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Effects of linseed oil and natural or synthetic vitamin E supplementation in lactating ewes' diets on meat fatty acid profile and lipid oxidation from their milk fed lambs

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1 # Effects of linseed oil and natural or synthetic vitamin E supplementation of lactating ewe diets on fatty acid profile and lipid oxidation of suckling lambs meat

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17 **ABSTRACT**

18 The objective of this study was to evaluate the effects of dietary linseed oil and vitamin
19 E, synthetic or natural, on the lipid composition of ewe milk, intramuscular suckling
20 lamb fat and colour and lipid oxidation of lamb meat. Forty-eight Churra ewes with
21 their new-born lambs were separated into four groups and each group assigned to one of
22 the four dietary treatments. The dietary treatments were: Control (without added fat),
23 LO (with 3% linseed oil), LO-Syn E (LO plus 400 mg/kg TMR of synthetic vitamin E)
24 and LO-Nat E (LO plus 400 mg/kg TMR of natural vitamin E). All lambs were reared
25 exclusively on milk, slaughtered when they reached 12 kg live weight and samples from
26 the *Longissimus dorsi* muscle were taken. Milk yield and protein percentage were not
27 affected by diets containing only linseed oil compared to Control, whereas the milk fat
28 percentages increased in dairy ewes fed linseed oil plus vitamin E. Milk from LO, LO-
29 Syn E and LO-Nat E treatments had lower percentages of saturated fatty acids (FAs) but
30 higher percentages of monounsaturated FAs and polyunsaturated FAs (PUFAs) than
31 ewes fed the Control diet. Linseed oil supplementation caused an increase in *trans*-11
32 C18:1 (vaccenic acid, VA), *trans*-10 C18:1, *cis*-9, *trans*-11 C18:2 (rumenic acid, RA),
33 *trans*-10, *cis*-12 C18.2 and C18:3 n-3 (α-linolenic acid, ALA) in milk fat compared to
34 the Control. The addition of vitamin E to the LO diets did not influence significantly the
35 majority of milk fatty acids compared with the LO diet alone. The LO-Syn E treatment
36 resulted in higher percentages of RA and *trans*-10, *cis*-12 C18:2 than the LO-Nat E and
37 Control treatments. The FA patterns of suckling lamb meat were similar to milk from
38 their respective dams, without affecting lamb performance. *Trans*-10 C18:1, VA, RA,
39 trans-10, *cis*-12 C18:2 and ALA levels were higher in intramuscular lamb fat from
40 treatments with linseed oil, however, no statistically significant differences were
41 observed in these FA due to vitamin E supplementation or the type of supplemented
42 vitamin E (synthetic vs. natural). Supplementing ewe diets with vitamin E (LO vs. LO-
43 Syn E and LO-Nat E) increased the vitamin E content in both milk and meat.
44 Treatments with vitamin E (LO-Syn E and LO-Nat E) kept the value of the lipid
45 oxidation constant below the acceptability threshold for lamb meat. In conclusion, the
46 use of linseed oil in lactating ewe diets increased the content of healthy FA, like VA,
47 RA and ALA in milk and meat, and even though vitamin E supplementation only had a
48 limited effect on milk and meat fatty acid profiles, it clearly affected colour and lipid
49 oxidation of suckling lamb meat.

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51 *Keywords:* suckling lamb; fatty acid; milk, meat, linseed oil; vitamin E

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53 **1. Introduction**

54 In recent years, there has been a growing interest in identifying strategies to enhance
55 the concentration of healthy fatty acids in ruminant foods (meat and milk), such as
56 conjugated linoleic acid (CLA) and n-3 polyunsaturated fatty acids (PUFAs). Till now
57 the dietary inclusion of PUFA-rich lipids has been the most commonly investigated
58 nutritional strategy (Raes, De Smet & Demeyer, 2004; Wood et al., 2008).

59

60 Current research has been focused on improving the fatty acid profile of suckling
61 lamb meat, owing to its importance as a traditionally consumed food in European
62 Mediterranean regions. Suckling lambs, covered by a protected geographical indication
63 (PGI), are reared with their dams, fed exclusively on maternal milk and slaughtered
64 after a suckling period of 30-35 days. As suckling lambs are considered to be functional
65 nonruminants, maternal milk enrichment with health-promoting FAs by supplementing
66 ewe diets with fat from appropriate sources could be a good strategy for naturally
67 enhancing these FA levels in suckling lamb meat (Manso, Bodas, Vieira, Mantecon &
68 Castro, 2011). In this regard, vegetable oil supplementation has been used in order to
69 increase rumenic acid (RA) and PUFA n-3. However, increases in dietary PUFA intake
70 appear to affect the rumen environment and thus, the biohydrogenation pathways of
71 linoleic and linolenic acid (ALA) This results in a shift in intermediate FAs
72 characterized by an increased formation of *trans*-10, *cis*-12 C18:2 and *trans*-10 C18:1
73 instead of *cis*-9, *trans*-11 C18:2 and *trans*-11 C18:1 (Shingfield, Bernard, Leroux &
74 Chilliard, 2010). *Trans*-10, *cis*-12 CLA has possible detrimental effects on human
75 health and has been shown to decrease the mammary synthesis of *de novo* FAs and
76 induce milk fat depression (Toral et al., 2010a). In contrast, *cis*-9, *trans*-11 CLA is more
77 desirable because of its anticarcinogenic and other health-promoting properties (Lock,
78 Kraft, Rice & Bauman, 2009).

79

80 Some studies have indicated a possible role for high doses of vitamin E in preventing
81 shifts in PUFA biohydrogenation pathways (Pottier et al., 2006; Juárez et al., 2011),
82 thus minimizing any negative effect of plant oil on milk production, milk fat yield
83 and/or milk fatty acid composition. Vitamin E could act either as an inhibitor of bacteria

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84 producing *trans*-10 C18:1 or as an electron acceptor for *Butyrivibrio fibrisolvens*
85 (Pottier et al., 2006). Hou, Wang, Wang & Liu (2013) have reported that vitamin E
86 could affect CLA content and the accumulation of biohydrogenation intermediates in
87 rumen fluid.

88

89 On the other hand, it is well known that increasing the content of unsaturated fatty
90 acids in muscle cell membranes increases their susceptibility to oxidation (Wood et al.,
91 2004). Therefore, the addition of antioxidants to animal diets has emerged as a strategy
92 for increasing the commercial value of meat, and one of the most widely used
93 antioxidants in this regard is vitamin E. Vitamin E supplementation of lamb and ewe
94 diets (Capper et al., 2005; Ripoll, Joy & Muñoz, 2011; Kasapidou et al., 2012) is
95 usually carried out by using a synthetic source of α-tocopherol (all-rac-α-tocopheryl-
96 acetate), due to its stability and lower cost in animal feeds (Vagni, Saccone, Pinotti &
97 Baldi, 2011). However, the use of natural solutions to minimize oxidative rancidity and
98 increase meat shelf-life is of growing interest due to consumer demand for natural
99 products and their willingness to pay a price premium for natural foods. In view of the
100 foregoing, another vitamin E source to consider is natural vitamin E (RRR-α-
101 tochopheryl-acetate) which is derived from vegetable oils and exhibits higher biological
102 activity than synthetic vitamin E (Lauridsen, Engel, Craig & Traber, 2002). Recent
103 studies in dairy cows have estimated that the relative bioavailability of vitamin E from
104 natural sources is 1.36 times greater than that of synthetic vitamin E (Weiss, Hogan &
105 Wyatt, 2009).

106

107 We can therefore hypothesize that dietary supplementation with polyunsaturated oils
108 could improve the fatty acid profiles of milk and suckling lamb meat. On the other
109 hand, vitamin E could affect CLA content and the accumulation of biohydrogenation
110 intermediates in milk and suckling lamb meat and prevent the adverse effects of PUFAs
111 on milk fat content, fatty acid profiles and oxidative stability of suckling lamb meat.

112

113 This study was undertaken because there are no specific studies comparing the
114 effects of supplementing ewe diets enriched in polyunsaturated fatty acids with different

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115 sources of vitamin E on milk and suckling lamb meat. The aim of this work was to
116 determine the effects of including linseed oil and vitamin E (natural or synthetic) in
117 early lactating ewe diets on the meat quality of their suckling lambs, with particular
118 reference to their fatty acid composition, and vitamin E content. An evaluation of the
119 colour and lipid oxidation of lamb meat stored under refrigerated display conditions
120 relative to maternal feeding was also conducted.

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123 **2. Material and methods**

124 *2.1. Animal and experimental diets*

125 The study was carried out with forty-eight pregnant Churra ewes (BW 63.6 ± 9.17
126 kg). The ewes were selected before lambing and fed on the same basal diet that they
127 would receive during the experimental period. Two days after lambing, each ewe, on the
128 basis of milk production, age, initial BW, prolificacy and parity in randomisation, was
129 assigned to one of four dietary treatments (12 ewes per treatment).

130 The experimental diets consisted of a total mixed ration (TMR) that varied according
131 to the inclusion of linseed oil (LO) and the type of vitamin E (synthetic or natural). The
132 four dietary treatments were: Control (without linseed oil), LO (with 3% linseed oil),
133 LO + Syn E (LO plus 400 mg/kg TMR of synthetic vitamin E) and LO + Nat E (LO
134 plus 400 mg/kg TMR of natural vitamin E). The ingredients and chemical composition
135 of the experimental diets are given in Table 1.

136 The experimental diets were fed ad libitum to each ewe during the whole
137 experimental period and fresh drinking water was always available. Diets were supplied
138 twice a day with forage and concentrate at a 45:55 ratio. The amount of diets offered
139 and of refusals were weighed daily and samples were collected for subsequent analyses.

140 The newborn lambs (12 lambs per treatment), covered by the protected geographical
141 indication (PGI) 'Lechazo de Castilla y León', were housed with their respective
142 mothers all day long and were fed exclusively by suckling throughout the whole
143 experimental period (27 ± 2.7 days). All animal handling practices followed the
144 Directive 2010/63/EU of the European Parliament and of the Council on the protection
145 of animals used for scientific purposes.

146

147 *2.2. Milk sampling and composition*

148 The ewes were milked once a day in a 2 x 24 low-line Casse system milking parlour,
149 with twelve milking units and two milkers. The milking machines (Alfa-Laval Iberia,
150 S.A., Madrid, Spain) were set to provide 180 pulsations per minute with a 50:50 ratio at
151 a vacuum level of 36 kPa. Milk production was recorded once a week by the oxytocine
152 technique: before milking each ewe was injected with 0.35 cc of oxytocin (Oxiton®,

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153 Laboratorios Ovejeros, S.A., Spain) and then immediately milked. Ewes were returned
154 to their paddock between the two milking sessions while the lambs were confined. Milk
155 samples were taken in milk collection jars. One sub-sample of milk was kept at 4ºC
156 until analysed for fat and protein, according to the International Dairy Federation (IDF,
157 2000), using a MilkoScan-400 analyser (Foss Electric, Hillerød, Denmark). Another
158 two sub-samples were stored at -80ºC for subsequent analysis of fatty acid and α-
159 tocopherol concentrations.

160

161 *2.3. Slaugther procedure, carcass and meat measurements*

162 Lambs were weighed twice a week until they reached the slaughter live weight
163 (approximately 12 kg). Then lambs were taken to a commercial EU-licensed abattoir,
164 stunned and slaughtered by section of the jugular vein in the neck. After slaughter, the
165 skin and all internal organs were removed and carcasses were immediately weighed (hot
166 carcass weight, HCW) and transferred to a cooler at 4ºC. After 24 hours, carcasses were
167 weighed again (cold carcass weight, CCW), and chilling losses were calculated as the
168 difference between HCW and CCW expressed as a proportion of the initial HCW.
169 Dressing percentage was calculated as the ratio of CCW to slaughter live weight. Two
170 samples of *m. Longissimus dorsi* (dissected between the $6^{th}$ and the $13^{th}$ rib) were stored
171 at -80ºC, one for fatty acid composition analyses and the other for α-tocopherol level
172 determination.

173

174 *2.4. Chemical analysis*

175 The chemical composition of the TMR was determined using the procedures
176 described by the AOAC (2003).

177 The chemical composition of meat was determined on m. *Longissimus dorsi* samples,
178 which were analysed for dry matter (AOAC official method 950.46), ash (AOAC
179 official method 920.153) and crude protein (AOAC official method 981.10).

