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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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ABSTRACT

Forty-eight Churra ewes with their new-born lambs were separated into four dietary treatments: 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 g/kg TMR of natural vitamin E). Linseed oil caused an increase in *trans*-11 C18:1 (VA), *trans*-10 C18:1, *cis*-9, *trans*-11 C18:2 (RA), *trans*-10, cis-12 C18:2 and C18:3 n-3 (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. *Trans*-10 C18:1, VA, RA, *trans*-10, *cis*-12 C18:2 and LA levels were higher in intramuscular lamb fat from treatments with linseed oil. No statistically significant differences were observed in these FA due to vitamin E supplementation or the type of vitamin E (synthetic vs. natural). Vitamin supplementation resulted in lipid oxidation levels below the threshold values for detection of rancidity in 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 in European Mediterranean regions has been focused on improving the fatty acid profile of suckling lamb meat, owing to its importance as a traditionally consumed food in that area. 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 the levels of these FA 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 shift in intermediate FAs is 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 unfavourable effects on human cholesterol level (Tricon et al., 2004) and has been shown to decrease *de novo* synthesis of FAs in the mammary gland and induce milk fat depression (Toral et al., 2010a). In contrast, *cis*-9, *trans*-11 CLA is more desirable because of its anticarcinogenic, antiatherosclerosis 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 bacterias producing *trans*-10 C18:1 or as an electron acceptor for *Butyrivibrio fibrisolvens* (Pottier et al., 2006). Hou, Wang, Wang & Liu (2013) have reported that

vitamin E could affect CLA content and the accumulation of biohydrogenation intermediates in rumen fluid.

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

The aim of this work was to determine the effects of including linseed oil and vitamin E (natural or synthetic) in early lactating ewe diets on the meat quality of their suckling lambs, with particular reference to muscle fatty acid composition, vitamin E content and its subsequent effect on colour and lipid oxidation. This work is a part of a series examining the relationship between ewe diet and milk fatty acid composition on suckling lamb fatty acid composition and *trans* fatty acid content.

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 selected ewes were fed on the same basal diet before and after parturition. The basal diet was supplied for two weeks before lambing and afterwards, 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.

Ewes were individually fed during the whole experimental period and each intake was recorded. The experimental diets were fed ad libitum to each ewe 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 in each ewe 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 exclusively milk fed during the entire 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

Twelve ewes per treatment were milked once a day during the entire experimental period 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.

Once a week, individual ewe milk production was recorded in second and third weeks of lactation and samples were taken in milk collection jars. For this, milk production was recorded by the oxytocine technique: in the morning before milking each ewe was injected with 0.35 cc of oxytocin (Oxiton®, Laboratorios Ovejeros, S.A., Spain) and then immediately milked. Ewes were returned to their paddock for six hours while the lambs were confined and after that milked again for milk sampling. One sub-sample of milk was kept at 4ºC until analysed for fat and protein, according to the International Dairy Federation (IDF, 2000), using a MilkoScan-400 analyser (Foss Electric, Hillerød, Denmark). Another two sub-samples were stored at -80ºC for subsequent analysis of fatty acid and α-tocopherol concentrations.

2.3. Slaugther procedure, carcass and meat measurements

Lambs were weighed twice a week until they reached the slaughter live weight (approximately 12 kg). Then lambs were taken to a commercial EU-licensed abattoir, stunned and slaughtered by section of the jugular vein in the neck. After exsanguination, dehiding and evisceration, carcasses were immediately weighed (hot carcass weight, HCW) and transferred to a cooler at 4ºC. After 24 hours, carcasses were weighed again (cold carcass weight, CCW), and chilling losses were calculated as the difference between HCW and CCW expressed as a proportion of the initial HCW. Dressing percentage was calculated as the ratio of CCW to slaughter live weight. Two samples of *m. Longissimus lumborum* (dissected between the 6th and the 13th rib) were stored at -80ºC, one for fatty acid composition analysis and the other for α-tocopherol level determination.

