Fatty acids and oxidative stability of meat from lambs fed carob-containing diets

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a b s t r a c t

Male Comisana lambs were individually stalled and, for 56 days, were fed concentrates with 60% barley (n = 8 lambs), or concentrates in which barley was partially replaced by 24% or 35% carob pulp (n = 9 lambs in each group). The intramuscular fatty acids were analyzed and the color stability, lipid and protein oxidation were measured in fresh meat overwrapped with polyvinyl chloride film at 0, 3 or 6 days of storage at 4 C in the dark. Carob pulp increased the concentration of polyunsaturated fatty acids (PUFA) in muscle, including the rumenic acid ($P < 0.01$), and reduced the saturated fatty acids ($P < 0.01$) and the n-6/n-3 PUFA ratio ($P = 0.01$). The meat did not undergo extensive oxidative deterioration and the diet did not affect the oxidative stability

parameters. Therefore, carob in lamb diet could increase PUFA in muscle without compromising meat oxidative stability.

Keywords: Lamb, Protein oxidation, Lipid oxidation, Carob pulp, PUFA, Phenolic compounds

1. Introduction

The increased consumption of foods with low level of saturated fatty acids (SFA) and high level of polyunsaturated fatty acids (PUFA), containing a low n-6/n-3 fatty acid ratio correlates with favorable human health conditions (Wood et al., 2003). Consequently, ruminant meat is not considered as a part of a healthy human diet due to its high content of SFA (Scollan et al., 2006). The fatty acid composition of meat from ruminants is in part a result of the dietary intake of the corresponding fatty acids (Wood et al., 2003), thus endeavors to modify the fatty acid composition and content of animal tissues through dietary strategies is in progress. These include, for instance, the dietary supplementation of PUFA-rich sources (Bessa et al., 2007; Scollan et al., 2006) or feeding animals with herbage (Luciano et al., 2009). On the other hand, the PUFA ingested by ruminants undergo a process in the rumen, known as biohydrogenation, leading to their progressive saturation. Therefore, feeding strategies that reduce the extent of the ruminal biohydrogenation might ultimately increase the deposition of PUFA in ruminant tissues (Buccioni, Decandia, Minieri, Molle, & Cabiddu, 2012; Wood et al., 1999). However, elevated levels of PUFA in the phospholipid fraction of cell membranes make the meat susceptible to oxidative changes (Luciano et al., 2011a). Oxidative processes shorten the shelf life of fresh meat and negatively affect the consumer acceptance as off-flavor and discoloration develop. Moreover, decreased oxidative stability also influences the meat proteins. Protein oxidation has a negative impact on the nutritive and sensory properties of meat due to oxidation of indispensable amino acids, to their reduced availability and digestibility, and to the reduction of meat tenderness (Lund, Heinonen, Baron, &

Estévez, 2011). Therefore, the oxidizable components of meat need to be protected from damages caused by the reactive oxygen species (ROS). The protection could be provided naturally through the deposition of antioxidant compounds derived from the feeds into the animal tissues. This is in accordance with the observation that the balance between the antioxidant and pro-oxidant components in meat determines the oxidative stability of lipids (Luciano et al., 2013), and correspondingly of proteins (Gravador et al., 2014). Dietary plant secondary metabolites, such as phenolics, saponins and essential oils, were hypothesized to enhance the transfer of the plant-derived PUFA into the ruminant tissues by inhibiting the complete biohydrogenation of the dietary PUFA, thus improving the quality of products (Vasta & Luciano, 2011). Phenolic-rich plant materials or extracts used as dietary supplement or added to meat products exerted antioxidant properties by inhibiting lipid and protein oxidation in meat (Gravador et al., 2014; Inserra et al., 2014; Jongberg, Tørngren, Gunvig, Skibsted, & Lund, 2013; Jongberg, Skov, Tørngren, Skibsted, & Lund, 2011; Nieto, Díaz, Bañón, & Garrido, 2010). Several underutilized feed resources, such as agro-industrial by-products, naturally contain high levels of these compounds. Among these, carob (Ceratonia siliqua) is a plant native to the Mediterranean areas where it is commercially available as a dry pulp, commonly used in animal feeding (Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). In this study, the effects of carob pulp as an alternative feed for growing lambs on the meat fatty acid composition, and, for the first time, on the storage stability of meat color, lipids and proteins under refrigerated and aerobic conditions, were investigated. Previous trials have demonstrated that when carob in lamb diets is equal or superior to 45% (as fed) the animal growth performances are compromised (Vasta et al., 2007; Priolo, Waghorn, Lanza, Biondi, & Pennisi, 2000). Therefore either 24% or 35% carob pulp was used in the diets in the current study in order to assure similar lamb growth performances compared to a conventional barley-based diet.

