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Effects of chestnut tannins on the meat quality, welfare, and antioxidant status of heat-stressed lambs

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ABSTRACT

A study was conducted to evaluate the effects of chestnut tannins (CT) on the meat quality, welfare and antioxidant status of heat-stressed lambs. Lambs in one group were raised at 20 °C and fed a basal diet (N), and three other groups (32 °C) were fed a basal diet with 0 (CT0), 5 (CT5), and 10 g (CT10) of CT/kg. Addition of CT increased the b^* and L^* values of meat and superoxide dismutase and glutathione peroxidase activity in the serum and liver of heat-stressed lambs. The malondialdehyde concentration in meat, serum, and liver of heat-stressed lambs was decreased by dietary CT supplementation. Lambs in the CT0 group had higher cortisol, T_3, and T_4 levels, creatine kinase activity, white blood cell count, neutrophil count, neutrophil:lymphocyte ratio and a lower lymphocyte count than that in the N and CT10 groups. In conclusion, the addition of CT improved meat quality, certain stress parameters, and the antioxidant status of heat-stressed lambs.

1. Introduction

In South China, high temperatures are common during the summer months and range from 30 to 40 °C (June through October). High ambient temperature is a major constraint on sheep production during hot summers (Liu, Cao, & Zhou, 2012; Sevi et al., 2001). When sheep are exposed to high ambient temperatures exceeding their thermal neutral zone (5 to 25 °C), compensatory physiological mechanisms are triggered, which permit the maintenance of vital functions by drastically changing biological functions of the animals (Marai, El-Darawany, Fadiel, & Abdel-Hafez, 2007). These changes in biological functions result in a decrease of feed intake, weight gain, meat quality, and health in sheep (Marai, Bahgat, Shalaby, & Abdel-Hafez, 2000; Shelton, 2000). Studies have shown that high ambient temperature initiates lipid oxidation in cell membranes and the detrimental effect of high ambient temperature could, in part, be the result of oxidative stress (Bollengier-Lee, Mitchell, Utomo, Williams, & Whitehead, 1998; Sahin & Kucuk, 2003). Lipid oxidation is a significant problem and produces an off-flavor, off-odor, and often warmed-over flavor in meat (Fernández, Pérez-Álvarez, & Fernández-López, 1997). Therefore, diets enriched with natural antioxidants which protect cells and tissues from lipoperoxidative damage induced by an excess of free radicals may be used to alleviate the negative effects of high ambient temperatures (Liu, Dong, Tong, & Zhang, 2011; Tuzcu et al., 2008).

Tannins are a complex group of water-soluble polyphenolic compounds that are formed from the metabolism of plants. They consist of one or more aromatic rings with one or more hydroxyl groups, which can combine with free radicals to form resonance-stabilized phenoxyl radicals. This structure confers strong antioxidant properties (Rice-Evans, Miller, & Paganga, 1996). Several studies evaluated in vitro antioxidant activities of chestnut tannins (CT; extracted from chestnut wood, *Castanea sativa*, which is rich in hydrolysable tannins) (Barreira, Ferreira, Oliveira, & Pereira, 2008; Dong et al., 2015) and in vivo antioxidant activities of tannins observed in rabbits (CT, Liu et al., 2011), broilers (Dong et al., 2015), and pigs (Ranucci et al., 2015). Moreover, tannins which are present in several feed resources used for livestock feeding have been reported to affect several aspects of ruminant nutrition and product quality (Tabacco et al., 2006; Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). Luciano et al. (2009) reported that quebracho tannins improved meat color stability in sheep because of their antioxidant properties. Priolo and Vasta (2007) reviewed that the effects of tannins on small ruminant meat color could be due to a reduced microbial biosynthesis of vitamin B_{12} which was a precursor of the synthesis of haeme pigments, and the ruminal biosynthesis of odor-active compounds could be affected by tannins. However, no information is available on the potential beneficial role of CT for heat-stressed lambs. The objective of this study was to compare the growth performance,

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meat quality, welfare, and antioxidant status of lambs fed with or without CT in a heat stress environment.

2. Materials and methods

2.1. Animals, diets and management

The use of the animals and the experimental procedures were approved by the Animal Care Committee at the Institute of Animal Science in the Chinese Academy of Agricultural Sciences in Beijing, China. The research was conducted at the Ecological Research Station for Grassland Farming, Chinese Academy of Sciences, Jilin, China.

A total of 40 healthy male Ujumqin lambs (a local breed; average body weight = 26.5 ± 1.02 kg; four-month-old) were randomly assigned into four groups (10 lambs per group). Lambs in each group were individually housed in pens (1.0 × 1.5 m, animal density of 1.5 m^2 per lamb) in one of two environmentally controlled rooms both maintained at approximately 50% relative humidity. One group was housed in a room kept at 20 °C (Temperature–humidity index, THI = 19.1), while the other three remaining groups in the other room were maintained at 32 °C (THI = 29.3) throughout the experimental period to simulate a heat stress environment. The temperature and humidity in environmentally controlled room were controlled by a synthesized equipment (Beijing Kooland Technologies Co., Ltd., Beijing, China) including air compressor, electric heater, humidifier, temperature and humidity sensor, fan, and air duct. The synthesized equipment was controlled by computer software. The THI was calculated according to the equation of Marai, Ayyat, and Abd El-Monem (2001): THI = db °C - {(0.31–0.31 × RH) (db °C - 14.4)}, where db °C is the dry bulb temperature (°C) and RH is the relative humidity (RH%)/100.