2.4. Feed and muscle chemical composition

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

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

2.5. Milk and meat fatty acid composition

Fatty acid composition of fat from milk and muscle samples was determined by gas chromatography (GC Turbo 3400 *CX*, Varian Inc., Palo Alto, CA). Fat was extracted from milk and meat by using the method described by Nudda et al. (2008). The m. *Longissimus lumborum* 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. Fatty acids were identified by comparing retention times of peaks with those of external methyl ester standards (Component FAME Mix; Supelco, Bellefonte, PA, U.S.A.). The PUFA-2, a non conjugated 18:2 isomer mixture of individual PUFA, all *cis*-5,8,11,14,17 C20:5 (eicosapentaenoic acid, EPA), all *cis*-4,7,10,13,16,19 C22:6 (docosahexaenoic acid, DHA), all *cis*-5,8,11,14 C20:4 (arachidonic acid, ARA), all *cis*-6,9,12 C18:3, and all *cis*-9,12,15 C18:3 (Matreya Inc., Pleasant Gap, PA, USA) were used to identify PUFA. Branched fatty acid methyl ester (BCFA) were identified using GLC-110 MIX (Matreya Inc. Pleasant Gap, Pa., USA) and individual BCFA methyl ester standards, methyl 14-methylpentadecanoate (*iso* C16:0), methyl 12-methyltetradecanoate (anteiso C15:0), methyl 12-tridodecanoate (*iso* C14:0) and methyl 14-methylhexadodecanoate (anteiso C17:0) (Matreya Inc. Pleasant Gap, Pa., USA). High purity individual isomers of conjugated linoleic acid (CLA; *cis*-9, *trans*-11 and *trans*-10, *cis*-12; Matreya Inc., Pleasant Gap, PA, U.S.A.) were used to identify most CLA isomers of interest. Additional CLA mixture standard (Nu-Check-Prep, Inc., Elysian, MN, U.S.A.), and published isomeric profiles (Kramer et al., 2004) were used to identify the others CLA isomers. The identification of C18:1 isomers was based on

individual commercial standard (Supelco, Bellefonte, PA, USA) and on chromatograms published by Kramer et al. (2008). Fatty acids were expressed as a proportion of identified total FAME (% of FAME).

2.6. Milk and muscle vitamin E concentration

For vitamin E analysis in ewe milk, vitamin E (in duplicated) 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). Briefly, milk and meat were saponified using methanolic KOH at 80°C for 20 min, and vitamins were extracted with n-hexane. 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.

2.7. Muscle colour evaluation and lipid oxidation

The *Longissimus thoracis et lumborum* 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 thoracis et lumborum* was divided into slices (25 mm thick), stored in polyethylene white 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, using a Minolta CM-2600d spectrophotometer (Minolta, Tokyo, Japan) in the CIE L*a*b* space under D65 illuminant, 10º observer visual angle, 11 mm aperture for illumination and 8 mm for measurement, and SCI mode conditions. The lightness (L*), redness (a*) and yellowness (b*) were recorded (in triplicated) and the hue angle (H*) and croma (C*) indices were calculated as $H^* = \tan^{-1}(b^*/a^*)*57.29$, expressed in degrees and $C^* = (a^{*2}+b^{*2})^{1/2}$.

The extent of lipid oxidation was assessed (in duplicate), using the method of Maraschiello, Sarraga & Garcia Reguiero (1999). Briefly, meat samples were mixed

with 10% trichloroacetic acid, centrifuged and filtered. The filtrate was vortexed with thiobarbituric acid; homogenized and incubated at 97°C for 20 min in water. The absorbance was measured at 532nm by spectrophotometry and a standard calibration curve was created with increasing concentrations of 1,1,3,3,tetramethoxypropane (99%), the precursor of malonaldehyde (MDA), thiobarbituric acid and water. Thiobarbituric acid reactive substance (TBARS) were expressed as μg MDA g−1 meat.

