Glutamine improves heat stress–induced oxidative damage in the broiler thigh muscle by activating the nuclear factor erythroid 2–related 2/Kelch-like ECH-associated protein 1 signaling pathway

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ABSTRACT The aim of the present study was to evaluate the effect of glutamine (Gln) on modulating heat stress–induced oxidative damage in the broiler thigh muscle through nuclear factor erythroid 2–related 2/Kelch-like ECH-associated protein 1 (Nrf2-Keap1) pathway. Three-hundred 22-day-old Arbor Acres broilers were reallocated into 5 groups: a control group (24°C/21°C) fed with basal diet and 4 heat stress (HS) groups (34°C/21°C for 8 h/D) fed with basal diet containing 0, 0.5, 1.0, and 1.5% Gln. This experiment lasted 21 D. Heat stress decreased (P,0.05) pH, redness, and Gln levels, and increased (P,0.05) luminance, water loss rate, and cooking loss (CL) values of the thigh meat. Compared with the HS group, supplementation with 1.5% Gln increased (P,0.05) pH, redness, and Gln levels, but decreased (P,0.05) luminance and CL values in the thigh meat. There were significant decreases (P,0.05) in glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and Nrf2 levels, but significant increases (P,0.05) in the malondialdehyde (MDA) and Keap1 levels of the thigh muscle after HS treatment. Compared with the HS group, supplementation with 1.0, and 1.5% Gln decreased (P,0.05) MDA and Keap1 levels; supplementation with 1.5% Gln increased (P,0.05) GSH, GSH-Px, T-AOC, CAT, SOD, and Nrf2 levels in the thigh muscle of heat-stressed broilers. Furthermore, HS decreased (P,0.05) Nrf2, SOD, CAT, and GSH-Px mRNA expression levels, but increased (P,0.05) Keap1 mRNA level in the thigh muscle of broiler. Dietary supplementation with 1.5% Gln increased (P,0.05) Nrf2, GSH-Px, CAT, and SOD mRNA expression levels, but decreased (P,0.05) Keap1 mRNA level in the thigh muscle of heat-stressed broilers. In conclusion, dietary Gln improved the resistance of heat-stressed broiler muscles to oxidative damage possibly through reversing the muscle Gln level and inducing the expression of the Nrf2-Keap1 pathway.

Key words: glutamine, heat stress, meat quality, antioxidant capacity, Nrf2-Keap1 signaling pathway

INTRODUCTION

With the development of high-density feeding modes and the deleterious influence of high temperatures during the summer, broilers as heat-sensitive animals are easily susceptible to heat stress in the production process (Lara and Rostagno, 2013). This condition decreases poultry production performance by causing heat exhaustion, which poses a great threat to broiler production (Quinteiro-Filho et al., 2010; Bai et al., 2019). A method commonly used to mitigate the negative effects of heat stress involves regulating dietary additives (Zhang et al. 2015). Because of the growing awareness of the importance of food safety and health, amino acids as constituents of animal proteins are a good choice for regulation based on animal metabolism and nutritional requirements.

Poultry meats are rich in polyunsaturated fatty acids. Therefore, heat stress aggravates muscle oxidation and lipid peroxidation, resulting in pale, soft, exudative meat (Tang et al., 2013; Xing et al., 2015; Zhang et al., 2017). Heat stress also increases the secretion of catecholamines, which increases the content of superoxide free radicals and hydrogen peroxide (Song and King, 2015). The

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production of excessive reactive oxygen species damages the antioxidant system of the body and induces oxidative stress (Belhadj et al., 2016; Zhang et al., 2018). The nuclear factor erythroid 2–related 2/Kelch-like ECH–related molecules, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Zhang et al., 2018).

Glutamine (Gln), a conditional essential amino acid, is the most abundant amino acid in broiler skeletal muscles. Glutamine, an important nutrient for the conversion of some amino acids and biological macromolecules, is a source of energy for some rapidly dividing cells. Gln is well known to have positive effects on cellular responses to environmental and oxidative stress (Bryan et al., 2013; Zhang et al., 2018). Activation of the Nrf2-Keap1 pathway increases antioxidant response element–related molecules, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Zhang et al., 2018).

