Dietary glutamine improves meat quality, skeletal muscle antioxidant capacity and glutamine metabolism in broilers under acute heat stress

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ABSTRACT
This study investigated the effects of glutamine (Gln) on meat quality, skeletal muscle antioxidant capacity and Gln metabolism in heat-stressed broilers. Three hundred 42-day-old broilers were randomly divided into five groups: a control group (23 ± 1°C), which was fed basal diet, and four experimental groups (34 ± 1°C), supplemented with 0, 5, 10, and 20 g Gln/kg of basal diet. The experiment lasted for 24 h. Compared with the control group, acute heat stress caused a significant reduction (p < .05) in meat pH, water-holding capacity (WHC), gumminess and hardness, and a significant increase (p < .05) in cooking loss (CL) and lightness (L*) values. However, dietary Gln (20 g/kg) increased (p < .05) meat pH, WHC, gumminess and hardness, but decreased (p < .05) meat CL and L* values in the acute heat-stressed group. In breast and thigh muscles, the acute heat stress group exhibited significantly (p < .05) higher concentrations of malondialdehyde (MDA), but significantly (p < .05) lower levels of Gln, glutamate and glutaminase than the control group; dietary 20 g/kg Gln significantly decreased (p < .05) MDA concentrations, while it increased (p < .05) glutathione, glutathione peroxidas, T-AOC, Gln, glutamate, and glutaminase levels in acute heat-stressed groups. Gln could increase meat quality by improving antioxidative capacity and Gln metabolism in heat-stressed broilers.

1. Introduction

The quality and palatability of broiler meat are increasingly concern to consumers worldwide. Acute heat stress as a result of high ambient temperatures in summer can significantly decrease chicken meat quality by inducing oxidative rancidity, which reduces the nutrition, flavour, appearance and consistency of meat products (Murakami et al. 2013; Wang et al. 2013; Chand et al. 2014; Hu, Bai, Shah, Dai et al. 2016). Thus, improving the oxidative stability and oxidative status of meat is considered to be one of the best ways to alleviate these detrimental effects.

Many natural antioxidants have recently received great interest in the animal nutrition and feed industry because of their impacts on meat quality characteristics (Murakami et al. 2007; Zhang et al. 2015; Wan et al. 2016; Song et al. 2017; Wan et al. 2018). In addition, synthetic antioxidants are considered to improve the meat quality of heat-stressed broilers; however, the negative effects of these agents on the animals (particularly, at high doses) has been questioned (Vossen et al. 2011). Amino acids, which are the basic components of proteins, also play a key role in maintaining both human and animal health, some of which have specific functions that include oxidative stability and antistress actions (Dai et al. 2011).

Glutamine (Gln), which is commonly considered a conditionally essential amino acid, is currently receiving a lot of interest in the field of animal nutrition (Bartell & Batal 2007). This amino acid is a precursor for the synthesis of glutamate (Glu) and nucleotides, particularly glutathione (GSH), which is one of the vital antioxidants in most cells and tissues. Under physiological conditions, sufficient quantities of Gln are synthesized in the liver and skeletal muscle. However, under heat and oxidative stress, its concentrations in the tissues decrease precipitously to meet the additional requirements of the animal, which results in energy metabolism disturbances and immunosuppression. This effect can be lessened by absorbing exogenous Gln from a Gln-rich diet (Hu, Bai, Shah, Dai et al. 2016).

Gln supplementation can increase survival, growth performance and gut-barrier function in livestock under injury and stress states (Dai et al. 2011; Zhong et al. 2012). Furthermore, previous studies by our group indicated that dietary supplementation with Gln significantly improves performance, serum parameters and carcass characteristics of broilers during heat exposure, suggesting that Gln may have potential functions in improving antioxidation and Gln metabolism in broilers (Dai et al. 2011, 2012; Hu, Bai, Shah, Dai et al. 2016; Hu, Bai, Shah, Wen et al. 2016). Few published studies are available regarding the effect of dietary Gln on Gln metabolism and oxidative status of acute heat-stressed broilers. Therefore, the objective of this research was to determine the effects of supplementary Gln on meat texture and quality, skeletal muscle antioxidant stability and Gln metabolism in broilers under acute heat stress.
2. Materials and methods

2.1. Broilers’ management and diet

The broiler experiment was performed at Anhui Science and Technology University, China, and approved by the Animal Ethics Committee. Following a 3-day adaptation period, three hundred 42-day-old Arbour Acres broilers were randomly divided into five groups: a control group (normal thermal environment: 23 ± 1°C; relative humidity, 45–50%), which was fed a basal diet, and four experimental groups (acute heat stress conditions: 34 ± 1°C; relative humidity, 60–65%), which were supplemented with 0, 5, 10, or 20 g Gln/kg of basal diet. The acute heat stress lasted for 24 h. There were six replicates per group of 10 birds per cage. The formulation of the basal diet (Table 1) was designed according to the nutrient requirements suggested by the National Research Council (1994). Both feed and water were provided ad libitum.

