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Antioxidant status, colour stability and myoglobin resistance to oxidation of *longissimus dorsi* muscle from lambs fed a tannin-containing diet

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ABSTRACT

Fourteen lambs were individually penned for 60 days. Seven lambs (C) were fed a barley-based concentrate, while 7 animals (T) received the same concentrate supplemented with 8.96% (dry matter basis) quebracho (*Schinopsis lorentzii*) extract. Tannin supplementation extended colour stability of fresh *longissimus dorsi* muscle (LM) over 7 days of aerobic storage with lower changes in hue angle over time in LM from T-fed animals compared to C-fed lambs ($P = 0.032$). Muscle from T-fed lambs had lower metmyoglobin percentages (MMb%) than that from C-fed animals after 3 days of storage ($P = 0.05$) and tended to have slower rates of change in $(K/S)_{572} \div (K/S)_{525}$ values ($P = 0.07$). Compared to the C diet, tannin supplementation increased the concentration of total phenols in muscle (+31.29%, $P = 0.001$) and resulted in a higher muscle antioxidant capacity measured by FRAP (+16.81%, $P = 0.026$) and TEAC (+24.81%, $P = 0.001$) tests. Furthermore, feeding tannins resulted in lower MMb% after nitrite-induced oxidation measured both at the meat surface (−10%, $P = 0.012$) and in a meat extract (−8.22%, $P = 0.004$).

1. Introduction

Among the major meat quality parameters, colour can be considered a key attribute, due to its direct impact on consumer's impression of product freshness and wholesomeness. Specifically, meat discolouration, due to the oxidation of myoglobin (Mb) to metmyoglobin (MMb) over time of storage or retail display, leads to significant product discards (McKenna et al., 2005). Generally, an enhanced antioxidant status in muscle results in a lower myoglobin oxidation extent and this often corresponds to an improved meat colour stability. For example, dietary antioxidants, such as vitamin E, are able to extend meat oxidative stability by improving the overall muscle's antioxidant status, by lowering the formation of some main oxidation markers and by extending, in turn, meat colour stability (Descalzo et al., 2007).

Several compounds are able to exert antioxidant effects in biological substrates and animal tissues. Among the most known natural antioxidants, the interest in phenolic compounds has been growing in recent years. Phenolics form one of the most numerous and chemically heterogeneous groups of plant secondary compounds, ranging from simple molecules – such as phenolic acids and flavonoids – to the highly polymerised tannins (Hagerman et al., 1998). The antioxidant properties of small phenolics have been extensively studied (Scalbert, Morand, Manach, & Rémésy, 2002). Although the influence of the molecule's complexity on the antioxidant ability of polyphenols is not fully clear, in some instances, the free radical scavenging activity of polyphenols was shown to be positively related to the number of hydroxyl groups in their molecules and to their polymerisation degree, that making tannins potentially important antioxidants (Hagerman et al., 1998). Besides in vitro assays suggesting their antioxidant activities, the direct addiction of purified phenolics and of polyphenol-rich plant extracts to muscle model systems was shown to delay metmyoglobin formation (Hayes et al., 2009).

Due to their almost ubiquitous distribution in the plant kingdom, the contribution of phenolic compounds to the dietary intake of antioxidants, which is estimated to be higher than that of vitamin E, is of great interest for human nutrition (Hollman & Katan, 1998). Moreover, tannins, in particular, are present in several feed resources used for livestock feeding and have been shown to affect several aspects of ruminants' nutrition and products quality (Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). The dietary administration of polyphenol- and tannin-rich plants and plant extracts has been shown to improve the oxidative stability of meat from different farming animals (Larraín, Krueger, Richards, & Reed, 2008; Nieto, Díaz, Bañón, & Garrido, 2010). The direct antioxidant activity of a dietary compound would assume its absorption along the gastrointestinal tract and its deposition in the tissues. Monomeric phenolics can be absorbed through the intestine and found in plasma

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(Scalbert et al., 2002). There is also evidence for an active metabolism of condensed tannins by the intestinal microflora in humans and rats leading to their depolymerisation into smaller and absorbable molecules (Déprez et al., 2000). However, the fate of tannins in the animal organisms, their rate of absorption and their concentration in the tissues are still under debate (Scalbert et al., 2002). Furthermore, considering other effects of dietary phenolic compounds, such as their ability to modify muscle's fatty acid composition in ruminants (Vasta et al., 2009), or to increase the levels of antioxidant enzymes in rat tissues (Anila & Vijayalakshmi, 2003), it may be difficult to distinguish between their direct and indirect effects on the resistance of meat to oxidative damages.

In a recent study (Luciano, Monahan, Vasta, Biondi, et al., 2009), we have found a protective effect of dietary quebracho tannins against discolouration and myoglobin oxidation in minced lamb *semimembranosus* muscle, previously frozen, over extended storage in high oxygen modified atmosphere packs (MAP). However, in that study, it was not possible to further investigate possible mechanisms explaining the observed effects. Here we have measured, under aerobic storage conditions, the colour stability of fresh *longissimus dorsi* muscle steaks from the same animals used in the above experiment. Furthermore, we have coupled a series of assays to measure the muscle's overall antioxidant status and the resistance of myoglobin to oxidation. We hypothesise that dietary polyphenols might enhance both muscle overall antioxidant capacity and myoglobin resistance to oxidation, leading to an improvement in the colour stability of muscle.

