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The effect of quercetin dietary supplementation on meat oxidation processes and texture of fattening lambs

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ABSTRACT

Thirty two lambs were fed a total mixed ration (TMR) formulated either with palm oil (CTRL; 34 g palm oil kg^{-1} TMR) or whole flaxseed (+FS, 85 g flaxseed kg^{-1} TMR) alone or enriched with quercetin (+QCT, 34 g palm oil plus 2 g quercetin kg^{-1} TMR; +FS + QCT, 85 g flaxseed plus 2 g quercetin kg^{-1} TMR). Dietary flaxseed did not affect, in a significant manner, the lipid peroxidation of meat samples. Quercetin treatment reduced oxysterol content ($P < 0.05$) after 7 days of refrigerated storage of fresh meat, but did not affect significantly ($P > 0.05$) the level of lipid-derived volatiles in the headspace of the light-exposed stored cooked meat. Sensory evaluation showed flaxseed as being responsible for a negative effect on meat flavour, probably associated with a modification of the fatty acid profile whereas, unexpectedly, quercetin seemed to worsen meat tenderisation.

1. Introduction

The protection of meat against lipid oxidation during storage is indispensable in order to preserve the quality standards and shelf life of the product (Nieto, Díaz, Bañón, & Garrido, 2010). This objective has been approached in several studies by adding directly to the meat metal-chelating agents (Allen & Cornforth, 2010) or synthetic antioxidants such as butylated hydroxytoluene (BHT) (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008), whose possible harmful effects on human health are still controversial. This is the reason why the addition to meat of natural (no synthetic) antioxidants has been proposed (Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007; Sampaio, Saldanha, Soares, & Torres, 2012). Moreover recently several research works have been carried out on studying the effects of natural antioxidants or their sources when included in the diets of the animals (Brewer, 2011). This strategy is especially interesting because if antioxidants are deposited in the meat during the life of the animal the addition of exogenous products would not be required after slaughter. This alternative, perceived by the consumer as a high quality standard (Sebranek & Bacus, 2007), might be especially useful to prevent meat lipid oxidation when diets rich in polyunsaturated fatty acids (PUFAs) are administered to the animals, since these dietary components are prone to undergo oxidation processes.

In this sense, attention has been paid to phenolic compounds, a group of substances present in fruits, vegetables, nuts and seeds which have shown potent antioxidant effect as metal chelators or free-radical scavenging activities (Rice-Evans, Miller, & Paganga, 1997). However, results have been variable when antioxidants are included in the diet of the animals. For example, naringenin (aglycone fraction of naringin) has been demonstrated to accumulate in the liver but not in the muscle, so meat quality attributes have not been modified by this flavonoid when included in the diet of fattening lambs at 0.15% level (Bodas et al., 2012). On the other hand, carnosic acid (the main phenolic compound retained in animal tissues after the consumption of rosemary) has positive effects on meat quality (improved texture, low oxysterol content and low lipid oxidation) when feeding rosemary extract to fattening lambs (Morán, Andrés, Bodas, Prieto, & Giráldez, 2012). Regarding quercetin (another aglycone fraction), another phenolic compound with demonstrated antioxidant, antiviral and anticarcinogenic properties in monogastrics (Nair et al., 2002), there is not much information about the effectiveness of this compound when included in the diet of ruminants. Therefore, the aim of the present study was to investigate the texture and antioxidant properties of meat when flaxseed (rich in PUFAs) and/or quercetin were included in the diet of fattening lambs.

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2. Materials 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 stratification on the basis of body weight (average body weight (BW), 15.5 ± 2.12 kg), the lambs were allocated randomly to 8 different groups housed in different pens of four animals each (2 pens per dietary treatment). All of the groups were fed their corresponding total mixed ration (TMR) as described below: two control groups (CTRL, 4 animals per group; 34 g palm oil kg^{-1} TMR), two groups fed ground whole flaxseed (+FS, 4 animals per group; 85 g flaxseed kg^{-1} of TMR), two groups fed control diet plus quercetin (99%) extracted from *Sophora japonica* L. (Shaanxi Sciphar Biotechnology Co., Ltd., Xi'an, China) (+QCT, 4 animals per group; 34 g palm oil plus 2 g quercetin kg^{-1} TMR), and two groups fed whole ground flaxseed plus quercetin (+FS + QCT, 4 animals per group; 85 g flaxseed plus 2 g quercetin kg^{-1} TMR). The four TMRs were formulated to be isoenergetic and isoproteic. Chemical composition of TMR is shown in Table 1. All handling practises followed the recommendations of the European Council Directive 2010/63/EU for the protection of animals used for scientific purposes and all the animals were able to see and hear other animals.

After 7 days of adaptation to the basal diet, all of the lambs were fed the corresponding TMR alone (CTRL and +FS groups) or supplemented with quercetin (+QCT and +FS + QCT groups) ad libitum during the experimental period (at least 5 weeks for each lamb depending on the time required for each animal to reach the target BW). The TMRs were weighed and supplied ad libitum at 9:00 a.m. everyday, and fresh drinking water was always available. Samples of feed offered and orts (approximately 20% of total offered) were taken daily, pooled to an individual composite sample each week, oven-dried at 55 °C for at least 72 h, ground to pass through a 1-mm screen using a Willey mill (Arthur H. Thomas, Philadelphia, PA), and stored until analyses.

