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

S. Andrés a*, L. Huerga b, J. Mateo b, M. L. Tejido a, R. Bodas c, L. Morán a, N. Prieto a, L. Rotolo d, F. J. Giráldez a

a Instituto de Ganadería de Montaña (CSIC-Universidad de León). Finca Marzanas. E-24346 Grulleros, León, Spain
b Instituto de Ciencia y Tecnología de los Alimentos, Universidad de León. La Serna 56, E-24071 León, Spain
c Instituto Tecnológico Agrario, Junta de Castilla y León. Finca Zamadueñas. Ctra. Burgos, km. 119. E-47071 Valladolid, Spain
d Dipartimento di Scienze Zootecniche, Università degli Studi di Torino. Via Leonardo da Vinci, 44 Grugliasco E-10095 Torino, Italy

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CORRESPONDING AUTHOR: Sonia Andrés, Instituto de Ganadería de Montaña (CSIC-ULE). Finca Marzanas. E-24346 Grulleros, León, Spain. Tel. +34 987 307 054 Fax +34 987 317 161 E-mail: sonia.andres@eae.csic.es
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 oxysterols 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.

Keywords: quercetin; flaxseed; volatiles; oxysterols; TBARS; meat

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, Diaz, Bañon, & 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, Vaithiyathan, 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, 2001). 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, Prieto, Jordán, López-Campos, Giráldez, Morán, L., & Andrés, 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 oxysterols 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, Kandaswami, Mahajan, Chadha, Chawda, Nair, Kumar, Nair, & Schwartz, 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.

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. every day, and fresh drinking water was always available. Samples of feed offered and or ts (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 lumbarum (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, Tejido, Bodas, Morán, Prieto, Blanco, & Giráldez, 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 and frozen and stored at -50 °C for up to 2 months prior 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 ºC for 30 min the samples (LL) were weighed again and frozen at -30 ºC until texture profile analysis (TPA) according to the procedure described by Herrero, de la Hoz, Ordoñez, Herranz, Romero de Ávila, & Cambero (2008) with slight modifications: meat specimens were cubic (10 mm³) 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 ºC for 30 min, weighed again, and freeze-dried for oxysterols analysis. Cholesterol oxidation products (COPs), also called oxysterols, were determined according to the method proposed by Grau, Codony, Grimpa, Baucells, & Guardiola (2001). Briefly, lipids were extracted from 1 g of cooked and freeze-dried GM samples using a mixture chloroform/methanol (2:1, v/v) (Folch, Lees, & Sloane Stanley, 1957). 19-Hydroxycholesterol (19-HC) was used as an internal standard. Ten millilitres of 1.5 M methanolic KOH were then added and the mixture was kept in an orbital shaker for 20 h at room temperature under N₂ atmosphere.
and darkness to complete the cold saponification. The unsaponifiable 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, & 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-5ms 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 ºC, to 230 ºC at 15 ºC min⁻¹, to 290 ºC at 10 ºC min⁻¹, and to 292 ºC at 0.05 ºC min⁻¹; injector and transfer-line temperatures were 250 and 290 ºC, 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 quadruples temperatures of 230 and 180, 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-epoxycholesterol (β-CE), cholestanetriol (CT), 25-hydroxycholesterol (25-HC), and 7-ketocholesterol (7-KC) were identified by comparing their retention times and spectra with those of authentic standards (Steraloids, Inc., Wilton, New Hampshire, UK) and quantified using the internal standard.

2.5. Iron-induced TBARS and volatile compounds in stored cooked meat

After thawing the BF muscles at 10 ºC for approximately 12 h into the packaging, the muscles were cut into three sections perpendicular to the long axis: proximal (1.5 cm), central (the largest) and distal (1.5 cm) sections. The proximal and distal sections were discharged and two slices (2.5-cm thick) were cut from the central section. Each
slice was cooked as previously described and a slice was immediately used for analysis. The remaining slice was placed in impermeable polypropylene trays, covered by an oxygen-permeable polyvinylchloride film and stored at 5 °C for 3 days, while exposed to a light source (12 h per day) provided with a pair of twin linear fluorescent tubes (34 W).

