Effects of dietary α-lipoic acid on carcass characteristics, antioxidant capability and meat quality in Hainan black goats

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ABSTRACT
The effects of α-lipoic acid (LA) on the carcase characteristics, antioxidant capability and meat quality of Hainan black goat were investigated in the present study. Thirty goats (10.71 ± 0.42 kg) were randomly assigned with three levels of LA in the concentration diet (0, 300, 600 mg/kg). The goats fed for 70 days. The results showed that goats fed the diet containing 600 mg/kg LA had a significantly \( (p < .05) \) higher ADG and better feed conversion rate (FCR) compared with goats offered the basal diet without LA. There were no differences in carcase characteristics among the three treatments. For antioxidant enzymes, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and catalase (CAT) in serum significantly increased \( (p < .05) \) in goats fed with 600 mg/kg LA at day 70. The concentration of malondiadehhyde (MDA) was significantly lower \( (p < .05) \) in serum of goats that received 600 mg/kg LA than in the goats fed diet without LA. Addition level of LA had no significant impact on pH and intramuscular fat (IMF) of longissimus dorsi muscle tissue (LM) and semimembranosus muscle tissue (SM) in the goats \( (p > .05) \). However, increasing additional LA in the diet caused a decrease on drip loss and WBSF \( (p < .05) \). Treatment of goats with 600 mg/kg of LA has the lowest drip loss and WBSF. These results indicate that diet supplementation with LA may enhance antioxidant capability, improved the growth performance and meat quality in Hainan black goats.

INTRODUCTION
Hainan black goat, a meat goat breed, is raised in the south of China. This breed is not only tolerant to the warm and rainy climates of the region, and it is also a source of delicious meat (Wang et al. 2014). High temperature and high humidity may cause oxidative stress, and then leaded to lipid oxidation. The lipid oxidation is one of the primary causes for deterioration in muscle quality (Buckley et al. 1995). The previous studies indicated that the use of antioxidants is one of the most important ways to enhance the antioxidant capacity and prevent lipid oxidation of animal (Jiang et al. 2007). Also, there were studies showed that the supplementation of exogenous antioxidants in diet could improve the meat quality (Sohaib et al. 2012). Alpha-lipoic acid (LA) is a coenzyme involved in mitochondrial metabolism. The reduced form of LA, dihydrolipoic acid (DHLA), is a powerful mitochondrial antioxidant (Moini et al. 2002). LA and DHLA act as a potent redox couple that has a standard reduction potential (Shay et al. 2009). Both LA and DHLA are capable of scavenging a variety of reactive oxygen species, hydroxyl radicals and hypochlorous acid (Bustamante et al. 1998; Wada et al. 1997). Furthermore, LA and DHLA appear to regenerate other endogenous antioxidants such as glutathione (GSH), vitamin C, ubiquinone and vitamin E to protect the integrity of cell membranes (Bast & Haenen 2003; Biewenga et al. 1997).

The past studies mostly focussed on the effects of LA supplementation in the diets on the lipid stability and antioxidant capacity in the mice or broilers (Chen et al. 2011; Goraca et al. 2013; Shen et al. 2005). The studies showed that dietary LA did improve antioxidation and improve the meat quality. Structure of LA may change during digestion and metabolism in ruminants, and then the structure changes may cause the changes in antioxidative status. Whether or not the similar responses of LA could be observed in ruminants has not been confirmed. So, the objective of this
study was therefore to evaluate the effects of LA addition in diets on antioxidant capacity and meat quality in Hainan black goats.

Materials and methods

Animals and treatments

The experiment was approved by the Institutional Animal Care and Use Committee at Chinese Academy of Tropical Agricultural Sciences, Haikou, China and was conducted in accordance with the National Institute of Health guidelines for the care and use of experimental animals.

Thirty castrated Hainan black goats (three months old and 10.71 ± 0.42 kg BW) were randomly allocated to three diet treatments, and each treatment was replicated five times. The three diet treatments were concentrate diet supplemented with 0 (control), 300, or 600 mg/kg DL-α-lipoic acid (Sigma Chemical, St. Louis, MO), respectively. Physical composition of concentrate, chemical composition of concentrate and King grass (Pennisetum purpureum × P.americanum cv. Reyan No.4) are listed in Table 1.