2.2. Slaughter procedure, packaging, and storage of meat samples

The animals were slaughtered on four different days, two lambs per group each day. The lambs were selected each day according to their weight (24.8 ± 1.05 kg) and slaughtered by stunning and exsanguination from the jugular vein; they were then eviscerated and skinned.

The *longissimus thoracis* (LT) *et lumborum* (LL), *gluteus medius* (GM), *biceps femoris* (BF), and *adductor magnus* (AM) muscles were removed from the right and left carcass sides. The AM muscle of both sides was frozen at −30 °C for sensory analysis. The LT samples of both sides were used for chemical analysis (Andrés et al., 2013). LL and GM muscles of both sides were cut into 2.5 cm-thick slices, placed on impermeable polypropylene trays, over-wrapped with an oxygen-permeable polyvinylchloride film (580 ml m^{-2} h^{-1}) and then stored under simulated retail display conditions [12 h daily fluorescent illumination (34 W) and 3 ± 1 °C] during 0, 7, and 14 days. Then, the samples were used either for texture and water holding capacity procedures (LL) or cholesterol oxidation analysis (GM).

Finally, BF muscles from the right or left side, at random, were vacuum packaged, frozen and stored at −50 °C for up to 2 months prior to cooking and subsequent analysis of iron-induced TBARS and volatile compounds.

2.3. Texture profile analysis (TPA) and water holding capacity (WHC)

The slices of LL after 0, 7, and 14 days under refrigerated storage condition were weighed and cooked in a double-sided griddle (preheated at 220 °C) until a core temperature of 75 °C was reached, following the guidelines for cooking procedures of AMSA (1995). After cooling at 4 °C for 30 min the samples (LL) were weighed again and frozen at −30 °C until texture profile analysis (TPA) according to the procedure described by Herrero et al. (2008) with slight modifications: meat specimens were cubic (10 mm^3) and the compression percentage of the initial height was 80%, with the compression axis perpendicular to the muscle fibre direction. The water holding capacity (WHC) was measured on LL samples via cooking loss, according to Honikel (1998).

2.4. Cholesterol oxidation

GM samples after 7 days of refrigerated storage were weighed and cooked as previously described for LL slices. Then, they were cooled at 4 °C for 30 min, weighed again, and freeze-dried for oxysterol analysis. Cholesterol oxidation products (COPs), also called oxysterols, were determined according to the method proposed by Grau, Codony, Grimpa, Baucells, and Guardiola (2001). Briefly, lipids were extracted from 1 g of cooked and freeze-dried GM samples using a mixture of chloroform/methanol (2:1, v/v) (Folch, Lees, & Stanley, 1957). 19-Hydroxycholesterol (19-HC) was used as an internal standard. Ten millilitres of 1.5 M methanolic KOH was then added and the mixture was kept in an orbital shaker for 20 h at room temperature under N_2 atmosphere and darkness to complete the cold saponification. The unsaponificable matter was extracted three times with diethyl ether in a separating funnel, and then purified by solid-phase extraction (SPE) according to the procedure described by Guardiola, Codony, Rafecas, and Boatella (1995). COPs were derivatised to trimethylsilyl (TMS) ethers prior to gas chromatographic (GC) analysis on a HP 6890 Series gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) provided with a mass selective detector (HP 5973), by splitless injection (HP 7683 Series injector) into a VF-5 ms CP8947 capillary column (50 m × 250 μm × 0.25 μm, Varian, Palo Alto, CA, USA). Chromatographic conditions were as follows: injection volume 1.0 μl; initial oven temperature 60 °C, to 230 °C at 15 °C min^{-1}, to 290 °C at 10 °C min^{-1}, and to 292 °C at 0.05 °C min^{-1}; and injector and transfer-line temperatures were 250 and 290 °C, respectively. Helium was used as a carrier gas at a flow rate of 0.5 ml min^{-1}. The mass spectrometer operated in electron impact mode with electron energy of 69.9 eV, an emission current of 34.6 μA, a source and quadruple temperatures of 230 °C and 180 °C, respectively, and scanned from m/z 40 to m/z 400. The oxysterols 7α-hydroxycholesterol (7α-HC), 7β-hydroxycholesterol (7β-HC), 5,6α-epoxycholesterol (α-CE), 5,6β-

Table 1
Ingredients (g kg^{-1}) and chemical composition (g kg^{-1} dry matter) of the experimental total mixed rations.

	CTR[a]	+FS[b]	+QCT[c]	+FS + QCT[d]
Barley	417	421	417	421
Soybean meal	187	149	187	149
Corn	170	170	170	170
Barley straw	149	149	149	149
Flaxseed	0	85	0	85
Palm oil	34	0	34	0
Soybean hulls	17	0	17	0
Min–vit. premix	26	26	26	26
Quercetin	0	0	2	2
Dry matter	920	926	921	926
Crude protein	162	162	158	165
Neutral detergent fibre	263	240	264	238
Acid detergent fibre	128	116	129	111
Ash	85	60	84	63

[a] Control group (no antioxidants, 34 g palm oil kg^{-1} TMR).
[b] Flaxseed group (85 g flaxseed kg^{-1} TMR).
[c] Quercetin group (34 g palm oil plus 2 g quercetin kg^{-1} TMR).
[d] Flaxseed plus quercetin group (85 g flaxseed plus 2 g quercetin kg^{-1} TMR).