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Antioxidant effects of liquorice (*Glycyrrhiza uralensis*) extract during aging of *longissimus thoracis* muscle in Tan sheep

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ABSTRACT

The study was conducted to investigate the potential of liquorice extract (LE) from *Glycyrrhiza uralensis* as a dietary supplement for sheep to improve antioxidant capacity of meat. Fifty Tan sheep were randomly allocated to five groups with LE supplementation at levels of 0, 1000, 2000, 3000 and 4000 mg/kg feed. After 120 days, the *longissimus thoracis* muscle was sampled and conditioned for 0, 2, 4, 6 and 8 days at 4 °C. The results revealed that LE scavenged free radical in a dose–response manner in vitro. Supplementation with LE in animal diet increased ($P < 0.05$) antioxidant content and radical scavenging activity while it decreased ($P < 0.05$) reactive oxygen species (ROS) and thiobarbituric acid reactive substance (TBARS) levels of meat. Dietary LE supplementation can improve antioxidant capacity of meat, and the optimum dosage range of LE supplementation appeared to be 3000 to 4000 mg/kg feed.

1. Introduction

Oxidative damage is the main non-microbial factor to induce meat quality deterioration and reduce shelf life of meat products (Descalzo et al., 2005; Terevinto, Ramos, Castroman, Cabrera, & Saadoun, 2010). Reactive oxygen species (ROS), molecular culprits, induce oxidative damage in biologically essential molecules such as protein and lipid, by donating a hydrogen atom or electron (Duan, Zhang, Li, & Wang, 2006; Fisch, Böhm, Wright, & König, 2003; Moyo, Oyedemi, Masika, & Muchenje, 2012; Nakamura, Watanabe, Miyake, Kohno, & Osawa, 2003). Protein oxidation in meat modifies the amino acid residues leading to the loss of sulphydryl groups, generation of oxidized derivatives and formation of cross-links (Terevinto et al., 2010), whereas lipid oxidation produces conjugated dienes, hydroperoxides and aldehydes (Raharjo & Sofos, 1993). In particular, malondialdehyde (MDA), an aldehydic product, is widely employed as a marker of oxidative deterioration (Descalzo & Sancho, 2008; Gray, Gomaa, & Buckley, 1996). Accumulation of oxidation products in meat foods adversely impacts color, flavor, odor, texture and nutritive value (Devatkal, Narsaiah, & Borah, 2010; Luciano et al., 2009; Terevinto et al., 2010). Therefore, it is essential to prevent oxidative damage in meat.

For improving the oxidative stability of meat products, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) have been widely used, because they are powerful yet inexpensive. However, more and more consumer concern over preservative safety and toxicity has led to a growing interest in natural sources of antioxidants (Devatkal et al., 2010; O'Grady, Maher, Troy, Moloney, & Kerry, 2006; Vasta & Luciano, 2011). Over the past five years, some trials have been conducted on natural, plant-based products like green tea leaves (Zhong et al., 2009), herbs (Karami, Alimon, Sazili, Goh, & Ivan, 2011), vegetables (Huang et al., 2011), fruits (Devatkal et al., 2010) and spices (Nieto, Estrada, Jordán, Garrido, & Bañón, 2011; Rojas & Brewer, 2008), which contain high levels of antioxidant components, such as polyphenol, flavonoid and polysaccharide. When fed to animals, these components can be delivered to the muscle and assist with the native defense system to counteract the action of pro-oxidants (Descalzo & Sancho, 2008; Lahucky, Nuernberg, Kovac, Bucko, & Nuernberg, 2010; Moyo et al., 2012).