The in-vitro iron-induced lipid oxidation was measured in duplicate on the BF slice sampled immediately after the cooking procedure. The procedure described by Mercier, Gatellier, & Renerre (2004) was followed for sample preparation and incubation of sample solutions, with incubation times of 0 (immediate measurement without incubation), 1, 4, and 6 hours and incubation temperature of 37 °C. Afterwards, the thiobarbituric acid reactive substances (TBARS) assay with 1 ml of incubated sample solution was carried out according to the method proposed by Nam & Ahn (2003).

The status of lipid oxidation of cooked meat samples (BF) after the refrigerated storage period was evaluated by determining volatile compounds in the headspace of meat. In order to set the initial point (day 1 of storage), an aliquot of the muscle slice sampled after cooking was immediately used for analysis. Thereafter, volatiles in the headspace after 48 hours of refrigerated storage of cooked meat (day 3), under the abovementioned refrigeration conditions, also were measured. Volatile analyses were performed in duplicate following the procedure of Vieira, Fernández-Diez, Mateo, Bodas, Soto, & Manso (2012). Quantification was based on the comparison of peak areas of the volatile compounds to the areas obtained from known amounts of undecane previously dissolved in hexane and analysed under the same conditions as described for the volatile compounds of the samples.

2.6. Sensory evaluation
The muscle *adductor magnus* (AM) of both hind legs was chosen for sensory evaluation. Sensory analysis was carried out by 24 consumers in only one session. The muscles were defrosted at 4 ºC for 48 h and dissected and cut into steaks 20 mm thick. The steaks were cooked a pre-warmed clam-shell grill to an internal temperature of 75º C in the geometric centre of the steak (measured by a Digi-Sense thermocouple probe, Cole-Parmer Instrument Company, Vernon Hills, IL, USA), after which all fat and connective tissue was trimmed and the muscle cut into blocks of 2×1×1 cm. The blocks were wrapped in pre-labelled foil (the blocks from each animal were coded with the same alphabetical letter), placed in a heated incubator and then given to the assessors in a random order chosen by a random number generator. All consumers participated in two blind preference tests in which they received two meat samples on a coded paper plate. Each consumer tasted and evaluated their preferences for the meat samples from lambs fed (1) CTRL vs +FS, and (2) CTRL vs +QCT diets, and provided the reasons for their choices (ISO 5495:2005).

### 2.7. Statistical analysis

Data of cooking loss, texture, COPs content, iron-induced TBARS values and volatile compound levels were subjected to a two-way analysis of variance, using the MIXED procedure of SAS (SAS, 1999) according to the following model:

\[
y_{ijk} = \mu + F_{Si} + Q_{CTj} + (FS\times QCT)_{ij} + B_k + S_l + (FS\times QCT\times B)_{ijk} + \epsilon_{ijklm}
\]

where \(y_{ijk}\) is the dependent variable, \(\mu\) is the overall mean, \(FS\) is the effect of flaxseed addition, \(QCT\) is the effect of quercetin addition, \(FS\times QCT\) is the effect of the interaction between quercetin and flaxseed, \(S\) is the effect of slaughter day (block), \(B\) is the effect of batch (block), \(B\times FS\times QCT\) the interaction between treatments and batch, which was used as experimental error to test the effects of treatments, and \(\epsilon_{ijklm}\) is the
residual error. Least square means were generated and separated using the PDIF option of SAS for main or interactive effects, with the level of significance being determined at $P < 0.05$. Data from the sensorial analysis were assessed according to ISO 5495:2005 and also analyzed using the chi-square test (Stone & Sidel, 1993) of the FREQ procedure in SAS (SAS, 1999).

3. Results and discussion

3.1. Water holding capacity (WHC) and texture profile analysis (TPA)

Table 2 summarises the cooking loss and texture profile analysis (TPA) of lamb meat samples (LL) during the refrigerated storage period. As can be observed, a lack of significant differences in the LL samples when cooking losses were measured after 0, 7, and 14 days of refrigerated storage was observed (Table 2). Regarding TPA, trends towards significantly greater values for hardness were detected in the LL meat samples from lambs being fed quercetin (0 days of refrigerated storage) and flaxseed (7 days of refrigerated storage), respectively. However, neither statistical differences nor a trend toward significantly different values was observed for chewiness, which is a property calculated from hardness, elasticity and cohesiveness (Table 2).