180 Fatty acid composition of fat from milk and muscle samples was determined by gas
181 chromatography (GC Turbo 3400 *CX*, Varian Inc., Palo Alto, CA). Fat was extracted
182 from milk and meat by using the method described by Nudda et al. (2008). The
183 *Longissimus dorsi* samples from the left half of the carcass were lyophilized and finely

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184 ground before fat extraction. About 20 mg of extracted lipids were added with 1 ml of
185 hexane containing nonadecanoic acid (C19:0) methyl ester (Sigma-Aldrich Inc., St.
186 Louis, MO, USA) as internal standard (0.5 mg/ml). The mixture was esterified by base-
187 catalyzed methylation using 500 µl of sodium methoxide in methanol (Sigma-Aldrich
188 Inc., St. Louis, MO, USA) according to the standard FIL-IDF procedure (FIL-IDF,
189 1999). The FAME was separated in a capillary column (CP-select CB for FAME; 100
190 m × 0.32 mm i.d., 0.25 µm film thickness, Varian Inc., Palo Alto, CA) and quantified
191 using the internal standard. The injector and FID (flame-ionization detector)
192 temperatures were 255 °C. For all samples the temperature programme was as follows:
193 75 °C for 1 min, increased by 8 °C/min to 165 °C, held for 35 min, increased by 5.5
194 °C/min to 210 °C, held for 1 min, and finally increased by 15 °C/min to 240 °C and held
195 for 15 min. The split ratio was 1:40 and helium was the carrier gas with a pressure of 37
196 psi. The relative amount of each fatty acid (% of total FAME) is reported as a percentage of
197 the total peak area for all fatty acids.

198 The *Longissimus dorsi* muscle from the right half of the suckling lamb carcass, was
199 dissected and used to assess the changes in meat colour and fat oxidation (thiobarbituric
200 acid reactive substances; TBARS). Meat samples were stored under refrigerated display
201 conditions (4ºC and fluorescent light) until they were analysed. The *m. Longissimus*
202 *dorsi* was divided into slices (25 mm thick), stored in polyethylene trays covered by an
203 oxygen-permeable PVC film and randomly assigned to one of the storage periods: 0, 5,
204 9 and 12 days. After each storage period, colour was measured in three different
205 locations on the top cut muscular surfaces of the slices, according to the CIE *L*a*b**
206 space using a portable spectrophotometer Minolta CM-2002 (Konica-Minolta Sensing,
207 Japan). The extent of lipid oxidation was assessed (in duplicate), using the method of
208 Maraschiello, Sarraga & Garcia Reguiero, 1999).

209 For vitamin E analysis in ewe milk, vitamin E was extracted from the milk using a
210 method adapted from the procedure of Czauderna & Kowalczyk (2007), and for lamb
211 meat using the method of Sampels, Pickova & Wiklund (2004). Subsequently,
212 separation of vitamin E was carried out by HPLC (Rodas Mendoza, Morera Pons,
213 Castellote Bargalló & López-Sabater, 2003) using a Separation Module (Walters 2690;
214 Waters Corporation, Milford, MA), equipped with a Photodiode Array (Waters 996)
215 detector and a C18 column, 250 x 3.00 mm i.d. (OmniSpher 5; Varian Inc., Palo Alto,

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216 CA, USA). Elution was performed with 100% methanol as the mobile phase at a flow
217 rate of 1 ml/min, with the column kept at 50ºC during analysis.

218

219 *2.5. Statistical analysis*

220 Statistical procedures were conducted using the SAS 9.2. software package (SAS
221 Inst. Inc., Cary, NC, USA) and the statistical significance of the differences were
222 defined as P values < 0.05. Average daily gain was estimated by regression of live
223 weight against time, using the REG procedure. Data regarding milk yield and
224 composition as well as colour and TBARS of lamb meat were analysed by repeated
225 measurements analyses using the MIXED procedure and included the fixed effects of
226 the experimental diet (D), time of sampling (T) and their interaction (D x T). The rest of
227 the parameters were statistically analysed by one-way analysis of variance using the
228 general linear model (PROC GLM). Within this analysis, the following contrasts were
229 carried out: (i) Control vs LO and LO-Syn E and LO-Nat E (ii) Control vs LO, (iii) LO
230 vs LO-Syn E and LO-Nat E, (iv) LO-SynE vs LO-Nat E. The CORR procedure was
231 used to calculate the correlation coefficients of the FA, TBARS and vitamin E content
232 in milk and meat. Differences were declared significant for P < 0.05 and tendencies for
233 P < 0.10.

234

235 **3. Results**

236 As shown in Table 2, dry matter intake, milk and protein yields were not modified by
237 dietary treatment and LO treatment did not significantly increase milk fat content and
238 yield compared to Control. However, dietary supplementation of linseed oil plus
239 vitamin E (LO-Syn E and LO-Nat E) decreased the protein content (P < 0.05) and
240 increased (P < 0.05) the milk fat content and yield with the same results (P > 0.05),
241 irrespective of whether diets were supplemented with synthetic or natural vitamin E.

242

243 Lamb performance, carcass characteristics and meat chemical composition are
244 shown in Table 3. No differences attributable to any experimental treatments were
245 observed for animal performance and carcass characteristics (P > 0.05), but fat content

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246 was affected significantly, increasing both with LO supplementation and natural vitamin
247 E.

248

249 The fatty acid profiles of milk fat from ewes of the different experimental treatments
250 are given in detail in Table 4. There were large differences in milk FA profiles due to
251 linseed oil supplementation (Control vs. LO and LO-Syn E and LO-Nat E), whereas the
252 effects of supplementing with vitamin E (LO vs LO-Syn E and LO-Nat E), whether
253 synthetic or natural, (LO-Syn E vs. LO-Nat E) were limited. With LO diets milk
254 percentages of short ($P < 0.01$) and medium-chain FAs ($P < 0.001$) decreased and long-
255 chain FAs increased ($P < 0.001$) compared with the Control diet. Dietary inclusion of
256 linseed oil decreased the total SFA percentage ($P < 0.001$) with a concomitant increase
257 in the MUFA ($P < 0.01$) and PUFA concentrations ($P < 0.001$).

258

259 With reference to individual saturated and monounsaturated fatty acids, milk from
260 ewes supplemented with LO had lower percentages of C8:0, C10:0, C12:0 ($P < 0.01$)
261 and C16:0 ($P < 0.01$) and higher percentages of *trans*-6/7/8 ($P < 0.001$), *trans*-9 (P <
262 0.1)*,* *trans*-10 ($P < 0.05$) and *trans*-11 C18:1 ($P < 0.001$) than Control. However, no
263 statistically significant differences ($P > 0.05$) in these FA were observed due to vitamin
264 E supplementation.

265

266 The percentage of linoleic acid (*cis*-9*,* *cis*-12 C18:2) and its isomers, such as *cis*-9
267 *trans*-11 C18:2 (RA) and *trans*-10*,* *cis*-12 C18:2, were higher ($P < 0.01$) in treatments
268 supplemented with linseed oil (LO, LO-Syn E and LO-Nat E) than in Control. The LO-
269 Syn E treatment resulted in a higher percentage of RA (1.46 vs. 0.97, $P < 0.01$) and
270 *trans*-10*,* *cis*-12 C18:2 (0.07 vs. 0.03, $P < 0.05$) than LO-Nat E treatments.

271

272 The proportion of ALA in milk increased 2-fold with the LO diet compared with the
273 Control diet. Eicosapentaenoic acid (C20:5 n-3, EPA), docosapentaenoic acid (C22:5 n-
274 3, DPA) and docosahexaenoic acid (C22:6 n-3, DHA) contents were extremely low, as
275 commonly occurs in ruminant milk. Although milk from ewes fed with linseed oil had

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276 lower concentrations of EPA (P < 0.05), DPA (P < 0.05) and DHA (P < 0.01), the
277 concentration of total n-3 PUFAs was higher (P < 0.001) in milk from ewes
278 supplemented with LO. The n-6/n-3 ratio was highest in milk fat from Control ewes (*P*
279 < 0.01); no differences (P > 0.05) were observed between LO diets due to vitamin E
280 supplementation.

281

282 Suckling lamb meat FA patterns were similar to those from the lactating dam milk
283 (Table 5), with C16:0 and *cis*-9 C18:1 being the most abundant FA in intramuscular fat.
284 Control lambs registered the highest concentrations of total saturated FA (P < 0.01) and
285 the lowest (P < 0.01) of total monounsaturated FA. Accordingly, milk fatty acid
286 composition of short (P < 0.01) and medium-chain FAs (P < 0.001) decreased and long-
287 chain FAs increased (P < 0.001) in intramuscular fat with diets containing LO compared
288 with the control diet.

289

290 With reference to individual fatty acids, C10:0, C12:0 (P < 0.01), C14:0 (P < 0.05)
291 and C16:0 (*P* < 0.01) were higher in Control lambs, and linseed oil supplementation
292 was accompanied by significant increases in *trans* C18:1, *trans* C18:2 and C18:3 n-3. In
293 particular, *trans*-10 C18:1 (P < 0.05), VA (P < 0.001), RA (P < 0.001), *trans*-10*, cis*-12
294 C18:2 (P < 0.001) were higher in LO diets and C18:3 n-3 (P < 0.001) in intramuscular
295 fat. Even so, no statistically significant differences (P > 0.05) were observed in these FA
296 due to vitamin E supplementation whether synthetic or natural.

297

298 PUFA n-3 content, including long chain PUFAs (LCFA, C > 20), was not affected
299 by the LO diet. Meat from treatments supplemented with vitamin E had higher levels of
300 EPA (P < 0.05), DPA (P < 0.10) and DHA (P < 0.10) and these fatty acid levels were
301 even higher (P < 0.05) when the type of vitamin supplemented was synthetic. The n-
302 6/n-3 ratio was the lowest (P < 0.01) in intramuscular fat from linseed oil treatments and
303 no differences (P > 0.05) were observed due to vitamin E supplementation of the ewe
304 diet, irrespective of the origin of the vitamin E.

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306 Vitamin E concentration in milk and in intramuscular fat was influenced by dietary
307 treatments (Table 6). Supplementing the ewe diet with vitamin E (LO vs. LO-Syn E and
308 LO-Nat E) increased the vitamin E content in milk ($P < 0.001$) and meat ($P < 0.05$) and
309 these vitamin E levels were positively correlated ($r = 0.75$, $P < 0.001$). Although milk
310 from LO-Nat E treatment had more vitamin E than milk from LO-Syn E ($P < 0.001$), no
311 such differences were found in suckling lambs meat ($P > 0.05$) as a result of the type of
312 vitamin E used.

313

314 There were no differences between Control and LO diets with respect to colour
315 variables and their evolution ($P > 0.05$). However, although LO diets showed a similar
316 evolution for all these variables, L*, b* and H* had higher values and a* lower values
317 than diets supplemented with vitamin E (LO-Syn E and LO-Nat E). No differences were
318 found between LO-Syn-E and LO-Nat-E ($P > 0.05$).

319

320 Initial L*, a*, b* and H* values of LO lambs were similar to those of Control lambs
321 ($P > 0.05$), whereas vitamin supplemented (LO-Nat E and LO-Syn E) lambs had a
322 significantly lower L* and b* and higher a* than LO and Control (Figure 1a, 1c and 1d;
323 $P < 0.05$) irrespective of the type of vitamin E supplement. From this point on, all
324 samples increased their L* values until day 9, and then from day 9 to day 12 the
325 lightness values of LO, LO-Syn E and LO-Nat E samples decreased. Likewise, a* and
326 H* values evolved in a similar way in all treatments studied (Control, LO, LO-Nat E
327 and LO-Syn E), as can be seen in Figures 1c and 1d.

328

329 With reference to TBARS (Figure 2) there was a significant interaction between
330 treatment and time ($P < 0.001$) even though there were no significant differences
331 between treatments on day 0 ($P > 0.05$). Nevertheless, treatments with vitamin E (LO-
332 Syn E and LO-Nat E), kept TBARS values constantly low (0.1 – 0.6 mg MDA/kg
333 muscle), while treatments without vitamin E (Control and LO) produced values above
334 1.0 mg MDA/kg muscle and even reached values greater than 2.0 over time in storage.
335 LO had higher TBARS values ($P < 0.05$) at 5 and 12 days than the Control treatment. In
336 general, the relationship between TBARS and meat fatty acid was not strong, but

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337 TBARS values at 5 and 9 days were negatively correlated with the level of vitamin E in
338 meat ($r = - 0.44$, $P < 0.05$; $r = - 0.56$, $P < 0.05$).