2. Materials and methods

2.1. Animal management, dietary treatments, slaughter and sampling

91 The trial was conducted at an experimental farm of the University of Catania (Italy). The
92 experimental protocol used was approved by the University of Catania in which the animals were
93 handled by specialized personnel following the European Union Guidelines (2010/63/ EU
94 Directive). The trial involved 26 male Comisana lambs, born in the same farm in November 2011.
95 Lambs were weaned at age 60 days and were given free access to commercial starter concentrate
96 and alfalfa hay until the start of the experiment. At age 90 days (average weight 20.3 kg ± 4.4 kg),
97 the lambs were randomly assigned to three groups, individually penned and fed a total mixed diet
98 according to three dietary treatments: Control (n = 8), Carob 24 (n = 9) or Carob 35 (n = 9). Lambs
99 in the Control treatment were the same used by Inserra et al. (2014) and Gravador et al. (2014). The
100 lambs were adapted to the experimental diets over 10 days, during which the pre-experimental diet
101 was gradually replaced with the experimental diets. Then, for 56 days of age, animals were given
102 the experimental diets. The Control diet included 60% barley, 20% dehydrated alfalfa, 9% soybean
103 meal and 11% wheat bran. In the Carob 24 diet, the percentage of barley was reduced to 33% and
104 24% carob pulp was included, while in the Carob 35 diet the 60% barley was reduced to 23% and
105 35% carob pulp was included. To balance the three diets for protein level, the amount of soybean
106 meal was increased with increasing proportion of carob pulp among the ingredients. No vitamin and
107 minerals were added to the diets. The ingredients and the chemical composition of the experimental
108 diets are reported in Table 1. All the ingredients were ground using feed mill to pass a 5-mm screen
109 mesh and thoroughly mixed. Over the duration of the trial, the lambs had access to the diets from
110 09.00 h to 18.00 h and fresh water was always available. Refusals were removed and weighed daily
111 to determine the voluntary dry matter intake (DMI). The live weight of each lamb was recorded
112 weekly at 09.00 h, before supplying feeds to calculate the average daily weight gain (ADG).
113 Samples of the feeds offered were collected 4 times during the trial, vacuum-packed and stored at
114 30 C for analyses. The lambs were slaughtered at age 158 days in a commercial abattoir, where they
115 had access to the experimental diets and water until approximately 15 min before slaughter. The
116 lambs were stunned by captive bolt and exsanguinated. The carcasses were weighed and halved,

and the left longissimus dorsi muscle (LD) from the 10th to 13th rib was excised within 20 min after the slaughter and was immediately vacuum packed and stored at 30 C until the analyses of fatty acid composition. The right half of the carcass was stored at 4 C for 24 h, after which LD was excised and pH was measured (pH meter Orion 9106). As it was not possible to keep the carcasses refrigerated at the slaughterhouse for more that 24 h, the LD was vacuum packed and stored for 4 days at 4 C to simulate meat ageing before the analyses of meat oxidative stability, described below.

2.2. Chemicals

All chemicals used were reagent grade, and water was deionized and filtered by the Milli-Q method.

2.3. Analyses of feed samples

All the analyses on feeds were performed in the pooled feed samples collected throughout the trial period. The methods of Van Soest, Robertson, and Lewis (1991) were used for the neutral detergent fiber and acid detergent fiber measurements, while the AOAC (1995) methods were used for the analyses of crude protein (CP; method 984.13) and crude fat (Ether extract; method 935.38). For the analysis of total phenols and tannins in the feeds, samples were first extracted following the method described by Makkar, Blümmel, Borowy, and Becker (1993) with minor modifications. Briefly, 200 mg of finely ground feeds was extracted with 5 ml of diethyl ether containing 1% acetic acid to remove the pigments and the supernate was discarded. For the extraction of total phenolic compounds, 10 ml of 70% (v/v) acetone were added and samples were subjected to ultrasonic treatment for 30 min in a cold water bath. Samples were then extracted for 2 h using a rotating device and then centrifuged at 2500 g for 10 min at 4 C. The supernate was collected for subsequent analyses. The above extraction procedure was repeated. The residue from the acetone extraction was further extracted using a modification of the method described by Silanikove et al.

(2006). Briefly, 9 ml of citrate–phosphate buffer containing 0.5 mg/ml of urea (pH 4.7) was added to the residue, and samples were incubated at 90 C for 2 h. The clear supernate was collected following centrifugation at 2500 g for 20 min. In all the above extracts, total phenols were determined using the Folin–Ciocalteu reagent, and the total tannins were measured after the removal of the tannins using polyvinylpyrrolidone (Makkar et al., 1993). The concentration of total phenols and total tannins in feeds was calculated as the sum of the concentration measured in each extract. A calibration curve using standard solutions of tannic acid (TA) was plotted and results were expressed as g of TA equivalents/kg of feed (on a dry matter basis).

2.4. Fatty acid analyses of feed and muscle lipids

The fatty acids in concentrate mixtures were determined using the method of Gray, Rumsby, and Hawke (1967). The total intramuscular lipids in the LD were extracted following the method of Folch, Lees, and Stanley (1957). Briefly, a 5 g portion of the muscle was homogenized twice in chloroform/methanol solution (2:1, vol/vol), filtered, transferred into a separatory funnel, and mixed with saline solution (0.88% KCl). The chloroform lipid fraction was washed with water/methanol solution (1:1, vol/vol), filtered and evaporated through a rotary evaporator. An aliquot of 100 mg lipid extract was methylated with 1 ml hexane and 0.05 ml 2 N methanolic KOH (IUPAC, 1987). Nonanoic acid (C9:0) was used as internal standard. The gas chromatographic analysis was carried

out on a Varian model Star 3400 CX instrument equipped with a CP 88 capillary column (length: 100 m, i.d.: 0.25 mm, film thickness: 0.25 lm). Helium was used as a carrier gas at a flow rate of 0.7 ml/min. The oven temperature was programmed at 140 C and held for 4 min, then increased to 220 C at the rate of 4 C/ min. The split–splitless injector temperature was 220 C and the FID detector temperature was 260 C. The injection rate was 120 ml/min with injection volume of 1 ll. Retention time and area of each peak were computed using the Varian Star 3.4.1 software. The identification of each fatty acid peak was done by comparison of the retention time with the retention times of known mixtures of standard fatty acids (37 component FAME mix, 18919-1 AMP, Supelco,

166 Bellefonte, PA) run under the same operating conditions. Fatty acids were expressed as percentage
167 of total fatty acids.

168 *2.5. Meat oxidative stability measurements*

169 After ageing under vacuum, three slices (2 cm thick) were prepared from each LD, using one slice
170 for each of the 3 days storage: day 0 (2 h), day 3, and day 6. The slices were placed on polystyrene
171 trays, overwrapped with a commercial PVC film (declared oxygen transmission rate: 6500
172 cm3/m2/24 h at 23 C) and kept refrigerated at 4 C in the dark. Lipid oxidation, protein oxidation
173 and color descriptors were measured after 2 h (day 0) and, subsequently, after 3 and 6 days of
174 refrigerated storage. Measurements were performed as follows.

