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Carnosic acid dietary supplementation at 0.12% rates slows down meat discoloration in *gluteus medius* of fattening lambs

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ABSTRACT

Thirty-two Merino lambs fed barley straw and a concentrate alone (CONTROL) or enriched with vitamin E (VITE006) or carnosic acid (CARN006; CARN012) were used to assess the effect of these antioxidant compounds on meat quality attributes. The animals were slaughtered after being fed for at least 5 weeks with the experimental diets. The *longissimus lumborum* samples of VITE006, CARN006 and CARN012 groups showed higher values ($P<0.001$) of L^* (lightness) through the complete storage period under modified atmosphere when compared to the CONTROL group. Moreover, the VITE006 and CARN012 samples revealed lower discoloration when compared to the CONTROL group, these differences being more apparent in a less color stable muscle such as *gluteus medius* ($P<0.05$ for hue after 14 days of refrigerated storage). Meat sensory traits were not significantly affected by carnosic acid and microbiological analyses were not conclusive at the doses administered.

1. Introduction

The shelf life of meat and meat products is seriously shortened by two main factors: microbial spoilage and color stability, the last one being closely related to lipid peroxidation (Young & West, 2001). Therefore, any finding focused on delaying either of these processes is highly relevant for the meat industry. However, the high quality meat demanded by the consumer in developed countries (Boleman et al., 1997) must be free of chemically synthesized additives, so the addition of synthetic food stabilizers to meat in order to extend the shelf life of these products in the market does not seem to be the best choice. A suitable alternative is the inclusion of natural compounds (plant origin) in animal feedstuff, thus avoiding any further manipulation of the meat.

In this context, especial attention has been paid to the antimicrobial and antioxidant effects promoted by rosemary (*Rosmarinus officinalis* L.), an herb commonly used as a flavoring agent. The bioactive properties of this herb have been attributed to the phenolic compounds in rosemary plants (Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, & Legarreta, 2009). These compounds have demonstrated antimicrobial and antioxidant activities when added to food as additives (McBride, Hogan, & Kerry, 2007), but they have also shown beneficial effects on eggs, milk and meat products when rosemary is included in the diet of the animal (Botsoglou, Govaris, Giannenas, Botsoglou, & Papageorgiou, 2007; Galobart, Barroeta, Baucells, Codony, & Ternes, 2001; Jordán, Moñino, Martínez, Lafuente, & Sotomayor, 2010; Nieto, Díaz, Bañón, & Garrido, 2010). In this last sense, the main phenolic compound retained in animal tissues after the consumption of rosemary is carnosic acid (Moñino, Martínez, Sotomayor, Lafuente, & Jordán, 2008), so it can be hypothesized that the antimicrobial and antioxidant properties observed in meat quality are mainly due to the increment of this phenolic compound at this level. However, the amount of carnosic acid which must be fed to the animals to have beneficial effects on meat quality has not yet been quantified. It must also be considered that the concentration of phenolic compounds in the plants varies depending on the maturity stage or climatic conditions, mainly conditions of drought (Munné-Bosch, Mueller, Schwarz, & Alegre, 2001). This is the reason why feeding rosemary extracts with a known richness of carnosic acid instead of intact plants will allow recommendations to be established about the amount of rosemary which should be fed to the animals according to its levels of carnosic acid.

Therefore, the aim of the present study was to investigate the shelf-life extension of meat (antimicrobial properties and color stabilization) when two different doses of carnosic acid (from rosemary extract) were included in the diet of lambs. Likewise, vitamin E (one of the most frequently used antioxidants in animal nutrition) was included in another group as a positive control.

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2. Material and methods

2.1. Animals and diets

Two weeks before the commencement of the trial, 32 male Merino lambs were treated with Ivermectin (Ivomec, Merial Labs, Barcelona, Spain) and vaccinated against enterotoxaemia (Miloxan, Merial Labs, Barcelona, Spain).