339

340 **4. Discussion**

341 *4.1. Animal performance*

342 In agreement with previous experiments, dairy ewe milk yield during nursing (Casals
343 et al., 2006) and milking (Toral et al., 2010 b) was not modified by supplementation
344 with additional fat. As a positive milk yield response to fat supplementation has only
345 been observed when energy limiting diets were used as Control, no differences were
346 expected between Control and LO diets in this study. Milk yield was only numerically
347 elevated in treatments with linseed oil, probably due to the higher energy content of
348 these diets because there were no differences in dry matter intake.

349

350 Milk protein yield was not affected by oil supplementation. The lower milk protein
351 content caused by vitamin E supplemented diets could be related to a dilution effect
352 resulting from milk yield rather than from reduced availability of amino acids in the
353 mammary gland or protein insulin resistance as previously reported by Pulina, Nudda,
354 Battacone & Cannas (2006).

355

356 Because no differences were found in dry matter intake between treatments, an
357 increase in milk fat yield and content would be expected from ewes assigned to the
358 linseed oil treatment (LO, LO-Syn E, LO-Nat E) compared with Control ewes without
359 fat-supplemented diets. Even so, LO treatment only caused a significant increase in
360 milk fat yield and content compared with non-supplemented ewes (Control treatment)
361 when LO diets were supplemented with vitamin E (LO-Syn E and LO-Nat E).

362

363 Shingfield & Griinari, (2007) suggested that *trans*-10 C18:1 and *trans*-10*, cis*-12
364 C18:2 are associated with changes in rumen lipid metabolism and could contribute
365 toward inhibiting milk fat synthesis in the udder. It has been reported that vitamin E

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366 may play a role in preventing the *trans*-10 shift in rumen biohydrogenation pathways
367 and subsequently alleviate a diet-induced low milk fat syndrome (Pottier et al., 2006).

368

369 Our results are in agreement with those of Gómez-Cortés et al. (2008) who suggested
370 that the response of sheep to supplementation with high concentrations of lipids, rich in
371 PUFAs, and the generation of *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 isomers
372 involved in milk fat depression did not significantly change milk fat in ewes.

373

374 It was reported that vitamin E may be involved in preventing the *trans*-10 shift in
375 rumen biohydrogenation pathways and thereby alleviate a diet induced low milk fat
376 syndrome (Pottier et al., 2006). Our results are consistent with the results reported by
377 Bell, Griinari & Kennelly (2006) in cows, who proposed that vitamin E
378 supplementation could mitigate the effect of vegetable oil supplementation on milk fat
379 depression (MFD), increasing both milk fat percentage and yield. However, the FA
380 profile was unaltered and remained characteristic of MFD, with increases in *trans*-10
381 C18:1 and *trans*-10, *cis*-12 C18:2 content in milk fat. Thus, other vitamin E
382 mechanisms, different from changing biohydrogenation pathways as reported in cows
383 (Pottier et al., 2006; Bell et al., 2006), must also be preventing milk fat depression.
384 These additional mechanisms should be investigated in lactating ewes to explain why
385 LO and vitamin E supplemented diets produce higher levels of milk fat compared to
386 Control diets.

387

388 The fact that suckling lambs were fed exclusively on maternal milk and that the milk
389 yield did not limit lamb growth may explain the similarity between lamb performance
390 and carcass characteristics due to linseed oil, vitamin E supplementation and the type of
391 vitamin E (synthetic or natural). Similar results were reported by Manso et al. (2011)
392 and Capper, Wilkinson, Mackenzie & Sinclair (2007) in suckling lambs fed with
393 different oils and supplemented with vitamin E respectively.

394

395 *4.2. Milk fatty acid composition*

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396 Milk fatty acid composition was strongly modified by LO supplementation, with
397 significant reductions in short-chain fatty acids (SCFAs) and some of the medium-chain
398 fatty acids (MCFAs) in milk. These fatty acids are synthesized *de novo* in the mammary
399 gland, and their marked decrease in milk from ewes fed linseed oil could be attributed to
400 a dilution effect generated by a greater uptake of long-chain fatty acids (LCFAs) in the
401 udder. On the other hand, the presence of some of these LCFAs in the mammary gland
402 could inhibit the activity of lipogenic enzymes involved in *de novo* synthesis
403 (Palmquist, 2006).

404

405 As already observed by Bodas et al. (2010), the lower levels of saturated fatty acids
406 (SFAs) and higher levels of MUFAs and PUFAs present in milk fat in the LO
407 treatments were the result of the fatty acid composition of the linseed oil and the
408 incomplete biohydrogenation of the dietary PUFAs. Manso et al. (2011) observed that
409 the high levels of linoleic and linolenic acid in linseed oil are manifested by increases in
410 these fatty acids in the milk of LO treated ewes compared with the Control.

411

412 In spite of the difficulty of increasing ALA levels in ruminant milk because of the
413 relatively low transfer rate of this FA from diet into milk (Palmquist, 2006), the
414 concentration of ALA in milk fat from diets with linseed oil increased 2.0 (LO), 1.9
415 (LO-Syn E) and 1.7-fold (LO-Nat E) compared to the Control diet. These increases
416 were similar to those reported in previous experiments with linseed oil (Manso et al.,
417 2011) and extruded linseed (Mele et al., 2011). However, Gomez-Cortes, Bach, Luna,
418 Juarez & de la Fuente (2009) observed an increase of 5.3-fold in ALA concentration
419 when ewe diets were supplemented with 6% of extruded linseed. The lower ALA
420 concentration in milk reported in this study can be attributed to rumen biohydrogenation
421 that results in lower ALA transference to the mammary gland.

422

423 LO supplementation reduced the milk fat percentage of arachidonic acid (C20:4 n-6,
424 AA), as described by Ferlay et al. (2010), suggesting an inhibitory effect of C18:3 n-3
425 or its metabolites on synthesis or milk secretion of this n-6 FA.

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426

427 With regard to the very long chain n-3 PUFAs, their concentrations were extremely
428 low in all groups and were in line with those reported in ewes supplemented with
429 extruded linseed (Gomez-Cortés et al. 2009) or linseed oil (Bodas et al., 2010).

430

431 The decrease of the n-6/n-3 ratio to below 4.0 in milk fat when ewes were
432 supplemented with linseed oil (LO, LO-Syn E and LO-Nat E), can be considered
433 positive from a nutritional point of view (Simopoulos, 2008).

434

435 With respect to the intermediaries in the biohydrogenation processes of linoleic and
436 linolenic acid, most increased their levels with the LO diets. Milk *trans*
437 monounsaturated FAs increased in diets with LO because of alterations in the rumen
438 metabolism which inhibited the last stages of biohydrogenation. In particular, the
439 proportion of *trans*-10 C18:1, an intermediate metabolite of a partial, incomplete and
440 altered ruminal biohydrogenation of linoleic and linolenic acid in milk fat, increased in
441 linseed oil treatments instead of *trans*-11 C18:1. *Trans*-10 C18:1 is associated with an
442 enrichment of the diet with unsaturated FAs, like linseed oil, and is related to increases
443 in the *trans*-10*, cis*-12 CLA content of milk fat (Toral et al., 2010 b).

444

445 Milk fat concentration of RA increased 2.8, 3.2 and 2.1-fold with LO, LO-Syn E and
446 LO-Nat E supplementation, respectively. The strong correlation between VA and RA
447 calculated in the current research (r = 0.86, P < 0.001) confirms the substrate-product
448 relationship for $\Delta^9$*-desaturase*. RA in ewe milk fat is not only formed by direct
449 isomerization of linoleic acid in the rumen, but also originates mainly from endogenous
450 synthesis from VA via $\Delta^9$*-desaturase* in the mammary gland (Bichi et al., 2012). The
451 ALA supplied by the linseed oil diet is a direct precursor of the VA produced in the
452 rumen, and therefore a 4.1, 4.0 and 2.8-fold increase in VA milk fat concentrations from
453 LO, LO-Syn E, LO-Nat E diets, respectively has been observed, which is used for
454 endogenous synthesis of RA in the mammary gland. Our results are in agreement with
455 other studies which reported an increase in VA and RA concentrations in the milk of

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456 ewes fed linseed oil (Bodas et al., 2010) extruded linseed (Gomez-Cortes et al., 2009)
457 and whole linseed (Zhang, Mustafa & Zhao, 2006).

458

459 Vitamin E supplementation had a limited effect on milk fatty acid profiles and most
460 of the biohydrogenation intermediates in ewes. Several authors stated that vitamin E
461 supplementation did not affect the proportions of unsaturated dietary fatty acids and
462 most biohydrogenation intermediates in the rumen, suggesting that vitamin E was
463 neither a limiting factor for rumen BH nor a modulator of BH pathways (Chikunya et
464 al., 2004; Zened, Troegeler-Meynadier, Najar & Enjalbert 2012). In contrast, as
465 discussed above, vitamin E could alter ruminal PUFA biohydrogenation in dairy (Bell et
466 al., 2006) and beef cattle (Juarez et al., 2011). The mechanism by which α-tocopherol
467 may alter biohydrogenation is unclear, so the modification of rumen microbial
468 populations and/or dynamics leading to FA hydrogenation might be involved (Hou et
469 al., 2013).

470

471 Despite the limited effect of vitamin E supplementation on milk fatty acids, there
472 were some statistical differences between fatty acid levels of LO-Syn E and LO-Nat E
473 treatments (Table 3). In agreement with the current study, research conducted *in vitro*
474 on dairy cattle showed that synthetic vitamin E supplementation increased the
475 proportions of *cis*-9, *trans*-11 C18:2 and *trans*-10, *trans*-12 C18:2 in the ruminal fatty
476 acid profile compared with natural vitamin E supplementation, which suggests that
477 differences in CLA percentages were not due to differences in isomerization efficiency
478 (Zened et al., 2012).

479

480 *4.3. Intramuscular fatty acid composition*

481 In suckling lambs the rumen is not functional, so there is no biohydrogenation of the
482 milk FAs before they are absorbed by the intestine. Therefore, the milk FA profile of
483 the lactating dams had a significant effect on the meat fatty acid profile. Because there
484 were no differences in growth rates and fat deposition in lambs during treatment,
485 differences in the intramuscular FA profile could only be due to milk fatty acid
486 composition.

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487

488 The presence of lower levels of SCFAs, MCFAs and SFAs and higher levels of
489 LCFAs and MUFAs in suckling lamb intramuscular fat in the LO treatments is
490 explained by differences in milk fatty acid composition. However, in spite of higher
491 PUFA levels in milk from LO diets, no differences were found in total intramuscular
492 PUFA content. The major presence of PUFA in intramuscular fat is due to the greater
493 proportion of phospholipids. As phospholipids are the constituents of cell membranes,
494 their composition is less influenced by diet, because large changes in the FA profile of
495 these membranes would alter their properties and other physiological functions (Juárez
496 et al., 2010).

497

498 As reported in the case of milk, *trans* monounsaturated fatty acid levels were more
499 elevated in suckling intramuscular fat from LO treatments. The levels of *trans*-10
500 C18:1, VA and RA in intramuscular fat increased 3.3, 4,6 and 3,2-fold, respectively
501 with LO supplementation compared with the Control diet. Dietary RA from milk would
502 not be the only source of RA in tissues, as it would also be partly derived from
503 endogenous synthesis from VA by the action of $\Delta^9$*-desaturase* in the animal tissue
504 (Raes et al., 2004). Hence, a significant positive correlation was observed between RA
505 and VA levels in intramuscular fat ($r = 0.94$, $P < 0.001$), stronger than in milk ($r = 0.86$,
506 $P < 0.001$).

507

508 Ewe diet supplementation with vitamin E did not have any effect on VA and RA
509 content in intramuscular fat. This pattern was similar to that reported by Capper et al.
510 (2007) in plasma from suckling lambs, where a ewe diet was not only supplemented
511 with fish oil but also with vitamin E (500mg/kg). Likewise in lambs, Kasapidou et al.
512 (2012) did not find any significant differences in *trans* C18:1 and RA intramuscular
513 content when the lamb diet was supplemented with vitamin E (500 mg/kg).