2.8. 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. Data regarding feed intake were analysed by general lineal model (12 ewes per treatment) using GLM procedure of SAS. Average daily gain of each lamb (12 lambs per treatment) was estimated by regression of live weight against time, using the REG procedure. Data regarding milk yield and composition of each ewe (12 ewes per treatment) as well as colour and TBARS of each 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) with 12 data per treatment. 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

3.1. Animal performance

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

3.2. Fatty acid composition

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

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

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

The proportion of ALA in milk increased 2-fold with the LO diet compared with the Control diet. Eicosapentaenoic acid (C20:5 n-3, EPA), docosapentaenoic acid (C22:5 n-

3, DPA) and docosahexaenoic acid (C22:6 n-3, DHA) contents were extremely low, as commonly occurs in ruminant milk. Although milk from ewes fed with linseed oil had lower concentrations of EPA ($P < 0.05$), DPA ($P < 0.05$) and DHA ($P < 0.01$), the concentration of total n-3 PUFAs was higher ($P < 0.001$) in milk from ewes supplemented with LO. The n-6/n-3 ratio was highest in milk fat from Control ewes ($P < 0.01$); no differences ($P > 0.05$) were observed between LO diets due to vitamin E supplementation.

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

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

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

3.3. Vitamin E concentration

Vitamin E concentration in milk and in intramuscular fat was influenced by dietary treatments (Table 6). Supplementing the ewe diet with vitamin E (LO vs. LO-Syn E and

LO-Nat E) increased the vitamin E content in milk ($P < 0.001$) and meat ($P < 0.05$) and these vitamin E levels were positively correlated (r = 0.75, $P < 0.001$). Although milk from LO-Nat E treatment had more vitamin E than milk from LO-Syn E ($P < 0.001$), no such differences were found in suckling lambs meat ($P > 0.05$) as a result of the type of vitamin E used.

3.4. Muscle colour

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

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

3.5. Muscle lipid oxidation (TBARS)

With reference to TBARS (Figure 2) there was a significant interaction between treatment and time ($P < 0.001$) even though there were no significant differences between treatments on day 0 ($P > 0.05$). Nevertheless, treatments with vitamin E (LO-Syn E and LO-Nat E), kept TBARS values constantly low (0.1 – 0.6 mg MDA/kg muscle), while treatments without vitamin E (Control and LO) produced values above 1.0 mg MDA/kg muscle and even reached values greater than 2.0 over time in storage. LO had higher TBARS values ($P < 0.05$) at 5 and 12 days than the Control treatment. In general, the relationship between TBARS and meat fatty acid was not strong, but TBARS values at 5 and 9 days were negatively correlated with the level of vitamin E in meat (r = - 0.44, $P < 0.05$; r = - 0.56, $P < 0.05$).

4. Discussion

4.1. Animal performance

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

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

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

Shingfield & Griinari, (2007) suggested that *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 are associated with changes in rumen lipid metabolism and could contribute toward inhibiting milk fat synthesis in the udder. However, our results are in agreement with those of Gómez-Cortés et al. (2008) who suggested that the response of sheep to supplementation with high concentrations of lipids, rich in PUFAs, and the generation of these isomers involved in milk fat depression did not significantly change milk fat in ewes.

It was reported that vitamin E may be involved in preventing the *trans*-10 shift in rumen biohydrogenation pathways and thereby alleviate a diet induced low milk fat syndrome (Pottier et al., 2006). Our results are consistent with the results reported by Bell, Griinari & Kennelly (2006) in cows, who proposed that vitamin E

supplementation could mitigate the effect of vegetable oil supplementation on milk fat depression (MFD), increasing both milk fat percentage and yield. However, the FA profile was unaltered and remained characteristic of MFD, with increases in *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 content in milk fat. Thus, other vitamin E mechanisms, different from changing biohydrogenation pathways as reported in cows (Pottier et al., 2006; Bell et al., 2006), must also be preventing milk fat depression. These additional mechanisms should be investigated in lactating ewes to explain why LO and vitamin E supplemented diets produce higher levels of milk fat compared to Control diets.