175 *2.5.1. Lipid oxidation measurement*

176 The 2-thiobarbituric acid reactive substances (TBARS) were quantified using the method described
177 by Siu and Draper (1978) with some modifications. An aliquot of 2.5 g finely chopped meat
178 trimmed of visible fat and connective tissues was homogenized in a 50 ml centrifuge tube with 12.5
179 ml distilled water using a tissue homogenizer (HeidolphDiax 900, HeidolphElektro GmbH & Co.
180 KG, Kelheim, Germany) for 2 min at 9500 rpm while immersed in an ice bath. An aliquot of 12.5
181 ml 10% (w/v) trichloroacetic acid (TCA) was added and mixed thoroughly using a vortex mixer,
182 and subsequently the sample was filtered through a Whatman No. 541 filter paper. To a 4.0 ml
183 aliquot of the filtrate in screwcapped glass tube, 1.0 ml of 0.06 M thiobarbituric acid solution
184 (TBA) was mixed prior to incubation for 90 min in a water bath at 80 C. Tubes were cooled to room
185 temperature, and the absorbance at 532 nm (UV–Vis spectrophotometer UV-1601, Shimadzu Co.,
186 Milan, Italy) was measured. A calibration curve was plotted using 0 nmol/ml–65.0 nmol/ml 1,1,3,3-
187 tetraethoxypropane (TEP) in 5% TCA.

188 *2.5.2. Protein oxidation measurements*

189 Measurements of myofibrillar protein (MPI) oxidation were performed as described by Gravador et
190 al. (2014). The MPI from the meat samples were isolated and were stored as freeze driedlyophilized
191 extracts at 20 C until analyzed. The MPI powder was used in the following protein oxidation
192 measurements: (1) levels of radicals by electron spin resonance (ESR), (2) oxidation of thiols
193 determined by derivatization with Ellman's reagent, (3) formation of protein cross-links examined
194 by SDS–PAGE, and (4) levels of protein carbonyls determined through derivatization with 2,4-
195 dinitrophenylhydrazine (DNPH). The protein concentration in the lyophilized MPI was quantified
196 using the Pierce BCA (bicinchoninic acid) Protein Kit Assay (Thermo Specific, Pierce
197 Biotechnology Rockford, IL, USA).

198 *2.5.3. Color stability and myoglobin oxidation measurements*

199 At each day of storage, a Minolta CM-2022 spectrophotometer (d/8 geometry; Minolta Co., Ltd.,
200 Osaka, Japan) was used to measure the meat color descriptors lightness (L*), redness (a*),
201 yellowness (b*), Chroma (C*), and Hue angle (H*), as well as the reflectance spectra from 400 to 700
202 nm, which were used to calculate the metmyoglobin percentage (MMb%) as described by
203 Krzywicki (1979). Measurements were done on two different areas on the meat surface and the
204 mean values were calculated. The spectrophotometer was set in the specular components excluded
205 (SCE) mode and the illuminant A and 10 standard observer were used.

206 *2.6. Statistical analyses*

207 The one-way ANOVA test was used to determine the effects of dietary treatment (Diet: Control,
208 Carob 24, or Carob 35) on animal growth performances and intramuscular fatty acid composition.
209 The General Linear Model with repeated measures was used to analyse the data of meat color
210 stability, lipid oxidation (TBARS) and protein oxidation (radical intensity, thiol content and protein
211 carbonyls concentration) over time of refrigerated storage. In the mixed statistical model, the
212 individual lamb was considered as a random effect, while the dietary treatment (Diet: Control,

213 Carob 24, or Carob 35), storage time (Time: 0, 3, or 6 days), and the Diet xTime interaction were
214 considered as fixed effects.

215 The Tukey's test was used for multiple comparisons of the means. All analyses were done using the
216 Minitab Software Version 16 (Minitab Inc., State College, PA).

217 **3. Results**

218 *3.1. Chemical composition of the diets, fatty acid intake and lamb growth performance*

219 The composition of the diets fed to the animals is reported in Table 1. The three diets contained
220 similar crude protein, ether extract and neutral detergent fiber, while the inclusion of carob pulp
221 appeared to increase the acid detergent fiber content. The concentration of total phenols and tannins
222 increased with increasing proportion of carob pulp in the diet. The Carob 24 and Carob 35 diets had
223 higher percentages of stearic acid (SA; C18:0) and of linolenic acid (LNA; C18:3n-3) compared to
224 the Control. With regard to the daily intake of the individual fatty acids, the intake of SA was higher
225 for lambs in the Carob 24 and Carob 35 groups in comparison to the Control treatment (on a dry
226 matter basis: 0.54 and 0.37 vs. 0.10 g/d; $P < 0.01$; data not shown). Furthermore, the daily intake of
227 LNA by lambs from the Control diet (1.13 g/d) was lower as compared to lambs from the Carob 24
228 and Carob 35 groups (2.68 and 2.69 g/d, respectively; $P < 0.01$; data not shown). The dietary
229 treatment did not affect the average daily gain, dry matter intake or feed efficiency of the lambs
230 (Table 2).

