant capacity [7]. Despite limitations such as low water solubility and susceptibility to conversion into ammonia and pyroglutamic acid, GLN' s unique antioxidant properties have enabled its widespread application in production. However, the mechanism underlying GLN' s enhancement of antioxidant capacity remains unclear, particularly regarding its effects on oxidative stress in weaned piglets. Therefore, this study established an oxidative stress model by intraperitoneal injection of bacterial lipopolysaccharide (LPS) to investigate the effects of GLN on oxidative stress in early-weaned piglets and elucidate the underlying mechanisms.

1.1 Experimental Materials

GLN was purchased from Wuhan Yuancheng Gongchuang Technology Co., Ltd. with 99.5% purity. Bacterial LPS (E. coli serotype O55:B5) was purchased from Sigma Chemical Company (USA). RNA extraction reagent Trizol was purchased from Invitrogen.

1.2 Experimental Animals and Management

Twenty-four healthy crossbred (Duroc × Landrace × Large White) weaned piglets at 28 days of age with initial body weight of (6.24 ± 0.25) kg (half male and half female) were randomly allocated into three groups with eight replicates per group and one piglet per replicate. The control and stress groups were fed basal diets, while the GLN group received basal diets supplemented with 1% GLN. The experimental period lasted 30 days. Basal diets were formulated according to NRC (2012) standards, and diet composition and nutrient levels are shown in Table 1 . On days 22, 25, 28, and 30, piglets in the stress and GLN groups were injected intraperitoneally with 100 g LPS per kg body weight, while control piglets received the same volume of sterile saline [3-4]. The experiment was conducted at the animal facility of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Piglets were housed individually in cages, and immunization and disinfection procedures followed conventional pig farm protocols. Feed intake and body weight were recorded daily. Piglets were fed three times daily at 07:30, 12:00, and 18:30 with free access to feed and water. Powdered feed was provided with the principle of no leftover feed in troughs. Pens were cleaned twice daily to maintain hygiene. The facility used natural ventilation with periodic disinfection throughout the experimental period.

1.3 Sample Collection

Blood samples were collected via precaval venipuncture before the first LPS (or saline) injection and after the final injection using heparin-anticoagulated

vacuum tubes. Samples were left at room temperature for 15 minutes, then centrifuged at 3,000 r/min for 15 minutes to separate serum, which was stored at -80°C until analysis. Twelve hours after the final LPS (or saline) injection, piglets were slaughtered after fasting. Intestinal segments (duodenum, jejunum, and ileum) were collected. Approximately 10 cm segments of duodenum, jejunum, and ileum were gently opened with scissors, rinsed with ice-cold saline to remove intestinal contents, and blotted with filter paper to remove surface moisture. Intestinal mucosa was scraped with glass slides, wrapped in aluminum foil, immediately snap-frozen in liquid nitrogen, and then transferred to -80°C storage for subsequent analysis [8].

1.4.1 Serum Antioxidant Indices

Serum catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) activities, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were measured using colorimetric methods with assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.2 Primer Design

Primers for CAT, manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (CuZnSOD), glutathione peroxidase 1 (GPX1), and glutathione peroxidase 4 (GPX4) genes were designed using Primer Premier 5.0 software based on porcine gene sequences from GenBank, with -actin as the internal reference gene. Primer specificity was confirmed using NCBI Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). All primers were synthesized by Shanghai Sangon Biotech Co., Ltd. The real-time quantitative PCR (RT-qPCR) primer sequences are shown in Table 2 .

1.4.3 Expression of Oxidative Stress-Related Genes in Intestinal Mucosa

1.4.3.1 Total RNA Extraction and Reverse Transcription

Total RNA was extracted using Trizol and RNAiso Plus (TaKaRa, 9109) reagents. RNA purity and concentration were measured using a NanoDrop ND-2000 spectrophotometer (USA). RNA with OD260 nm/OD280 nm ratio of 1.8–2.2 was considered pure. RNA quality was assessed by 1% agarose gel electrophoresis, with a 28S rRNA to 18S rRNA grayscale ratio of 2:1 indicating good quality. Reverse transcription to synthesize cDNA was performed using a TaKaRa reverse transcription kit (TaKaRa, RR047A).

1.4.3.2 Real-Time Fluorescence Quantitative PCR

The 10 L qPCR reaction mixture contained: 5.0 L SYBR® Premix Ex TaqTM II, 0.2 L forward primer (10 mol/L), 0.2 L reverse primer (10 mol/L), 3.6 L

RNase-free dH2O, and 1.0 L cDNA. The reaction program was 95°C for 5 s and 60°C for 30 s for 40 cycles. The melting curve program was 95°C for 15 s, 60°C for 15 s, and 95°C for 15 s.