3.2. Cholesterol oxidation

Regarding oxysterol content in meat samples, these compounds can be absorbed through the intestinal tract into the blood stream, thus increasing the susceptibility of the consumer to coronary heart disease (Valenzuela, Sanhueza, & Nieto, 2003). Since the main source of oxysterols in meat is heat processing, these substances were determined in cooked meat samples (cooked GM after 7 days of refrigerated storage of raw muscles, previously used to measure the WHC by cooking loss). Table 3 presents the
corresponding results of oxysterols content for each group. As can be observed, dietary flaxseed did not affect significantly the oxysterols content of meat samples, whereas three of these compounds (7α-HC, 7β-HC, and 7-KC) were significantly reduced in the meat of the lambs being fed quercetin (+QCT and +FS+QCT) when compared to the CTRL and +FS groups (Table 3).

The effectiveness of quercetin in reducing oxysterols content in cooked meat samples found in the present study is in agreement with the results previously described by other authors for other natural antioxidants such as vitamin E supplemented either to pigs (Eder, Müller, Kluge, Hirche, & Brandsch, 2005) or chickens (Grau et al., 2001) or carnosic acid supplemented to lambs (Morán et al., 2012).

It has been suggested that hydroperoxides of polyunsaturated fatty acids formed during lipid oxidation might be necessary to initiate cholesterol oxidation, so oxysterols content might be synergistically increased by unsaturated fat (Smith, 1987). In this regard, in the present study total oxysterol content (Table 3) was greater when compared to the meat from lambs being fed diets with no fats (∑COPs ranging from 1.428 to 3.022 µg g⁻¹ cooked meat; Morán et al., 2012). Moreover, oxysterol levels (Table 3) were lower than those reported in pork from animals fed unsaturated fats (linseed and sunflower oils added to the diet at 2% rates), where meat was cooked after being stored at 4 °C for 9 days (∑COPs ranging from 6.07 to 12.39 µg g⁻¹ cooked meat; Rey, Kerry, Lynch, López-Bote, Buckley, & Morrissey, 2001). Also, all lamb meat samples (even those of the CTRL group) showed very low levels of CT and 25-HC, which have been described as atherogenic oxysterols responsible for acute injury to the endothelium (Taylor, Peng, Werthessen, Tham, & Lee, 1979; Peng, Taylor, Hill, & Morin, 1985). These important differences in the oxysterol content of meat when lambs and pigs are
fed unsaturated fats might be explained by the particularity of the ruminant gut, since
the biohydrogenation process at rumen level undergone by the unsaturated fatty acids
consumed by the lambs might have protected meat against cholesterol oxidation during
refrigerated storage and later on during the cooking procedure. This fact might also
explain the lack of significant differences observed in the present study between the
groups being fed palm oil (CTRL and +QCT lambs) and flaxseed (+FS and +FS+QCT
lambs).

3.3. Iron-induced TBARS and volatile compounds in stored cooked meat

Results on lipid oxidation of cooked meat generated via Fenton reaction (Fe^{2+}/H_{2}O_{2})
and quantified by the TBARS assay are shown in Table 4. This assay (iron-induced
TBARS) has been considered as suitable to assess the animals’ diet influence on meat
oxidation processes (Mercier et al., 2004). In the present study, meat from animals fed
quercetin supplemented diets (+QCT and +FS+QCT groups) showed lower iron-
induced TBARS values after 4 hours of incubation (QCT, \( P = 0.028 \)), than meat from
animals fed without this flavonoid (CTRL and +FS groups). This means that quercetin
increased the lipid resistance to iron-induced oxidation. The effect was not significant
after 6 h of incubation, probably as a consequence of the high intra-group variability
observed in this parameter. Regarding dietary flaxseed this component did not affect
significantly the lipid peroxidation of meat samples, probably due to the presence of
antioxidants such as vitamin E in the oil of the seeds or as a consequence of the
biohydrogenation process at rumen level undergone by the unsaturated fatty acids
consumed by the lambs.