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

4.2. Milk and meat fatty acid composition

In suckling lambs the rumen is not functional, so there is no biohydrogenation of the milk FAs before they are absorbed by the intestine. Therefore, the milk FA profile of the lactating dams had a significant effect on the FA profile of meat from their suckling lambs.

As already observed by Manso et al. (2011) the lower levels of SFAs and MUFAs in suckling lamb intramuscular fat in the LO reflected the differences observed in milk FA composition and were the result of the fatty acid composition of the linseed oil. 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 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).

In spite of the difficulty of increasing ALA levels in ruminant milk because of the relatively low transfer rate of this FA from diet into milk (Palmquist, 2006), the concentration of ALA in milk fat from diets with linseed oil increased 2.0 (LO), 1.9 (LO-Syn E) and 1.7-fold (LO-Nat E) compared to the Control diet. This ALA milk content increased the ALA proportion 1.56 (LO), 1.95 (LO-Syn E) and 1.69-fold (LO-Nat E) in intramuscular fat ($r = 0.51$, $P < 0.05$). Consistent with the foregoing, Manso et al. (2011) reported a 2.0-fold increase in ALA in intramuscular fat of suckling lambs when their dams had been fed a diet supplemented with linseed oil (3% DM).

As it has been previously reported for total PUFAs, increases in long chain fatty acids (LCFA) take place mainly in the phospholipid rather than in the trygliceride fraction (Jerónimo, Alves, Prates, Santos-Silva & Bessa, 2009), so the failure of LCFA n-3 (EPA, DPA and DHA) levels to increase in the present study could be related to a lower proportion of phospholipids relative to the triglyceride fraction.

LO supplementation reduced the meat fat percentage of arachidonic acid (C20:4 n-6, AA). As it has been described by Ferlay et al. (2010) an inhibitory effect of C18:3 n-3 or its metabolites on synthesis or milk secretion of this n-6 FA could explain this result.

With respect to the intermediaries in the biohydrogenation processes of linoleic and linolenic acids, most increased their levels in milk and intramuscular fat 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 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, compared with the Control. Furthermore, RA in intramuscular fat increased 3.2-fold with LO supplementation. The strong correlation between VA and RA calculated in the current research ($r = 0.86$, $P < 0.001$ in milk; $r = 0.94$, $P < 0.001$ in meat) confirms the substrate-product relationship for Δ^9-*desaturase* (Bodas et al., 2010; 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. In tissues dietary RA from milk would not be the only source of RA, 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).

The decrease of the n-6/n-3 ratio to below 4.0 in milk and meat 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). According to recommendations of EFSA (2010) human *trans* FA intake should be as low as possible due to their association with coronary heart diseases. In all our experimental treatments with LO, with or without vitamin E supplementation, meat *trans* FA profile were acceptable, mainly due to their low *trans*-10 C18:1 concentrations improving the intake of FA like VA, RA associated with health benefits.

Some studies have indicated a possible role for high doses of vitamin E in preventing shifts in PUFA biohydrogenation pathways (Chikunya et al., 2004; Zened, Troegeler-Meynadier, Najar & Enjalbert 2012). The mechanism by which α-tocopherol may alter biohydrogenation is unclear, so the modification of rumen microbial populations and/or dynamics leading to FA hydrogenation might be involved (Hou et al., 2013).

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

In spite of the lack of higher levels of n-3 PUFAs in milk from LO-Syn E treatment, we observed significant increases in n-3 PUFAs, including EPA, DPA and DHA in suckling lamb meat from LO-Syn E treatment compared to LO-Nat E. That could be ascribed mostly to differences in intramuscular fat content, with the resulting variation in the triglycerides to phospholipid ratio, rather than to a higher protective effect of synthetic vitamin E against PUFA peroxidation. On the other hand, Kasapidou et al. (2012) reported that dietary vitamin E supplementation did not affect EPA and DHA meat content in lambs.