514

515 As with milk fat, linseed oil supplementation of the ewe diet increased the ALA
516 proportion 1.56 (LO), 1.95 (LO-Syn E) and 1.69-fold (LO-Nat E) in intramuscular fat.



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517 The ALA presence in suckling lamb muscle depends on the ALA content in the milk (r
518 = 0.51, P < 0.05), which, in turn, is related to the dietary composition of their dams.
519 Consistent with the foregoing, Manso et al. (2011) reported a 2.0-fold increase in ALA
520 in intramuscular fat of suckling lambs when their dams had been fed a diet
521 supplemented with linseed oil (3% DM).

522

523 Kasapidou et al. (2012) observed that vitamin E supplementation did not affect the
524 ALA content in intramuscular fat in lambs. However, Juarez et al. (2011) reported that
525 the inclusion of high levels of vitamin E in the lamb diet resulted in higher levels of
526 ALA when the diet was supplemented with linseed, which could indicate that vitamin E
527 somehow modifies C18:3 biohydrogenation.

528

529 Linseed oil supplementation was shown to increase intramuscular fat levels of long
530 chain n-3 fatty acids in suckling lambs (Manso et al., 2011). Nevertheless, in the present
531 experiment, linseed oil inclusion in the ewe diet did not produce any increase in long
532 chain n-3 PUFAs in the suckling lamb intramuscular fat. The lack of increase in EPA,
533 DPA and DHA levels in LO lambs compared to Control can probably be explained by
534 the higher intramuscular fat content and a consequently higher triglyceride to
535 phospholipid ratio. Since increases in long chain fatty acids take place mainly in the
536 phospholipid rather than in the trygliceride fraction (Jerónimo, Alves, Prates, Santos-
537 Silva & Bessa, 2009), the failure of LCFA n-3 to increase in the present study could be
538 related to a lower proportion of phospholipids relative to the triglyceride fraction.

539

540 The significant increase in n-3 PUFAs, including EPA, DPA and DHA in suckling
541 lamb meat from LO-Syn E treatment could be ascribed mostly to differences in
542 intramuscular fat content rather than to a higher protective effect of synthetic vitamin E
543 against PUFA peroxidation. On the other hand, Kasapidou et al. (2012) reported that
544 dietary vitamin E supplementation did not affect EPA and DHA meat content in lambs.

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546 Although there were no differences between n-3 PUFA and n-6 PUFA levels in
547 intramuscular fat of suckling lambs from Control ewes and ewes fed linseed oil, the
548 n6/n3 ratio was considerably lower in lambs from LO, LO-Syn E and LO-Nat E
549 treatments, and their values (LO: 3.65, LO-Syn E: 3.31 and LO-Nat E: 3.40) remained
550 below 4, the nutritionally recommended threshold (Simopoulus, 2008).

551

552 With reference to vitamin E supplemented groups, the very long chain PUFAs were
553 only increased when synthetic vitamin E was provided. Since these differences occurred
554 despite the lack of difference in milk fatty acid composition between types of vitamin
555 E, this result is probably related to the different intramuscular fat content of natural and
556 synthetic vitamin E supplemented groups with the resulting variation in the triglycerides
557 to phospholipid ratio, as discussed above.

558

559 *4.4. Vitamin E, colour and lipid oxidation*

560 Total vitamin E levels of ewe milk did not differ significantly between Control and
561 LO treatment, probably due to the fact that differences in total vitamin E between these
562 two diets were insufficient to affect the amounts in the milk. Even so, Capper et al.
563 (2005) observed a decrease in vitamin E content in milk fat when the ewe diet was
564 supplemented with fish oil. This could be because the animal's need for vitamin E as a
565 cellular antioxidant, is positively correlated with the oxidative challenge faced by the
566 animal as a result of fatty acid supply, therefore causing milk concentration of vitamin E
567 to fall. The increase in milk vitamin E concentrations conferred by supranutritional
568 vitamin E supplementation within the current study agrees with the results published by
569 Capper et al. (2005).

570

571 Data from the present study showed that concentrations of vitamin E in milk were
572 2.73 times greater for ewes fed the natural vitamin E (LO-Nat E treatment) than for
573 ewes fed the synthetic vitamin E (LO-Syn E treatment). This could be owing to the fact
574 that the RRR form (natural vitamin E) is preferentially taken up or transferred from
575 plasma to milk (Vagni et al., 2011). In this sense, Meglia, Jenkens, Lauridsen & Waller
576 (2006) and Weiss et al. (2009) observed a 1.24 and 1.43-fold greater concentration of

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577 vitamin E, respectively, in milk from cows fed with RRR supplement (natural vitamin
578 E) compared to cows fed the all-rac supplement (synthetic vitamin E).

579

580 As expected, the vitamin E concentration of suckling lamb meat mirrored maternal
581 milk vitamin E concentrations, with the highest amounts being recorded in vitamin E
582 supplemented ewes (LO-Syn E and LO-Nat E). Vitamin E concentrations in meat from
583 linseed oil supplemented ewe diets and the Control diet were statistically the same.
584 Muscle vitamin E levels were positively correlated with those in the maternal milk ($r =$
585 $0.75$, $P < 0.001$), a finding in agreement with Kasapidou et al. (2012) who also showed
586 that muscle vitamin E levels increased in line with dietary vitamin E levels.

587

588 The vitamin E content and fatty acid composition of meat affect its colour stability
589 (Lopez-Bote, Daza, Soares & Berges, 2001; Kasapidou et al., 2012). It is advisable to
590 evaluate meat colour in terms of lightness (L*) and hue angle (H*), because these are
591 the real parameters of colour that human evaluators are able to understand (Ripoll et al.,
592 2008).

593

594 Lightness from suckling lamb muscle increased until day 9 of storage (Fig. 1a),
595 according to several authors who reported increases in L* over time in suckling lamb
596 meat (Osorio, Zumalacarregui, Cabeza, Figueira & Mateo (2008). In agreement with
597 Vieira et al. (2012), there were no differences in colour measurements of suckling lamb
598 meat between LO and Control treatments. The increase of α-tocopherol in lamb muscle
599 because of ewe dietary vitamin E supplementation (LO-Syn E and LO-Nat E) could
600 significantly lower L* values in meat (Ripoll et al., 2011). These authors suggested that
601 vitamin E should modify lightness by means of water holding capacity, thus preventing
602 high short-term lightness values due to superficial moisture. Meat oxidation reduces the
603 water-holding capacity between muscle myofibrils, which increases juice loss from the
604 meat and as a result meat lightness (Elisabeth & Steven, 2005). All treatments in the
605 present study produced meat with L* values greater than 34, the acceptable threshold
606 for fresh lamb meat colour (Khliji, Van de Ven, Lamb, Lanza & Hopkins, 2010).
607 Lopez-Bote et al. (2001) proposed 3.2 mg/kg as the concentration of vitamin E required

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608 in light lambs to have a significant impact on L* stability. However, our results showed
609 that with a lower concentration of vitamin E in suckling lamb muscle (LO-Syn E: 1.3
610 and LO-Nat E: 1.53 mg vitamin E/kg muscle) colour parameters could be positively
611 affected.

612

613 Like the L* values, H* values were also affected by ewe dietary vitamin E and time
614 in storage (Fig. 1d). The inclusion of linseed oil in ewe diets (LO) did not have any
615 effect on the H* value of their suckling lambs, as observed by Juárez et al. (2011) in
616 beef fed linseed. The higher vitamin E content in suckling lamb muscle could also
617 reduce H* values, compared with lambs from treatments without additional vitamin E.
618 H* values in LO-Syn E and LO-Nat E remained below 59, the acceptability limit for
619 lamb meat proposed by Ripoll et al. (2011) throughout storage display.

620

621 As expected, the oxidative processes in muscle (TBARS) were significantly affected
622 by dietary treatment ($P < 0.001$) and storage display ($P < 0.001$). The lack of difference
623 in TBARS values at day 0 (non-aged), can be explained by taking into account that
624 compounds that contribute to oxidised flavour development are mainly formed during
625 storage (Ahn, Grün & Mustapha, 2007). Nevertheless, at days 5 and 12 of storage, LO
626 treated lamb meat registered higher TBARS values than the Control samples. Increasing
627 the ALA content in meat (LO: 0.97 vs. Control: 0.62; $P < 0.001$) has been shown to
628 result in higher levels of oxidation due to the higher susceptibility of this n-3 PUFA
629 (Wood et al., 2004).

630

631 During storage LO-Syn E and LO-Nat E treatments kept oxidized lamb meat far
632 below the limiting acceptability threshold (1 mg MDA/kg muscle; Ripoll et al., 2011),
633 demonstrating that vitamin E counteracts fatty acid oxidation and consequently
634 increases the shelf life of meat. The negative correlation between TBARS values at 5
635 and 9 days of storage and vitamin E concentrations in muscle ($r = -0.44$, $P < 0.05$; $r =$
636 $0.56$, $P < 0.05$) support this statement. Lauzurica et al. (2005) maintained TBARS
637 values around 0.5 at 12 days in the meat of lambs fed on a diet enriched with 500 mg of

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638 vitamin E/kg. At day 9 of storage, Lopez-Bote et al. (2001) reported TBARS values of
639 0.45 mg MDA/kg muscle in lambs fed on a diet enriched with 1000 mg of vitamin E/kg.

640

641 **Conclusions**

642 To conclude, it can be said that the use of linseed oil as a supplement in lactating ewe
643 diets modified the milk fat FA profile, and consequently the meat FA profile of their
644 suckling lambs, by increasing the content of healthy FAs in meat, like VA, RA and
645 ALA. Ewe diet supplementation with natural or synthetic vitamin E only had a limited
646 effect on animal performance, milk and fatty acid profiles, however it clearly affected
647 the lipids and colour stability of suckling lamb meat from ewes fed with linseed oil.

648

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657 Ahn, J., Grün, I.U., & Mustapha, A. (2007). Effects of plant extracts on
658 microbial growth, color change, and lipid oxidation in cooked beef. *Food*
659 *Microbiology* 24, 7-14.

660 AOAC, (2003). *Official Method of Analysis of the Association of Official*
661 *Agricultural Chemists.*17th Ed. AOAC International, Gaitherburg (United States
662 of America)

663 Bell, J.A., Griinari, J.M., & Kennelly, J.J. (2006). Effect of Safflower Oil,
664 Flaxseed Oil, Monensin, and Vitamin E on Concentration of Conjugated
665 Linoleic Acid in Bovine Milk Fat. *Journal of Dairy Science,* 89, 733-748.

666 Bichi, E. Toral, P.G. Hervás, G., Frutos, P., Gómez-Cortés, P., Juárez, M., & De
667 la Fuente, M.A. (2012). Inhibition of Δ9-desaturase activity with sterculic acid:
668 effect on the endogenous synthesis of cis-9 18:1 and cis-9 trans-11 18:2 in dairy
669 sheep. *Journal of Dairy Science*, 95, 5242-5252.

670 Bodas, R., Manso, T., Mantecon, A.R., Juarez, M., De la Fuente, M.A., &
671 Gomez-Cortes, P. (2010). Comparison of the Fatty Acid Profiles in Cheeses
672 from Ewes Fed Diets Supplemented with Different Plant Oils. *Journal of*
673 *Agricultural and Food Chemistry* 58, 10493-10502.

674 Capper, J.L., Wilkinson, R.G., Kasapidou, E., Pattinson, S.E., Mackenzie, A.M.,
675 & Sinclair, L.A. (2005). The effect of dietary vitamin E and fatty acid
676 supplementation of pregnant and lactating ewes on placental and mammary
677 transfer of vitamin E to the lamb. *British Journal of Nutrition* 93, 549-557.

678 Capper, J.L., Wilkinson, R.G., Mackenzie, A.M., & Sinclair, L.A. (2007). The
679 effect of fish oil supplementation of pregnant and lactating ewes on milk
680 production and lamb performance. *Animal* 1, 889-898.

681 Casals, R., Caja, G., Pol, M.V., Such, X., Albanell, E., Gargouri, A., & Casellas,
682 J. (2006). Response of lactating dairy ewes to various levels of dietary calcium
683 soaps of fatty acids. *Animal Feed Science and Technology* 131, 312-332.