2.2. Sample collection

After 24 h of acute heat stress, a chick from each replicate was randomly selected for sampling. The broilers were slaughtered via cervical dislocation, following which the breast (pectoralis major) and thigh (leg) muscles were excised. The above muscles (about 1 g) were homogenized in the hand-held glass homogenizer (Shengtai Co. Ltd., China) with 10 mM phosphate buffer (pH 7.4) on ice for 10 minutes. This homogenate was then centrifuged at 10 000 rpm for 8 min at 2–6°C, and the supernatant was harvested for biochemical analysis. The remaining muscles (pectoralis major and leg muscles) were also stored immediately at 2–4°C (24 h) for meat quality and texture analyses.

2.3. Meat quality analyses

The pH of the breast and thigh muscles was determined using a precise pH meter (SevenCompact™; Mettler-Toledo Co. Ltd, Switzerland) that had been calibrated at pH 4.0 and 7.0 at room temperature (Dai et al. 2009).

2.4. Texture analyses

A texture profile analysis (TPA) was performed using a Texture Analyser (CT3; Brookfield Co. Ltd, USA) under the TPA model. The TPA of the meat was determined as previously described by Chan et al. (2011) and Linares et al. (2014). Samples of the breast and thigh muscles were refrigerated overnight and then cut into 10 mm × 10 mm × 5 mm cubes and cooked for 30 min in steam (approximately 98°C). Double compression cycles of 50% compression, with a test speed of 3 mm/s were then performed in the TPA test. When the analysis was finished, the CT3 software (Texture Pro CT Version 1.8) calculated values for springiness (expressed as g), chewiness (expressed as mJ), cohesiveness and hardness (expressed as g).

2.5. Biochemical measurements

The following parameters were measured in triplicate for each muscle sample using assay kits purchased from the Nanjing Jiancheng Bioengineering Institute, China: malondialdehyde (MDA assay kit, A003-1), catalase (CAT assay kit, A007-1), GSH (reduced glutathione assay kit, A006-1), glutathione peroxidase (GSH-Px assay kit, A005), total antioxidant capability (T-AOC assay kit, A015), Gln (Gln assay kit, A073), Glu (Glu assay kit, A074), glutaminase (GLS assay kit, A124) and glutamine synthetase (GS assay kit, A047). The concentration of protein in each homogenate was also measured using a total protein quantitative assay kit (A045-2; Jiancheng Bioengineering Institute, China). Cooking loss (CL) was calculated by weighing the meat samples (about 4 g; 4 cm × 1 cm × 0.5 cm) and wrapped in plastic bags. Then, the samples were cooked for 30 min in steam (approximately 98°C). The individual samples were then cooled to room temperature and reweighed. CL was then calculated as the difference between the two weights (Dai et al. 2012; Wan et al. 2016).

Drip loss (DL) was measured by weighing individual pieces of meat samples (about 3 g; 3 cm × 1 cm × 0.5 cm) and then placing them in plastic bags (minimizing the contact between the inside surface of the bags and the muscle samples), suspending them by a steel wire hook and keeping them at 2–4°C. After 48 h, the muscle samples were wiped and reweighed. DL was then calculated as the percentage of the initial weight (Dai et al. 2012; Wan et al. 2016).

Water-holding capacity (WHC) was measured by Lee et al. (2012). Each muscle sample (approximately 1.0 g) was placed between 16 pieces of 125-mm filter paper (8 pieces of filter paper above and another 8 below the sample) and pressed at 2000 psi for 1 min. The WHC value was the weight of the sample after pressing as a percentage of the weight before pressing.

Meat colour was detected with a chromameter (Chroma Meter CR-300; Minolta Co. Ltd, Japan) to measure the International Commission on Illumination (CIE) LAB values of lightness (L*), redness (a*) and yellowness (b*) (Dai et al. 2009). The blooming time of the chromameter is 15 min. The colour of each meat sample was detected twice.