[INSERT TABLE 4 NEAR HERE, PLEASE]
A total of 24 volatile compounds were detected in the headspace of the cooked meat samples, with 21 of them being identified, and thus assigned to the following chemical families (the number of compounds is shown between brackets): straight-chain aliphatic hydrocarbons (3), aldehydes (5), ketones (1) and alcohols (3), and terpenic (3), benzenic (5) and furanic (1) compounds. Among the identified compounds the presumably lipid-derived compounds were hexane, heptane, octane, pentanal, hexanal, heptanal, octanal, nonanal, 2,3-octanodione, 2-octen-1-ol and 2-pentyl-furan (Frankel, 1982; Mottram, 1998). Among them, only the straight-chain aliphatic aldehydes are shown in Table 5 as the predominant group. The relevance of the aliphatic aldehydes in headspace of the lamb meat studied (approximately 50 percent of the total volatile compounds; not shown in Table 5) was in agreement with that found in other studies (Vasta & Priolo, 2012; Vieira et al., 2012). The presence of aldehydes in ruminant meat headspace is mainly attributed to lipid oxidation/degradation taking place during cooking and storage of cooked meat (Shahidi & Pegg, 1994a,b; Sivadier, Ratel, Bouvier, & Engel, 2008).

At day 1 (just after cooking the meat samples), meat from the lambs being fed flaxseed (+FS and +FS+QCT groups) showed a trend towards significantly lower values of pentanal, hexanal, and octanal ($P < 0.1$) when compared to the meat from the lambs being fed no flaxseed (CTRL and +QCT diets). Those aldehydes are typically derived from the two main unsaturated fatty acids in meat, i.e. oleic acid, 18:1, and linoleic acid, 18:2n-6 (Elmore, Campo, Enser, & Wood, 2002; Frankel, 1982; Zanardi, Novelli, Nanni, Ghiretti, Delbono, Campanini, Dazzi, Madarena, & Chizzolini, 1998). As observed in this study, it has already been proven that diet can affect the levels of lipid-derived volatiles of cooked meat (Elmore, Mottram, Enser, & Wood, 2000; Vasta & Priolo, 2006; Vasta, Ventura, Luciano, Andronico, Pagano, Scerra, Biondi, Avondo, &
The differences found in the present study might be attributed to diet-related changes in fatty acid and natural antioxidant contents and/or to compositional or structural changes in meat affecting the formation of lipid-derived volatiles during cooking and their release to headspace.

In meat exposed to oxidation during storage (at day 3 of storage), the levels of aldehydes were dramatically increased with respect to day 1 (Table 5). In this sense, strong increases in lipid-derived compounds, resulting in oxidized flavours, have been reported in stored cooked meat after hours of cooking (Kingston, Monahan, Buckley, & Lynch, 1998). However, in the present study no significant effects ($P > 0.05$) of dietary treatment on the levels of straight-chain aliphatic aldehydes (Table 5), or the rest of lipid-derived compounds (data not shown in tables) were found at day 3 of storage. Thus, considering hexanal (or the group of aldehydes) in meat headspace as oxidation index (Shahidi & Pegg, 1994a,b), neither dietary flaxseed exerted a negative effect, nor quercetin a positive effect on oxidation status of the light-exposed stored cooked meat.

The effect of the addition of quercetin or flaxseed to diet on lipid oxidation stability of cooked meat differs between the two analysis carried out: a significant effect on iron-induced TBARS values of recently cooked meat (Table 3), and no significant effect on hexanal accumulation at day 3 of light-exposed refrigerated storage of cooked meat. A reason for this discrepancy could be attributed to methodological differences between both procedures that can modify the lipid oxidation process, i.e., catalysis pathway, temperature, time. One other possible reason might be an eventual time-related degradation of quercetin in stored cooked meat.

3.4. Sensory evaluation
When meat from the lambs fed the CTRL and +FS diets were compared, 83.3% of the consumers preferred the CTRL samples describing them as less strong flavoured, being this percentage statistically different from the percentage of consumers who preferred the +FS samples \( (P = 0.001, \text{Table 6}) \). The use of flaxseed in lambs’ diet has been related to high \( \alpha \)-linolenic content \( (18:3n-3) \) in meat (Bas, Berthelot, Pottier, & Normand, 2007; Elmore et al., 2000). In this sense, Sañudo, Enser, Campo, Nute, María, Sierra, & Wood (2000) found a negative correlation between flavour liking and \( \alpha \)-linolenic content \( (18:3n-3) \) in lamb meat. Moreover, the CTRL samples were preferred (preference test) over the +QCT ones in 17 out of 24 cases \( (P = 0.041) \). The +QCT samples were judged as less tender, thus in agreement with the TPA results (Hardness, LL d 0; Table 2). This result was unexpected, since it has been described that some dietary antioxidants preserve the activity of calpain during the ageing process, thus improving meat tenderisation (Morán et al., 2012).