231 *3.2. Intramuscular fat content and fatty acid composition*

232 The use of carob pulp as a feed alternative did not affect the total intramuscular fat content of the
233 longissimus dorsi muscle (LD) with a mean value of 3.26 mg/100 g muscle. However, the fatty acid
234 profiles were affected by the inclusion of carob in the diet. The concentration of saturated fatty
235 acids (SFA) in the LD from Control-fed lambs was higher compared to Carob 24 and Carob

35 ($P < 0.01$) treatments. Similarly, the content of monounsaturated fatty acids (MUFA) was higher in the Control-fed lamb muscle than in Carob 24 or in Carob 35 groups ($P < 0.01$). Conversely, the concentration of polyunsaturated fatty acids (PUFA) was lower in the muscle of Control-fed lambs than in the muscle of both the Carob 24 and Carob 35 lambs ($P < 0.01$). Furthermore, the n-6/n-3 PUFA ratio was significantly higher in the muscle of Control lambs than in muscle of Carob 24 and Carob 35 ($P < 0.05$) lambs. On the level of individual fatty acids, the muscle from lambs in the carob pulp-fed lambs had a lower concentration of stearic acid (SA; C18:0) as compared to the Control treatment ($P < 0.01$). The concentrations of linoleic acid (LA; C18:2 n-6) and of alpha-linolenic acid (LNA; C18:3n-3) were affected by the dietary treatments ($P < 0.01$; Table 3). Specifically, higher LA concentrations were found in the muscle of animals in the Carob 24 and Carob 35 groups as compared to the muscle of animals in the Control group ($P < 0.01$). In the same manner, the LNA content in the muscle of animals in the Carob 24 and Carob 35 dietary groups was higher than in the Control ($P < 0.01$). Additionally, the content of LNA in the muscle of Carob 24 animals was lower than in the muscle of Carob 35 animals ($P < 0.05$). The concentration of rumenic acid (C18:2 cis-9 trans-11) was significantly higher in the muscle from lambs fed Carob 24 or Carob 35 diet as compared to the muscle from lambs fed Control diet ($P < 0.01$), while the level of vaccenic acid (C18:1 trans-11) was not affected by the dietary treatment ($P > 0.05$). On the other hand, a markedly higher concentration of stearic acid was found in the LD from lambs in the Control group than in Carob 24 ($P < 0.01$) or in Carob 35 ($P < 0.05$) group. Similarly, significantly higher oleic acid (C18:1 cis-9) concentration was found in muscle from Control-fed lambs in comparison with the carob pulp-fed lambs ($P < 0.01$). Additionally, there was a significantly higher lauric acid (C12:0) content in the muscle of Control lambs than in Carob 35 lambs ($P < 0.05$), similar to what was found for the concentration of heptadecenoic acid (C17:1; $P < 0.05$). The intramuscular content of eicosapentaenoic acid (EPA; C20:5n-3) in the muscle of Carob 24 and Carob 35 lambs was higher compared to the Control treatment ($P < 0.01$). Also, the concentration of

docosahexaenoic acid (DHA; C22:6n-3) in the LD of lambs fed the Carob 35 diet tended to be greater as compared to the Control animals ($P = 0.051$).

3.3. Oxidative stability of meat under aerobic storage conditions

The concentration of TBARS in meat increased for all the dietary treatments with time of storage ($P < 0.01$), with values from 0.16 to 2.04 mg MDA/kg meat (malondialdehyde equivalents). However, the TBARS levels in the meats were not affected by the dietary treatment ($P > 0.05$), nor by the Diet x Time interaction ($P > 0.05$; Table 4).

The stability of myofibrillar proteins toward oxidation during storage was measured by determination of the level of protein radicals by electron spin resonance spectroscopy (ESR), as well as by determination of the concentration of protein thiols, carbonyl compounds and myosin-heavy chains cross-links (MHC-CL). The protein radical signal intensity was affected by the time of storage ($P < 0.05$) and tended to be affected by the dietary treatment ($P = 0.081$). A significant Diet x Time interaction ($P < 0.01$) was found. The radical intensity increased from 3 to 6 days of storage in the MPI from lambs fed the Control diet ($P < 0.05$), while it remained stable in the MPI from lambs fed the carob-supplemented diets (Fig. 1). The thiol concentration decreased significantly from 60.10 to 51.78 nmol thiols/mg proteins from day 0 to day 6 ($P < 0.01$; Table 4), which corresponds to oxidation of about 8 nmol thiols/mg proteins or 14% oxidation, but no significant difference was observed between the different diets. The measurement of thiol concentrations was complemented with the detection of myosin heavy chains cross-links (MHC-CL) in the MPI, but no MHC-CL could be detected in the gels of the MPI of meat from Control and Carob 35 stored for 0 day and 6 days (data not shown). A significant increase in the levels of protein carbonyl compounds from day 0 to day 6 ($P < 0.01$; Table 4) was observed in all the MPI. The increase in protein carbonyl concentrations accounted for 14.7%, 18.6%, 15.4% in Control, Carob 24 and Carob 35 meat samples, respectively. Overall, the dietary treatment did not exert effects on the protein

oxidation parameters (P > 0.05; Table 4). The Diet x Time interaction (Table 4) demonstrated no significant effects on either thiol oxidation or carbonyl formation in the MPI. Regarding meat color stability, the lightness descriptor (L*) showed a tendency to change with time of storage (P = 0.062) and was significantly affected by the dietary treatment (P < 0.05). The meat from Carob 24 was lighter than meat from Carob 35 (P < 0.01), while the meat from Control tended to be lighter (higher L* values) than the meat from Carob 35 (P = 0.06). The redness descriptor (a*) in meat decreased with time of storage (P < 0.01) but was not affected by the diet (P > 0.05). The yellowness (b*) was significantly increased in meat over time of storage (P < 0.01) and was influenced by the dietary treatment (P < 0.01), but no Diet x Time interaction was found (P > 0.05; Table 4). The Carob 35 meat samples were less yellow than Control and Carob 24 (P < 0.01) meat samples. A less saturated (lower C* values) meat color was measured in meat from Carob 35 fed animals compared to Carob 24 and Control-fed animals (P < 0.01), and values overall decreased significantly with storage duration (P < 0.01). Both the hue angle (H*) and metmyoglobin increased significantly with time of storage (P < 0.01), but were not affected by the dietary treatments (P > 0.05).