The aim of this work was to determine if the effect of heat stress on meat quality and oxidative damage of meat can be alleviated by Gln supplementation. Moreover, the effect and mechanism of action of Gln on oxidative stress in broilers under heat stress. Therefore, the aim of this work was to determine if the effect of heat stress on meat quality and oxidative damage can be alleviated by Gln supplementation. Moreover, regulation of the antioxidant and Nrf2-Keap1 pathway was investigated to elucidate a possible mechanism for Gln-induced protection of meat against oxidation under heat stress.

MATERIALS AND METHODS

Animals and Experimental Design

The experimental animal protocol was approved by the Animal Care and Use Committee of Anhui Science and Technology University. Specifically, 400 1-day-old Arbor Acres broilers were obtained from the Anhui Science and Technology University Farm (Chuzhou, People’s Republic of China). Broilers (aged 1–21 D) were placed in an environmentally controlled room at 32 to 35°C for the first week and then gradually reduced by 3.5°C per week to a final temperature of 24°C. On day 22, 300 broilers (50% males and females) were randomly divided into the following 5 groups with 6 replicate cages of 10 broilers per cage (50% males and females; there were no differences in initial weight among 5 groups): the control (CON) group was fed basal diet; heat stress (HS) treatment groups, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln, were circuitous heat-stressed groups and fed the basal diet with 0, 0.5, 1.0, and 1.5% Gln, respectively. Broilers from the CON group were kept under normal-temperature conditions at 24°C (humidity: 45–55%) for 24 h/D, whereas broilers in the heat-stressed groups were kept under hot-temperature conditions at 34°C (humidity: 60–70%) for 8 h/D (9:00–17:00) and at 24°C (humidity: 45–55%) for 16 h/D. All experimental chickens were placed in a stereoscopic cage (120 cm × 70 cm × 40 cm) and enjoyed a 12-h light regimen (10 lux) every day. The plastic feeders (diameter: 25 cm; feed weight: 2 kg) and drinkers (3 L water) were used for free access to water and feed. The growth performance was measured according to the “Technical regulation for performance testing of meat-type chicken (NY/T 828-2004).” The basal diet (Table 1) was corn–soybean meal, and the nutrient level of the formulation was designed according to the NRC (1994).

Sample Collection

On day 42, 3 broilers from each replicate were euthanized by dislocation, manual exsanguination, and pulling of feathers. The thigh muscle samples were divided into 2 portions: 1 was stored at 4°C for meat quality analysis, whereas the other was stored at −80°C for biochemical, mRNA, and protein assays.

Meat Quality

Meat quality was determined 45 min after euthanasia. The pH value of the thigh muscle was measured using a pH meter (Mettler Toledo, Zurich, Switzerland) as per the procedure described by Hu et al. (2016a). The luminance (L*), redness (a*), and yellowness (b*) of the thigh muscle were measured using a colorimeter (Minolta, Tokyo, Japan) as described by Hu et al. (2016a).

| Table 1. Composition and nutrient levels of the basal diets1. |
|-------------------------------------------------------------|
| Ingredients (g/kg)                                         |
|-------------------------------------------------------------|
| Corn                                                       | 585 |
| Starch                                                    | 10  |
| Soybean meal                                              | 320 |
| Fish meal                                                 | 20  |
| Soybean oil                                               | 35  |
| CaHPO4·2H2O                                               | 15  |
| Limestone                                                 | 9   |
| Salt                                                       | 3   |
| DL-Methionine                                             | 1   |
| Vitamin and trace mineral premix2                         | 2   |
| Total                                                     | 1000|
| Nutritional composition                                   |     |
| ME (MJ/kg)                                                | 12.73|
| Crude protein (g/kg)                                      | 203.0|
| Lysine (g/kg)                                             | 10.8|
| Methionine + cysteine (g/kg)                              | 7.6 |
| Ca (g/kg)                                                 | 8.9 |
| Available P (g/kg)                                        | 4.2 |