1.5 Statistical Analysis

Relative gene expression levels from RT-qPCR data were calculated using the 2^(-ΔΔCt) method [9]. All data were analyzed using one-way ANOVA with SPSS 13.0 software. Duncan' s multiple comparison test was used for significant differences, with $P < 0.05$ considered statistically significant. All data are expressed as "mean ± standard deviation."

2.1 Effects of GLN on Serum Antioxidant Capacity of Weaned Piglets

As shown in Table 3 , serum antioxidant capacity indices did not differ significantly among groups before LPS challenge ($P > 0.05$). Table 4 shows that after LPS challenge, serum MDA content in the stress group was significantly higher than in the control group ($P < 0.05$), while serum MDA content in the GLN group was significantly lower than in both the stress and control groups ($P < 0.05$). Serum SOD activity did not differ significantly between control and stress groups ($P > 0.05$), but was significantly lower in the GLN group compared to both control and stress groups ($P < 0.05$). Serum GSH-Px, CAT activities, and T-AOC did not differ significantly among groups ($P > 0.05$), though GSH-Px and CAT activities were numerically lower while T-AOC was numerically higher in the GLN group compared to the other groups.

2.2 Effects of GLN on Expression of Oxidative Stress-Related Genes in Intestinal Mucosa of Weaned Piglets

As shown in Table 5 , after LPS challenge, the relative expression levels of CAT and CuZnSOD genes in duodenal mucosa were significantly higher in the GLN group than in the stress group ($P < 0.05$), but did not differ significantly from the control group ($P > 0.05$). No significant differences were observed among groups in MnSOD, GPX1, and GPX4 gene expression ($P > 0.05$), though expression levels were numerically higher in the GLN group compared to the stress group.

Table 6 shows that after LPS challenge in jejunal mucosa, the GLN group had the highest relative expression levels of CAT, MnSOD, and GPX1 genes, which were significantly higher than both control and stress groups ($P < 0.05$), while the stress group had the lowest expression. CuZnSOD gene expression did not differ significantly among groups ($P > 0.05$), though it was numerically higher in the GLN group. GPX4 gene expression was significantly higher in the GLN group than in both control and stress groups ($P < 0.05$), and significantly higher in the control group than in the stress group ($P < 0.05$).

As shown in Table 7 , after LPS challenge in ileal mucosa, CAT gene expression was significantly lower in both GLN and stress groups compared to the control group ($P < 0.05$). MnSOD gene expression was highest in the GLN group, significantly higher than in both control and stress groups ($P < 0.05$). No significant differences were observed in GPX1 gene expression among groups ($P > 0.05$). CuZnSOD gene expression was significantly higher in the GLN group than in the control group ($P < 0.05$). GPX4 gene expression was significantly higher in both GLN and stress groups compared to the control group ($P < 0.05$).

3.1 Effects of GLN on Serum Antioxidant Indices of Weaned Piglets

MDA, SOD, GSH-Px, CAT, and T-AOC are all components of the animal antioxidant system. MDA is a small molecular product generated during the chain termination phase of lipid peroxidation reactions, and its content can indirectly reflect free radical production and the degree of lipid peroxidation in tissues. SOD is an important antioxidant enzyme with special physiological activity and serves as the primary substance for scavenging free radicals in vivo. It catalyzes the dismutation of superoxide anion radicals, counteracts and blocks oxygen radical-induced cellular damage, and repairs damaged cells in a timely manner. CAT primarily catalyzes the decomposition of hydrogen peroxide (H O) into molecular oxygen and water, eliminating H O from the body and protecting cells from H O toxicity, making it a key enzyme in the biological defense system. GSH-Px is an important enzyme that catalyzes H O decomposition throughout the body. It specifically catalyzes the reduction of H O by GSH, generating water and thereby reducing lipid peroxidation of polyunsaturated fatty acids in cell membranes and decreasing free radical production to protect membrane structure and function [10-11].

Studies have shown that GLN, as a precursor for synthesizing the antioxidant GSH, can maintain and increase reduced GSH levels in the body and promote synthesis of GSH-Px precursors [12], thus achieving free radical scavenging. Research also indicates that endotoxins can reduce SOD, CAT, and GSH-Px activities [13-15].

The current study showed no significant differences in serum antioxidant indices among groups before LPS challenge. After LPS challenge, serum MDA content increased significantly in the stress group compared to the control group, while SOD, CAT activities, and T-AOC showed decreasing trends. These results are consistent with previous studies and may be attributed to reduced efficiency of GSH-Px synthesis in LPS-challenged weaned piglets, necessitating exogenous supplementation to meet normal requirements. Denno et al. [16] reported that dietary GLN supplementation significantly increased serum GSH content in rats. Bian et al. [17] demonstrated that exogenous GLN supplementation significantly increased intestinal mucosal GSH content and CAT activity while reducing MDA content. Huang et al. [7] found that dietary supplementation with 0.5% and 0.8% GLN significantly increased blood GSH-Px activity and reduced MDA content

in yellow-feathered broilers under high temperature [(33±2)°C] at 28 and 35 days of age, without significantly affecting SOD activity. The present study showed that dietary GLN supplementation significantly reduced serum MDA content, consistent with previous findings, indicating that GLN can alleviate LPS-induced oxidative stress in weaned piglets to some extent.