4. Discussion

The intramuscular fat content was not affected by the dietary treatment, which could be partially explained considering that the diets were formulated to be isonitrogenous and isoenergetic and that the animal growth performances were unaffected by substituting part of the barley in the diet with up to 35% carob pulp. It has been reported that the inclusion of carob pulp into a concentrate-based diet at higher levels than those used in the present experiment (45–56%) can compromise the growth performances of lambs and kids, resulting in the reduction of the intramuscular fat content (Priolo et al., 2000; Silanikove et al., 2006; Vasta et al., 2007).

The intramuscular fatty acid composition was clearly affected by the inclusion of carob in the diet, whereby higher concentrations of PUFA, lower concentrations of SFA and a lower (more beneficial) ratio n-6/n-3 PUFA were found in the muscle from the carob-fed lambs as compared to the Control group. Some of these results could be explained considering that the dietary treatment

affected the intake of fatty acids of the lambs. For example, the inclusion of carob pulp in the diet led to a higher daily intake of ALA in comparison to the Control diet. This may explain the higher concentrations of long chain n-3 PUFA (EPA and DHA) in the muscle from lambs in both the Carob 24 and Carob 35 groups as compared to the Control group, as the synthesis of these fatty acids in animal tissues requires ALA as a substrate (Wood et al., 1999). In ruminants, the deposition of some fatty acids in the muscle depends not only on the intake of the different fatty acids but also on the extent of the ruminal biohydrogenation of the ingested PUFA. In the present study, a possible effect of dietary carob pulp on the ruminal biohydrogenation could contribute to explaining some of the observed results. For example, although the carob-supplemented diets provided a higher daily intake of stearic acid than the Control diet, the muscle of lambs supplemented with carob pulp had a lower stearic acid content. Considering that stearic acid is the final product of the ruminal biohydrogenation, this result suggests that this process might have been inhibited to some extent in the rumen of animals fed carob pulp-containing diets. This is in agreement with the results found by Vasta et al. (2007), where meat from animals fed a carob-containing diet had less stearic acid compared to a control dietary treatment. A further indication of the possible effect of dietary carob pulp upon ruminal biohydrogenation was given by the results obtained with rumenic acid in muscle, which was increased by feeding carob. The Butyrivibrio fibrisolvens in the rumen is responsible for the isomerization of LA to rumenic acid, which is an intermediate of the biohydrogenation whose final product is stearic acid (Vasta et al., 2010). Therefore, it could be speculated that dietary carob inhibited to some extent the complete ruminal biohydrogenation pathway, leading to the accumulation of rumenic acid. Another intermediate of the ruminal biohydrogenation, produced by the same microorganisms, is vaccenic acid (C18:1 trans-11). If the

last step of ruminal biohydrogenation was inhibited by carob pulp in the diet, the muscle from Carob 24 and Carob 35-fed animals would have been expected to possess a higher vaccenic acid concentration than the Control treatment, which was not the case in the present study. However, it should be stressed that, while the vaccenic acid in the muscle originates solely from its synthesis in the rumen during the biohydrogenation, rumenic acid can be also synthesized endogenously in the muscle from vaccenic acid through a reaction catalyzed by the D9-desaturase enzyme (Bessa et al., 2007; Vasta et al., 2007). Taken together, these results lead to suppose that: (1) the conversion of rumenic acid to vaccenic acid was slowed down, or (2) the vaccenic acid was converted back to rumenic acid more efficiently in the muscle from carob-fed lambs. Considering that in the rumen, the bacteria that convert LA to rumenic acid are also responsible for the further conversion of rumenic acid to vaccenic acid, the second hypothesis appears to be more likely. A possible effect of dietary carob pulp on the ruminal biohydrogenation of PUFA could be justified taking into account that carob has been reported to contain phenolic compounds, including condensed tannins (Vasta, Makkar, Mele, & Priolo, 2009; Vasta et al., 2007; Silanikove et al., 2006), which is in agreement with the fact that, in our study, the content of total phenols and tannins in the diets increased with increasing proportion of carob among the ingredients of the diet. Several results from in vivo and in vitro studies provided evidence of the inhibitory effect of purified tannins and of tannin-rich plant extracts upon the ruminal biohydrogenation, which leads to the accumulation of PUFA in animal tissues (Vasta & Luciano, 2011). Regarding the results of meat oxidative stability, it was observed that the secondary products of lipid oxidation in meat, measured as TBARS values, increased with time of storage but did not reach values (about 4 mg MDA/kg of meat) that were reported to indicate a perceivable rancidity in meat (Soldatou, Nerantzaki, Kontominas, & Savvaidis, 2009). It has been reported that aldehydes derived from lipid peroxidation, such as 4-hydroxynonenal and malondialdehyde (MDA), can cross-link with proteins and phospholipids and this covalent bond formed limits their quantification, resulting in the underestimation of the actual amount (Zhao, Chen, Zhu, & Xiong, 2012). This could be a reason for the low TBARS values measured across the

storage period. Increasing the concentration of PUFA in the muscle, which in the case of the present study was obtained by feeding lambs with carob pulp, can be associated to a reduced oxidative stability of meat lipids (Luciano et al., 2013, 2011a). However, the results of the current study showed that the extent of lipid oxidation was not affected by the dietary treatment. It has been often reported that dietary phenolic compounds, including tannins, can confer on the meat a superior resistance to oxidative deterioration, although the exact mechanisms of action have not yet been fully elucidated (Vasta & Luciano, 2011). Jerónimo et al. (2012) hypothesized that condensed tannins could indirectly improve meat oxidative stability through the interaction with other antioxidant compounds present in muscle. Polyphenol rich plant extract incorporated in the basal diet of rats enriched with n-3 PUFA showed the ability to recycle or spare the vitamin E, and to enhance the activity of antioxidant enzymes, such as catalase (Gladine et al., 2007). Moreover, the oxidative stability of broiler meat was also improved through dietary supplementation of phenolics which resulted in the enhancement of cellular antioxidant enzyme activity (Delles, Xiong, True, Ao, & Dawson, 2014). It could be speculated that, in the present study, the phenolic antioxidant compounds contained in carob pulp might have exerted antioxidant effects that resulted in an increased resistance of meat to oxidation. Therefore, despite the higher levels of oxidizable PUFA consequent to carob feeding, oxidative damages in meat from carob-fed animals could have been minimized, being comparable to the meat from the Control-fed animals with a lower content of PUFA. With regards to protein oxidation, a possible antioxidant effect of carob pulp in the diet could explain the observation that the protein radical intensity increased after 6 days of storage in the MPI from Control-fed lambs, but neither the Carob 24 nor Carob 35 treatments showed considerable increase in radical signal intensity. The loss of thiols observed in this study across time of storage (ca. 10 nmol/mg protein) was smaller compared to other studies (Nieto, Jongberg, Andersen, & Skibsted, 2013; Zakrys- Waliwander, O'Sullivan, O'Neill, & Kerry, 2012). The level of thiol oxidation is linked to the formation of disulfide cross-links in the myosin heavy chains