The lambs in the N group kept at 20 °C were fed the basal diet, and each of the other three groups kept at 32 °C were fed the basal diet with 0 (CT0), 5 (CT5), and 10 (CT10) g of CT/kg of dry matter. The diets were formulated according to feeding standards for meat-producing sheep and goats (NY/T 816-2004, Ministry of Agriculture, China; Table 1). Dry matter was measured by oven drying at 105 °C overnight (AOAC cod. 930.15, 1995). Diet samples were first dried at 65 °C for determination of air DM. Thereafter, crude protein and crude fat of diets were analyzed. Crude protein was measured by a Kjeldahl nitrogen analysis (AOAC cod. 954.01, 1995). Crude fat was extracted with diethyl ether using a Soxhlet apparatus (AOAC cod. 945.16, 1995). Neutral detergent fiber and acid detergent fiber content were determined by the method of Van Soest, Robertson, and Lewis (1991) using heat-stable amylase (A3306, Sigma, St. Louis, MO) and sodium sulfite for neutral detergent fiber determination, and expressed without residual ash. Gross energy content was determined using the values for heat of combustion by an adiabatic bomb calorimeter (Parr Instrument Co., 1970). The CT (purchased from SilvaTeam, San Michele di Mondovì, Italy, http://silvateam.com) is extracted from chestnut wood by a heat and low-pressure treatment process according to the manufacturer's information; only the water-soluble fraction is retained and subsequently dehydrated. The product is commercially available as a fine brown powder. Chemical composition of the CT batch used in this experiment consisted of 751 g/kg tannins (153 g/kg condensed tannins), 221 g/kg nontannin, 35 g/kg water, and 17 g/kg insolubles (pH 3.41, 0.1 mg/mL solution) on a fresh matter basis. The total tannin content, expressed as tannic acid equivalents, was measured according to the Folin–Ciocalteu method (Makkar, Bluemmel, Borowy, & Becker, 1993). The condensed tannin content was determined as described by Porter, Hrstich, and Chan (1986).

The experiment lasted for 56 days. The daily light illumination period in the environmental rooms was 10 h (0700 to 1700 h), and ventilation was maintained throughout this trial. The feed and water were offered ad libitum and refilled at 0700 and 1700 h daily. The residual feed in the pens was collected.

Table 1
Ingredients and chemical composition of the basal diet.

Item	Basal diet
Ingredient (g/kg diet)	
Aneurolepidium chinense	300
Ground corn	410
Extruded-expelled soybean meal, 410 g/kg CP	150
Wheat bran	100
Sodium chloride	20
Minerals and vitamins[1]	20
Chemical composition (analyzed)	
Dry matter (g/kg FM)	908
Crude protein (g/kg DM)	178
Crude fat (g/kg DM)	35
Neutral detergent fiber (g/kg DM)	653
Acid detergent fiber (g/kg DM)	401
Gross energy (MJ/kg DM)	22.01

[1] Minerals and vitamins were purchased from a commercial company (Continental Grain Corp., Beijing, China), and contained (per kg) 24,000 IU vitamin A, 2500 IU vitamin D_3, 4800 IU vitamin E, 32 g Ca, 11 g P, 5 g S, 65 mg Zn, 50 mg Mn, 120 mg Fe, 25 mg Cu, 0.9 mg Co, 0.8 mg Se.

2.2. Growth performance and sample collection

Feed offered and refused was recorded daily, and daily samples were collected to measure dry matter intake (DMI). Body weight was individually recorded on days 1 and 56 of the experiment to calculate the average daily gain (ADG) and feed efficiency (FE).

On day 56, blood samples were collected by jugular venipuncture into evacuated tubes from all lambs. Each lamb was sampled in less than 1 min to minimize handling stress. Blood from each lamb was collected into 2 vacutainer tubes containing coagulant (silicon dioxide). One tube was kept at 4 °C for a maximum of 2 h before arriving at the laboratory for routine hematological measurements. The other tube was allowed to clot at room temperature (25 °C) for 45 min and centrifuged (Himac CR22G2; Hitachi Koki Co., Ltd., Tokyo, Japan) at 3500 rpm for 15 min at 4 °C. Separated serum was stored at −20 °C for subsequent analysis.

After sampling blood, all lambs were electrically stunned and humanely slaughtered by exsanguination under commercial procedures in accordance with policies of the Animal Ethics Committee of Chinese Academy of Agricultural Sciences. The lambs were hung to remove the skin, head (at the occipito-atlantal joint), fore feet (at the carpal-metacarpal joint), hind feet (at the tarsalmetatarsal joint), gastrointestinal tract and other visceral organs. Hot carcasses were weighed, and dressing percentage was calculated and expressed as a percentage of live weight. Liver samples were rinsed in saline, frozen in liquid nitrogen, and then stored at −80 °C for subsequent analysis of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and malondialdehyde (MDA).

After 24 h of chilling (4 °C), the carcasses were halved into sides, and the *longissimus thoracis et lumborum* (LTL) muscle was excised. The LTL muscle taken from the left side was divided into two parts. The fore part was used to measure cooking loss, color, and pH_{24}; the hind part was frozen and used to measure shear force. The LTL muscle taken from the right side was vacuum-packed and frozen at −20 °C until the thiobarbituric-acid reacting substances (TBARS) were measured.

2.3. Analysis of the blood constituents

An automatic blood cell analyzer (Coulter LH 750, Beckman Coulter, USA) was used to analyze quantities of red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), neutrophil (NEU), and lymphocyte (LYM). The serum was used to determine total protein (TP) content using an automatic biochemistry analyzer (Synchron CX5 PRO, Beckman Coulter, USA). The concentration of glucose and the activity of creatine kinase (CK) in the serum samples were determined with a