Feeding and management

Animals were given a fifteen-day adaptation period during which they were treated with Ivermectin against internal and external parasites. Concentrate-based diet were fed twice daily (08:30 and 15:00), and followed by the fresh King grass. Water was freely available to the goats. Feeding allocations and refusals to eat were recorded daily for each replicate. During the experimental period of 70 days, animals were stalled in individual pens. Animals were weighed monthly before morning feeding.

Blood collection and assay of antioxidant indices in serum

Approximately, 10 ml of blood was collected from 15 goats (one goat selected at random from each replicate) through a jugular venipuncture and brought to the laboratory for analysis. Blood collection occurred in the morning of day 70 before water and feed were offered. To separate out the serum, the test tubes with blood samples were kept in a slant position for 45 min, followed by centrifugation at 700 g for 15 min. Serum samples were stored in 2-ml plastic vials at −20°C to measure glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), catalase (CAT) and malondiadehdyte (MDA) levels using colorimetric methods with a spectrophotometer (UV-2600, Shimadzu Corporation, Japan). The assay were conducted with the assay kits obtained from Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China) and the procedures accordingly.

Slaughter procedure, carcase measurements and dissection

Goats were weighed on two consecutive days prior to slaughter to obtain the final live weight. Goats were fasted for 16 h, with free access to water and weighed again to obtain the slaughter weight. The slaughter was performed during two consecutive days. All goats were stunned before slaughter. Fifteen goats were slaughtered for carcase measurements, and another fifteen goats were slaughtered for meat property determination. After slaughtering, the head was removed at the atlanto-occipital joint and fore and hind feet removed at the carpus-metacarpal and tarsus-metatarsal joints. Hot carcases were weighed. Carcases were then chilled at 4°C for 24h. They were then re-weighed and cold carcase weights were obtained. Dressing percentage was determined as the rate of cold carcase weight over slaughter weight. The cold carcases were separated into meat, fat and bone, and meats were weighed. Net meat percentage of cold carcase was determined as the ratio of meat weight over cold carcase (Majdoub-Mathlouthi et al. 2013).

Table 1. The rations and their chemical compositions of concentrate and King grass (Pennisetum purpureum × P.americanum cv. Reyan No.4).

| Ingredients, g/100 g DM | Concentrate | King grass |
|------------------------|-------------|------------|
| Maize                  | 66.90       | –          |
| Soy bean meal          | 16.00       | –          |
| Wheat bran             | 2.00        | –          |
| Rice bran              | 8.00        | –          |
| Palm oil               | 1.00        | –          |
| Limestone              | 0.20        | –          |
| Sodium chloride        | 1.40        | –          |
| Sodium Bicarbonate     | 0.50        | –          |
| Premix*                | 4.00        | –          |
| Nutrition level, g/100 g DM* | 87.67 | 20.59 |
| Dry matter             | 135.00      | 92.00      |
| Crude protein          | 15.10       | 7.36       |
| NDF                    | 22.41       | 69.99      |
| ADF                    | 12.60       | 47.24      |
| Calcium                | 1.04        | 0.35       |
| Phosphorous            | 0.51        | 0.26       |

*The premix provides the following per kg of diet: VA 15000 IU, VD 5000 IU, VE 50 mg, Fe 9 mg, Cu 12.5 mg, Zn 100 mg, Mn 130 mg, Se 0.3 mg, I 1.5 mg, Co 0.5 mg.

bThe nutrient levels are measured values not including DE.
Samples collection and determination of muscle property

Approximately, 50 g of longissimus dorsi muscle tissue (LM, 5th lumbar vertebra region) and 50 g of semimembranosus muscle tissue (SM) were removed from the left sides of carcases and stored at −20 °C for subsequent intramuscular fat (IMF) determination. Approximately, 50 g of LM and SM samples were thawed for 16 h at 4 °C. External fat and connective tissue were removed prior to homogenisation. Samples were placed in a drying oven at 65 °C for at least 48 h and then IMF was extracted in petroleum ether using an automated extraction system (Gerhardt, Germany).

Two 2.5-cm thick chops of LM and SM were obtained. The first chop was used for pH measurement approximately 45 min and 24 h post-mortem using a penetrating electrode (Mettler Toledo) attached to a portable pH-meter (FG2, Shanghai, China). The pH probe was calibrated with pH 4 and pH 7 standard buffer solutions. The chop was then stored at 4 °C for 24 h before the final pH measurement. The second chop was weighed, placed in a Whirlpak bag, suspended in a 4 °C cooler for 24 h, and then reweighed. Chop drip loss was calculated based on the weight loss, and drip loss was expressed as percentage.