684 Chikunya, S., Demirel, G., Enser, M., Wood, J.D., Wilkinson, R.G., & Sinclair,
685 L.A. (2004). Biohydrogenation of dietary n-3 PUFA and stability of ingested
686 vitamin E in the rumen, and their effects on microbial activity in sheep. *British*
687 *Journal of Nutrition* 91, 539-550.

688 Czauderna, M., & Kowalczyk, J. (2007). Alkaline saponification results in
689 decomposition of tocopherols in milk and ovine blood plasma. *Journal of*
690 *Chromatography* B 858, 8-12.

691 Elisabeth, H.L., & Steven, M.L. (2005). Mechanisms of water holding capacity
692 of meat: The role of postmortem biochemical and structural changes. *Meat*
693 *Science* 71, 194-204.

694 Ferlay, A., Martin, B., Lerch, S., Gobert, M., Pradel, P., & Chilliard, Y. (2010).
695 Effects of supplementation of maize silage diets with extruded linseed, vitamin
696 E and plant extracts rich in polyphenols, and morning v. evening milking on
697 milk fatty acid profiles in Holstein and Montbeliarde cows. *Animal* 4, 627-640.

post-print

698 FIL-IDF, (1999). *Milk Fat. Preparation of fatty acid methyl esters*. International
699 Standard ISO 182. International Dairy Federation, Brussels (Belgium).

700 Folch, J., Lees, M., & Sloane-Stanley, G.H. (1957). A simple method for
701 isolation and purification of total lipids from animal tissues. *Journal of*
702 *Biological Chemistry* 226, 497-509.

703 Gomez-Cortes, P., Bach, A., Luna, P., Juarez, M., & de la Fuente, M.A. (2009).
704 Effects of extruded linseed supplementation on n-3 fatty acids and conjugated
705 linoleic acid in milk and cheese from ewes. *Journal of Dairy Science* 92, 4122-
706 4134.

707 Gómez-Cortés, P., Frutos, P., Mantecón, A.R., Juárez, M., de la Fuente, M.A., &
708 Hervás, G. (2008). Milk Production, Conjugated Linoleic Acid Content, and In
709 Vitro Ruminal Fermentation in Response to High Levels of Soybean Oil in
710 Dairy Ewe Diet. *Journal of Dairy Science* 91, 1560-1569.

711 Hou, J., Wang, F., Wang, Y., & Liu, F. (2013). Effects of vitamin E on the
712 concentration of conjugated linoleic acids and accumulation of intermediates of
713 ruminal biohydrogenation in vitro. *Small Ruminant Research* 111, 63-70.

714 International Dairy Federation (2000). International IDF standard 141C:2000.
715 *Determination of milk fat, protein and lactose content. Guidance on the*
716 *operation of mid-infrared instruments.* International Dairy Federation, Brussels,
717 Belgium.

718 Jerónimo, E., Alves, S.P., Prates, J.A.M., Santos-Silva, J., & Bessa, R.J.B.
719 (2009). Effect of dietary replacement of sunflower oil with linseed oil on
720 intramuscular fatty acids of lamb meat. *Meat Science* 83, 499-505.

721 Juárez, M., Dugan, M.E.R., Aalhus, J.L., Aldai, N., Basarab, J.A., Baron, V.S.,
722 & McAllister, T.A. (2011). Effects of vitamin E and flaxseed on rumen-derived
723 fatty acid intermediates in beef intramuscular fat. *Meat Science* 88, 434-440.

724 Juarez, M., Horcada, A., Alcalde, M.J., Aldai, N., Polvillo, O., Valera, M., &
725 Molina, A. (2010). Fatty acid composition of lamb fat depots as an origin
726 discriminator. *Spanish Journal of Agricultural Research* 8, 976-980.

727 Kasapidou, E., Wood, J.D., Richardson, R.I., Sinclair, L.A., Wilkinson, R.G., &
728 Enser, M. (2012). Effect of vitamin E supplementation and diet on fatty acid
729 composition and on meat colour and lipid oxidation of lamb leg steaks displayed
730 in modified atmosphere packs. *Meat Science* 90, 908-916.

731 Khliji, S., Van de Ven, R., Lamb, T.A., Lanza, M., & Hopkins, D.L. (2010).
732 Relationship between consumer ranking of lamb colour and objective measures
733 of colour. *Meat Science* 85, 224-229.

734 Kittessa, S.M., Peake, D., Bencinni, R., & Williams, A.J. (2003). Fish oil
735 metabolim in ruminants III. Transfer of n-3 polyunsaturated fatty acids (PUFA)
736 from tuna oil sheep´s milk. *Animal Feed Science and Technology* 108, 1-14.

post-print

737 Lauridsen, C., Engel, H., Craig, A.M., & Traber, M.G. (2002). Relative
738 bioactivity of dietary RRR- and all-rac-alpha-tocopheryl acetates in swine
739 assessed with deuterium-labeled vitamin E. *Journal of Animal Science* 80, 702-
740 707.

741 Lauzurica, S., de la Fuente, J., Díaz, M.T., Álvarez, I., Pérez, C., & Cañeque, V.
742 (2005). Effect of dietary supplementation of vitamin E on characteristics of lamb
743 meat packed under modified atmosphere. *Meat Science* 70, 639-646.

744 Lock, A.L., Kraft, J., Rice, B.H., & Bauman, D.E. (2009). Biosynthesis and
745 biological activity of rumenic acid: a natural CLA isomer. In: Trans Fatty Acids
746 in Human Nutrition (F. Destaillats, J.L. Sébédio, F. Dionisi and J.M. Chardigny,
747 eds.). pp. 195-230. The Oily Press, Brigwater (United Kingdom).

748 Lopez-Bote, C.J., Daza, A., Soares, M., & Berges, E. (2001). Dose-response
749 effect of dietary vitamin E concentration on meat quality characteristics in light-
750 weight lambs. *Animal Science* 73, 451-457.

751 Manso, T., Bodas, R., Vieira, C., Mantecon, A.R., & Castro, T. (2011). Feeding
752 vegetable oils to lactating ewes modifies the fatty acid profile of suckling lambs.
753 *Animal* 5, 1659-1667.

754 Maraschiello, C., Sarraga, C., & Garcia Reguiero, J.A. (1999). Glucation
755 peroxidase activity, TBARS, and α-tocoferol in meat from chickens fed diets.
756 *Journal of Agricultural Food Chemistry* 47, 867-872.

757 Meglia, G.E., Jenkens, S.K., Lauridsen, C., & Waller, K.P. (2006). α-Tocopherol
758 concentrations and stereoisomer composition in plasma and milk from dairy
759 cows fed natural or synthetic vitamin E around calving. *Journal of Dairy Science*
760 73, 227-234.

761 Mele, M., Contarini, G., Cercaci, L., Serra, A., Buccioni, A., Povolo, M., Conte,
762 G., Funaro, A., Banni, S., Lercker, G., & Secchiari, P. (2011). Enrichment of
763 Pecorino cheese with conjugated linoleic acid by feeding dairy ewes with
764 extruded linseed: Effect on fatty acid and triglycerides composition and on
765 oxidative stability. *International Dairy Journal* 21, 365-372.

766 Nudda, A., Palmquist, D.L., Battacone, G, Fancellu, S., Rassu, S.P.G., & Pulina,
767 G. (2008). Relationships between the contents of vaccenic acid, CLA and n−3
768 fatty acids of goat milk and the muscle of their suckling kids. *Livestock Science*
769 118, 195-203.

770 Osorio, M.T., Zumalacarregui, J.M., Cabeza, E.A., Figueira, A., & Mateo, J.
771 (2008). Effect of rearing system on some meat quality traits and volatile
772 compounds of suckling lamb meat. *Small Ruminant Research* 78, 1-12.

773 Palmquist, D.L. (2006). Milk fat: Origin of fatty acids and influence of
774 nutritional factors thereon. In: *Advanced Dairy Chemistry*, Vol. 2, Lipids. (P.F.
775 Fox and P.L.H. McSweeney, eds.). pp 43-92. Springer, New York (United States
776 of America).

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777 Pottier, J., Focant, M., Debier, C., De Buysser,G., Goffe, C., Mignolet, E.,
778 Froidmont, E., & Larondelle, Y. (2006). Effect of dietary vitamin E on rumen
779 biohydrogenation pathways and milk fat depression in dairy cows fed high-fat
780 diets. *Journal of Dairy Science* 89, 685-692.

781 Pulina, G., Nudda, A., Battacone, G., & Cannas, A. (2006). Effects of nutrition
782 on the contents of fat, protein, somatic cells, aromatic compounds, and
783 undesirable substances in sheep milk. *Animal Feed Science and Technology* 131,
784 255-291.

785 Raes, K., De Smet, S., & Demeyer, D. (2004). Effect of dietary fatty acids on
786 incorporation of long chain polyunsatureted fatty acids and conjugated linoleic
787 acid in lamb, beef and pork meat: a review. *Animal Feed Science and*
788 *Technology* 113, 199-221.

789 Ripoll, G., Joy, M., & Muñoz, F. (2011). Use of dietary vitamin E and selenium
790 (Se) to increase the shelf life of modified atmosphere packaged light lamb meat.
791 *Meat Science* 87, 88-93.

792 Ripoll, G., Joy, M., Muñoz, F., & Albertí, P. (2008). Meat and fat colour as a
793 tool to trace grass-feeding systems in light lamb production. *Meat Science* 80,
794 239-248.

795 Rodas Mendoza, B., Morera Pons, S., Castellote Bargalló, A.I., & López-
796 Sabater, M.C. (2003). Rapid determination by reversed-phase high-performance
797 liquid chromatography of Vitamins A and E in infant formulas. *Journal of*
798 *Chromatography* A 1018, 197-202.

799 Sampels, S., Pickova, J., & Wiklund, E. (2004). Fatty acids, antioxidants and
800 oxidation stability of processed reindeer meat. *Meat Science* 67, 523-532.

801 Shingfield, K.J., Bernard, L., Leroux, C., & Chilliard, Y. (2010). Role of trans
802 fatty acids in the nutritional regulation of mammary lipogenesis in ruminants.
803 *Animal* 4, 1140-1166.

804 Shingfield, K.J., & Griinari, J.M. (2007). Role of biohydrogenation
805 intermediates in milk fat depression. *European Journal of Lipid Science and*
806 *Technology* 109, 799-816.

807 Simopoulos, A.P. (2008). The importance of the omega-6/omega-3 fatty acid
808 ratio in cardiovascular disease and other chronic diseases. *Experimental Biology*
809 *and Medicine* 233, 674-688.

810 Toral, P.G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juarez, M., & de la
811 Fuente, M.A. (2010 a). Milk fatty acid profile and dairy sheep performance in
812 response to diet supplementation with sunflower oil plus incremental levels of
813 marine algae. *Journal of Dairy Science* 93, 1655-1667.

814 Toral, P.G., Frutos, P., Hervas, G., Gomez-Cortes, P., Juarez, M., & de la
815 Fuente, M.A. (2010 b). Changes in milk fatty acid profile and animal
816 performance in response to fish oil supplementation, alone or in combination
817 with sunflower oil, in dairy ewes. *Journal of Dairy Science* 93, 1604-1615.

post-print

818 Vagni, S., Saccone, F., Pinotti, L., & Baldi, A. (2011). Vitamin E
819 Bioavailability: Past and Present Insights. *Food and Nutrition Sciences* 2, 1088-
820 1096.

821 Vieira, C., Fernández-Diez, A., Mateo, J., Bodas, R., Soto, S., & Manso, T.
822 (2012). Effects of addition of different vegetable oils to lactating dairy ewes' diet
823 on meat quality characteristics of suckling lambs reared on the ewes' milk. *Meat*
824 *Science* 91, 277-283.

825 Weiss, W.P., Hogan, J.S., & Wyatt, D.J. (2009). Relative bioavailability of all-
826 rac and RRR vitamin E based on neutrophil function and total α-tocopherol and
827 isomer concentration in periparturient dairy cows and their calves. *Journal of*
828 *Dairy Science* 92, 720-731.

829 Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I.,
830 Hughes, S.I., & Wittington, F.M. (2008). Fat deposition, fatty acid composition
831 and meat quality: A review. *Meat Science* 78, 343-358.

