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# Effect of dietary carnosic acid on meat quality from suckling lambs

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ABSTRACT

In order to elucidate the influence of dietary carnosic acid on the quality of suckling-lamb meat, twenty-four lambs were fed *ad libitum* daily with milk replacer (MR) alone (control group, CTRL), enriched with carnosic acid (CARN, 0.096 g $kg^{-1}$ live weight, LW), or vitamin E (VITE, 0.024 g $kg^{-1}$, LW), the last group being considered as a positive control. Animals were slaughtered at the intended body weight (11–12 kg LW). *Longissimus thoracis* muscles were used to asses proximate composition of meat, whereas different muscles (*longissimus lumborum* and *gluteus medius*) were sliced and kept refrigerated during 0, 7, and 14 days to determine water holding capacity, thiobarbituric acid reactive substances (TBARS), and cholesterol oxidation products (COPs) in cooked meat samples. *Biceps femoris* muscles were used for the analysis of volatile compounds on precooked meat after 1 and 7 days of storage. The results indicate that, at the dose used, carnosic acid dietary supplementation seemed to be less effective than vitamin E reducing lipid oxidation of suckling-lambs meat.



## 1. Introduction

Suckling-lamb meat is a high quality edible product on the basis of its tenderness, juiciness and palatability (Gorraiz et al., 2000). This is the reason why in markets of the European Mediterranean area is common the presence of meat from suckling lambs '*lechales*' with a slaughter age between 25 and 45 days and a carcass weight of up to 7 kg (Sañudo et al., 2007; Bernabéu and Tendero, 2005).

After a few days of colostrums feeding, suckling lambs are fed milk-replacer (MR) in high production sheep dairy farms. These MRs usually include different lipids of vegetable sources in the formulation, so the meat from lambs fed MR has higher levels of $n=6$ fatty acids than that corresponding to the lambs fed ewe's milk (Napolitano et al., 2002; Osorio et al., 2007). It is well known that the level of fatty acid unsaturation and lipid oxidation are closely related factors which, at the end, may promote undesirable color and flavor and a loss of nutritional value of meat (Addis et al., 1989; Gray et al., 1996). Consequently, any finding focused on delaying this process is relevant for the suckling lamb meat industry.

One of the most common methods to reduce the oxidation in meat and meat products is the use of different synthetic antioxidants (Decker and Xu, 1998) such as butylated hydroxytoluene (BHT). This compound is usually included in the MR of suckling lambs as food additive, but also transmitted to the meat of the animals where it exerts

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its antioxidant effect. However, the effect of this compound on the health of the consumer is still controversial. In this sense, the inclusion of natural compounds (plant origin) in animal feedstuff seems to be a suitable approach to reduce lipid oxidation of meat (Brewer, 2011). The antioxidant properties of rosemary, mainly attributed to the phenolic diterpene carnosic acid (Moñino et al., 2008), have been studied on meat when rosemary is included in the diet of lambs (Morán et al., 2012a, 2012b, 2013) or dams (Nieto et al., 2010, 2011). However, to our knowledge, no studies have tested the effect of rosemary or carnosic acid when included in the diet of suckling lambs fed MR.

Therefore, the aim of the present study is to determine if the inclusion of carnosic acid in the diet (MR) of suckling lambs at the daily dose of 0.096 g $kg^{-1}$ of live weight (LW) may reduce the lipid oxidation of the meat. A negative control group (animals with no antioxidants in the MR), and a positive control group (animals whose MR was supplemented with a daily dose of 0.024 g of vitamin E $kg^{-1}$ LW), were used to compare the results obtained.

## 2. Materials and methods

### 2.1. Animals and diets

Twenty-four Assaf lambs were used in this experiment. Just after parturition the lambs were separated from the ewes for being fed hand-milked calostrum of their mothers for 2 days. Afterwards, lambs were fed two more days with milk replacer (MR) (Cordevit, Lemasa, León, Spain) comprised of skim milk powder 50%, milk whey, cocoa vegetal oil, animal fats, whey protein powder and yeasts; dry matter (DM) 960 g $kg^{-1}$, crude protein (CP) 235 g $kg^{-1}$ DM, Ether Extract (EE) 260 g $kg^{-1}$ DM, and ash 70 g $kg^{-1}$ DM. This MR was formulated without BHT.

Then the lambs were stratified on the basis of body weight (average BW, 5.95 ± 0.766 kg), and allocated randomly to one of three different groups ($n = 8$ per dietary treatment); a control group (CTRL), a second group (CARN) fed a daily dose of 0.096 g of a commercial carnosic acid preparation per kg of LW (Shaanxi Sciphar Biotechnology Co., Ltd., Xi'an, China; carnosic acid concentration of 470 g $kg^{-1}$), and a third group (VITE) fed a daily dose of 0.024 g of vitamin E $kg^{-1}$ LW (α-tocopheryl acetate 50%, Industrias de Alimentación Animal, Spain).