Two 5 × 2.5 × 2.5 cm chops of LM and SM were obtained from the right side of the carcase along the direction of the muscle fibres, and stored at −20 °C for 24 h. Then they were thawed for 16 h at 4 °C, and then cooked to an internal temperature of 70 °C in a thermostatic water-bath set at 80 °C. After removal from the water bath, LM and SM chops (two chops in every muscle) were allowed to cool to 4 °C, and then two 5 × 1 × 1 cm chops from each chop were cut parallel to the orientation of the muscle fibre. Each chop was then sheared four times at a crosshead speed of 1 mm/s using a Texture Measurement System (Food Technology Corporation). Warner Bratzler shear force (WBSF) for individual chops were averaged for each sample, and then were averaged five replicates for each dietary treatment (Guo et al. 2011).

Statistical analysis

Data were analysed by one-way ANOVA with the assumption of homogeneity of variance using SAS software (SAS Institute 1990). For growth and carcase data, initial body weight and cold carcase weight were included as covariates, respectively. Results were expressed as mean and pooled standard error (n = 5). Significant differences were evaluated by Tukey’s comparison test at p < .05.

Results

Growth performance and carcase characteristics

In the present study, addition of LA to goat’s concentrate feed had no significant impact on live body weight and feed intake (p > .05) (Table 2). However, goats offered concentrate feed with 600 mg/kg of LA had higher average daily gain (ADG) (p < .05) and better feed conversion ratio (FCR) (p < .05) than those goats fed concentrate feed without LA.

There were no differences in slaughter weight, carcase weight, dressing percentage, net meat weight and net meat percentage of cold carcase among the three treatments (Table 3).

Antioxidative property

Table 4 shows the content of MDA and the activities of GSH-Px, T-AOC, SOD and CAT in the serum of goats. Treatment of goats with 600 mg/kg of LA caused an increase (p < .05) of GSH-Px, T-AOC, SOD and CAT compared with the control. The levels of MDA were significantly decreased (p < .05) in LA addition treatments compared with the control.

Meat quality

In the present study, addition level of LA had no significant impact on pH and IMF of LM and SM in the goats (p > .05) (Table 5). However, with the increasing addition level of LA in the diet, drip loss and WBSF reduced (p < .05). Treatment of goats with 600 mg/kg of LA has the lowest drip loss and WBSF.

Discussion

Effects of LA supplementation on growth performance and carcase characteristics of goats

Addition of exogenous antioxidants in diet could enhance the antioxidant capacity, alleviate oxidative stress and improve meat quality. The present study confirms that LA supplementation in diet could improve the performance and carcase characteristics of goats.

Table 2. Effects of α-lipoic acid levels of diets on growth performance in Hainan black goats (n = 5).

| α-Lipoic acid in the diet, mg kg⁻¹ dry feed | 0 | 300 | 600 | SEM | p-value |
|-------------------------------------------|---|-----|-----|-----|---------|
| Live weight, kg                           | 16.72| 17.00| 16.75| 0.15| .92     |
| Initial weight, kg                        | 10.67| 11.14| 10.32| 0.11| .62     |
| Final weight, kg                          | 14.50| 15.14| 14.42| 0.12| .90     |
| ADG, g/d                                  | 69b | 75b  | 77b  | 2   | .04     |
| Feed intake, g/d                          | 206.27| 214.04| 209.99| 1.00| .37     |
| Concentrate, g/kg DM                     | 201.07| 204.73| 195.50| 1.20| .27     |
| King grass, g/kg DM                      | 407.34| 418.77| 405.50| 1.86| .36     |
| Total feed intake, g/kg DM               | 6.00a| 5.59b | 5.29  | 0.09| .02     |

ADG: average daily gains; FCR: feed conversion rate.