After random stratification on the basis of body weight (average body weight (BW), 15.2 ± 0.749 kg), the lambs were allocated to four different groups: a control group (CONTROL), a group fed vitamin E (α-tocopheryl acetate) at a rate of 0.6 g kg^{-1} of feed concentrate (VITE006, equivalent to 900 IU of vitamin E kg^{-1} of feed concentrate), a third group fed a similar dose as the previous group (0.6 g carnosic acid kg^{-1} of feed concentrate, CARN006) of carnosic acid (Shaanxi Sciphar Biotechnology Co., Ltd, Xi'an, China) and the fourth group fed a double dose of carnosic acid (1.2 g carnosic acid kg^{-1} of feed concentrate, CARN012). The animals were then individually penned. All handling practices followed the recommendations of the European Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes, and all of the animals were able to see and hear other lambs.

After 7 days of adaptation to the basal diet comprised concentrate (50% barley, 20% soybean meal, 15% maize, 8% wheat, 4% molasses and 3% mineral premix; chemical composition: dry matter (DM) 888 g kg^{-1}, crude protein (CP) 178 g kg^{-1} DM, neutral detergent fiber (NDF) 134 g kg^{-1} DM, and ash 56 g kg^{-1} DM) and barley straw (DM 913 g kg^{-1}, CP 35 g kg^{-1} DM, NDF 757 g kg^{-1} DM, ash 55 g kg^{-1} DM), all of the lambs were fed barley straw and the corresponding concentrate feed alone (CONTROL group) or supplemented with either vitamin E or carnosic acid. The concentrate (35 g kg^{-1} BW day^{-1}) and forage (200 g day^{-1}) were weighed and supplied in separate feeding troughs at 9:00 a.m. every day, and fresh drinking water was always available.

2.2. Slaughter procedure, packaging, storage and sampling

The animals were slaughtered on four different days, two lambs per group each day. The lambs were selected each day according to their weight (25 kg intended body weight) and slaughtered by stunning and exsanguination from the jugular vein; they were then eviscerated and skinned. The hot carcass of each lamb was weighed, chilled at 4 °C for 24 h and weighed again. The pH value from the *longissimus thoracis* muscle at the sixth rib was determined in triplicate at 0 h, 45 min and at 24 h post-mortem before the muscle was removed from the carcass, using a pH meter equipped with a penetrating glass electrode (pH meter Metrohm® 704, Switzerland).

The left hind leg was removed and stored at −30 °C until sensory evaluation. Moreover, the *longissimus thoracis* (LT) *et lumborum* (LL) and *gluteus medius* (GM) muscles were removed from the right and left carcass sides. The LT samples were used for chemical analysis in accordance with the methods described by the Association of Official Analytical Chemists (AOAC, 2003), whereas the LL and GM muscles were cut into slices 2.5 cm thick, placed on impermeable polypropylene trays and wrapped with ML40-G bags (Krehalon; Proveedora Hispano Holandesa S.A., Barcelona, Spain), which were immediately modified-atmosphere packaged (MAP) using a tabletop Multivac A300 packaging machine (Multivac Verpackungsmaschinen, Wolfertschwenden, Germany). The air in the bags was replaced by a commercial gas blend intended for red and poultry meats consisting of 35% CO_2, 35% O_2 and 30% N_2, with a gas:meat volume ratio of about 2:5:1. The ML40-G bags had O_2 and CO_2 transmission rates of 20 and 100 ml m^{-2} 24 h^{-1}, respectively, at 23 °C and 80% relative humidity. All packages were stored under simulated retail display conditions [12 h daily fluorescent illumination (34 W) and 3 ± 1 °C] and the air temperature was monitored using a Testo175-T2 data logger (Instrumentos Testo S.A., Cabrils, Barcelona, Spain). The meat in these polypropylene trays was used to study the extract-release volume (ERV, on LT muscle), microbial spoilage (on LT muscle), the rate of discoloration (on LL and GM muscles) and the water holding capacity (WHC, on LL muscle).