All the lambs were fed *ad libitum* with MR using an automatic feeder. In order to provide the antioxidant supplements, every day all the lambs were separated from the automatic lamb feeder at approximately 9:00 a.m. After 2 h each lamb was fed with a feeding bottle containing the corresponding dose of supplement suspended in reconstituted MR (no more than 25 mL of MR). No antioxidants were included in the feeding bottle of the CTRL lambs. All handling practices during the rearing of lambs followed the recommendations of the European Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes.

### 2.2. Slaughter procedure, packaging, storage and sampling

The animals were slaughtered when the lambs reached the intended body weight (11–12 kg LW) 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. During chilling of the carcass, the pH value of *longissimus thoracis* muscle was determined at the sixth rib (right carcass) at 0 h, 45 min and 24 h post-mortem, using a pH meter equipped with a penetrating electrode (pH meter Metrohm® 704, Switzerland). Then, the *longissimus thoracis* (LT) *et lumborum* (LL), *gluteus medius* (GM), and *biceps femoris* (BF) muscles were removed from the right and left carcass sides at 24 h post-mortem.

The LT was immediately frozen at −20 °C and lyophilized in order to determinate the chemical composition. The LL and GM muscles were cut into 2.5-cm thick slices, placed on impermeable polypropylene trays and wrapped with an oxygen-permeable polyvinylchloride film (oxygen permeability of 580 mL $m^{-2}$ $h^{-1}$). The packaged meat was then stored under simulated retail display conditions (12 h daily illumination and 3 ± 1° C). A slice from each muscle was sampled from the trays at 0, 7, and 14 days of storage. Sampled meat slices were used to study the water holding capacity (WHC) *via* cooking losses (LL samples), thiobarbituric acid reactive substances (TBARS; raw GM samples), and cholesterol oxidation products (COPs; cooked GM samples).

Furthermore, BF was cut into three sections perpendicular to the long axis: proximal (1.5 cm long), central (the largest) and distal (1.5 cm) sections. Proximal and distal sections were discarded and the central section was cut into two slices (2 cm thick). Slices were cooked in a double-sided griddle plate (preheated at 220 °C) until a core temperature of 70 °C was reached (AMSA, 1995). After 30 min at 20 °C the slices were packaged in trays similar to those described previously for LL and GM muscle samples, and then used for the analysis of volatile compounds after 1 and 7 days of refrigerated storage (3 ± 1° C in darkness).

### 2.3. Proximate composition and WHC

Proximate composition was determined in fresh LT samples (24 h post-mortem) in accordance with the methods described by the Association of Official Analytical Chemists (AOAC, 2003). Finally, different slices of fresh LL muscle were used to determine WHC *via* cooking losses (CL) after 0, 7, and 14 days of refrigerated storage according to Honikel (1998).

### 2.4. Meat oxidation

TBARS content was determined on fresh GM samples stored during 0, 7, and 14 days in accordance with the methodology previously described in Morán et al. (2012a). The results were expressed as μg of malonaldehyde (MDA) $g^{-1}$ meat.

COPs, also called oxysterols, were determined according to the method proposed by Grau et al. (2001) on cooked GM samples stored for 7 days. Briefly, lipids were extracted from 1 g of cooked and freeze-dried GM samples according to Aldai et al. (2010), using 19-hydroxycholesterol (19-HC) as an internal standard. 10 mL of 1.5 N methanolic KOH was then added and the mixture was kept in an orbital shaker for 20 hours at room temperature under $N_2$ atmosphere and darkness to complete the cold saponification. The unsaponificable material was extracted, purified and cholesterol oxides were derivatized to trimethylsilyl (TMS) ethers according to Guardiola et al. (1995). Finally the following COPs: 7α-hydroxycholesterol (7α-HC), 7β-hydroxycholesterol (7β-HC), 5,6α-epoxycholesterol (α-CE), 5,6β-epoxycholesterol (β-CE), cholestanetriol (CT), 25-hydroxycholesterol (25-HC) and 7-ketocholesterol (7-KC), were analyzed by gas chromatographic (GC) according to Morán et al. (2012a).

Volatile compounds of cooked meat stored under refrigeration during 1 and 7 days were determined by static headspace-gas chromatography coupled to mass spectrometry. Samples were blended in a small food processor. Three grams of sample, 5 ml of water and 0.07 g of NaCl were then placed in 20-ml headspace vials, sealed with magnetic screw caps with silicone/PTFE septa (Agilent Technologies, Santa Clara, CA, USA), and the vials were placed in a headspace tray for volatile analysis. Analysis of volatile compounds was performed using the equipment according to the procedure described by Vieira et al. (2012).

### 2.5. Statistical analysis

COPs data were subjected to one-way analysis of variance with the diet as the only source of variation using the general linear models (GLM) procedure of SAS package (SAS, 1999). WHC, TBARS and volatile compounds were subjected to two-way analysis of variance, with the dietary treatment, day and treatment by day interaction as sources of variation, using the GLM procedure of SAS. The pH was analyzed as a repeated measures design using the MIXED procedure of SAS with treatment and time as fixed effects and individual lamb as the experimental unit. In all the cases least square means were generated and separated using the LSMEANS/PDIFF option of SAS for main or interactive effects, with the level of significance being determined at $P < 0.05$.