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 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, 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, 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.

[INSERT TABLE 1 NEAR HERE, PLEASE]

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

98 (+QCT and +FS+QCT groups) *ad libitum* during the experimental period (at least 5
99 weeks for each lamb depending on the time required for each animal to reach the target
100 BW). The TMRs were weighed and supplied *ad libitum* at 9:00 a.m. every day, and
101 fresh drinking water was always available. Samples of feed offered and orts
102 (approximately 20% of total offered) were taken daily, pooled to an individual
103 composite sample each week, oven-dried at 55 °C for at least 72 h, ground to pass
104 through a 1-mm screen using a Willey mill (Arthur H. Thomas, Philadelphia, PA), and
105 stored until analyses.

106 *2.2. Slaughter procedure, packaging, and storage of meat samples*

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

111 The *longissimus thoracis* (LT) *et lumborum* (LL), *gluteus medius* (GM), *biceps*
112 *femoris* (BF), and *adductor magnus* (AM) muscles were removed from the right and left
113 carcass sides. The AM muscle of both sides was frozen at -30 ºC for sensory analysis.
114 The LT samples of both sides were used for chemical analysis (Andrés, Tejido, Bodas,
115 Morán, Prieto, Blanco, & Giráldez, 2013). LL and GM muscles of both sides were cut
116 into 2.5 cm-thick slices, placed on impermeable polypropylene trays, over-wrapped
117 with an oxygen-permeable polyvinylchloride film (580 ml m^{-2} h^{-1}) and then stored
118 under simulated retail display conditions [12 h daily fluorescent illumination (34 W)
119 and 3±1 ºC] during 0, 7, and 14 days. Then, the samples were used either for texture and
120 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 ºC 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 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 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 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, & 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 previouly 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 ºC. 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 sesion. 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:

$$y_{ijk} = \mu + FS_i + QCT_j + (FS\times QCT)_{ij} + B_k + S_l + (FS\times QCT\times B)_{ijk} + \varepsilon_{ijklm}$$

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, S is the effect of slaughter day (block), B is the effect of batch (block), B×FS×QCT the interaction between treatments and batch, which was used as experimental error to test the effects of treatments, and ε_{ijk} is the

218 residual error. Least square means were generated and separated using the PDIFF option
219 of SAS for main or interactive effects, with the level of significance being determined at
220 $P < 0.05$. Data from the sensorial analysis were assessed according to ISO 5495:2005
221 and also analyzed using the chi-square test (Stone & Sidel, 1993) of the FREQ
222 procedure in SAS (SAS, 1999).

223 **3. Results and discussion**

224 *3.1. Water holding capacity (WHC) and texture profile analysis (TPA)*

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

234 [INSERT TABLE 2 NEAR HERE, PLEASE]

235 *3.2. Cholesterol oxidation*

236 Regarding oxysterol content in meat samples, these compounds can be absorbed
237 through the intestinal tract into the blood stream, thus increasing the susceptibility of the
238 consumer to coronary heart disease (Valenzuela, Sanhueza, & Nieto, 2003). Since the
239 main source of oxysterols in meat is heat processing, these substances were determined
240 in cooked meat samples (cooked GM after 7 days of refrigerated storage of raw
241 muscles, previously used to measure the WHC by cooking loss). Table 3 presents the

242 corresponding results of oxysterols content for each group. As can be observed, dietary
243 flaxseed did not affect significantly the oxysterols content of meat samples, whereas
244 three of these compounds (7α-HC, 7β-HC, and 7-KC) were significantly reduced in the
245 meat of the lambs being fed quercetin (+QCT and +FS+QCT) when compared to the
246 CTRL and +FS groups (Table 3).

247 [INSERT TABLE 3 NEAR HERE, PLEASE]

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

253 It has been suggested that hydroperoxides of polyunsaturated fatty acids formed
254 during lipid oxidation might be necessary to initiate cholesterol oxidation, so oxysterols
255 content might be synergistically increased by unsaturated fat (Smith, 1987). In this
256 regard, in the present study total oxysterol content (Table 3) was greater when compared
257 to the meat from lambs being fed diets with no fats (∑ COPs ranging from 1.428 to
258 3.022 μg g^{-1} cooked meat; Morán et al., 2012). Moreover, oxysterol levels (Table 3)
259 were lower than those reported in pork from animals fed unsaturated fats (linseed and
260 sunflower oils added to the diet at 2% rates), where meat was cooked after being stored
261 at 4 ºC for 9 days (∑ COPs ranging from 6.07 to 12.39 μg g^{-1} cooked meat; Rey, Kerry,
262 Lynch, López-Bote, Buckley, & Morrissey, 2001). Also, all lamb meat samples (even
263 those of the CTRL group) showed very low levels of CT and 25-HC, which have been
264 described as atherogenic oxysterols responsible for acute injury to the endothelium
265 (Taylor, Peng, Werthessen, Tham, & Lee, 1979; Peng, Taylor, Hill, & Morin, 1985).
266 These important differences in the oxysterol content of meat when lambs and pigs are

267 fed unsaturated fats might be explained by the particularity of the ruminant gut, since
268 the biohydrogenation process at rumen level undergone by the unsaturated fatty acids
269 consumed by the lambs might have protected meat against cholesterol oxidation during
270 refrigerated storage and later on during the cooking procedure. This fact might also
271 explain the lack of significant differences observed in the present study between the
272 groups being fed palm oil (CTRL and +QCT lambs) and flaxseed (+FS and +FS+QCT
273 lambs).

