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Quercetin dietary supplementation of fattening lambs at 0.2% rate reduces discolouration and microbial growth in meat during refrigerated storage

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ABSTRACT

Thirty-two Merino lambs fed barley straw and a concentrate formulated either with palm oil (CTRL group) plus quercetin (QCT group) or flaxseed (FS group) plus quercetin (FS-QCT group) were used to assess the effects of this flavonoid on meat quality attributes. The animals were slaughtered after being fed for at least 5 weeks with the experimental diets. Chemical composition of *longissimus thoracis* (LT) muscle was not different among treatments. The *longissimus lumborum* (LL) samples of QCT and FS-QCT groups revealed lower discolouration (hue angle) when compared to the CTRL and FS lambs ($P<0.05$), whereas extract release volume (ERV) and microbiological data jointly suggest that flaxseed and quercetin may reduce the growth of microbial populations responsible for meat spoilage in quadriceps femoris (QF).

1. Introduction

Protective lipid oxidation during storage of meat is indispensable in order to preserve the quality standards and the shelf life of this product (Nieto, Díaz, Bañón, & Garrido, 2010). This objective has been approached in several studies by adding nitrite directly to the meat (Krause, Sebranek, Rust, & Mendonca, 2011), metal-chelating agents (Allen & Cornforth, 2010) or synthetic antioxidants such as BHT (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008), whose possible harmful effects on human health are still controversial. That is the reason why increasing numbers of studies have examined dietary additions of natural (no synthetic) antioxidants (Bodas et al., 2012; Morán, Andrés, Bodas, Prieto, & Giráldez, 2012; Morán, Rodríguez-Calleja, et al., 2012; Nieto et al., 2010). This strategy is especially interesting because if antioxidants are deposited in the meat during the life of the animal no addition of exogenous products would 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 peroxidation when diets rich in polyunsaturated fatty acids (PUFAs) are administered to the animals.

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 effects as metal chelators or free-radical scavenging activities (McBride, Hogan, & Kerry, 2007). Most of these compounds also have shown antimicrobial properties when added directly to the meat as additives (McBride et al., 2007). However, results have been different when included in the diet of the animals. For example, naringenin (aglycone fraction of naringin) has demonstrated to be accumulated 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% rates (Bodas et al., 2012). On the other hand, carnosic acid (the main phenolic compound retained in animal tissues after the consumption of rosemary) has demonstrated positive effects in meat quality (colour, antioxidant status) when feeding rosemary extract to fattening lambs (Moñino, Martínez, Sotomayor, Lafuente, & Jordán, 2008; Morán, Andrés, et al., 2012; Morán, Rodríguez-Calleja, et al., 2012). Regarding quercetin, another phenolic compound with demonstrated antioxidant properties, to our knowledge, no studies have evaluated the effects of including this flavonol in the diet of fattening lambs.

Therefore, the aim of the present study was to investigate the shelf-life extension of meat (antimicrobial properties and colour stabilization) when flaxseed (rich in PUFAs) and/or quercetin were included in the diet of fattening lambs.

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).

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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 (each housed in a different batch, 2 batches per dietary treatment). All of the groups were fed their corresponding total mixed ration (TMR) as described below: two control groups (CTRL, 34 g palm oil kg^{-1} of TMR), two groups fed flaxseed (FS) at a rate of 85 g kg^{-1} of TMR, two groups fed quercetin 99% extract from *Sophora japonica* L. (Shaanxi Sciphar Biotechnology Co., Ltd., Xi'an, China) at a rate of 2 g kg^{-1} TMR (QCT), and two groups (FS-QCT) fed flaxseed plus quercetin (85 and 2 g kg^{-1} of TMR, respectively). The four TMRs were formulated to be isoenergetic and isoproteic. Ingredients and chemical composition of TMR are shown in Table 1. 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 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 depending on the target BW of the animals). The TMR was weighed and supplied *ad libitum* at 9:00 a.m. every day, 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, 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, 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 (24.8 ± 1.05 kg) 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 *longissimus thoracis* (LT) *et lumborum* (LL), and *quadriceps femoris* (QF) muscles were removed from the right and left carcass sides. The LT samples of both sides were used for chemical analysis in accordance with the methods described by the Association of Official Analytical Chemists (AOAC, 2003), whereas the LL muscle of both sides was cut into slices 2.5 cm thick, placed on impermeable polypropylene trays, over-wrapped with an oxygen-permeable polyvinylchloride film and then stored under simulated retail display conditions [12 h daily fluorescent illumination (34 W) and 3 ± 1 °C]. The meat in these polypropylene trays was used to study the rate of discolouration (on LL muscle) during 14 days of refrigerated storage. The QF muscle of both sides was cut into bigger portions (75 g approximately each portion), packed and wrapped (equally to the LL muscle); then QF was used to measure the extract-release volume (ERV), and microbial spoilage after 0, 3 and 7 days of refrigerated storage.

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

	CTRL	FS	QCT	FS-QCT
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

CTRL: control group (no antioxidants, 34 g palm oil kg^{-1} of TMR); FS (85 g flaxseed kg^{-1} of TMR); QCT (CTRL plus 2 g quercetin kg^{-1} of TMR); FS-QCT (85 g flaxseed plus 2 g quercetin kg^{-1} of TMR).

2.3. Physicochemical analysis

The same slice of LL for each animal was unpackaged and measured for colour parameters on days 0, 1, 3, 7, 9 and 14. The L* (lightness), a* (redness) and b* (yellowness) values (Centre Internationale de l'Eclairage, 1986) were used to determine the meat colour of the muscles using a chromameter (Minolta® Chroma Meter 2002, Germany). The aperture diameter was 8 mm and illuminant D65 and 10° standard observer were used. The colorimeter was previously calibrated with a pure white colour tile. The hue angle (h*), which defines colour (0° is red; 90° is yellow), was calculated as arctangent (b*/a*), and the chroma (C*), a measure of colour 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, 3, and 7) in QF muscle (Rodríguez-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.

2.4. Microbiological analysis

Twenty-five grammes of QF muscle from each tray (0, 3, and 7 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., mould and yeast (MY) and *Enterobacteriaceae* (EC) were determined and confirmed as described elsewhere (Rodríguez-Calleja, García-López, Santos, & Otero, 2005; Rodríguez-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 7 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. MY colonies were determined after 5 days of incubation 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. Statistical analysis

Data of carcass characteristics, pH values, chemical composition, and ERV were subjected to a two way analysis of variance, using the GLM procedure of SAS (SAS Inst. Inc.) according to the following model:

$$y_{ijk} = \mu + FS_i + QCT_j + (FS * QCT)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the dependent variable, μ is the overall mean, FS is the effect of flaxseed addition, QCT is the effect of quercetin addition, FS*QCT is the effect of the interaction between quercetin and flaxseed, and ε_{ijk} is the residual error.

Microbiological counts were transformed and expressed as log CFU g^{-1} (CFU, colony-forming units) before calculating the descriptive statistics. These data were analysed as a repeated measures