T-AOC serves as a comprehensive indicator of antioxidant system function, reflecting the overall compensatory capacity of the antioxidant system to external stimuli and the status of free radical metabolism [18]. The present results showed that dietary GLN supplementation increased serum T-AOC by 2.14-fold compared to the stress group, suggesting that GLN can improve the antioxidant capacity of weaned piglets. Interestingly, serum GSH-Px, SOD, and CAT activities were lower in the GLN group than in control and stress groups. Previous studies have shown that exogenous GLN supplementation enhances GSH-Px and CAT activities to alleviate LPS-induced oxidative stress [18]. The contrasting results in this study may be due to GLN improving overall antioxidant capacity, such that piglets receiving GLN had elevated basal antioxidant levels. When challenged with E. coli LPS, these piglets did not experience severe oxidative stress or inflammatory responses, and consequently these enzyme activities did not show compensatory increases.

3.2 Effects of GLN on Expression of Oxidative Stress-Related Genes in Intestinal Mucosa

GPX1 and GPX4 are two important selenium-containing proteins belonging to the GSH-Px family of isoenzymes that play crucial roles in scavenging oxygen free radicals, resisting lipid peroxidation, and protecting cellular components from oxidative damage. GPX1 was the first selenium-containing enzyme discovered in mammals [19] and is the most abundant selenoprotein in most cells, considered a major antioxidant protein [20]. GPX4 exhibits high activity against phospholipid hydroperoxides and is an important antioxidant enzyme for membrane components. It works synergistically with vitamin E and is the only antioxidant enzyme in mammals that can directly reduce phospholipids, protecting host cells from damage mediated by intracellular and membrane lipid protein hydroperoxides (LOOHs) [21]. Studies have shown that high GPX4 gene expression in intestinal tissue indicates elevated GPX4 activity and enhanced antioxidant capacity [21].

The present results showed that after LPS challenge, piglets exhibited stress responses, and GLN supplementation increased GPX1 and GPX4 gene expression in duodenal, jejunal, and ileal mucosa compared to the stress group, with significant increases in GPX1 and GPX4 expression in jejunal mucosa. These findings indicate that dietary GLN promotes GPX1 and GPX4 gene expression in small intestinal mucosa, thereby enhancing antioxidant capacity.

CuZnSOD and MnSOD are highly inducible genes that are highly responsive to oxidative stress and regulate their own synthesis and secretion. CuZnSOD is an

important metalloenzyme for defense against oxidative damage that specifically scavenges superoxide anion radicals and plays a crucial role in maintaining oxygen radical balance [22]. MnSOD is highly responsive to oxidative stress and catalyzes the conversion of superoxide to H O and oxygen molecules in mitochondria, thereby removing harmful reactive oxygen species (ROS) from organelles [22]. The present results showed that after LPS challenge, GLN supplementation increased CuZnSOD and MnSOD gene expression in small intestinal mucosa compared to the stress group, indicating that GLN promotes expression of these genes and enhances responsiveness to oxidative stress. CAT is an enzymatic scavenger that catalyzes H O decomposition into oxygen and water, protecting cells from H O toxicity. The CAT gene regulates CAT synthesis and secretion. In this study, GLN significantly increased CAT gene expression in duodenal and jejunal mucosa after LPS challenge but had no significant effect in ileal mucosa, suggesting that GLN promotes CAT gene expression in the duodenum and jejunum to increase CAT activity, but is less effective in the ileum.

This study established an oxidative stress model using E. coli LPS and examined the effects of dietary 1% GLN supplementation on oxidative stress in weaned piglets. The results demonstrated that GLN significantly reduced serum MDA content and SOD activity, decreased serum GSH-Px and CAT activities, increased serum T-AOC, and enhanced expression of GPX1, GPX4, CuZnSOD, MnSOD, and CAT genes in small intestinal mucosa (except for ileal CAT). These findings indicate that dietary GLN supplementation can alleviate LPS-induced oxidative stress responses, improve antioxidant capacity, and thereby enhance disease resistance in weaned piglets.

We thank Professor YAO Kang and Dr. HE Liuqin from the Institute of Subtropical Agriculture, Chinese Academy of Sciences, for their valuable comments on the manuscript.

