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

74 texture and antioxidant properties of meat when flaxseed (rich in PUFAs) and/or

75 quercetin were included in the diet of fattening lambs.

76 **2. Materials and Methods**

77 *2.1. Animals and diets*

78   Two weeks before the commencement of the trial, 32 male Merino lambs were

79 treated with Ivermectin (Ivomec, Merial Labs, Barcelona, Spain) and vaccinated against

80 enterotoxaemia (Miloxan, Merial Labs, Barcelona, Spain).

81   After stratification on the basis of body weight (average body weight (BW), 15.5 ±

82 2.12 kg), the lambs were allocated randomly to 8 different groups housed in different

83 pens of four animals each (2 pens per dietary treatment). All of the groups were fed

84 their corresponding total mixed ration (**TMR**) as described below: two control groups

85 (**CTRL**, 4 animals per group; 34 g palm oil kg^{-1} TMR), two groups fed ground whole

86 flaxseed (+**FS,** 4 animals per group; 85 g flaxseed kg^{-1} of TMR), two groups fed control

87 diet plus quercetin (99%) extracted from *Sophora japonica L.* (Shaanxi Sciphar

88 Biotechnology Co., Ltd, Xi'an, China) (+**QCT,** 4 animals per group; 34 g palm oil plus

89 2 g quercetin kg^{-1} TMR), and two groups fed whole ground flaxseed plus quercetin

90 (+**FS+QCT,** 4 animals per group; 85 g flaxseed plus 2 g quercetin kg^{-1} TMR). The four

91 TMRs were formulated to be isoenergetic and isoproteic. Chemical composition of

92 TMR is shown in Table 1. All handling practises followed the recommendations of the

93 European Council Directive 2010/63/EU for the protection of animals used for scientific

94 purposes and all the animals were able to see and hear other animals.

95   [INSERT TABLE 1 NEAR HERE, PLEASE]

96   After 7 days of adaptation to the basal diet, all of the lambs were fed the

97 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).

121   Finally, BF muscles from the right or left side, at random, were vacuum packaged

122   and frozen and stored at -50 ºC for up to 2 months prior cooking and subsequent

123   analysis of iron-induced TBARS and volatile compounds.

124   *2.3. Texture profile analysis (TPA) and water holding capacity (WHC)*

125   The slices of LL after 0, 7, and 14 days under refrigerated storage condition were

126   weighed and cooked in a double-sided griddle (preheated at 220 °C) until a core

127   temperature of 75 °C was reached, following the guidelines for cooking procedures of

128   AMSA (1995). After cooling at 4 ºC for 30 min the samples (LL) were weighed again

129   and frozen at -30 ºC until texture profile analysis (TPA) according to the procedure

130   described by Herrero, de la Hoz, Ordoñez, Herranz, Romero de Ávila, & Cambero

131   (2008) with slight modifications: meat specimens were cubic (10 mm^3) and the

132   compression percentage of the initial height was 80%, with the compression axis

133   perpendicular to the muscle fibre direction. The water holding capacity (WHC) was

134   measured on LL samples via cooking loss, according to Honikel (1998).

135   *2.4. Cholesterol oxidation*

136   GM samples after 7 days of refrigerated storage were weighed and cooked as

137   previously described for LL slices. Then, they were cooled at 4 ºC for 30 min, weighed

138   again, and freeze-dried for oxysterols analysis. Cholesterol oxidation products (COPs),

139   also called oxysterols, were determined according to the method proposed by Grau,

140   Codony, Grimpa, Baucells, & Guardiola (2001). Briefly, lipids were extracted from 1 g

141   of cooked and freeze-dried GM samples using a mixture chloroform/methanol (2:1, v/v)

142   (Folch, Lees, & Sloane Stanley, 1957). 19-Hydroxycholesterol (19-HC) was used as an

143   internal standard. Ten millilitres of 1.5 M methanolic KOH were then added and the