Table 1. Ingredients and composition of diets (g/kg, as-fed basis).

| Ingredients | g/kg | Chemical composition |
|-------------|------|----------------------|
| Ground yellow maize | 567.6 | AMEa (MJ/kg) 12.46 |
| Maize starch | 10.0 | Lysine 11.6 |
| Soybean meal | 360.0 | Methionine 4.4 |
| Soybean oil | 35.0 | Methionine + cystine 7.7 |
| Dicalcium phosphate | 11.0 | Available phosphorus 3.8 |
| Ground limestone | 10.5 | Crude protein 203.7 |
| Iodized salt | 3.0 | Calcium 8.8 |
| DL-Methionine | 1.0 | |
| Micronutrientsb | 1.9 | |

aProvided per kilogram of diet: Vitamin A (as all-trans retinol acetate), 10,000 IU; Vitamin B6, 3.0 mg; Vitamin K (as menadione sodium bisulphate), 2.0 mg; Vitamin E (as all-rac-alpha tocopheryl acetate), 20 IU; cholecalciferol, 2600 IU; Vitamin B12, 0.014 mg; riboflavin, 6.0 mg; thiamine (as thiamin mononitrate), 1.6 mg; calcium pantothenate, 20 mg; iron (from iron sulphate), 80 mg; selenium (from sodium selenite), 0.15 mg; niacin, 30 mg; copper (from copper sulphate), 8 mg; folic acid, 0.8 mg; zinc (from zinc sulphate), 40 mg; iodine (from potassium iodide), 0.35 mg; choline (as choline chloride), 500 mg; biotin, 0.12 mg.
bAME = apparent metabolizable energy
2.6. Statistical analysis

Data were analysed by conducting analysis of variance (ANOVA) procedures for a completely randomized design using the GLM procedure in SPSS (2008) version 18.0. The replicate was the experimental unit (6 pens per group with ten broilers per pen for growth performance measurements and 6 pens per group with 1 broiler per pen for some other measurements). Tukey’s multiple range tests were then used to determine any marked statistical differences between various groups. p < .05 was considered statistically significant. Values are expressed as means with a pooled SEM for each of the five treatment groups.

3. Results and discussion

3.1. Meat quality

The effects of acute heat stress and Gln supplementation on breast and thigh meat quality in chickens are presented in Table 2. Both the breast and thigh meat of the acute heat-stressed broilers exhibited significantly higher (p < .05) CL values but lower (p < .05) pH and WHC values than the control group (Table 2). In contrast, the addition of 20 g/kg Gln significantly increased (p < .05) the pH and WHC values compared with the acute heat-stressed group (0 g/kg Gln); no significant differences in CL, DL, WHC and pH values were observed between the control and the 20 g/kg Gln-supplemented group (Table 2).

In the breast meat, broilers under acute heat stress (0 g/kg Gln) had higher (p < .05) \( L^* \) values but lower \( b^* \) values than the control group (Table 2). However, compared with the acute heat-stressed broilers, the addition of 20 g/kg Gln in the diet significantly increased the \( L^* \) values and increased (p < .05) the \( b^* \) values (Table 2). For the thigh meat, only the \( L^* \) values were significantly (p < .05) higher in the acute heat-stressed group than in the control group (Table 2). However, 20 g/kg Gln supplementation (p < .05) significantly reduced the \( L^* \) values compared with those in the acute heat-stressed group (Table 2).

The pH value is directly related to the acid content of the meat and thus affects meat quality. Generally, meat pH is positively correlated with WHC and \( a^* \) values but negatively correlated with CL and \( L^* \) values. Lower WHC and higher DL values have been shown to reduce the flavour and soluble nutrients in meat (Lee et al. 2012), resulting in the formation of tasteless, hard and dry meat. Furthermore, \( L^* \) value is important for estimating the pale, soft, exudative (PSE) occurrence in chicken meat. It has previously been demonstrated that high temperatures have detrimental effects on broiler meat quality (Khan et al. 2012; Wan et al. 2018). Dai et al. (2012) suggest that the cyclic heat stress reduced the meat quality of broiler, but the addition of Gln could improve it. Similarly, the acute heat stress significantly decreased the meat pH and WHC, but increased the meat \( L^* \) values. However, it was also found that a diet that was supplemented with 15 or 20 g/kg Gln had beneficial effects on broiler meat pH, CL, WHC and \( L^* \) values during heat stress.

3.2. TPA characteristics

In breast meat, the gumminess and hardness were significantly lower (p < .05) in the acute heat-stressed group than in the control group (Table 3). However, the addition of 20 g/kg Gln significantly increased (p < .05) these parameters in heat-stressed broilers (Table 3). There were no significant differences (p > .05) in springiness, chewiness and cohesiveness between the control group and the Gln addition groups (Table 3).