4. Conclusions

According to our results, at the doses used in the present study dietary flaxseed does not seem to affect the lipid peroxidation of meat samples (M. gluteus medius and M. biceps femoris) from fattening lambs, whereas quercetin dietary supplementation reduced oxysterols content after 7 days of refrigerated storage of fresh meat. However, it must be stressed that the use of both quercetin and flaxseed in lambs’ diet might modify the sensory quality of recently cooked meat in a negative manner.

Acknowledgements

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INSERT TABLE 6 NEAR HERE, PLEASE]
Bodas, and Nuria Prieto have a JAE-Doc contract and Lara Morán was supported by a JAE-Predoc grant under the programme ‘Junta para la Ampliación de Estudios’ (CSIC-European Social Fund).

References


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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>CTR(^a)</th>
<th>+FS(^b)</th>
<th>+QCT(^c)</th>
<th>+FS+QCT(^d)</th>
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<tbody>
<tr>
<td>Barley</td>
<td>417</td>
<td>421</td>
<td>417</td>
<td>421</td>
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<tr>
<td>Soybean meal</td>
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<td>187</td>
<td>149</td>
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<tr>
<td>Corn</td>
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<td>170</td>
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<tr>
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<td>Quercetin</td>
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<td>2</td>
</tr>
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<td>Dry matter</td>
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<tr>
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<tr>
<td>Ash</td>
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<td>60</td>
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</tbody>
</table>

\(^a\) 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).
Table 2. Cooking loss (% of lost water) and texture profile analysis (hardness, expressed as N, cohesiveness, springiness, chewiness) of meat samples (*longissimus lumborum*) after 0, 7 or 14 days of light-exposed refrigerated storage (raw) and subsequent cooking.

<table>
<thead>
<tr>
<th>Dietary treatments (n = 8)</th>
<th>Cooking loss</th>
<th>Hardness</th>
<th>Cohesiveness</th>
<th>Springiness</th>
<th>Chewiness</th>
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<tr>
<td></td>
<td>CTRL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+FS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+QCT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+FS+QCT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>sed&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 d</td>
<td>19.5</td>
<td>20.6</td>
<td>19.7</td>
<td>19.9</td>
<td>4.55</td>
</tr>
<tr>
<td>7 d</td>
<td>19.2</td>
<td>20.7</td>
<td>21.2</td>
<td>22.0</td>
<td>1.68</td>
</tr>
<tr>
<td>14 d</td>
<td>16.4</td>
<td>16.0</td>
<td>15.9</td>
<td>16.5</td>
<td>3.19</td>
</tr>
<tr>
<td>0 d</td>
<td>172</td>
<td>177</td>
<td>189</td>
<td>186</td>
<td>12.8</td>
</tr>
<tr>
<td>7 d</td>
<td>179</td>
<td>184</td>
<td>175</td>
<td>187</td>
<td>13.5</td>
</tr>
<tr>
<td>14 d</td>
<td>161</td>
<td>159</td>
<td>159</td>
<td>148</td>
<td>12.4</td>
</tr>
<tr>
<td>0 d</td>
<td>0.489</td>
<td>0.462</td>
<td>0.445</td>
<td>0.463</td>
<td>0.0222</td>
</tr>
<tr>
<td>7 d</td>
<td>0.466</td>
<td>0.463</td>
<td>0.451</td>
<td>0.457</td>
<td>0.0175</td>
</tr>
<tr>
<td>14 d</td>
<td>0.465</td>
<td>0.443</td>
<td>0.451</td>
<td>0.444</td>
<td>0.0205</td>
</tr>
<tr>
<td>0 d</td>
<td>0.502</td>
<td>0.499</td>
<td>0.461</td>
<td>0.492</td>
<td>0.0330</td>
</tr>
<tr>
<td>7 d</td>
<td>0.431</td>
<td>0.455</td>
<td>0.472</td>
<td>0.471</td>
<td>0.0282</td>
</tr>
<tr>
<td>14 d</td>
<td>0.457</td>
<td>0.461</td>
<td>0.445</td>
<td>0.441</td>
<td>0.0345</td>
</tr>
<tr>
<td>0 d</td>
<td>42.3</td>
<td>41.1</td>
<td>40.0</td>
<td>42.4</td>
<td>5.39</td>
</tr>
<tr>
<td>7 d</td>
<td>36.3</td>
<td>38.5</td>
<td>37.5</td>
<td>40.6</td>
<td>5.03</td>
</tr>
<tr>
<td>14 d</td>
<td>35.3</td>
<td>32.9</td>
<td>32.5</td>
<td>29.7</td>
<td>4.86</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control group (no antioxidants, 34 g palm oil kg<sup>-1</sup> TMR).
<sup>b</sup> Flaxseed group (85 g flaxseed kg<sup>-1</sup> TMR).
<sup>c</sup> Quercetin group (34 g palm oil plus 2 g quercetin kg<sup>-1</sup> TMR).
<sup>d</sup> Flaxseed plus quercetin group (85 g flaxseed plus 2 g quercetin kg<sup>-1</sup> TMR).
<sup>e</sup> Standard error of the difference.
<sup>f</sup> Probability of significant effects of flaxseed (FS), quercetin (QCT), and their interaction (FS×QCT).
Table 3. Oxysterols content in meat (µg g⁻¹ meat; M. gluteus medius) after 7 days of refrigerated storage (raw) and subsequent cooking.