832 Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M.,
833 Kasapidou, E., Sheard, P.R., & Enser, M. (2004). Effects of fatty acids on meat
834 quality: a review. *Meat Science* 66, 21-32.

835 Zened, A., Troegeler-Meynadier, A., Najar, T., & Enjalbert, F. (2012). Effects of
836 oil and natural or synthetic vitamin E on ruminal and milk fatty acid profiles in
837 cows receiving a high-starch diet. *Journal of Dairy Science* 95, 5916-5926.

838 Zhang, R.H., Mustafa, A.F., & Zhao, X. (2006). Effects of feeding oilseeds rich
839 in linoleic and linolenic fatty acids to lactating ewes on cheese yield and on fatty
840 acid composition of milk and cheese. *Animal Feed Science and Technology* 127,
841 220-233.

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**Table 1.** Ingredients and chemical composition of the experimental diets

| | Diets[1] | | | |
|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E |
| Ingredients, % as feed | | | | |
| Dehydrated alfalfa | 35.5 | 34.4 | 34.4 | 34.4 |
| Cereal straw | 9.07 | 9.07 | 9.07 | 9.07 |
| Soybean meal | 15.6 | 15.2 | 15.2 | 15.2 |
| Corn grain | 10.7 | 10.4 | 10.4 | 10.4 |
| Oat grain | 9.39 | 9.11 | 9.11 | 9.11 |
| Barley grain | 7.11 | 6.89 | 6.89 | 6.89 |
| Beet pulp | 7.11 | 6.89 | 6.89 | 6.89 |
| Molasses | 4.54 | 4.43 | 4.43 | 4.43 |
| Linseed oil[2] | | 2.61 | 2.61 | 2.61 |
| Vitamin mineral premix | 1.00 | 1.00 | 1.00 | 1.00 |
| Chemical composition, %DM | | | | |
| DM | 88.6 | 88.9 | 89.9 | 87.9 |
| Ash | 7.78 | 7.63 | 7.73 | 7.69 |
| Crude Protein | 16.8 | 16.3 | 16.4 | 16.5 |
| NDF | 34.4 | 33.5 | 33.2 | 33.4 |
| ADF | 23.16 | 22.6 | 22.6 | 22.5 |
| Ether extract | 2.70 | 5.56 | 5.44 | 5.61 |

[1]Diets supplemented without linseed oil and vitamin E (Control), with linseed oil (LO), with linseed oil and 400 mg/kg of synthetic vitamin E (LO-Syn E) and with linseed oil and 400 mg/kg of natural vitamin E (LO-Nat E); [2] Fatty acid composition (%): C12:0, < 0.01; C14:0, 0.10; C15:0, < 0.01; C16:0, 6.20; C16:1, 0.10; C18:0, 4.90; C18:1, 21.90; C18:2, 14.80; C18:3, 51.30: C20:0, 0.20; C22:0, 0.10.

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852 **Table 2.** Milk production and chemical composition of milk

| | Diets[1] | | | | | P value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E | SED | D | T | D x T |
| Intake g DM $day^{-1}$ | 2283 | 2287 | 2413 | 2224 | 132.2 | ns | ns | ns |
| Yield, g/day | | | | | | | | |
| Milk | 2174 | 2203 | 2357 | 2491 | 223.4 | ns | ns | ns |
| Fat | 123.6 [a] | 128.1 [a] | 156.5 [ab] | 174.9 [b] | 19.02 | * | ns | ns |
| Protein | 95.7 | 100.1 | 100.7 | 109.4 | 10.29 | ns | ns | ns |
| Composition, % | | | | | | | | |
| Fat | 5.67[a] | 6.16 [ab] | 6.71 [b] | 6.74 [b] | 0.385 | * | * | ns |
| Protein | 4.54 [a] | 4.56 [a] | 4.28 [b] | 4.39 [ab] | 0.102 | * | *** | ns |

853
854 SED: standard error of difference.
855 [1]Diets supplemented without linseed oil and vitamin E (Control), with linseed oil (LO),
856 with linseed oil and 400 mg/Kg of synthetic vitamin E (LO-Syn E) and with linseed oil
857 and 400 mg/kg of natural vitamin E (LO-Nat E)
858 [2]Effects caused by experimental diet (D), time on diet (T), and their interaction (D x T)
859 [a,b]: Different letters indicate significant differences ($P < 0.05$)
860 † $P < 0.10$, * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$
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**Table 3.** Animal performance, carcass characteristics and meat chemical composition of suckling lambs

| | Diets[1] | | | | | | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E | RSD | P value | 1 | 2 | 3 | 4 |
| Animal performance | | | | | | | | | | |
| Birth body weight (kg) | 4.22 | 4.19 | 4.38 | 4.13 | 0.628 | ns | ns | ns | ns | ns |
| Slaughter weight (kg) | 12.81 | 12.37 | 12.84 | 12.19 | 1.184 | ns | ns | ns | ns | ns |
| Average daily gain (g animal$^{-1}$ day$^{-1}$) | 310 | 293 | 314 | 286 | 41.0 | ns | ns | ns | ns | ns |
| Carcass characteristics | | | | | | | | | | |
| Hot carcass weight (kg) | 7.04 | 6.79 | 7.12 | 6.59 | 0.719 | ns | ns | ns | ns | † |
| Cold carcass weight (kg) | 6.88 | 6.65 | 6.97 | 6.45 | 0.707 | ns | ns | ns | ns | † |
| Chilling losses (%) | 2.24 | 2.06 | 2.17 | 2.12 | 0.667 | ns | ns | ns | ns | ns |
| Dressing percentage (%) | 46.23 | 46.25 | 45.74 | 47.19 | 2.120 | ns | ns | ns | ns | ns |
| Kidney knob fat (g) | 216 | 209 | 245 | 206 | 73.8 | ns | ns | ns | ns | ns |
| Omental fat (g) | 118 | 118 | 139 | 134 | 41.0 | ns | ns | ns | ns | ns |
| Meat chemical composition | | | | | | | | | | |
| Moisture | 75.46 | 74.84 | 75.92 | 74.75 | 1.044 | ns | ns | ns | ns | † |
| Protein | 19.56 | 19.72 | 20.36 | 20.39 | 0.636 | † | † | ns | † | ns |
| Total Fat | 2.79 | 3.55 | 2.38 | 3.52 | 0.755 | * | ns | † | ns | * |
| Ash | 1.42 | 1.36 | 1.29 | 0.98 | 0.173 | * | ns | * | * | * |

RSD: residual standard desviation

[1]Diets supplemented without linseed oil and vitamin E (Control), with linseed oil (LO), with linseed oil and 400 mg/kg of synthetic vitamin E (LO-Syn E) and with linseed oil and 400 mg/kg of natural vitamin E (LO-Nat E)

[2]Orthogonal contrasts were (1) effect of linseed oil supplementation (Control vs. linseed oil diets), (2) effect of Control diet vs. LO diet, (3) effect of vitamin E addition (LO diet vs. LO-Syn E and LO-Nat E diets), (4) effect of LO-Syn E diet vs. LO-Nat E diet.

† $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

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874 **Table 4.** Milk fatty acid profile (g/100 g of total fatty acid methyl esters)

| | Diets[1] | | | | RSD | P value | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E | | | 1 | 2 | 3 | 4 |
| ***Saturated (SFA)*** | | | | | | | | | | |
| C4:0 | 2.78 | 2.93 | 3.19 | 3.01 | 0.263 | † | * | ns | ns | ns |
| C6:0 | 2.18 | 1.88 | 1.89 | 1.64 | 0.319 | † | * | ns | ns | ns |
| C8:0 | 2.27 | 1.76 | 1.71 | 1.40 | 0.401 | * | ** | * | ns | ns |
| C10:0 | 7.38 | 5.38 | 5.26 | 4.27 | 1.453 | * | ** | * | ns | ns |
| C11:0 | 0.08 | 0.05 | 0.04 | 0.03 | 0.031 | † | * | ns | ns | ns |
| C12:0 | 4.46 | 3.41 | 3.30 | 2.80 | 0.855 | * | ** | * | ns | ns |
| C13:0 *iso* | 0.01 | 0.02 | 0.01 | 0.01 | 0.006 | ns | ns | ns | ns | ns |
| C13:0 *anteiso* | 0.02 | 0.01 | 0.03 | 0.02 | 0.009 | * | ns | ns | ** | ns |
| C13:0 | 0.08 | 0.06 | 0.05 | 0.05 | 0.023 | † | * | ns | ns | ns |
| C14:0 *iso* | 0.10 | 0.06 | 0.06 | 0.06 | 0.019 | ** | *** | *** | ns | ns |
| C14:0 | 9.74 | 8.76 | 9.17 | 8.40 | 1.109 | ns | † | ns | ns | ns |
| C15:0 *iso* | 0.20 | 0.15 | 0.16 | 0.15 | 0.040 | ns | * | † | ns | ns |
| C15:0 *anteiso* | 0,36 | 0.32 | 0.29 | 0.29 | 0.049 | † | * | ns | ns | ns |
| C15:0 | 0.88 | 0.75 | 0.68 | 0.70 | 0.106 | * | ** | * | ns | ns |
| C16:0 *iso* | 0.31 | 0.30 | 0.21 | 0.20 | 0.115 | ns | ns | ns | ns | ns |
| C16:0 | 24.40 | 20.50 | 21.53 | 20.95 | 1.775 | ** | ** | ** | ns | ns |
| C17:0 *iso* | 0.45 | 0.39 | 0.38 | 0.38 | 0.043 | * | ** | * | ns | ns |
| C17:0 *anteiso* | 0.50 | 0.40 | 0.38 | 0.39 | 0.054 | ** | ** | ** | ns | ns |
| C17:0 | 0.77 | 0.57 | 0.53 | 0.63 | 0.148 | † | * | * | ns | ns |
| C18:0 | 12.72 | 12.65 | 13.35 | 15.10 | 2.288 | ns | ns | ns | ns | ns |
| C22:0 | 0.11 | 0.12 | 0.13 | 0.11 | 0.019 | ns | ns | ns | ns | ns |
| C24:0 | 0.05 | 0.05 | 0.05 | 0.04 | 0.013 | ns | ns | ns | ns | ns |
| ***Monounsaturated (MUFA)*** | | | | | | | | | | |
| C10:1 | 0.18 | 0.12 | 0.12 | 0.10 | 0.048 | * | ** | * | ns | ns |
| *cis-9* C14:1 | 0.11 | 0.10 | 0.09 | 0.10 | 0.026 | ns | ns | ns | ns | ns |
| C16:1 n-9 | 0.26 | 0.29 | 0.26 | 0.29 | 0.042 | ns | ns | ns | ns | ns |
| C16:1 n-7 | 0.51 | 0.47 | 0.44 | 0.58 | 0.082 | * | ns | ns | ns | * |
| *trans-6+7+8* C18:1 | 0.24 | 0.80 | 0.77 | 0.95 | 0.270 | ** | *** | ** | ns | ns |
| *trans-9* C18:1 | 0.22 | 0.34 | 0.49 | 0.31 | 0.194 | ns | † | ns | ns | ns |
| *trans-10* C18:1 | 0.46 | 3.52 | 2.37 | 3.47 | 2.301 | ns | * | * | ns | ns |
| *trans-11* C18:1 (VA) | 1.16 | 3.55 | 4.66 | 3.28 | 1.175 | *** | *** | ** | ns | † |
| *cis-9* C18:1 | 20.54 | 18.85 | 17.61 | 20.16 | 3.717 | ns | ns | ns | ns | ns |
| *cis-10 + trans 15* C18:1 | 0.15 | 0.99 | 0.62 | 0.57 | 0.705 | ns | † | † | ns | ns |
| *cis-11* C18:1 | 0.68 | 1.12 | 1.02 | 1.21 | 0.285 | * | ** | * | ns | ns |
| *cis-12* C18:1 | 0.35 | 0.84 | 0.80 | 0.71 | 0.177 | *** | *** | *** | ns | ns |
| *cis-13* C18:1 | 0.04 | 0.19 | 0.17 | 0.22 | 0.118 | † | * | * | ns | ns |
| *cis-15* C18:1 | 0.13 | 0.13 | 0.13 | 0.13 | 0.035 | ns | ns | ns | ns | ns |
| C22:1 n-9 | 0.02 | 0.08 | 0.09 | 0.08 | 0.020 | *** | *** | *** | ns | ns |
| *cis-15* C24:1 | 0.02 | 0.01 | 0.01 | 0.01 | 0.006 | ns | ns | ns | ns | ns |
| *cis-15* C24:1 | 0.02 | 0.01 | 0.01 | 0.01 | 0.006 | ns | ns | ns | ns | ns |