2.3. Color, extract release volume (ERV) and water holding capacity (WHC)

On each sampling day, the concentrations of CO_2 and O_2 inside each tray were determined using a CheckMate 9900 (PBI Dansensor, Denmark). After opening the packages, a slice of fresh meat from each muscle was measured for color parameters on days 0, 3, 7, 9 and 14. The L* (lightness), a* (redness) and b* (yellowness) values (CIE, 1986) were used to determine the meat color of the muscles using a chromameter (Minolta® Chroma Meter 2002, Germany). The hue angle (h*), which defines color (0° is red; 90° is yellow), was calculated as arctangent (b*/a*), and the chroma (C*), a measure of color intensity (0 is dull; 60 is vivid), was computed as $\sqrt{(a^{*2} + b^{*2})}$ (Young & West, 2001). Also, the extract release volume (ERV) was measured (on days 0, 7 and 14) as previously described (Rodriguez-Calleja, Santos, Otero, & García-López, 2004). Briefly, minced lamb (15 g) was mixed with 60 ml of the extraction reagent (0.2 M KH_2PO_4 and 0.2 M NaOH; pH 5.8) and homogenized for 2 min. The homogenate was filtered through Whatman no. 1 paper and the ERV was recorded as the volume collected in 15 min.

The water holding capacity (WHC) was measured on LL muscle via cooking losses, according to Honikel (1998).

2.4. Microbiological analysis

Twenty-five grams of LL muscle from each tray (0, 7 and 14 days) was placed into sterile Stomacher bags, rinsed with peptone water (1:5 dilution) and the rinsate was then diluted tenfold. The numbers of total viable bacteria at 4.5 °C (TVB), *Pseudomonas* spp., lactic acid bacteria (LAB) and *Enterobacteriaceae* (EC) were determined and confirmed as described elsewhere (Rodríguez-Calleja, García-López, Santos, & Otero, 2005; Rodriguez-Calleja et al., 2004). Briefly, TVB were determined by the pour plate technique on Plate Count Agar (PCA; Oxoid, Basingstoke, UK) incubated at 4.5 °C for 14 days. *Pseudomonas* spp. numbers were determined after 2 days of incubation at 25 °C on a *Pseudomonas* agar base (Oxoid) to which a CFC (cetrimide, fucidin, cephaloridine; Oxoid) supplement was added. The oxidase test (Oxidase Touch sticks, Oxoid) was performed on randomly selected colonies and only oxidase-positive colonies were counted as *Pseudomonas* spp. The LAB colonies were enumerated on overlaid plates of MRS (de Man, Rogosa and Sharpe; Oxoid) agar following 3 days of incubation at 30 °C. *Brochothrix thermosphacta* was enumerated on streptomycin sulfate cycloheximide thallous acetate agar (STAA, Oxoid) after incubation for 2 days at 25 °C. Overlaid plates of Violet Red Bile Glucose agar (VRBGA, Oxoid) were used for EC counts after 24 h incubation at 37 °C.

2.5. Sensory evaluation

The muscle *vastus intermedius* (VI) of the left hind leg was chosen for sensory evaluation because it is considered a high value retail cut—lamb leg roast, chump (Johansen, Aastveit, Egelandsdal, Kvaal, & Røe, 2006). Sensory analysis was carried out by 24 consumers. The left hind leg was defrosted at 4 °C for 48 h and the VI was dissected and cut into steaks 20 mm thick. The steaks were cooked a pre-warmed clam-shell grill to an internal temperature of 74 °C in the geometric center of the steak (measured by a Digi-Sense thermocouple probe, Cole-Parmer Instrument Company, Illinois, USA), after which all fat and connective tissue were trimmed and the muscle cut into blocks of 2 × 1 × 1 cm. The blocks were wrapped in pre-labeled foil (the blocks from each animal were coded with the same alphabetical letter), placed in a