<table>
<thead>
<tr>
<th>Dietary treatments (n = 8)</th>
<th>P-value¹</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTRLᵃ</td>
<td>+FSᵇ</td>
</tr>
<tr>
<td>7α-HCᵍ 0.79ᵇ</td>
<td>0.78ᵇ 0.59ᵃ</td>
<td>0.59ᵃ</td>
</tr>
<tr>
<td>7β-HCʰ 1.52ᶜ</td>
<td>1.44ᵇ 1.02ᵃᵇ</td>
<td>0.84ᵃ</td>
</tr>
<tr>
<td>α-CEⁱ 2.24</td>
<td>2.01 1.79</td>
<td>1.55</td>
</tr>
<tr>
<td>β-CEʲ 0.40</td>
<td>0.43 0.38</td>
<td>0.33 0.095</td>
</tr>
<tr>
<td>CTᵏ</td>
<td>0.01 0.01 0.00</td>
<td>0.01 0.013</td>
</tr>
<tr>
<td>25-HC¹ 0.11</td>
<td>0.06 0.16</td>
<td>0.08 0.048</td>
</tr>
<tr>
<td>7-KCᵐ 1.09ᵇ</td>
<td>1.07ᵇ 0.80ᵃ</td>
<td>0.66ᵃ</td>
</tr>
<tr>
<td>Σ COPsⁿ 6.17ᵇ</td>
<td>5.80ᵇ 4.44ᵃ</td>
<td>3.74ᵃ</td>
</tr>
</tbody>
</table>

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).
e Standard error of the difference.
f Probability of significant effects of flaxseed (FS), quercetin (QCT), and their interaction (FS×QCT).
g 7α-hydroxycholesterol.
h 7β-hydroxycholesterol.
i 5,6α-epoxycholesterol.
j 5,6β-epoxycholesterol.
k Cholestanetriol.
l 25-hydroxycholesterol.
m 7-ketocholesterol.
n Cholesterol oxidation products.
aᵇ Different superscripts in the same row indicate statistical differences (P < 0.05) between treatments.
Table 4. Values of iron-induced thiobarbituric acid reactive substances (TBARS; mg kg\(^{-1}\)) in recently cooked meat (M. biceps femoris) after 0, 1, 4 and 6 hours of incubation.