1The basal diet was designed according to the NRC (1994) and Bai et al. (2019).
2Provided per kilogram of diet: vitamin A: 10,000 IU; cholecalciferol: 2600 IU; vitamin E: 20 IU; vitamin K2: 2.0 mg; riboflavin: 6.0 mg; thiamin: 1.6 mg; vitamin B6: 3.0 mg; vitamin B12: 0.014 mg; niacin: 30 mg; choline (as choline chloride): 500 mg; folic acid: 0.8 mg; biotin: 0.12 mg; calcium pantothenate: 20 mg; Fe [from Fe2(SO4)3·H2O]: 80 mg; Zn (from ZnSO4·7H2O): 40 mg; Cu (from CuSO4·5H2O): 8 mg; Se (from Na2SeO3): 0.15 mg; I (from IK): 0.35 mg.
was calculated as follows: (initial weight-final weight)/initial weight × 100%.

To assess cooking loss (CL), the muscle samples were weighed (initial weight), placed in a Petri dish, and steamed in an aluminum pot of boiling water for 30 min. Then, the surface juice was gently wiped off using an absorbent paper, followed by cooling and reweighing (final weight). The CL (%) was calculated as follows: (initial weight-final weight)/initial weight × 100%.

The water loss rate (WLR) was measured using a pressure gravimetric method. The muscle sample initial weight was determined. Then, the sample was placed between the 18 layers of the filter paper in a compressor and pressed with a pressure of 2000 psi for 1 min. This meat sample was reweighed immediately (final weight), and the WLR (%) was calculated as follows: (initial weight-final weight)/initial weight × 100%.

**Homogenate of the Thigh Muscle**

Under cold conditions in an ice water bath, 10% of the tissue homogenate was prepared using physiological saline (0.9%) at a weight (g)-to-volume (mL) ratio of 1:9. The homogenate supernatant was obtained by centrifugation (3500 rpm) for 10 min. The protein concentration of the supernatant was measured using a bicinchoninic acid kit (Beyotime Institute of Biotechnology, Shanghai, China).

**Determination of Gln Concentration in the Thigh Muscle**

The concentration of Gln was determined in the homogenate supernatant of the thigh muscle using a Gln measurement kit (Jiancheng Bioengineering Research Institute, Nanjing, China).

**Determination of Redox State and Antioxidants in the Thigh Muscle**

The levels of malondialdehyde (MDA), GSH, glutathione peroxidase (GSH-Px), SOD, CAT, and total antioxidant capacity (T-AOC) were assayed in the homogenate supernatant of the thigh muscle using their respective commercial assay kits purchased from Jiancheng Bioengineering Research Institute (Nanjing, China).

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**Determination of Nrf2 and Keap1 in the Thigh Muscle**

The Nrf2 and Keap1 protein levels in the homogenate supernatant of the thigh muscle were detected using a chicken Nrf2 and Keap1 enzyme-linked immunosorbent assay kit (Jiancheng Bioengineering Research Institute, Nanjing, China). The coefficient of variance of replicates was <10%.

**Gene Expression**

Total RNA of the thigh muscle was isolated using the RNAprep pure tissue kit (TianGen, Beijing, China). The mRNA expression levels of Nrf2, Keap1, SOD, CAT, GSH-Px, and β-actin (reference gene) were measured by quantitative real-time polymerase chain reaction (PCR) technique with the primers shown in Table 2. The quantitative real-time PCR was performed using the TB Green Premix Ex Taq (Takara, Dalian, China). The PCR reaction and program were performed as per the method of Zhang et al. (2018), and the mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

**Statistics Analysis**

The data, including those of the CON group, were analyzed by a one-way analysis of variance using SPSS (2008), version 18.0, software (SPSS Inc., Chicago, IL). Duncan’s test was used to compare statistical differences among the HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups. $P < 0.05$ was considered significant.

**RESULTS**

**Meat Quality**

As shown in Table 3, significant decreases ($P < 0.05$) in pH and $a^*$ values, but significant increases ($P < 0.05$)
in L*, WLR, and CL values of the thigh meat, were observed in the HS group compared with the CON group. Compared with the HS group, supplementation with 1.0% Gln significantly increased (P < 0.05) a* value, but decreased (P < 0.05) L*, WLR, and CL values; supplementation with 1.5% Gln significantly increased (P < 0.05) a* and pH values, but decreased (P < 0.05) L* and CL values in the thigh meat of heat-stressed broilers.