(continued)

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879 **Table 4.** Continued

| | Diets[1] | | | | | | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E | RSD | P value | 1 | 2 | 3 | 4 |
| ***Polyunsaturated (PUFA)*** | | | | | | | | | | |
| *trans-9, trans-*12 C18:2 | 0.09 | 0.21 | 0.17 | 0.20 | 0.061 | * | ** | ** | ns | ns |
| *cis-9, trans-11* CLA | 0.46 | 1.31 | 1.46 | 0.97 | 0.448 | ** | ** | ** | ns | † |
| *trans-9, cis-7* CLA+ C20:0 | 0.26 | 0.27 | 0.29 | 0.28 | 0.028 | ns | † | ns | ns | ns |
| *trans-10, cis-12* CLA | 0.01 | 0.05 | 0.07 | 0.03 | 0.031 | * | ** | * | ns | * |
| *cis-11, cis-13* CLA | 0.01 | 0.15 | 0.15 | 0.14 | 0.028 | *** | *** | *** | ns | ns |
| *trans-11, trans-13* CLA | 0.01 | 0.08 | 0.09 | 0.10 | 0.034 | *** | *** | ** | ns | ns |
| *trans-9, trans-11* CLA + C20:1 | 0.06 | 0.15 | 0.15 | 0.14 | 0.037 | ** | *** | *** | ns | ns |
| C18:2 n-6 (LA) | 2.79 | 4.08 | 3.93 | 3.82 | 0.792 | * | ** | * | ns | ns |
| C18:3 n-6 (γ-linolenic acid) | 0.08 | 0.03 | 0.04 | 0.01 | 0.038 | * | ** | * | ns | ns |
| C18:3 n-9 | 0.02 | 0.03 | 0.03 | 0.03 | 0.013 | ns | * | † | ns | ns |
| C18:3 n-4 | 0.00 | 0.03 | 0.03 | 0.02 | 0.013 | ** | ** | ** | ns | ns |
| C18:3 n-3 (ALA) | 0.52 | 1.08 | 0.98 | 0.89 | 0.188 | *** | *** | *** | ns | ns |
| C18:4 n-3 | 0.02 | 0.04 | 0.03 | 0.03 | 0.017 | ns | ns | † | ns | ns |
| C20:2 n-6 | 0.02 | 0.03 | 0.02 | 0.02 | 0.009 | ns | ns | ns | ns | ns |
| C20:3 n-9 | 0.11 | 0.08 | 0.08 | 0.07 | 0.013 | *** | *** | *** | ns | ns |
| C20:3 n-6 | 0.04 | 0.03 | 0.03 | 0.03 | 0.009 | ** | ** | † | ns | ns |
| C20:4 n-6 (AA) | 0.23 | 0.13 | 0.12 | 0.12 | 0.033 | *** | *** | *** | ns | ns |
| C20:3 n-3 | 0.00 | 0.02 | 0.01 | 0.01 | 0.004 | ** | *** | *** | ns | ns |
| C20:4 n-3 | 0.00 | 0.01 | 0.00 | 0.01 | 0.005 | * | ns | * | * | ns |
| C20:5 n-3 (EPA) | 0.05 | 0.04 | 0.04 | 0.04 | 0.008 | ns | * | ns | ns | ns |
| C22:2 n-6 | 0.06 | 0.06 | 0.06 | 0.05 | 0.015 | ns | ns | ns | ns | ns |
| C22:4 n-6 | 0.03 | 0.02 | 0.01 | 0.01 | 0.009 | * | ** | * | ns | ns |
| C22:5 n-3 (DPA) | 0.12 | 0.09 | 0.08 | 0.10 | 0.023 | † | * | * | ns | ns |
| C22:6 n-3 (DHA) | 0.06 | 0.04 | 0.04 | 0.04 | 0.011 | ** | ** | * | ns | ns |
| SCFA | 14.87 | 12.13 | 12.20 | 10.45 | 2.199 | * | ** | * | ns | ns |
| MCFA | 43.16 | 36.56 | 37.59 | 36.00 | 3.350 | ** | ** | ** | ns | ns |
| LCFA | 41.97 | 51.31 | 50.20 | 53.55 | 5.080 | ** | ** | ** | ns | ns |
| SFA | 69.84 | 60.54 | 62.40 | 60.63 | 4.091 | ** | *** | *** | ns | ns |
| MUFA | 25.08 | 31.40 | 29.65 | 32.18 | 3.580 | * | ** | ** | ns | ns |
| PUFA | 5.07 | 8.06 | 7.95 | 7.20 | 1.001 | *** | *** | *** | ns | ns |
| PUFA n-3 | 0.78 | 1.33 | 1.29 | 1.13 | 0.193 | *** | *** | *** | ns | ns |
| PUFA n-6 | 3.26 | 4.37 | 4.20 | 4.06 | 0.771 | † | * | * | ns | ns |
| ***Ratios*** | | | | | | | | | | |
| 14:1 desaturase index[3] | 0.01 | 0.01 | 0.01 | 0.01 | 0.002 | ns | ns | ns | ns | ns |
| 16:1 desaturase index[3] | 0.03 | 0.03 | 0.03 | 0.04 | 0.004 | ** | * | † | ns | ** |
| 18:1 desaturase index[3] | 0.65 | 0.71 | 0.68 | 0.67 | 0.038 | ns | † | * | ns | ns |
| CLA desaturase index[3] | 0.29 | 0.27 | 0.24 | 0.23 | 0.036 | * | * | ns | * | ns |

880

881 RSD: residual standard desviation; [1]Diets supplemented without linseed oil and vitamin
882 E (Control), with linseed oil (LO), with linseed oil and 400 mg/kg of synthetic vitamin
883 E (LO-Syn E) and with linseed oil and 400 mg/kg of natural vitamin E (LO-Nat E);
884 [2]Orthogonal contrasts were (1) effect of linseed oil supplementation (Control vs. linseed

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885 oil diets), (2) effect of Control diet vs. LO diet, (3) effect of vitamin E addition (LO diet
886 vs. LO-Syn E and LO-Nat E diets), (4) effect of LO-Syn E diet vs. LO-Nat E diet.
887 [3]14:1 desaturase index = C14:1/(C14:0 + C14:1); desaturase index = C18:1/ (C18:0 +
888 C18:1); CLA desaturase índex = *cis*-9, *trans*-11 C18:2/ (*cis*-9, *trans*-11 C18:2 + *trans*-
889 11 C18:1) ; † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
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891 **Table 5.** FA composition (g/100 g of total fatty acids) of intramuscular fat of lambs
892 suckling from ewes receiving diets supplemented with or without linseed oil and with
893 synthetic or natural vitamin E.

| | Diets[1] | | | | | | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E | RSD | P value | 1 | 2 | 3 | 4 |
| ***Saturated (SFA)*** | | | | | | | | | | |
| C8:0 | 0.01 | 0.01 | 0.01 | 0.01 | 0.004 | † | * | ns | ns | ns |
| C10:0 | 0.37 | 0.29 | 0.20 | 0.26 | 0.078 | ** | ** | † | ns | ns |
| C11:0 | 0.01 | 0.01 | 0.01 | 0.01 | 0.003 | * | † | ns | ** | ns |
| C12:0 | 0.74 | 0.54 | 0.37 | 0.52 | 0.165 | ** | ** | * | ns | ns |
| C13:0 *iso* | 0.00 | 0.01 | 0.00 | 0.00 | 0.009 | ns | ns | ns | ns | ns |
| C13:0 *anteiso* | 0.02 | 0.01 | 0.01 | 0.01 | 0.004 | ** | ** | * | ns | ns |
| C13:0 | 0.03 | 0.03 | 0.02 | 0.02 | 0.006 | ** | ** | † | † | ns |
| C14:0 *iso* | 0.04 | 0.02 | 0.02 | 0.02 | 0.009 | ** | *** | ** | ns | ns |
| C14:0 | 6.85 | 6.35 | 5.29 | 6.12 | 0.891 | * | * | ns | ns | ns |
| C15:0 *iso* | 0.09 | 0.08 | 0.06 | 0.07 | 0.018 | * | * | ns | † | ns |
| C15:0 *anteiso* | 0.15 | 0.11 | 0.09 | 0.11 | 0.025 | ** | ** | * | ns | ns |
| C15:0 | 0.48 | 0.39 | 0.32 | 0.37 | 0.070 | ** | ** | * | ns | ns |
| C16:0 *iso* | 0.19 | 0.15 | 0.13 | 0.14 | 0.029 | ** | ** | * | ns | ns |
| C16:0 | 24.02 | 22.43 | 20.55 | 21.68 | 1.412 | ** | ** | † | † | ns |
| C17:0 *iso* | 0.48 | 0.42 | 0.37 | 0.37 | 0.038 | *** | *** | ** | * | ns |
| C17:0 *anteiso* | 0.49 | 0.39 | 0.37 | 0.35 | 0.054 | ** | *** | ** | ns | ns |
| C17:0 | 0.96 | 0.72 | 0.73 | 0.74 | 0.213 | ** | *** | *** | ns | ns |
| C18:0 | 13.52 | 13.47 | 13.32 | 14.31 | 1.052 | ns | ns | ns | ns | ns |
| C22:0 | 0.03 | 0.02 | 0.02 | 0.02 | 0.006 | * | ** | * | ns | ns |
| C24:0 | 0.10 | 0.06 | 0.10 | 0.06 | 0.029 | * | † | * | ns | * |
| ***Monounsaturated (MUFA)*** | | | | | | | | | | |
| C10:1 | 0.01 | 0.01 | 0.01 | 0.01 | 0.004 | ns | † | ns | † | ns |
| *cis-9* C14:1 | 0.26 | 0.23 | 0.20 | 0.20 | 0.036 | * | * | ns | ns | ns |
| C16:1 n-9 | 0.31 | 0.33 | 0.30 | 0.33 | 0.047 | ns | ns | ns | ns | ns |
| C16:1 n-7 | 1.77 | 1.62 | 1.53 | 1.51 | 0.223 | ns | † | ns | ns | ns |
| *trans-6+7+8* C18:1 | 0.16 | 0.50 | 0.41 | 0.59 | 0.142 | *** | *** | *** | ns | * |
| *trans-9* C18:1 | 0.19 | 0.41 | 0.32 | 0.42 | 0.076 | *** | *** | *** | ns | † |
| *trans-10* C18:1 | 0.30 | 1.00 | 1.13 | 1.38 | 0.686 | † | * | † | ns | ns |
| *trans-11* C18:1 | 0.67 | 3.09 | 3.10 | 3.66 | 0.908 | *** | *** | *** | ns | ns |
| *cis-9* C18:1 | 32.82 | 32.44 | 31.88 | 31.64 | 2.810 | ns | ns | ns | ns | ns |
| *cis-10* + *trans 15* C18:1 | 0.55 | 0.27 | 0.35 | 0.17 | 0.254 | † | * | † | ns | ns |
| *cis-11* C18:1 | 1.02 | 0.98 | 1.11 | 1.05 | 0.111 | ns | ns | ns | † | ns |
| *cis-12* C18:1 | 0.33 | 1.08 | 0.95 | 0.95 | 0.180 | *** | *** | *** | ns | ns |
| *cis-13* C18:1 | 0.06 | 0.15 | 0.13 | 0.16 | 0.038 | ** | *** | *** | ns | ns |
| *cis-15* C18:1 | 0.18 | 0.18 | 0.17 | 0.16 | 0.041 | ns | ns | ns | ns | ns |
| *cis-15* C24:1 | 0.01 | 0.01 | 0.01 | 0.01 | 0.004 | ns | ns | ns | ns | ns |
| ***Polyunsaturated (PUFA)*** | | | | | | | | | | |
| *trans-9, trans-12* C18:2 | 0.09 | 0.29 | 0.19 | 0.23 | 0.046 | *** | *** | *** | ** | ns |
| *cis-9, trans-11* CLA | 0.50 | 1.62 | 1.54 | 1.63 | 0.386 | *** | *** | *** | ns | ns |
| *trans-9, cis-7* CLA + C20:0 | 0.17 | 0.14 | 0.14 | 0.15 | 0.025 | ns | * | * | ns | ns |
| *trans-10, cis-12* CLA | 0.00 | 0.05 | 0.06 | 0.06 | 0.021 | *** | *** | *** | ns | ns |
| *cis-11, cis-13* CLA | 0.02 | 0.13 | 0.12 | 0.15 | 0.033 | *** | *** | *** | ns | ns |
| *trans-11, trans-13* CLA | 0.03 | 0.03 | 0.03 | 0.05 | 0.021 | ns | ns | ns | ns | ns |
| *trans-9, trans-11* CLA + C20:1 | 0.12 | 0.15 | 0.16 | 0.16 | 0.025 | * | ** | † | ns | ns |
| C18:2 n-6 (LA) | 5.97 | 5.61 | 7.23 | 5.74 | 0.947 | * | ns | ns | † | * |
| C18:3 n-6 (γ-linolenic acid) | 0.07 | 0.05 | 0.08 | 0.07 | 0.017 | * | ns | † | ** | ns |