(MHC-CL; Jongberg et al., 2013; Zakrys- Waliwander et al., 2012; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007), and in this study no MHC-CL was observed in the MPI of muscle from both Carob-fed and Control-fed lambs, which agrees with the low degree of oxidation of thiols. The MHC-CL is usually attributed to the formation of reducible disulfide bonds by oxidation of cysteine residues resulting in reduced meat tenderness (Zakrys-Waliwander et al., 2012; Lund et al., 2011, 2007). The carbonyl residues, on the other hand, are formed through metal-catalyzed reactions involving the side chains of some amino acids like arginine, lysine and proline (Lund et al., 2011). In this study the carbonyl concentration in meat was 2.09 nmol/mg protein after 6 days. This carbonyl concentration is lower than what was obtained by Santé-Lhoutellier, Engel, Aubry, and Gatellier (2008), who reported an initial concentration of carbonyls in lamb meat of about 2.0 nmol carbonyls/mg proteins, which increased by 31.4% and 13.0% in aerobically stored meat from animals fed concentrates or pasture, respectively. The oxidation of myoglobin to metmyoglobin, which causes browning in meat, has been found to be delayed by the dietary supplementation of antioxidant compounds or by feeding on pasture (Nieto et al., 2010; Luciano et al., 2009). In terms of meat color stability, the oxidation of myoglobin over time of storage or display is generally associated with a corresponding decrease in the redness and saturation color parameters (a^* and C^* values, respectively) and with increases of the hue angle (H^*) descriptor (Luciano et al., 2011b; Khliji, Van de Ven, Lamb, Lanza, & Hopkins, 2010). This is in agreement with the results found in the present study which showed that, within 6 days of storage, the a^* and C^* values both decreased while the H^* values increased. The meat from Control fed lambs showed a tendency to be more red (higher a^* values), which might partially account for the lower C^* values measured in meat from Carob 35-fed lambs, as the C^* descriptor results from a combination of the a^* and b^* coordinates. Overall, the results of all the meat oxidative stability parameters measured in the present study indicate that meat underwent oxidative deterioration at a rather low rate, which is evident the current results are compared with those obtained in studies in which meat was subjected to higher oxidative challenges during the monitoring period, such as mincing and/or high-oxygen

411 modified atmosphere packaging (HiOx-MAP). Aerobic packaging, as used in the present study,
412 only contains about 20% O2, while HiOx-MAP contains at least 70% O2, thus enhancing the
413 reactions of oxygen with the muscle components. Mincing increases the release of pro-oxidative
414 compounds and impairs the antioxidant defenses of muscle by disruption of cell membranes and
415 depletion of antioxidant compounds (Luciano et al., 2009). Refrigerated raw minced lamb meat
416 packed in HiOx-MAP had levels of TBARS in the range 0.50–18.00 mg MDA/kg meat (Luciano et
417 al., 2009), while raw whole slice of LD in HiOx-MAP had about 0.02–8.43 mg MDA/ kg meat
418 (Nieto et al., 2010) over 14 days of storage. In the present study, the range of TBARS values
419 measured (0.16–2.04 mg MDA/kg meat) indicated that lipid oxidation proceeded at a slow rate. A
420 similar conclusion can be reached based on the results of protein oxidation found in the present
421 study. Beef held in Hi-Ox-MAP for 9 days with antioxidants had 3.3–3.7 nmol carbonyls/mg
422 proteins, while without antioxidants the levels were 4.0–4.8 nmol carbonyls/ mg proteins (Jongberg
423 et al., 2011), which are all higher than the values of 2.09 nmol/mg protein found in the present
424 study. In addition, MHC-CL readily formed in meats in HiOx-MAP stored for up to 14 days such as
425 pork patties (Nieto et al., 2013), pork and beef slices (Zakrys-Waliwander et al., 2012; Lund et al.,
426 2007), or beef patties (Jongberg et al., 2011), than similar meat stored in either vacuum or aerobic
427 package, as was the case of our study. Similarly, for the color stability parameters, pronounced
428 increases in H′ values and metmyoglobin percentages (from 16 to 70 and from 30% to 65%,
429 respectively) were reported over time of storage of minced lamb meat in HiOx-MAP (Luciano et
430 al., 2009). The increase in H′ values and MMb% in this study were lower than the above values,
431 which demonstrates that meat samples did not undergo severe meat browning.

