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

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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ABSTRACT

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

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

1. Introduction

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

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

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

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

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.

121

122

2. Material and methods

2.1. Animal and experimental diets

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

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

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

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

2.2. Milk sampling and composition

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

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

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

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

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

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

2.5. Statistical analysis

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

3. Results

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

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

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

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.

305

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

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

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*

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.

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

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

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

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

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.

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

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

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

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

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.

545

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

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 ($r = 0.75$, $P < 0.001$), 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

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

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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844 **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

845
846 [1]Diets supplemented without linseed oil and vitamin E (Control), with linseed oil (LO),
847 with linseed oil and 400 mg/kg of synthetic vitamin E (LO-Syn E) and with linseed oil
848 and 400 mg/kg of natural vitamin E (LO-Nat E); [2] Fatty acid composition (%): C12:0, <
849 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;
850 C18:2, 14.80; C18:3, 51.30: C20:0, 0.20; C22:0, 0.10.
851

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 † $P < 0.10$, * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$
861

862 **Table 3.** Animal performance, carcass characteristics and meat chemical composition of
863 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	*	*	*

864
865 RSD: residual standard desviation
866 [1]Diets supplemented without linseed oil and vitamin E (Control), with linseed oil (LO),
867 with linseed oil and 400 mg/kg of synthetic vitamin E (LO-Syn E) and with linseed oil
868 and 400 mg/kg of natural vitamin E (LO-Nat E)
869 [2]Orthogonal contrasts were (1) effect of linseed oil supplementation (Control vs. linseed
870 oil diets), (2) effect of Control diet vs. LO diet, (3) effect of vitamin E addition (LO diet
871 vs. LO-Syn E and LO-Nat E diets), (4) effect of LO-Syn E diet vs. LO-Nat E diet.
872 † $P < 0.10$, * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$
873

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

	Diets[1]						Contrast[2]			
	Control	LO	LO-Syn E	LO-Nat E	RSD	P value	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

875 (continued)
876
877
878

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

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
890

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

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 **$P < 0.01$, ***$P < 0.001$
906

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
917

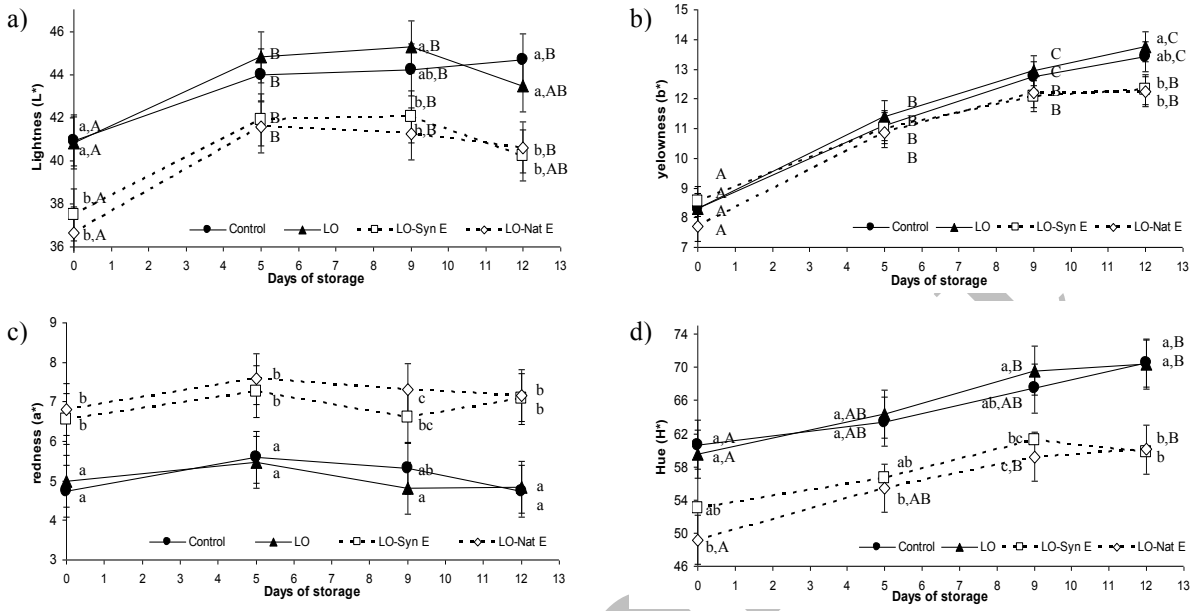
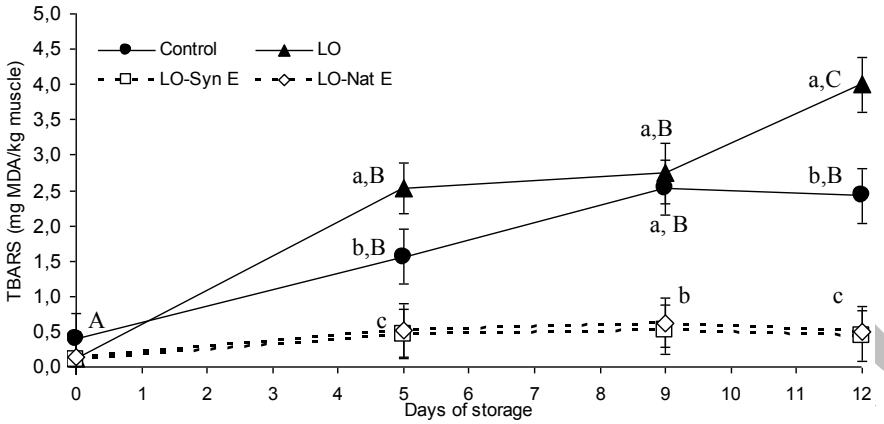


Figure 1. Effect of ewe treatments on the development of lightness (a), yellowness (b), redness (c) and hue (d) in suckling lamb *Longissimus dorsi* muscle samples stored at refrigerated display conditions for 12 days in polyethylene trays by an oxygen-permeable PVC film. Different minuscule letters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

927



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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