Liquorice, an extract from the root *of Glycyrrhiza species*, is widely distributed in the middle-east, Mediterranean area and north of China. It contains high levels of glycyrrhizin, flavonoids and polysaccharides. Previous studies paid close attention to glycyrrhizin for its antiphlogosis and ability to relieve asthma (Ram et al., 2006; Toshihiro, 2000), but most flavonoids are discarded in the process of extracting glycyrrhizin to cause economic waste products. Flavonoids exert many physiological actions including those of an antioxidant, because they can scavenge free radials (Cook & Samman, 1996; Formica & Regelson, 1995; Sestili, Guidarelli, Dachà, & Cantoni, 1998), chelate metal ions (Belinky, Aviram, Fuhrman, Rosenblat, & Vaya, 1997), inhibit enzyme activity involved in the formation of free radical (Gong & Chen, 2003; Valdameri et al., 2010), and modulate signal transduction pathways to affect antioxidase expression (Williams, Spencer, & Rice-Evans, 2004). All of

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these actions can help to improve oxidation resistance. Belinky et al. (1997) reported that glabridin, an isoflavan isolated from *Glycyrrhiza glabra*, reduced the susceptibility of low density lipoprotein (LDL) to oxidation induced by copper ion and 2,2′-azobis dihydrochloride (AAPH). Laura et al. (2011) also demonstrated the antioxidant capacity of flavonoid extracted from *G. glabra*. Currently, as an antioxidant, liquorice flavonoid has been used in health and skin care products for humans. However, there is little or no data on the antioxidant impact of liquorice flavonoid on meat when supplemented to animals.

As antioxidant enzymes in meat would gradually lose their activity postmortem, some non-enzymatic antioxidants might play a key role in protecting meat from oxidation. We have previously reported that liquorice extract (LE, containing 16.4% total flavonoids) could improve meat color during postmortem aging and we speculated that it was possibly caused by the antioxidant activity of LE (Zhang et al., 2013). The higher meat quality may result in higher price received, thereby making economic sense. Therefore, the objective of the present study was to investigate the potential of LE as a dietary supplement to sheep for improving antioxidant capacity of meat. Toward this end, we tested the antioxidant properties of LE in vitro, and then assessed the effects of different levels of dietary LE supplementation on antioxidant capacity of fresh lamb meat during 8 days postmortem aging.

2. Materials and methods

2.1. Animals and diets

The feeding trial was conducted with Tan sheep, a local breed that is celebrated for its meat in Ningxia, China, where the meat is authorized by the government as a geographically symbolic product. In our experiment, all procedures were undertaken following the guidelines of the China Agricultural University Animal Care and Use Committee on animal ethics.

Fifty Tan male sheep at 20–30 days after weaning, with average initial body weight of 17.56 ± 0.39 kg and the same genetic background, from the Nuanquan farm, Ningxia, China, were randomly assigned into five groups (N = 10) for a 120 day of feeding trial before slaughter. The sheep were housed individually in pens (1.1 m long × 1.0 m wide), allowed visual contact with each other and had water supplied ad libitum. During the feeding period, each sheep was fed the basal diet twice daily (08:00 and 18:00 h).

The basal diet shown in Table 1 was formulated according to NRC feeding standard (NRC, 2007). The concentration of metabolizable energy was calculated from the ingredient values based on the feeding standard of meat-producing sheep and goats (NY/T816-2004). Concentrations of crude protein, ether extract, calcium and phosphorus in the basal diet were determined as described in AOAC (1990), while neutral detergent fiber and acid detergent fiber levels were measured according to Van Soest, Robertson, and Lewis (1991). Corn silage (20 mm long) was supplied by the Nuanquan farm, Ningxia, China. Diets were offered as separate concentrate and forage, and the amount was adjusted according to the bodyweight of the sheep.

Table 1
Ingredients and chemical composition of the basal diet (on dry matter basis).