274 *3.3. Iron-induced TBARS and volatile compounds in stored cooked meat*

275 Results on lipid oxidation of cooked meat generated via Fenton reaction (Fe^{2+}/H_2O_2)
276 and quantified by the TBARS assay are shown in Table 4. This assay (iron-induced
277 TBARS) has been considered as suitable to assess the animals' diet influence on meat
278 oxidation processes (Mercier et al., 2004). In the present study, meat from animals fed
279 quercetin supplemented diets (+QCT and +FS+QCT groups) showed lower iron-
280 induced TBARS values after 4 hours of incubation (QCT, $P = 0.028$), than meat from
281 animals fed without this flavonoid (CTRL and +FS groups). This means that quercetin
282 increased the lipid resistance to iron-induced oxidation. The effect was not significant
283 after 6 h of incubation, probably as a consequence of the high intra-group variability
284 observed in this parameter. Regarding dietary flaxseed this component did not affect
285 significantly the lipid peroxidation of meat samples, probably due to the presence of
286 antioxidants such as vitamin E in the oil of the seeds or as a consequence of the
287 biohydrogenation process at rumen level undergone by the unsaturated fatty acids
288 consumed by the lambs.

289 [INSERT TABLE 4 NEAR HERE, PLEASE]

290 A total of 24 volatile compounds were detected in the headspace of the cooked meat
291 samples, with 21 of them being identified, and thus assigned to the following chemical
292 families (the number of compounds is shown between brackets): straight-chain aliphatic
293 hydrocarbons (3), aldehydes (5), ketones (1) and alcohols (3), and terpenic (3), benzenic
294 (5) and furanic (1) compounds. Among the identified compounds the presumably lipid-
295 derived compounds were hexane, heptane, octane, pentanal, hexanal, heptanal, octanal,
296 nonanal, 2,3-octanodione, 2-octen-1-ol and 2-penthyl-furan (Frankel, 1982; Mottram,
297 1998). Among them, only the straight-chain aliphatic aldehydes are shown in Table 5 as
298 the predominant group. The relevance of the aliphatic aldehydes in headspace of the
299 lamb meat studied (approximately 50 percent of the total volatile compounds; not
300 shown in Table 5) was in agreement with that found in other studies (Vasta & Priolo,
301 2012; Vieira et al., 2012). The presence of aldehydes in ruminant meat headspace is
302 mainly attributed to lipid oxidation/degradation taking place during cooking and storage
303 of cooked meat (Shahidi & Pegg, 1994a,b; Sivadier, Ratel, Bouvier, & Engel, 2008).

304 [INSERT TABLE 5 NEAR HERE, PLEASE]

305 At day 1 (just after cooking the meat samples), meat from the lambs being fed
306 flaxseed (+FS and +FS+QCT groups) showed a trend towards significantly lower values
307 of pentanal, hexanal, and octanal ($P < 0.1$) when compared to the meat from the lambs
308 being fed no flaxseed (CTRL and +QCT diets). Those aldehydes are typically derived
309 from the two main unsaturated fatty acids in meat, i.e. oleic acid, 18:1, and linoleic acid,
310 18:2n-6 (Elmore, Campo, Enser, & Wood, 2002; Frankel, 1982; Zanardi, Novelli,
311 Nanni, Ghiretti, Delbono, Campanini, Dazzi, Madarena, & Chizzolini, 1998). As
312 observed in this study, it has already been proven that diet can affect the levels of lipid-
313 derived volatiles of cooked meat (Elmore, Mottram, Enser, & Wood, 2000; Vasta &
314 Priolo, 2006; Vasta, Ventura, Luciano, Andronico, Pagano, Scerra, Biondi, Avondo, &

315 Priolo, 2012). The differences found in the present study might be attributed to diet-
316 related changes in fatty acid and natural antioxidant contents and/or to compositional or
317 structural changes in meat affecting the formation of lipid-derived volatiles during
318 cooking and their release to headspace.

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

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

337 *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, Table 6). The use of flaxseed in lambs' diet has been related to high α-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 α-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).

[INSERT TABLE 6 NEAR HERE, PLEASE]

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.

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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).

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.