References

[1] LI Yongyi. Protective effects and mechanisms of tea polyphenols on oxidative stress in piglets [D]. PhD dissertation. Ya' an: Sichuan Agricultural University, 2011.
[2] YU Guojiang, FENG Huizhong, ZHENG Pengfei, et al. Diagnosis and treatment of piglet transport syndrome [J]. China Swine Industry, 2009, 4(9): 43–44.
[3] WANG Lei, LIU Jian, HOU Yongqing, et al. Effects of -ketoglutarate on intestinal mucosal morphology and function in piglets under chronic LPS stress [J]. Acta Veterinaria et Zootechnica Sinica, 2010, 41(1): 46–52.
[4] LIU Jian, HOU Yongqing, DING Binying, et al. Effects of -ketoglutarate on protein synthesis and antioxidant capacity in jejunal mucosa of weaned piglets challenged with lipopolysaccharide [J]. Chinese Journal of Animal Science, 2010, 46(11): 35–38.
[5] LIN Hai. Nutritional and physiological responses of poultry under heat stress

[M]//LI Defa. Advances in Animal Nutrition Research. Beijing: China Agricultural Science and Technology Press, 2004: 237-248.
[6] CURI R, LAGRANHA CJ, DOI SQ, et al. Molecular mechanisms of glutamine action [J]. Journal of Cellular Physiology, 2005, 204(2): 392-401.
[7] HUANG Guanqing, LIN Hongying. Effects of glutamine on antioxidant capacity of yellow-feathered broilers under high temperature environment [J]. China Feed, 2006(20): 24-26.
[8] DAHLQVIST A. Method assay intestinal disaccharidases [J]. Analytical Biochemistry, 1964, 7(1): 18-25.
[9] SCHMITTGEN TD, LIVAK KJ. Analyzing real-time PCR data by the comparative CT method [J]. Nature Protocols, 2008, 3(6): 1101-1108.
[10] CHEN Jin, ZHOU Xiaoqiu, FENG Lin. Effects of glutamine on intestinal antioxidant capacity in animals [J]. Feed Industry, 2009, 30(2): 55-58.
[11] CAO Jingran, XIE Ying, LI Hui, et al. Role and mechanism of glutamine in oxidative stress diseases [J]. Clinical Misdiagnosis & Mistherapy, 2013, 26(9): 102-104.
[12] YANG Caimei, XU Weidan, CHEN Anguo. Effects of glycyl-L-glutamine on growth performance and intestinal absorption function in weaned piglets [J]. Chinese Journal of Animal Science, 2005, 41(8): 6-8.
[13] ZHANG HJ, GUO YM, TIAN YD, et al. Dietary conjugated linoleic acid improves antioxidant capacity in broiler chicks [J]. British Poultry Science, 2008, 49(2): 213-221.
[14] GHOSH B, HANEVOLD CD, DOBASHI K, et al. Tissue differences in antioxidant enzyme gene expression in response to endotoxin [J]. Free Radical Biology and Medicine, 1996, 21(4): 533-538.
[15] ABD-ALLAH ARA, HELAL GK, AL-YAHYA AA, et al. Pro-inflammatory and oxidative stress pathways which compromise sperm motility and survival may be altered by L-carnitine [J]. Oxidative Medicine and Cellular Longevity, 2009, 2(2): 73-81.
[16] DENNO R, ROUNDS JD, FARIS R, et al. Glutamine-enriched total parenteral nutrition enhances plasma glutathione in the resting state [J]. Journal of Surgical Research, 1996, 61(1): 35-38.
[17] BIAN Lianquan, CHEN Jing, LIU Xianjun. Effects of glutamine on intestinal mucosal morphology and antioxidant properties in early-weaned piglets [J]. Journal of Shenyang Agricultural University, 2006, 37(6): 849-852.
[18] ZHENG Ronglian, HUANG Zhongyang. Free Radical Biology [M]. 3rd ed. Beijing: Higher Education Press, 2007.
[19] ROTRUCK JT, POPE AL, GANTHER HE, et al. Selenium: biochemical role as a component of glutathione peroxidase [J]. Science, 1973, 179(4073): 588-590.
[20] WANG Xiaoya. Antioxidant effects of selenoproteins [J]. Modern Agricultural Science and Technology, 2006(8): 183-184.
[21] SUN Q, KOJIMA H, KOMURA S, et al. Effect of selenium on human phospholipid hydroperoxide glutathione peroxidase expression and host cell susceptibility to lipid hydroperoxide-mediated injury [J]. Biochemistry Molecular Biology International, 1997, 42(5): 957-963.

[22] NOOR R, MITTAL S, IQBAL J. Superoxide dismutase–applications and relevance to human diseases [J]. Medical Science Monitor, 2002, 8(9): 210-215.

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