**Gln Concentration in the Thigh Muscle**

As shown in Figure 1, a significant decrease (P < 0.05) in Gln concentration of the thigh muscle was observed after HS treatment. Compared with the HS group, supplementation with 0.5, 1.0, and 1.5% Gln significantly increased (P < 0.05) the Gln concentration in the thigh muscle of heat-stressed broilers.

**Redox State and Antioxidants in the Thigh Muscle**

Table 4 shows the redox state and antioxidants in the thigh muscle. There were significant decreases (P < 0.05) in the GSH, SOD, GSH-Px, CAT, and T-AOC levels but significant increases (P < 0.05) in the MDA level of the thigh muscle after HS treatment. Compared with the HS group, supplementation with 0.5, 1.0, and 1.5% Gln significantly decreased (P < 0.05) MDA concentration; supplementation with 1.0 and 1.5% Gln significantly increased (P < 0.05) GSH, GSH-Px, and T-AOC levels; supplementation with 1.5% Gln significantly increased (P < 0.05) CAT and SOD levels in the thigh muscle of heat-stressed broilers.

**The mRNA Expression Levels of Antioxidant Enzymes**

There were significant decreases (P < 0.05) in the SOD, GSH-Px, and CAT mRNA expression levels of the thigh muscle after HS treatment (Figure 2). Compared with the HS group, supplementation with 1.0 and 1.5% Gln significantly increased (P < 0.05) the GSH-Px mRNA expression level; supplementation with 1.5% Gln significantly increased (P < 0.05) CAT and SOD mRNA expression levels in the thigh muscle of heat-stressed broilers (Figure 2).

**Expression of Nrf2-Keap1 Signaling Pathway–Related Proteins**

As shown in Figure 3, the Nrf2 protein level significantly decreased (P < 0.05), whereas that of Keap1 significantly increased (P < 0.05) in the thigh muscle after HS treatment. Compared with the HS group, supplementation with 1.0 and 1.5% Gln significantly increased (P < 0.05) the protein level of Nrf2, whereas supplementation with 0.5, 1.0, and 1.5% Gln significantly decreased

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**Table 3. Effect of heat stress and glutamine on meat quality of the thigh muscle in broilers.**

| Item       | CON                | HS                 | HS-0.5% Gln         | HS-1.0% Gln         | HS-1.5% Gln         | SEM     | P-value |
|------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------|---------|
| pH         | 6.19ab             | 5.95b              | 6.02b              | 6.05b              | 6.15a              | 0.021   | <0.001  |
| L*         | 48.28a             | 50.21a             | 49.10b             | 47.95a             | 48.38b             | 0.240   | 0.013   |
| a*         | 8.38b              | 6.81b              | 7.06b              | 7.03b              | 7.94a              | 0.121   | <0.001  |
| b*         | 8.06               | 7.59               | 7.71               | 7.85               | 8.02               | 0.161   | 0.884   |
| DL (%)     | 4.60               | 5.10               | 4.88               | 4.73               | 4.66               | 0.073   | 0.204   |
| CL (%)     | 37.27a             | 41.57b             | 41.18c             | 40.25c             | 39.01b             | 0.326   | <0.001  |
| WLR (%)    | 26.34ab            | 31.59c             | 29.66bc            | 28.34ab            | 29.19b             | 0.490   | 0.007   |

a–dValues without common superscripts in the same row differ significantly (P < 0.05); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups.

Abbreviations: a*, redness; b*, yellowness; CL, cooking loss; CON, control; DL, drip loss; HS, heat stress; L*, luminance; WLR, water loss rate.

1CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5, 1.0, and 1.5% Gln.

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**Figure 1.** Effect of heat stress and glutamine on glutamine concentration of thigh meat in broilers. a–dGroups without common letters differ significantly (P < 0.05); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups. CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed a basal diet; HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5, 1.0, and 1.5 Gln. CON, control; Gln, glutamine; HS, heat stress.
broilers (Figure 4). That of Keap1 in the thigh muscle of heat-stressed Nrf2 mRNA expression level but decreased (\( P < 0.05 \)) in the expression level of the Nrf2 gene, \( P < 0.05 \), in the thigh muscle after HS treatment. Compared with the HS group, supplementation with 1.0 and 1.5% Gln significantly increased (\( P < 0.05 \)) the Nrf2 mRNA expression level but decreased (\( P < 0.05 \)) that of Keap1 in the thigh muscle of heat-stressed broilers (Figure 4).