	Dietary treatments (n = 8)					*P*-value[f]		
	CTRL[a]	+FS[b]	+QCT[c]	+FS+QCT[d]	sed[e]	FS	QCT	FS×QCT
Cooking loss								
0 d	19.5	20.6	19.7	19.9	4.55	0.842	0.955	0.874
7 d	19.2	20.7	21.2	22.0	1.68	0.338	0.129	0.757
14 d	16.4	16.0	15.9	16.5	3.19	0.800	0.750	0.886
Hardness								
0 d	172	177	189	186	12.8	0.703	0.069	0.604
7 d	179	184	175	187	13.5	0.088	0.713	0.691
14 d	161	159	159	148	12.4	0.282	0.285	0.612
Cohesiveness								
0 d	0.489	0.462	0.445	0.463	0.0222	0.891	0.200	0.111
7 d	0.466	0.463	0.451	0.457	0.0175	0.765	0.306	0.697
14 d	0.465	0.443	0.451	0.444	0.0205	0.187	0.509	0.553
Springiness								
0 d	0.502	0.499	0.461	0.492	0.0330	0.657	0.254	0.418
7 d	0.431	0.455	0.472	0.471	0.0282	0.796	0.291	0.455
14 d	0.457	0.461	0.445	0.441	0.0345	0.807	0.677	0.865
Chewiness								
0 d	42.3	41.1	40.0	42.4	5.39	0.853	0.987	0.601
7 d	36.3	38.5	37.5	40.6	5.03	0.382	0.662	0.882
14 d	35.3	32.9	32.5	29.7	4.86	0.378	0.322	0.937

[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).

Table 3. Oxysterols content in meat (μg g^{-1} meat; M. *gluteus medius*) after 7 days of refrigerated storage (raw) and subsequent cooking.

	Dietary treatments (n = 8)					*P*-value[f]		
	CTRL[a]	+FS[b]	+QCT[c]	+FS+QCT[d]	sed[e]	FS	QCT	FS×QCT
7α-HC[g]	0.79[b]	0.78[b]	0.59[a]	0.59[a]	0.109	0.895	0.003	0.832
7β-HC[h]	1.52[c]	1.44[bc]	1.02[ab]	0.84[a]	0.329	0.688	0.025	0.809
α-CE[i]	2.24	2.01	1.79	1.55	0.336	0.293	0.121	0.957
β-CE[j]	0.40	0.43	0.38	0.33	0.095	0.412	0.105	0.474
CT[k]	0.01	0.01	0.00	0.01	0.013	0.858	0.411	0.611
25-HC[l]	0.11	0.06	0.16	0.08	0.048	0.063	0.218	0.710
7-KC[m]	1.09[b]	1.07[b]	0.80[a]	0.66[a]	0.173	0.366	0.001	0.624
∑ COPs[n]	6.17[b]	5.80[b]	4.44[a]	3.74[a]	0.855	0.314	0.001	0.922

[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.
[a,b] 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.

	Dietary treatment (n = 8)					*P*-value[f]		
	CTRL[a]	+FS[b]	+QCT[c]	+FS+QCT[d]	sed[e]	FS	QCT	FS×QCT
0 h	0.36	0.74	0.25	0.30	0.269	0.223	0.144	0.267
1 h	0.63	1.46	0.70	0.95	0.587	0.193	0.430	0.425
4 h	3.35[ab]	5.38[b]	1.93[a]	3.17[a]	1.481	0.203	0.028	0.670
6 h	7.57	9.93	5.06	8.78	2.574	0.200	0.159	0.670

[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).

	Dietary treatment (n = 8)					*P*-value[f]		
	CTRL[a]	+FS[b]	+QCT[c]	+FS+QCT[d]	sed[e]	FS	QCT	FS×QCT
Aldehydes in headspace								
Pentanal								
1 d	5.0	1.3	8.4	1.2	4.20	0.067	0.579	0.379
3 d	65.0	63.8	81.5	59.6	18.92	0.470	0.646	0.381
Hexanal								
1 d	89.9[ab]	65.7[a]	142.8[b]	51.9[a]	27.82	0.076	0.208	0.160
3 d	569.5	566.4	822.0	543.7	200.32	0.337	0.434	0.273
Heptanal								
1 d	8.8	6.4	11.4	6.9	3.42	0.464	0.419	0.647
3 d	29.4	29.3	42.9	28.8	10.75	0.349	0.416	0.295
Octanal								
1 d	4.7	2.9	8.4	1.0	3.92	0.065	0.576	0.258
3 d	17.0[a]	35.3[b]	43.0[b]	21.5[ab]	11.29	0.722	0.193	0.008
Nonanal								
1 d	9.4	7.2	11.2	6.3	2.79	0.165	0.625	0.485
3 d	25.9[a]	34.7[a]	51.5[b]	27.0[a]	12.37	0.559	0.206	0.038

[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,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.

Preference test	Preferences for CTRL (n = 24)	*P*-value
CTRL[a] vs +FS[b]	20	0.001
CTRL vs +QCT[c]	17	0.041

[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).