In thigh meat, the springiness, gumminess, chewiness and hardness were significantly lower (p < .05) in the acute heat-stressed group than in the control group, and the addition of Gln (20 g/kg) significantly increased (p < .05) all of these parameters in heat-stressed broilers (Table 4). There was no (p > .05) significant difference in cohesiveness between the control group and the Gln supplementation groups (Table 3).

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Table 2. Effects of dietary Gln on breast and thigh meat quality in broilers under acute heat stress.

| Item                  | Normal temperature | Acute heat stress |
|-----------------------|--------------------|-------------------|
|                       | Control            | 0 g/kg Gln        | 5 g/kg Gln        | 15 g/kg Gln       | 20 g/kg Gln       | SEM       |
| **Breast**            |                    |                   |                   |                   |                   |           |
| pH30min               | 5.93c              | 5.69a             | 5.70ab            | 5.75ab            | 5.85bc            | 0.057     |
| CL (%)                | 40.6b              | 44.6a             | 43.1ab            | 42.6ab            | 40.7b             | 1.28      |
| DL (%)                | 3.83               | 5.36              | 4.90              | 4.56              | 4.27              | 0.800     |
| WHC (%)               | 76.6a              | 71.9b             | 74.8ab            | 76.4a             | 76.2a             | 1.24      |
| **Thigh**             |                    |                   |                   |                   |                   |           |
| pH30min               | 6.30b              | 5.94a             | 6.03ab            | 6.11ab            | 6.29b             | 0.107     |
| CL (%)                | 44.5b              | 49.4a             | 47.7ab            | 45.6b             | 44.2b             | 1.28      |
| DL (%)                | 3.63               | 4.51              | 4.48              | 4.31              | 4.04              | 0.712     |
| WHC (%)               | 76.7a              | 71.8b             | 73.4ab            | 76.1a             | 76.9a             | 1.44      |

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Notes:

1. There were six replicates per group of 10 birds; A chick from each replicate was randomly selected for sampling (n = 6).
2. Gln = glutamine.
3. SEM = standard error of the mean.
4. The muscles were stored at 2–4°C (24 h) for meat quality analyses; CL = cooking loss; DL = drip loss; WHC = water-holding capacity; The value was the weight of the sample after pressing as a percentage of the weight before pressing.
TPA parameters are important for the broiler meat quality because they influence consumer acceptability. In general, textural characteristics such as springiness, gumminess, chewiness, cohesiveness and hardness directly reflect the firmness, tenderness and juiciness of the meat (Martinez et al. 2004; Chan et al. 2011). In the present study, acute heat stress reduced the springiness, gumminess, chewiness and hardness of broiler meat. These may cause by the PSE-meat of acute heat stressed broiler, which has been shown to have lower TPA parameters than normal chicken meat (Zhang & Barbut 2005). The addition of 20 g/kg Gln in the diet reversed these effects under acute heat stress, indicating that Gln would ameliorate meat quality in heat-stressed broilers.

### 3.3. MDA levels and antioxidant enzymes

Acute heat stress induces serious tissue deterioration and physiological dysfunction, which may lead to a decline in meat quality. It has previously been shown that hyperpyrexia can greatly increase the production of reactive oxygen species (ROS) and cause oxidative stress in tissues and cells (Lin et al. 2006; Yang et al. 2010). In broilers, the balance between the antioxidation system and the formation of ROS can also be disrupted by acute heat stress (Wan et al. 2016).

The accumulation of ROS in broiler skeletal muscle enhances lipid peroxidation, which can be monitored by detecting MDA concentrations (Jiang et al. 2009; Hu et al. 2015). In the present study, acute heat stress was found to significantly increase (p < 0.05) the MDA concentrations in the breast and thigh muscles compared with the control group (Table 4). However, dietary supplementation with 15 or 20 g/kg Gln resulted in significantly lower MDA levels in the breast and thigh muscles of heat-stressed broilers compared with the acute heat-stressed group (0 g/kg Gln). In addition, there was no significant difference in MDA concentrations between the control and the 20 g/kg Gln supplementation group (Table 4). Thus, it appears that Gln can enhance antioxidant ability, reducing the oxidative damage caused to the skeletal muscle under hot environmental conditions. The addition of 20 g/kg Gln also significantly increased the GSH concentrations and GSH-Px activity in the breast and thigh muscles of acute heat-stressed broilers (Table 4).