144   mixture was kept in an orbital shaker for 20 h at room temperature under N_2 atmosphere

145 and darkness to complete the cold saponification. The unsaponificable matter was

146 extracted three times with diethyl ether in a separating funnel, and then purified by

147 solid-phase extraction (SPE) according to the procedure described by Guardiola,

148 Codony, Rafecas, & Boatella (1995). COPs were derivatised to trimethylsilyl (TMS)

149 ethers prior to gas chromatographic (GC) analysis on a HP 6890 Series gas

150 chromatograph (Agilent Technologies, Santa Clara, CA, USA) provided with a mass

151 selective detector (HP 5973), by splitless injection (HP 7683 Series injector) into a VF-

152 5ms CP8947 capillary column (50 m × 250 μm × 0.25 μm, Varian, Palo Alto, CA,

153 USA). Chromatographic conditions were as follows: injection volume 1.0 μl; initial

154 oven temperature 60 ºC, to 230 ºC at 15 ºC min^{-1}, to 290 ºC at 10 ºC min^{-1}, and to 292

155 ºC at 0.05 ºC min^{-1}; injector and transfer-line temperatures were 250 and 290 ºC,

156 respectively. Helium was used as a carrier gas at a flow rate of 0.5 ml min^{-1}. The mass

157 spectrometer operated in electron impact mode with electron energy of 69.9 eV, an

158 emission current of 34.6 μA, a source and quadruples temperatures of 230 and 180,

159 respectively, and scanned from m/z 40 to m/z 400. The oxysterols 7α-

160 hydroxycholesterol (7α-HC), 7β-hydroxycholesterol (7β-HC), 5,6α-epoxycholesterol

161 (α-CE), 5,6β-epoxycholesterol (β-CE), cholestanetriol (CT), 25-hydroxycholesterol (25-

162 HC), and 7-ketocholesterol (7-KC) were identified by comparing their retention times

163 and spectra with those of authentic standards (Steraloids, Inc., Wilton, New Hamp-

164 shire, UK) and quantified using the internal standard.

165 *2.5. Iron-induced TBARS and volatile compounds in stored cooked meat*

166 After thawing the BF muscles at 10 ºC for approximately 12 h into the packaging,

167 the muscles were cut into three sections perpendicular to the long axis: proximal (1.5

168 cm), central (the largest) and distal (1.5 cm) sections. The proximal and distal sections

169 were discharged and two slices (2.5-cm thick) were cut from the central section. Each

170 slice was cooked as previouly described and a slice was immediately used for analysis.

171 The remaining slice was placed in impermeable polypropylene trays, covered by an

172 oxygen-permeable polyvinylchloride film and stored at 5 °C for 3 days, while exposed

173 to a light source (12 h per day) provided with a pair of twin linear fluorescent tubes (34

174 W).

175 The in-vitro iron-induced lipid oxidation was measured in duplicate on the BF slice

176 sampled immediately after the cooking procedure. The procedure described by Mercier,

177 Gatellier, & Renerre (2004) was followed for sample preparation and incubation of

178 sample solutions, with incubation times of 0 (immediate measurement without

179 incubation), 1, 4, and 6 hours and incubation temperature of 37 ºC. Afterwards, the

180 thiobarbituric acid reactive substances (TBARS) assay with 1 ml of incubated sample

181 solution was carried out according to the method proposed by Nam & Ahn (2003).

182 The status of lipid oxidation of cooked meat samples (BF) after the refrigerated

183 storage period was evaluated by determining volatile compounds in the headspace of

184 meat. In order to set the initial point (day 1 of storage), an aliquot of the muscle slice

185 sampled after cooking was immediately used for analysis. Thereafter, volatiles in the

186 headspace after 48 hours of refrigerated storage of cooked meat (day 3), under the

187 abovementioned refrigeration conditions, also were measured. Volatile analyses were

188 performed in duplicate following the procedure of Vieira, Fernández-Diez, Mateo,

189 Bodas, Soto, & Manso (2012). Quantification was based on the comparison of peak

190 areas of the volatile compounds to the areas obtained from known amounts of undecane

191 previously dissolved in hexane and analysed under the same conditions as described for

192 the volatile compounds of the samples.