4.3. Vitamin E

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

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

As expected, the vitamin E concentration of suckling lamb meat mirrored maternal milk vitamin E concentrations, with the highest amounts being recorded in vitamin E supplemented ewes (LO-Syn E and LO-Nat E). Vitamin E concentrations in meat from linseed oil supplemented ewe diets and the Control diet were statistically the same. 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 that muscle vitamin E levels increased in line with dietary vitamin E levels. The lack of statistically significant differences between LO-Nat E and LO-Syn E treatments in meat vitamin E concentration could be due to greater variability in meat samples than in milk. Furthermore, the smaller effect of LO-Nat E could be because the level of vitamin E deposition was approaching maximum. Several authors suggested that muscle from lambs (Kasapidou et al., 2012) could be saturated with "supranutritional" levels of vitamin E.

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

4.4. Muscle colour

Lightness from suckling lamb muscle increased until day 9 of storage (Fig. 1a), according to several authors who reported increases in L* over time in suckling lamb meat (Osorio, Zumalacarregui, Cabeza, Figueira & Mateo (2008). In agreement with Vieira et al. (2012), there were no differences in colour measurements of suckling lamb meat between LO and Control treatments. The increase of α-tocopherol in lamb muscle because of ewe dietary vitamin E supplementation (LO-Syn E and LO-Nat E) could significantly lower L* values in meat (Ripoll et al., 2011). These authors suggested that vitamin E should modify lightness by means of water holding capacity, thus preventing high short-term lightness values due to superficial moisture. Meat oxidation reduces the water-holding capacity between muscle myofibrils, which increases juice loss from the meat and as a result meat lightness (Elisabeth & Steven, 2005). Lopez-Bote et al. (2001) proposed 3.2 mg/kg as the concentration of vitamin E required in light lambs to have a significant impact on L* stability. However, our results showed that with a lower concentration of vitamin E in suckling lamb muscle (LO-Syn E: 1.3 and LO-Nat E: 1.53 mg vitamin E/kg muscle) colour parameters could be positively affected.

Like the L* values, H* values were also affected by ewe dietary vitamin E and time in storage (Fig. 1d). The inclusion of linseed oil in ewe diets (LO) did not have any effect on the H* value of their suckling lambs, as observed by Juárez et al. (2011) in beef fed linseed. The higher vitamin E content in suckling lamb muscle could also reduce H* values, compared with lambs from treatments without additional vitamin E.

4.5. Muscle lipid oxidation (TBARS)

As expected, the oxidative processes in muscle (TBARS) were significantly affected by dietary treatment ($P < 0.001$) and storage display ($P < 0.001$). The lack of difference in TBARS values at day 0 (non-aged), can be explained by taking into account that compounds that contribute to oxidised flavour development are mainly formed during storage (Ahn, Grün & Mustapha, 2007). Nevertheless, at days 5 and 12 of storage, LO

treated lamb meat registered higher TBARS values than the Control samples. Increasing the ALA content in meat (LO: 0.97 vs. Control: 0.62; $P < 0.001$) has been shown to result in higher levels of oxidation due to the higher susceptibility of this n-3 PUFA (Wood et al., 2004).

The negative correlation between TBARS values at 5 and 9 days of storage and vitamin E concentrations in muscle (r = -0.44, $P < 0.05$; r = 0.56, $P < 0.05$) support that vitamin E increases the shelf life of meat. Lauzurica et al. (2005) maintained TBARS values around 0.5 at 12 days in the meat of lambs fed on a diet enriched with 500 mg of vitamin E/kg. At day 9 of storage, Lopez-Bote et al. (2001) reported TBARS values of 0.45 mg MDA/kg muscle in lambs fed on a diet enriched with 1000 mg of vitamin E/kg.