432 **5. Conclusions**

433 This study demonstrated that including carob pulp up to 35% into a concentrate-based diet could be
434 a feasible strategy to improve lamb meat quality traits without compromising animal productivity.
435 Indeed, meat from lambs fed concentrates containing 24% or 35% carob pulp had a higher

concentration of PUFA, of the beneficial n-3 PUFA, such as LNA, EPA and DHA, of rumenic acid and a lower n-6/n-3 PUFA ratio as compared to meat from lambs fed conventional concentrates. Some of these results could be explained considering that carob pulp increased the intake of essential PUFA, such as LA and ALA. Furthermore, carob pulp could have reduced the ruminal biohydrogenation of PUFA to some extent, leading to a higher deposition of PUFA in the muscle. The increased concentration of PUFA in meat from lambs fed carob pulp did not compromise the oxidative stability of meat over storage in aerobic and refrigerated conditions for 6 days, as the extent of lipid and protein oxidation reactions and of color deterioration were unaffected by the dietary treatments. These results could partially be explained by the action of antioxidant phenolic compounds in the carob pulp.

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Table 1
Ingredients and chemical composition of the diets.

	Diets		
	Control	Carob 24	Carob 35
Ingredient[a]			
Barley	60	33	23
Carob pulp	0	24	35
Dehydrated alfalfa	20	20	17
Soybean meal	9	13	16
Wheat bran	11	10	9
Chemical composition[b]			
Dry matter (DM) (g/kg of fresh weight)	889	882	878
Crude protein (g/kg of DM)	180	196	192
Ether extract (g/kg of DM)	20	33	22
Neutral detergent fiber (g/kg of DM)	346	344	346
Acid detergent fiber (g/kg of DM)	137	180	227
Total phenols (g/kg of DM)	8.6	14.2	16.6
Total tannins (g/kg of DM)	1.6	3.4	4.5
Fatty acid composition (g/100g of total fatty acids)			
C12:0	0.61	0.10	0.23
C14:0	0.31	0.87	1.03
C16:0	22.22	17.82	17.75
C16:1	0.60	0.51	0.32
C18:0	1.27	4.17	3.05
C18:1 *cis*-9	14.62	13.12	11.81
C18:2 *cis*-9 *cis*-12	44.20	39.83	40.96
C18:3 *cis*-9 *cis*-12 *cis*-15	13.8	20.75	22.23

[a] Ingredient percentages are expressed on an as-fed basis.
[b] Results are the average of triplicate analyses performed on one sample pooled from sub-samples collected weekly.

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Table 2
Effect of the dietary treatment on lamb growth rate, feed intake and efficiency and ultimate pH of *longissimus dorsi* muscle.

	Dietary treatment (Diet)			SEM	*P*-value
	Control	Carob 24	Carob 35		
No. of lambs	8	9	9	–	–
Body weight at 100 d (kg)	19.3	18.7	18.6	0.72	0.923
Body weight at 157 d (kg)	29.6	29.9	28.3	1.08	0.797
Average daily gain, ADG (100–157 d) (g/d)	181	198	170	8.76	0.418
Dry matter intake, DMI (g/d)	749	843	809	30.60	0.485
Feed efficiency (g body weight gain/kg DMI)	240	235	209	6.86	0.126

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Table 3
Effects of dietary treatment on the intramuscular fat content and fatty acid composition of lamb *longissimus dorsi* muscle.

	Dietary treatment (Diet)			SEM	*P*-value
	Control	Carob 24	Carob 35		
No. of lambs	8	9	9	–	–
Total intramuscular fat, g/100 g of muscle	2.77	3.12	3.88	0.236	0.147
Fatty acid composition (g/100 g of total fatty acids)					
C10:0	0.40	0.38	0.29	0.028	0.210
C12:0	0.79[a]	0.65[ab]	0.51[b]	0.041	0.013
C14:0	4.64	3.78	3.62	0.217	0.129
C14:1 *cis*-9	0.12	0.15	0.17	0.009	0.126
C15:0	0.64	0.52	0.48	0.037	0.203
C15:1	0.19	0.21	0.26	0.020	0.317
C16:0	16.47	15.72	15.42	0.234	0.183
C16:1 *cis*-9	1.09	0.99	0.96	0.039	0.407
C17:0	1.05	0.96	0.92	0.031	0.254
C17:1 *cis*-9	0.40[b]	0.61[ab]	0.71[a]	0.046	0.014
C18:0	14.33[a]	13.00[b]	13.39[b]	0.174	0.003
C18:1 *cis*-9	28.40[a]	26.16[b]	25.00[c]	0.320	<0.001
C18:1 *trans*-11	1.29	1.39	1.47	0.037	0.141
C18:2 *trans*-9 *trans* 12	0.59	0.54	0.51	0.02	0.270
C18:2*n*-6 *cis*-9 *cis*-12	9.20[b]	12.51[a]	12.96[a]	0.418	<0.001
C18:3*n*-6 *cis*-6 *cis*-9 *cis*-12	0.28	0.22	0.33	0.027	0.241
C18:3*n*-3 *cis*-9 *cis*-12 *cis*-15	1.00[c]	2.11[b]	2.60[a]	0.150	<0.001
C18:2 *cis*-9 *trans*-11	0.70[b]	1.21[a]	1.52[a]	0.089	<0.001
C20:2*n*-6	0.23	0.20	0.17	0.015	0.404
C20:3*n*-6	0.47	0.35	0.38	0.024	0.107
C20:3*n*-3	0.14[a]	0.07[b]	0.13[a]	0.006	<0.001
C20:4*n*-6	8.67	8.21	8.49	0.301	0.833
C20:5*n*-3 EPA	0.69[b]	1.39[a]	1.39[a]	0.089	<0.001
C22:5*n*-3 DPA	1.94	1.78	1.76	0.101	0.737
C22:6*n*-3 DHA	0.71	1.15	1.35	0.112	0.058
Other FA	5.57	5.75	5.21	0.196	0.541
ΣSFA[x]	38.32[a]	35.00[b]	34.62[b]	0.471	0.001
ΣMUFA[y]	31.49[a]	29.51[b]	28.57[c]	0.281	<0.001
ΣPUFA[z]	24.62[b]	29.75[a]	31.59[a]	0.682	<0.001
n-6 PUFA	19.44[b]	22.04[a]	22.84[a]	0.453	0.003
n-3 PUFA	4.48[b]	6.50[a]	7.22[a]	0.307	<0.001
n-6/*n*-3	4.39[a]	3.44[b]	3.32[b]	0.162	0.010

[a,b,c]Within row different superscripts indicate differences between dietary treatments ($P < 0.05$) tested using the Tukey's adjustment for multiple comparisons.