193 *2.6. Sensory evaluation*

194   The muscle *adductor magnus* (AM) of both hind legs was chosen for sensory

195   evaluation. Sensory analysis was carried out by 24 consumers in only one sesion. The

196   muscles were defrosted at 4 ºC for 48 h and dissected and cut into steaks 20 mm thick.

197   The steaks were cooked a pre-warmed clam-shell grill to an internal temperature of 75º

198   C in the geometric centre of the steak (measured by a Digi-Sense thermocouple probe,

199   Cole-Parmer Instrument Company, Vernon Hills, IL, USA), after which all fat and

200   connective tissue was trimmed and the muscle cut into blocks of 2×1×1 cm. The blocks

201   were wrapped in pre-labelled foil (the blocks from each animal were coded with the

202   same alphabetical letter), placed in a heated incubator and then given to the assessors in

203   a random order chosen by a random number generator. All consumers participated in

204   two blind preference tests in which they received two meat samples on a coded paper

205   plate. Each consumer tasted and evaluated their preferences for the meat samples from

206   lambs fed (1) CTRL vs +FS, and (2) CTRL vs +QCT diets, and provided the reasons for

207   their choices (ISO 5495:2005).

208   *2.7. Statistical analysis*

209   Data of cooking loss, texture, COPs content, iron-induced TBARS values and

210   volatile compound levels were subjected to a two-way analysis of variance, using the

211   MIXED procedure of SAS (SAS, 1999) according to the following model:

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

213   where y_{ijk} is the dependent variable, μ is the overall mean, FS is the effect of flaxseed

214   addition, QCT is the effect of quercetin addition, FS×QCT is the effect of the

215   interaction between quercetin and flaxseed, S is the effect of slaughter day (block), B is

216   the effect of batch (block), B×FS×QCT the interaction between treatments and batch,

217   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 ($\sum$ 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 ($\sum$ 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

11

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]

12

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*

338   When meat from the lambs fed the CTRL and +FS diets were compared, 83.3% of

339 the consumers preferred the CTRL samples describing them as less strong flavoured,

340 being this percentage statistically different from the percentage of consumers who

341 preferred the +FS samples ($P = 0.001$, Table 6). The use of flaxseed in lambs' diet has

342 been related to high α-linolenic content (18:3n−3) in meat (Bas, Berthelot, Pottier, &

343 Normand, 2007; Elmore et al., 2000). In this sense, Sañudo, Enser, Campo, Nute,

344 María, Sierra, & Wood (2000) found a negative correlation between flavour liking and

345 α-linolenic content (18:3n−3) in lamb meat. Moreover, the CTRL samples were

346 preferred (preference test) over the +QCT ones in 17 out of 24 cases ($P = 0.041$). The

347 +QCT samples were judged as less tender, thus in agreement with the TPA results

348 (Hardness, LL d 0; Table 2). This result was unexpected, since it has been described that

349 some dietary antioxidants preserve the activity of calpain during the ageing process,

350 thus improving meat tenderisation (Morán et al., 2012).

351   [INSERT TABLE 6 NEAR HERE, PLEASE]

352 **4. Conclusions**

353   According to our results, at the doses used in the present study dietary flaxseed does

354 not seem to affect the lipid peroxidation of meat samples (M. *gluteus medius* and M.

355 *biceps femoris*) from fattening lambs, whereas quercetin dietary supplementation

356 reduced oxysterols content after 7 days of refrigerated storage of fresh meat. However,

357 it must be stressed that the use of both quercetin and flaxseed in lambs' diet might

358 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
$\sum$ 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).