In the same row, values with different letter superscripts mean significant difference (p < .05).
stress, and improve growth performance of animal (Jiang et al. 2007; Richard & Giovann 2010; Young et al. 2003). The effects of LA in diet on the growth performance of other animals were not consistent. Hamano et al. (1999) indicated that LA in the diet had no effect on the growth rates (body weight) in broilers. Yasin et al. (2012) reported that the lower concentration of LA (25 mg/kg) in the feed improved the growth performance of broilers, and the higher concentration of LA (150 mg/kg) suppressed the growth rate of broilers. Zhang et al. (2014) reported that broilers fed 100 mg/kg LA had lower AFI and ADG than that of no LA treatment. Schmidt et al. (2005) indicated that 8 mg to 16 mg of LA/kg of BW per d in diet had no significant effects on growth performance of finishing steer. Bai et al. (2012) reported that LA was effective in improving the performance of sows and their nursing piglets. There were few studies focussed on the effects of LA on the growth and antioxidant activity of goats. Our present study also indicated that 300 mg/kg to 600 mg/kg of LA in the diet had no significant impact on live body weight and feed intake, but improved the ADG and FCR of the goats. The results disagreed with the previous studies may be attributed to the fact that structure of LA may change during digestion and metabolism in ruminants, and then the structure changes may cause the changes in antioxidative status. Digestion and metabolism of LA in ruminants need further research.

The present research also indicated that LA supplementation did not affect carcase weight, dressing percentage, net meat weight and net meat percentage. The results agreed with the previous study by Schmidt et al. (2005), which indicated that 8 mg to 16 mg of LA/kg of BW per d in diet had no significant effects on carcase characteristics. El-Senousey et al. (2013) also indicated that dietary LA did not influence meat yield in broilers.

Table 3. Effects of LA level of diets on the carcase characteristics in Hainan black goats (n = 5).

| α-Lipoic acid in the diet, mg kg⁻¹ dry feed | 0 | 300 | 600 | SEM | p-value |
|-------------------------------------------|---|-----|-----|-----|---------|
| Slaughter weight, kg                      | 14.61 | 15.06 | 14.94 | 0.13 | .50     |
| Hot carcasse weight, kg                   | 6.70  | 6.85  | 6.65  | 0.06 | .67     |
| Cold carcasse weight, kg                  | 6.43  | 6.65  | 6.55  | 0.06 | .64     |
| Dressing percentage, %                    | 44.01 | 44.16 | 43.84 | 0.39 | .39     |
| Net meat weight, kg                       | 4.80  | 4.59  | 4.71  | 0.06 | .15     |
| Net meat percentage of cold carcasse, %   | 74.69 | 69.16 | 71.97 | 1.59 | .05     |

In the same row, values with different letter superscripts mean significant difference (p < .05).

Table 4. Effects of α-lipoic acid on the antioxidant capacity in the serum of Hainan black goats (n = 5).

| α-Lipoic acid in the diet, mg kg⁻¹ dry feed | 0 | 300 | 600 | SEM | p-value |
|-------------------------------------------|---|-----|-----|-----|---------|
| GSH-Px, U/ml                              | 162.14 | 193.59 | 213.44 | 6.87 | .01     |
| T-AOC, U/ml                               | 2.29a  | 2.33a  | 2.48a  | 0.09 | <.01    |
| SOD, U/ml                                 | 71.43b | 75.63b | 84.68b | 1.75 | <.01    |
| CAT, U/ml                                 | 2.18ab | 2.42ab | 2.73a  | 0.07 | .01     |
| MDA, nmol/ml                              | 6.19a  | 4.21a  | 3.08a  | 0.41 | <.01    |

In the same row, values with different letter superscripts mean significant difference (p < .05).

Table 5. Effects of LA level of diets on meat quality in Hainan black goats (n = 5).

| α-Lipoic acid in the diet, mg kg⁻¹ dry feed | 0 | 300 | 600 | SEM | p-value |
|-------------------------------------------|---|-----|-----|-----|---------|
| Longissimus dorsi muscle                  |   |     |     |     |         |
| Drip loss, %                              | 1.46a  | 1.33a  | 1.07b  | 0.12 | <.01    |
| pH45min                                   | 6.51  | 6.58  | 6.56  | 0.02 | .77     |
| pH24h                                     | 5.66  | 5.63  | 5.68  | 0.02 | .96     |
| IMF, %                                    | 6.09  | 6.22  | 6.79  | 0.22 | .13     |
| WBSF, N                                   | 129.19a | 95.14b | 86.38b | 13.06 | <.01    |
| Semimembranosus muscle                    |   |     |     |     |         |
| Drip loss, %                              | 1.83a  | 1.20b  | 1.15b  | 0.22 | <.01    |
| pH45min                                   | 6.56  | 6.75  | 6.75  | 0.06 | .37     |
| pH24h                                     | 5.52  | 5.78  | 5.80  | 0.09 | .09     |
| IMF, %                                    | 5.44  | 5.46  | 5.72  | 0.09 | .77     |
| WBSF, N                                   | 126.75a | 114.97b | 94.11b | 9.54 | <.01    |

IMF, intramuscular fat; WBSF, Warner-Bratzler shear force. In the same row, values with different letter superscripts mean significant difference (p < .05).