[x] ΣSFA = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0.

[y] ΣMUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1 *trans-11* + C18:1 *cis*-9.

[z] ΣPUFA = C18:2 *cis*-9 *trans*-11 + C18:2 *trans*-9 *cis*-12 + C18:2 *cis*-9 *cis*-12 + C18:3 *cis*-6 *cis*-9 *cis*-12 + C18:3 *cis*-9 *cis*-12 *cis*-15 + C20:2*n*-6 + C20:3*n*-3 + C20:4*n*-6 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3.

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Table 4
Effect of the dietary treatment and time of refrigerated storage of lamb meat on lipid oxidation, protein oxidation, and color parameters.

	Dietary treatment (Diet)			Days of storage (Time)			SEM	*P* values		
	Control	Carob 24	Carob 35	0	3	6		Diet	Time	Diet × Time
No. of lambs	8	9	9							
TBARS (mg MDA/kg meat)	1.15	1.32	1.20	0.16^{z}	1.44^{y}	2.04^{x}	0.127	0.857	<0.001	0.876
Free thiol (nmol/mg proteins)	57.73	55.60	56.80	60.10^{x}	58.12^{x}	51.78^{y}	0.643	0.290	<0.001	0.433
Carbonyl (nmol/mg proteins)	1.77	1.95	1.80	1.60^{y}	–	2.09^{x}	0.089	0.701	0.007	0.630
Lightness (L*)	48.52^{ab}	48.98^{a}	46.47^{b}	48.56	47.66	47.26	0.310	0.025	0.062	0.160
Redness (a*)	15.82	15.74	14.49	17.52^{x}	14.94^{y}	13.71^{y}	0.284	0.132	<0.001	0.912
Yellowness (b*)	13.16^{a}	13.23^{a}	11.43^{b}	11.65^{y}	13.00^{x}	13.10^{x}	0.207	0.003	<0.001	0.100
Saturation (C*)	20.65^{a}	20.65^{a}	18.52^{b}	21.05^{x}	19.82^{xy}	18.99^{y}	0.297	0.024	0.001	0.500
Hue angle (H*)	39.98^{ab}	40.13^{a}	38.44^{b}	33.46^{z}	41.07^{y}	43.70^{x}	0.549	0.133	<0.001	0.006
Metmyoglobin	44.82	47.05	44.30	35.33^{z}	48.25^{y}	52.53^{x}	0.013	0.155	<0.001	0.507

^{a,b}Within row different superscripts indicate differences between dietary treatments ($P < 0.05$) tested using the Tukey's adjustment for multiple comparisons.
^{x,y}Within row different superscripts indicate differences between days of storage ($P < 0.05$) tested using the Tukey's adjustment for multiple comparisons.

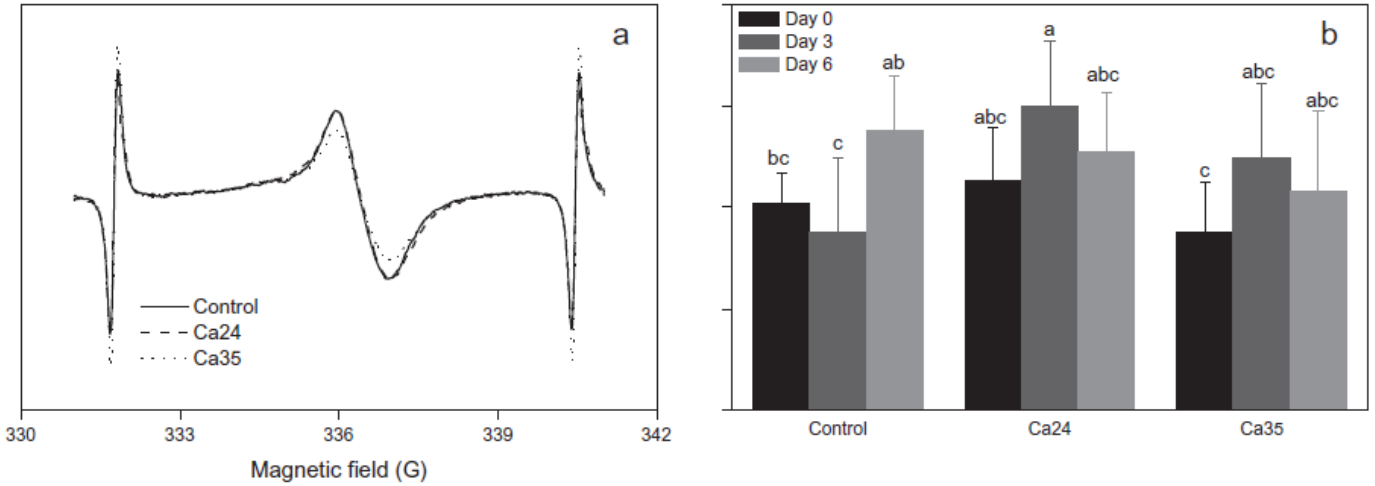

Fig. 1. Electron spin resonance (ESR) spectra (a) and radical signal intensity (RI) (b) of myofibrillar protein isolates (MPI) extracted from lamb meat. MPI samples were stored for 0, 3 and 6 days in the dark at 4 °C on polystyrene trays wrapped with oxygen-permeable PVC film. The lambs were fed with diets consisting of cereal-based concentrates (Control), or concentrates containing 24% or 35% carob pulp (Ca24 and Ca35, respectively). Values presented are means plus standard errors. Values with different superscripts are significantly different ($P < 0.05$).