2. Materials and methods

2.1. Animals and diets

A detailed description of the animals' management and of the dietary treatments has been published by Luciano, Monahan, Vasta, Biondi, et al. (2009). In brief, 14 male Comisana weaned lambs (45 days of age and average initial body weight (BW) of 21.3 kg ± 2.8 kg) were individually penned for the duration of the trial. After a 7-day period of adaptation to the experimental diets, seven lambs (control group; C) were fed a barley-based concentrate comprising, on an as fed basis, of 551 g/kg of barley, 300 g/kg of alfalfa hay, 130 g/kg of soybean meal and 19 g/kg of mineral and vitamin premix. The concentration of tocopheryl acetate in the premix was 35 mg/kg. The remaining seven lambs (Tannin group; T) received the same concentrate with the addition of a commercially available tannin-rich quebracho (*Schinopsis lorentzii*) extract (Figli di Guido Lapi S.p.a., Castelfranco di Sotto, Pisa, Italy). The inclusion of the extract was formulated to obtain a percentage of quebracho powder into the concentrate of 8.96% (dry matter basis). Individual dry matter intakes were recorded each day before morning feeding. Once a week animals were weighed and the daily rations were consequently modulated in order to achieve comparable growth rates between C and T groups.

2.2. Slaughter procedures and muscle sampling

After a 60-day experimental period, lambs were transported to a commercial abattoir where they had access to the experimental feeds until approximately 15 min before slaughter. Within 20 min of slaughtering, the left *longissimus dorsi* muscle (LM) was excised from the 6th to the 13th rib. One portion was immediately vacuum-packaged and stored at 4 °C for 24 h for subsequent ultimate pH measurement and for measurement of colour stability and metmyoglobin development of fresh LM steaks over aerobic storage. The remaining portion was immediately frozen in liquid nitrogen for 90 s, then wrapped in aluminium foil, vacuum-packaged and stored at −30 °C until required for measurement of muscle antioxidant status and myoglobin resistance to induced oxidation.

2.3. Colour stability and metmyoglobin formation measurement of fresh longissimus dorsi muscle

After 24 h of anaerobic storage at 4 °C, fresh LM were removed from the bags and muscle ultimate pH was measured using a pH-meter (Orion 9106, Orion Research Incorporated, Boston, MA) equipped with a penetrating electrode. Muscle slices (thickness: 3 cm) were placed on polystyrene trays, over-wrapped with a oxygen-permeable PVC film and stored in the dark at 4 °C. Hue angle (H^*) values and the reflectance (R) spectra were measured at the meat surface after 2 h of blooming and, then, after 24, 72, 96 and 168 h of refrigerated storage, using a Minolta CM-2022 spectrophotometer ($d/8°$ geometry; Minolta Co. Ltd. Osaka, Japan). Diffuse reflection was measured in SCE (Specular Component Excluded) mode, using illuminant A and 10° standard observer. For each sample, average values were calculated from triplicate readings made on non-overlapping zones of the steak. Metmyoglobin (MMb) percentages were estimated following the method of Krzywicki (1979). Furthermore, MMb accumulation at the meat surface, across the 7-day storage period, was followed by calculating the $(K/S)_{572} \div (K/S)_{525}$ ratio (Stewart, Zipser, & Watts 1965), where:

$$(K/S)_\lambda = (1 - R_\lambda)^2/2R_\lambda$$

2.4. Antioxidant status of longissimus dorsi muscle

The muscle overall antioxidant status was measured by the determination of its total phenolic compounds concentration, of its ferric reducing antioxidant power (FRAP assay) and of its radical scavenging ability (TEAC assay). All measurements were performed on the portion of the LM previously frozen in liquid nitrogen and stored, vacuum-packaged, at −30 °C.

2.4.1. Preparation of muscle extract for antioxidant status measurements

Aqueous muscle extracts were prepared according to the procedure described by Estévez, Ventanas, and Cava (2007) for the determination of the concentration of phenolics in meat with some modifications. Muscles were chopped into small cubes and, while still frozen, were minced using a blender. For each muscle, triplicate sub-samples (500 mg) of minced meat were placed into 50 ml centrifuge tubes and 10 ml of distiled water was added. Samples were then homogenised for 60 s at 9500 rpm using a Heidolph Diax 900 tissue homogenizer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) and the centrifuge tubes were constantly kept in a water/ice bath during the homogenisation. Samples were then subjected to sonication (cycle: 4 × 10%; power %: 0.31) for a total duration of the sonication of 6 min (with a break of 2 min after the first 3 min of sonication) using a Bandelin Sonoplus HD2070 sonicator (Bandelin Electronic GmbH & Co. KG, Berlin, Germany). The tubes were kept in a water/ice bath during the sonication. The sonicated homogenates were centrifuged at 3000g for 15 min at 4 °C (Thermo, model IEC CL31R; Thermo Fisher Scientific, Milan, Italy). Then, the supernatant was filtered through Whatman 541 filter paper.

2.4.2. Total phenolic compounds concentration in muscle

The concentration of total phenolics in LM was measured using the Folin–Ciocalteau reagent as described by Makkar, Blümmel, Borowy, and Becker (1993). Aliquots (500 μl) of the muscle extract were transferred into 15 ml centrifuge tubes and 500 μl of distiled