Expression of Nrf2-Keap1 Signaling Pathway–Related Genes

As shown in Figure 4, there was a significant decrease (\( P < 0.05 \)) in the expression level of the Nrf2 gene, whereas that of Keap1 significantly increased (\( P < 0.05 \)) in the thigh muscle after HS treatment. A pH that is too low can easily lead to the appearance of pale, soft, exudative meat, which is characterized by paleness and decreased WHC of the meat (Petracci, 2017). A pH that is too low could be detrimental to meat quality. It has been reported that a hot environment could be detrimental to meat quality such as trauma, long-term stress, and infection, the consumption of Gln increases in the broiler muscle. However, the endogenous Gln levels in the muscle cannot meet the requirements, leading to the need to exogenously supplement Gln in the diet (Jazideh et al., 2014; Dai et al., 2018; Hu et al., 2016b). Barekatain and Toghyani (2019) suggested that there is need for exogenous addition of Gln in broiler diet under long-term stress rather than under normal conditions. In the present study, heat stress markedly decreased (\( P < 0.05 \)) the Gln levels in the broiler thigh muscle, whereas exogenous Gln supplementation reversed this effect. This observation suggests that Gln deficiency in the body could be improved by exogenous supplementation to relieve the damage of heat stress.

Malondialdehyde is the stable end product of the lipid oxidation reaction, and its content reflects the degree of damage induced by free radicals in body fat (Wang et al., 2018).

**DISCUSSION**

Meat quality of broilers is a complex characteristic that is generally determined by meat color, water-holding capacity, and pH. It has been reported that a hot environment could be detrimental to meat quality (Gregory, 2010; Lara and Rostagno, 2013; Lu et al., 2017). Heat stress can depress postmortem energy metabolism and accelerate glycolysis and lactic acid formation, which leads to rapid pH decline (Lu et al., 2017). A pH that is too low can easily lead to the appearance of pale, soft, exudative meat, which is characterized by paleness and decreased WHC of the meat (Petracci et al., 2015; Hu et al., 2016a). In this experiment, the decreased (\( P < 0.05 \)) pH, \( a^* \), WLR, and CL and increased (\( P < 0.05 \)) L* values observed in the thigh meat after the high-temperature treatment were prevented by Gln supplementation. Similar results were also found by Hu et al. (2016a), who reported that dietary Gln increased the pH and WHC values, but decreased CL values of broiler thigh meat after acute heat stress. Preslaughter intervention by adding Gln to the diet may provide an attractive strategy for improving meat quality of broilers exposed to a hot environment.

Glutamine is not only an important amino acid in proteins and peptides but also the biosynthetic precursor of pyrimidine and purine nucleotides, nucleic acids, and amino sugars in vivo. Under pathological conditions such as trauma, long-term stress, and infection, the consumption of Gln increases in the broiler muscle. However, the endogenous Gln levels in the muscle cannot meet the requirements, leading to the need to exogenously supplement Gln in the diet (Jazideh et al., 2014; Dai et al., 2018; Hu et al., 2016b). Barekatain and Toghyani (2019) suggested that there is need for exogenous addition of Gln in broiler diet under long-term stress rather than under normal conditions. In the present study, heat stress markedly decreased (\( P < 0.05 \)) the Gln levels in the broiler thigh muscle, whereas exogenous Gln supplementation reversed this effect. This observation suggests that Gln deficiency in the body could be improved by exogenous supplementation to relieve the damage of heat stress.