<table>
<thead>
<tr>
<th>Dietary treatment (n = 8)</th>
<th>CTRL(^a)</th>
<th>+FS(^b)</th>
<th>+QCT(^c)</th>
<th>+FS+QCT(^d)</th>
<th>sed(^e)</th>
<th>FS</th>
<th>QCT</th>
<th>FS×QCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>0.36</td>
<td>0.74</td>
<td>0.25</td>
<td>0.30</td>
<td>0.269</td>
<td>0.223</td>
<td>0.144</td>
<td>0.267</td>
</tr>
<tr>
<td>1 h</td>
<td>0.63</td>
<td>1.46</td>
<td>0.70</td>
<td>0.95</td>
<td>0.587</td>
<td>0.193</td>
<td>0.430</td>
<td>0.425</td>
</tr>
<tr>
<td>4 h</td>
<td>3.35(^a)(^b)</td>
<td>5.38(^b)</td>
<td>1.93(^a)</td>
<td>3.17(^a)</td>
<td>1.481</td>
<td>0.203</td>
<td>0.028</td>
<td>0.670</td>
</tr>
<tr>
<td>6 h</td>
<td>7.57</td>
<td>9.93</td>
<td>5.06</td>
<td>8.78</td>
<td>2.574</td>
<td>0.200</td>
<td>0.159</td>
<td>0.670</td>
</tr>
</tbody>
</table>

\(^a\) 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).
\(^e\) Standard error of the difference.
\(^f\) Probability of significant effects of flaxseed (FS), quercetin (QCT), and their interaction (FS×QCT).
\(^a\)\(^b\) Different superscripts in the same row indicate statistical differences (\(P < 0.05\)) between treatments.
Table 5. Levels of straight-chain aliphatic aldehydes from headspace of cooked meat samples (M. biceps femoris), expressed as ng undecane/g, just after cooking (1 d) and at day 3 of light-exposed refrigerated storage (3 d).

<table>
<thead>
<tr>
<th>Aldehydes in headspace</th>
<th>Dietary treatment (n = 8)</th>
<th>P-value$^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTRL$^a$</td>
<td>+FS$^b$</td>
</tr>
<tr>
<td>Pentanal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 d</td>
<td>5.0</td>
<td>1.3</td>
</tr>
<tr>
<td>3 d</td>
<td>65.0</td>
<td>63.8</td>
</tr>
<tr>
<td>Hexanal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 d</td>
<td>89.9$^{ab}$</td>
<td>65.7$^a$</td>
</tr>
<tr>
<td>3 d</td>
<td>569.5</td>
<td>566.4</td>
</tr>
<tr>
<td>Heptanal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 d</td>
<td>8.8</td>
<td>6.4</td>
</tr>
<tr>
<td>3 d</td>
<td>29.4</td>
<td>29.3</td>
</tr>
<tr>
<td>Octanal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 d</td>
<td>4.7</td>
<td>2.9</td>
</tr>
<tr>
<td>3 d</td>
<td>17.0$^a$</td>
<td>35.3$^b$</td>
</tr>
<tr>
<td>Nonanal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 d</td>
<td>9.4</td>
<td>7.2</td>
</tr>
<tr>
<td>3 d</td>
<td>25.9$^a$</td>
<td>34.7$^a$</td>
</tr>
</tbody>
</table>

$^a$ 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).

$^e$ Standard error of the difference.

$^f$ Probability of significant effects of flaxseed (FS), quercetin (QCT), and their interaction (FS×QCT).

$^{ab, c}$ Different superscripts in the same row indicate statistical differences ($P < 0.05$) between treatments.
Table 6. Results from sensory analysis carried out to discriminate between cooked meat samples from lambs fed 1) CTRL and +FS diets and 2) CTRL and +QCT diets.

<table>
<thead>
<tr>
<th>Preference test</th>
<th>Preferences for CTRL (n = 24)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL&lt;sup&gt;a&lt;/sup&gt; vs +FS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20</td>
<td>0.001</td>
</tr>
<tr>
<td>CTRL vs +QCT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17</td>
<td>0.041</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control group (no antioxidants, 34 g palm oil kg<sup>-1</sup> TMR).
<sup>b</sup> Flaxseed group (85 g flaxseed kg<sup>-1</sup> TMR).
<sup>c</sup> Quercetin group (34 g palm oil plus 2 g quercetin kg<sup>-1</sup> TMR).