Ingredients	Concentration
Corn silage (%)	50.00
Corn (%)	27.81
Soybean meal (%)	13.00
Wheat bran (%)	4.93
Shelled sunflower meal (%)	2.11
Sodium chloride (%)	0.68
Calcium carbonate (%)	0.23
Calcium hydrophosphate (%)	0.11
Premix (%)[a]	1.13
Chemical composition	
Metabolizable energy (MJ/kg)[b]	8.95
Crude protein (%)	12.30
Ether extract (%)	5.08
Neutral detergent fiber (%)	48.77
Acid detergent fiber (%)	33.89
Calcium (%)	0.51
Phosphorus (%)	0.36
Total flavonoids (mg/kg)	19.00

[a] Per kilogram of premix: 100,000 IU vitamin A, 20,000 IU vitamin D_3, 60 IU vitamin E, 1 g Fe, 1 g Mn, 0.78 g Zn, 0.27 g Cu, 0.012 g Se, and 0.01 g I.
[b] All values are analyzed values except metabolizable energy.

LE, the yellow powder, from *Glycyrrhiza uralensis* containing 16.4% (on air dry basis) total flavonoids, was supplied by Shaanxi Sciphar Biotechnology Co. Ltd., China. Total flavonoid content was determined according to Moreno, Isla, and Sampietro (2000), while moisture, crude protein and crude ash were measured according to AOAC (1990). Neutral detergent fiber level was measured according to Van Soest et al. (1991). LE contained 7.7% moisture, 8.6% crude protein, 11.1% crude ash, 54.0% neutral detergent fiber and 0.3% vitamin E (VE). The control group (LE0) was fed a basal diet without LE supplementation, while the four experimental groups were fed basal diet with LE supplementation at levels of 1000 (LE1000), 2000 (LE2000), 3000 (LE3000) and 4000 (LE4000) mg/kg feed (on dry matter basis). The basal diet contained 19.0 mg total flavonoids per kg feed (on dry matter basis). In all five groups, <0.5% nutritional differences, such as energy and protein, due to LE supplementation were ignored.

2.2. Slaughter procedures and muscle sampling

Sheep were fasted for 12 h with free access to water and weighed immediately before slaughtering by the standard Halal procedures according to Santos et al. (2007). All sheep were electrically stunned and slaughtered by exsanguination. The dressed carcass comprised the body after removing the skin, head (at the occipital–atlantal joint), fore feet (at the carpal–metacarpal joint), hind feet (at the tarsal–metatarsal joint) and viscera. Warm carcass parameters were measured within 30 min post-slaughter. The carcasses were then separated into dissectible muscle, bone, fat and the remainder (major blood vessels, ligaments, tendons and thick connective tissue sheets associated with some muscles) under a controlled environment. Muscle cuts of approximately 200 g were dissected out from the *longissimus thoracis* muscle between 12th thoracic and 5th lumbar vertebrae from the right side of each carcass. 20 g each of muscle (4 cm long × 4 cm wide) was stored at −70 °C until determination of total flavonoid, VE and glutathione (GSH) contents. The remaining 180 g of muscle samples was cut into small slices (3 cm long × 3 cm wide), vacuum packaged and stored for 0, 2, 4, 6 and 8 days in the dark at 4 °C, after which they were stored at −70 °C until further analysis of antioxidant activity, including α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity, 2,2′-azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid (ABTS) free radical scavenging activity, ROS level, thiobarbituric acid reactive substance (TBARS) value and carbonyl protein content.

2.3. Analytical procedure

2.3.1. Antioxidant assay in vitro

DPPH and ABTS free radicals have been widely employed to evaluate antioxidant properties of natural antioxidants (Da Porto, Calligaris, Celotti, & Nicoli, 2000). In our experiment, 0, 1000, 2000, 3000 and 4000 mg of LE were separately mixed with 1 l of 80% ethanol before extraction at 4 °C for 24 h in the dark. DPPH and ABTS free radial scavenging activities of the extract samples (<5% insoluble ingredient was ignored) were measured in triplicate according to Moyo et al. (2012), using BHT (Analytical Reagent, Sigma Chemical Co., St. Louis, MO, USA) as a positive control, using the same concentrations (0, 1000, 2000, 3000 and 4000 mg/l) as for the LE.