Table 4. Effect of heat stress and glutamine on redox state and antioxidants of the thigh muscle in broilers.

| Item                  | CON    | HS     | HS-0.5% Gln | HS-1.0% Gln | HS-1.5% Gln | SEM     | P-value |
|-----------------------|--------|--------|-------------|-------------|-------------|---------|---------|
| MDA (mmol/mg protein) | 0.40a  | 0.66d  | 0.58b       | 0.52h,c     | 0.46h,c     | 0.020   | <0.001  |
| GSH (mg/g protein)    | 5.92a  | 3.33b  | 4.89h       | 5.87c       | 5.80c       | 0.312   | 0.023   |
| SOD (U/mg protein)    | 109.97a| 85.53a | 89.32b      | 96.53h,b,c  | 106.57h,b,c | 2.930   | 0.021   |
| GSH-Px (U/mg protein) | 25.88a | 19.36b | 23.08b      | 25.17a      | 26.91a      | 0.744   | 0.003   |
| CAT (U/mg protein)    | 3.80a  | 2.29b  | 2.79b       | 3.17b       | 3.85a       | 0.179   | 0.015   |
| T-AOC (U/mg protein)  | 2.84a  | 1.98b  | 2.74b       | 3.56a,c     | 3.40a       | 0.144   | 0.004   |

* Values without common superscripts in the same row differ significantly (\( P < 0.05 \)); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups.
1CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed a basal diet; HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5, 1.0, and 1.5% Gln.

Figure 2. Effect of heat stress and glutamine on the mRNA expression of antioxidant enzymes of thigh muscle in broilers. a,b,c,d Groups without common letters differ significantly (\( P < 0.05 \)); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups. CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed a basal diet; HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5, 1.0, and 1.5% Gln. SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase. The mRNA expression of each gene of the CON was set to be 1.
Effect of heat stress and Gln on the protein expression of Nrf2 and Keap1 of the thigh muscle in broilers. *a,b,c* Groups without common letters differ significantly ($P < 0.05$); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups. CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed a basal diet; HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5%, 1.0%, and 1.5% Gln. Nrf2, nuclear factor erythroid 2-related 2; Keap1, kelch-like ECH-associated protein 1. The protein expression of each protein of the CON was set to be 1. CON, control; Gln, glutamine; HS, heat stress.

Effect of heat stress and Gln on the mRNA expression of Nrf2 and Keap1 of the thigh muscle in broilers. *a,b,c* Groups without common letters differ significantly ($P < 0.05$); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups. CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed a basal diet; HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5%, 1.0%, and 1.5% Gln. Nrf2, nuclear factor erythroid 2-related 2; Keap1, kelch-like ECH-associated protein 1. The mRNA expression of each gene of the CON was set to be 1. CON, control; Gln, glutamine; HS, heat stress.

Figure 3. Effect of heat stress and Gln on the protein expression of Nrf2 and Keap1 of the thigh muscle in broilers. *a,b,c* Groups without common letters differ significantly ($P < 0.05$); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups. CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed a basal diet; HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5%, 1.0%, and 1.5% Gln. Nrf2, nuclear factor erythroid 2-related 2; Keap1, kelch-like ECH-associated protein 1. The protein expression of each protein of the CON was set to be 1. CON, control; Gln, glutamine; HS, heat stress.

Figure 4. Effect of heat stress and Gln on the mRNA expression of Nrf2 and Keap1 of the thigh muscle in broilers. *a,b* Groups without common letters differ significantly ($P < 0.05$); Duncan’s test was used to compare statistical differences among the CON, HS, HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln groups. CON = broilers were kept in the normal-temperature environment and fed a basal diet; HS = broilers were kept in the circular heat stress environment and fed a basal diet; HS-0.5% Gln, HS-1.0% Gln, and HS-1.5% Gln = broilers were kept in the circular heat stress environment and fed basal diets supplemented with 0.5%, 1.0%, and 1.5% Gln. Nrf2, nuclear factor erythroid 2-related 2; Keap1, kelch-like ECH-associated protein 1. The mRNA expression of each gene of the CON was set to be 1. CON, control; Gln, glutamine; HS, heat stress.
quality reduction in the broiler thigh muscle. Supplementation with exogenous Gln improved the resistance of heat-stressed broiler muscles to oxidative damage by increasing muscle Gln levels and activating the antioxidant defense mechanism. These Gln-induced effects may have been achieved by the enhancement of antioxidant activity through the Nrf2-Keap1 signaling pathway. These results suggested that Gln could serve as an antistress additive in broiler production to improve the muscle redox status and meat quality under hot environmental conditions.

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