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894 **Table 5.** Continued

| | Diets[1] | | | | | | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E | RSD | P value | 1 | 2 | 3 | 4 |
| ***Polyunsaturated (PUFA)*** | | | | | | | | | | |
| C18:3 n-9 | 0.06 | 0.03 | 0.04 | 0.03 | 0.013 | * | ** | ** | ns | ns |
| C18:3 n-4 | 0.00 | 0.02 | 0.02 | 0.02 | 0.008 | *** | *** | ** | ns | ns |
| C18:3 n-3 (ALA) | 0.62 | 0.97 | 1.21 | 1.05 | 0.219 | ** | *** | * | ns | ns |
| C18:4 n-3 | 0.03 | 0.03 | 0.03 | 0.03 | 0.010 | ns | ns | ns | ns | ns |
| C20:2 n-6 | 0.06 | 0.04 | 0.05 | 0.04 | 0.010 | ** | ** | ** | ns | * |
| C20:3 n-9 | 0.38 | 0.28 | 0.46 | 0.28 | 0.124 | † | ns | ns | ns | * |
| C20:3 n-6 | 0.20 | 0.12 | 0.19 | 0.12 | 0.048 | ** | * | ** | ns | * |
| C20:4 n-6 (AA) | 2.67 | 1.41 | 2.62 | 1.45 | 0.677 | ** | * | ** | † | ** |
| C20:3 n-3 | 0.03 | 0.02 | 0.03 | 0.02 | 0.008 | ns | ns | ns | ns | ns |
| C20:4 n-3 | 0.02 | 0.02 | 0.02 | 0.01 | 0.006 | ns | ns | ns | ns | ns |
| C20:5 n-3 (EPA) | 0.30 | 0.27 | 0.57 | 0.31 | 0.147 | ** | ns | ns | * | ** |
| C22:2 n-6 | 0.01 | 0.02 | 0.02 | 0.02 | 0.009 | ns | ns | ns | ns | ns |
| C22:4 n-6 | 0.26 | 0.14 | 0.22 | 0.13 | 0.057 | ** | ** | * | ns | * |
| C22:5 n-3 (DPA) | 0.77 | 0.51 | 0.87 | 0.56 | 0.235 | * | ns | † | † | * |
| C22:6 n-3 (DHA) | 0.41 | 0.25 | 0.51 | 0.26 | 0.161 | * | ns | ns | † | * |
| SCFA | 0.40 | 0.32 | 0.22 | 0.28 | 0.082 | ** | ** | † | ns | ns |
| MCFA | 36.89 | 33.83 | 30.36 | 32.57 | 2.400 | ** | ** | * | † | ns |
| LCFA | 62.71 | 65.85 | 69.42 | 67.14 | 2.456 | ** | ** | * | † | ns |
| SFA | 48.60 | 45.51 | 41.97 | 45.20 | 2.802 | ** | ** | † | ns | † |
| MUFA | 38.62 | 42.30 | 41.60 | 42.22 | 2.456 | † | ** | * | ns | ns |
| PUFA | 12.78 | 12.19 | 16.43 | 12.58 | 2.330 | * | ns | ns | † | ** |
| PUFA n-3 | 2.17 | 2.06 | 3.24 | 2.25 | 0.631 | * | ns | ns | * | * |
| PUFA n-6 | 9.24 | 7.38 | 10.41 | 7.58 | 1.599 | * | ns | † | † | ** |
| ***Ratios*** | | | | | | | | | | |
| n-6 / n-3 | 4.32 | 3.65 | 3.31 | 3.40 | 0.500 | ** | ** | * | ns | ns |
| 14:1 desaturase index[3] | 0.04 | 0.03 | 0.04 | 0.03 | 0.006 | ns | ns | ns | ns | ns |
| 16:1 desaturase index[3] | 0.08 | 0.08 | 0.08 | 0.08 | 0.009 | ns | ns | ns | ns | ns |
| 18:1 desaturase index[3] | 0.73 | 0.75 | 0.75 | 0.74 | 0.021 | ns | ns | ns | ns | ns |
| CLA desaturase index[3] | 0.43 | 0.35 | 0.34 | 0.31 | 0.037 | *** | *** | ** | ns | ns |

895

896 RSD: residual standard desviation; [1]Diets supplemented without linseed oil and vitamin
897 E (Control), with linseed oil (LO), with linseed oil and 400 mg/kg of synthetic vitamin
898 E (LO-Syn E) and with linseed oil and 400 mg/kg of natural vitamin E (LO-Nat E);
899 [2]Orthogonal contrasts were (1) effect of linseed oil supplementation (Control vs. linseed
900 oil diets), (2) effect of Control diet vs. LO diet, (3) effect of vitamin E addition (LO diet
901 vs. LO-Syn E and LO-Nat E diets), (4) effect of LO-Syn E diet vs. LO-Nat E diet
902 [3]14:1 desaturase index = C14:1/(C14:0 + C14:1); 16:1 desaturase index = C16:1/ (C16:0
903 + C16:1); 18:1 desaturase index = C18:1/ (C18:0 + C18:1); CLA desaturase índex =
904 *cis-9, trans-11* C18:2/ (*cis-9, trans-11* C18:2 + *trans-11* C18:1) ; † P < 0.10, * P < 0.05,
905 **P < 0.01, ***P < 0.001
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907 **Table 6.** Vitamin E concentrations in ewe milk and in intramuscular fat of suckling
908 lambs.

| | Diets[1] | | | | | | Contrast[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | LO | LO-Syn E | LO-Nat E | RSD | P value | 1 | 2 | 3 | 4 |
| Milk, µ/g | 0.08 | 0.09 | 0.53 | 1.45 | 0.253 | *** | *** | ns | *** | *** |
| *Longissimus dorsi*, µ/g | 0.88 | 0.91 | 1.30 | 1.53 | 0.373 | * | † | ns | * | ns |

909
910 RSD: residual standard desviation; [1]Diets supplemented without linseed oil and vitamin
911 E (Control), with linseed oil (LO), with linseed oil and 400 mg/kg of synthetic vitamin
912 E (LO-Syn E) and with linseed oil and 400 mg/kg of natural vitamin E (LO-Nat E);
913 [2]Orthogonal contrasts were (1) effect of linseed oil supplementation (Control vs. linseed
914 oil diets), (2) effect of Control diet vs. LO diet, (3) effect of vitamin E addition (LO diet
915 vs. LO-Syn E and LO-Nat E diets), (4) effect of LO-Syn E diet vs. LO-Nat E diet; † P <
916 0.10, * P < 0.05, **P < 0.01, ***P < 0.001
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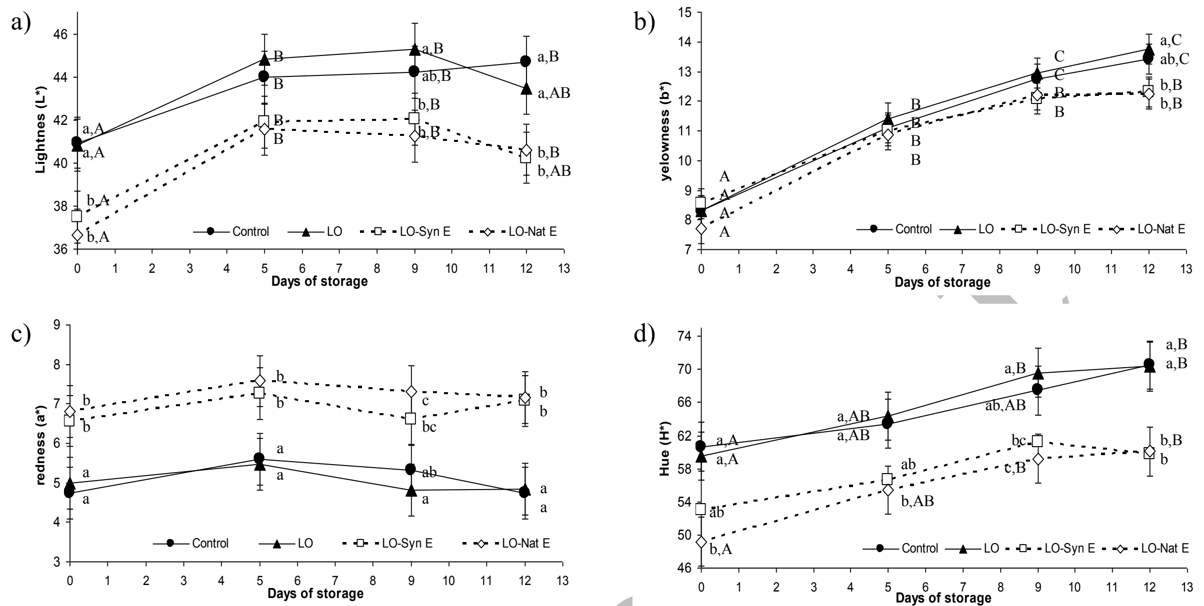
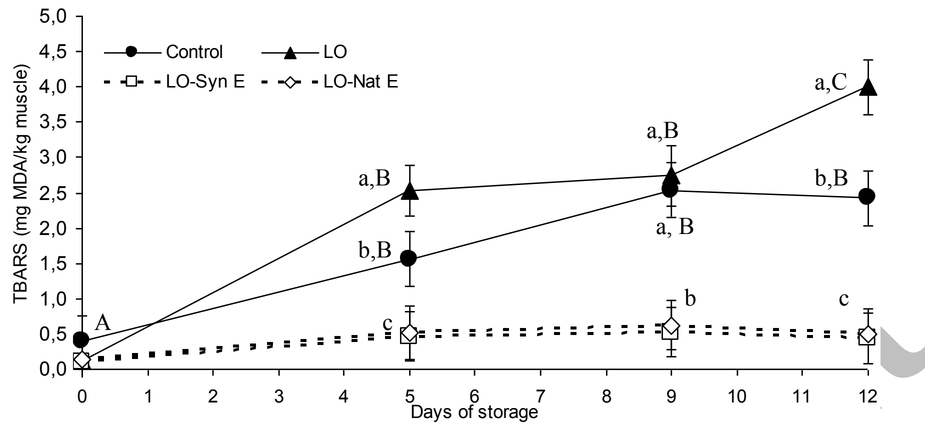



919
920 **Figure 1.** Effect of ewe treatments on the development of lightness (a), yellowness (b),
921 redness (c) and hue (d) in suckling lamb *Longissimus dorsi* muscle samples stored at
922 refrigerated display conditions for 12 days in polyethylene trays by an oxygen-
923 permeable PVC film. Different minuscule letters mean significant differences (P < 0.05)
924 between treatments within time and capital letters mean significant differences (P <
925 0.05) between time within treatment. The error bars represent standard error.
926

927




928
929
930 **Figure 2.** Effect of ewe treatments on the evolution of lipid oxidation during suckling
931 lamb meat display time (TBARS). Different minuscule letters mean significant
932 differences ($P < 0.05$) between treatments within time and capital letters mean
933 significant differences ($P < 0.05$) between time within treatment. The error bars
934 represent standard error.
935

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