Modern broiler meat is sensitive to oxidative deterioration as a result of acute stress due to its high levels of polyunsaturated fats. The antioxidation system and the formation of ROS can be disrupted by acute heat stress (Wan et al. 2016). The accumulation of ROS in broiler skeletal muscle enhances lipid peroxidation, which can be monitored by detecting MDA concentrations (Jiang et al. 2009; Hu et al. 2015). In the present study, acute heat stress was found to significantly increase (p < 0.05) the MDA concentrations in the breast and thigh muscles compared with the control group (Table 4). However, dietary supplementation with 15 or 20 g/kg Gln resulted in significantly lower MDA levels in the breast and thigh muscles of heat-stressed broilers compared with the acute heat-stressed group (0 g/kg Gln). In addition, there was no significant difference in MDA concentrations between the control and the 20 g/kg Gln supplementation group (Table 4). Thus, it appears that Gln can enhance antioxidant ability, reducing the oxidative damage caused to the skeletal muscle under hot environmental conditions. The addition of 20 g/kg Gln also significantly increased the GSH concentrations and GSH-Px activity in the breast and thigh muscles of acute heat-stressed broilers (Table 4).

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fatty acids. Gln is a conditionally essential amino acid that enhances the body’s antioxidative defence system by regulating GSH content and antioxidative enzymes, particularly during stress conditions (Amores-Sánchez & Medina 1999; Matés et al. 2002). It is beneficial to prevent ROS damage to metabolic enzymes, biomembranes and other key elements (DeBerardinis & Cheng 2010). The heat-stressed broilers that were fed a diet supplemented with Gln exhibited increased antioxidative enzyme activities and reduced peroxidation products in the muscles, which imply that Gln may improve meat quality by enhancing the antioxidative status of broilers under acute heat stress.

3.4. Gln, Glu, GLS and GS levels

Broilers that were exposed to acute heat stress exhibited significantly lower concentrations (p < .05) of Gln, Glu and GLS in the breast and thigh muscles and significantly higher (p < .05) GS activities in the thigh muscle compared with the control group (Table 5). However, supplementation with 20 g/kg Gln significantly increased (p < .05) the concentrations of Gln, Glu and GLS in the breast and thigh muscles and reduced (p < .05) GS activities in the thigh muscle of broilers under acute heat stress, with no significant difference in the levels of any of these between the 20 g/kg Gln supplementation group and the control group (Table 5).

Intracellular Gln and Glu are precursors of GSH which play an important role in the redox state of skeletal muscle (Amores-Sánchez & Medina 1999; Matés et al. 2002; DeBerardinis & Cheng 2010). It has previously been shown that the levels of Gln and Glu in the muscles decrease precipitously under hypercatabolic conditions, such as illness, injury and stress, and this was supported by the findings of the present study under acute heat stress (Table 5). These lower intramuscular Gln and Glu concentrations are usually accompanied by increased levels of oxidative stress (Amores-Sánchez & Medina 1999). Therefore, the unexpected variations in meat quality of broiler under acute heat stress (Tables 2 and 3) may be caused by changes in the metabolism of Gln and Glu (Table 5) and oxidative damage (Table 4) in the skeletal muscle. Dai et al. (2011) reported that dietary Gln could increase the concentrations of Gln and Glu in the serum of broilers during circular heat stress. The similar results were found in the present study, where 20 g/kg Gln in the diet restored their levels in the breast and thigh muscles. These findings imply that exogenous Gln should be added to the feed of broilers to satisfy the increased Gln requirement during acute heat stress conditions.

GLS and GS which are the major Gln metabolic enzymes catalyse Gln synthesis and utilization in broiler muscle. The detection of GLS and GS levels can provide an initial assessment of changes in Gln level. The breast and thigh muscles of acute heat-stressed broilers had higher GS activities and lower GLS concentrations than the control group in the present study. These results match the findings of Chen et al. (1993) and Dai et al. (2011) in broiler serum and rat kidneys, respectively. However, Lam et al. (1994) previously reported that physiological stress significantly decreases GS activities in rat livers. These differences may be due to tissue specificity (Matés et al. 2002).

4. Conclusion

In conclusion, acute heat stress decreased breast and thigh meat quality of broilers as a result of oxidative damage and lipid peroxidation. Dietary Gln could improve meat texture and quality through its positive effects on the antioxidative ability and Gln metabolism of the skeletal muscle in heat-stressed broilers. These results imply that Gln is an important additive to increase the meat quality of broilers during acute heat stress conditions.

Disclosure statement

No potential conflict of interest was reported by the authors.

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