Effects of LA supplementation on antioxidative property of goats

Hainan black goat, a meat goat breed, is raised in the south of China. This breed is tolerant to the warm and rainy climates of the region. High temperature and high humidity may cause oxidative stress though Hainan black goats have been adapted to the local environment and climate due to the domestication of long time.

Glutathione plays an important role in maintaining the integrity of the cell system and it is a sensitive marker of oxidative stress. Meanwhile, GSH is one of the most prominent non-enzymatic antioxidant which involved in several reactions in the body (Meister & Anderson 1983). Shay et al. (2009) indicated that LA
may act indirectly to maintain cellular antioxidant status by enhancing the synthesis of antioxidants such as reduced glutathione, vitamin C and vitamin E. In the present study, increased SOD, GSH-Px, T-AOC and CAT activity in serum in LA-fed goats were observed compared with control goats. Similar findings were reported by Srilatha et al. (2010) in broilers and by Kowluru et al. (2005) in rats. SOD, GSH-Px, T-AOC and CAT are the main part of body antioxidant defence systems against lipid peroxidation. The increase in the activities of these enzymes could reflect the positive effects of LA on this balanced antioxidant system, which suggested that diet LA improves the capacity of scavenging free radical in Hainan black goats.

MDA is formed as end product of lipid peroxidation and oxidative stress, and therefore the extent of lipid peroxidation by ROS can be monitored by MDA levels (Sumida et al. 1989). Hence, the reduced serum MDA concentrations in LA supplemented as compared with control goats indicated that oxidative stress was reduced by LA via improving the capacity of antioxidant system in Hainan black goats. These results were agreed with previously reported finding of other researchers (Bai et al. 2012; Hagen et al. 2002; Halici et al. 2012).

**Effects of LA supplementation on meat quality of goats**

Water-holding capacity (WHC) of meat is one of the most important factors of meat quality. A low WHC in muscles can increase the liquid outflow and lead loss of soluble nutrients and flavour. WHC of goat meat was evaluated in this study by measuring drip loss. The present study showed that the addition of LA in the diets decreased the drip loss of goat muscle in a dose-dependent manner. EI-Senousey et al. (2013) reported that 400 mg/kg to 1200 mg/kg LA in the diet-inhibited mRNA expression of COL3A1 gene in muscle and decreased muscle glycolysis early post-mortem, and then improved the WHC.

Generally, muscle pH values are reduced during the immediate post-mortem period; the rate of pH decline usually has remarkable effects on meat quality (Diaz et al. 2002). Feeding diets supplemented with LA effectively retarded early post-mortem pH decline, which showed that LA inhibited post-mortem glycolysis and increased pH in muscle of mice (Shen et al. 2005). In the present study, the muscle pH$_{24h}$ values were not influenced by LA addition in diet. However, the muscle pH$_{24h}$ values were within or near the acceptable range for goats (pH range: 5.6–5.8) (Pratiwi et al. 2007).

To our knowledge, the effect of LA on IMF of goat have not been studied, however, the effects of LA on abdominal fat in other animals have been evaluated. Shen et al. (2005) reported that LA (0.5% and 1.0%) decreased percentage weight of abdominal fat in mice. Zhang et al. (2009) also indicated that 900 mg/kg LA in the diet decreased the abdominal fat percentage. Reed (1973) thought that lipoamide dehydrogenase was the flavoprotein component of the α-keto acid, and which was involved in Krebs cycle promoting energy metabolism. The current study indicated that the LA addition in diet did not affect the IMF of goat. The results disagreed with the previous studies may be attributed to the fact that LA addition level in the diet and animal differences.

Shear force is indicative of meat tenderness, which has been noted as the most important factor in consumer perception of meat quality (Savell et al. 1989). LA decreased the sheer force of LM and SM in the goat in the present study, which was consistent with previous studies (Schmidt et al. 2005; Zhang et al. 2009).

**Conclusions**

In conclusion, the results presented in this study indicated that dietary LA could improve the ADG, feed conversion ratio, anti-oxidative ability and meat quality of Hainan black goats. Results showed that dietary LA improved meat quality of goat by decreasing drip loss and shear force value, and enhancing the meat tenderness.

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The authors of this manuscript have no financial or personal relationship with any other organisations which could influence the work on the compound in this manuscript.

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