According to Ripoll et al. (2011) TBARS were correlated with meat colour parameters. These correlations were negative for redness (r = -0.43, $P < 0.05$) and positive for hue (r = 0.48, $P < 0.05$) because the conversion of deoxymyoglobin (purple red) to metmyoglobine (brown).

The consumer´s perception and acceptability of lamb meat has been evaluated by several authors (Khliji, Van de Ven, Lamb, Lanza & Hopkins, 2010; Ripoll et al., 2011). L*, H* and TBARS values of meat from LO-Syn E and LO-Nat E treatments remained under the acceptability thresholds (34, 59 and 1 mg MDA/kg muscle, respectively) throughout storage display, demonstrating that vitamin E counteracts fatty acid oxidation and consequently improve the colour and the shelf life of meat.

Conclusions

The use of linseed oil as a supplement in lactating ewe diets modified the milk and meat FA profile of their suckling lambs by increasing the content of healthy FAs in meat, like VA, RA and ALA. Trans-10 FA profile was low and increased in milk and meat from LO treatments. Vitamin E supplementation (synthetic or natural) had a limited effect in preventing shifts in PUFA biohydrogenation pathways from ewes fed with linseed oil, however, it kept acceptable lipid oxidation, lightness and hue angle values in suckling lamb meat during all experimental storage. Further studies should be carried out in order to determine the optimum level of natural or synthetic dietary vitamin E supplementation to prolong the shelf life of suckling lamb meat.

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

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

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

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

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

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

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

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

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

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

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

European Food Safety Authority (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal, 8: 1461.

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

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

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

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

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

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

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

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

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

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

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

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

Kramer, J.K.G., Cruz-Hernandez, C., Deng, Z., Zhou, J., Jahreis, G., & Dugan, M.E.R. (2004). Analysis of conjugated linoleic acid and trans 18:1 isomers in synthetic and animal products. *American Journal of Clinical Nutrition* 79 (suppl), 1137S-45S.

Kramer, J.K.G., Hernández, M., Cruz-Hernández, C., Kraft, J., & Dunga, M.E.R. (2008).Combining results of two GC separations partly achieves determination of all cisand trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstratedusing Ag-ion SPE fractionation. *Lipids,* 43, 259–273

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

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

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

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

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

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

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

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

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

Palmquist, D.L. (2006). Milk fat: Origin of fatty acids and influence of nutritional factors thereon. In: *Advanced Dairy Chemistry*, Vol. 2, Lipids. (P.F.

Fox and P.L.H. McSweeney, eds.). pp 43-92. Springer, New York (United States of America).

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

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

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

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

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

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

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

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

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

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

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

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

Tricon S., Burdge, G.C., Kew, S., Banerjee, T., Russell, J.J., Jones, E.L., Grimble, R.F., Williams, C.M., Yaqoob, P., & Calder, P.C. (2004). Opposing effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on blood lipids in healthy humans. *American Journal of Clinical Nutrition* 80, 614-620.

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

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

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

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

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

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

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
Fatty acid profile (%)				
C14:0	0.52	0.25	0.25	0.25
C16:0	19.11	10.95	10.95	10.95
C16:1	0.35	0.19	0.19	0.19
C18:0	2.37	3.98	3.98	3.98
C18:1	21.73	21.90	21.90	21.90
C18:2	41.43	24.61	24.61	24.61
C18:3	12.67	37.26	37.26	37.26
C>20	1.82	0.86	0.86	0.86

[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

Table 2. Milk production and chemical composition of milk

	Diets[1]				Time[2]			*P* value[3]		
	Control	LO	LO-Syn E	LO-Nat E	1	2	SED	D	T	D x T
Intake g DM day^{-1}	2283	2287	2413	2224	2301	2303	132.2	ns	ns	ns
Yield, g/day										
Milk	2174	2203	2357	2491	2223	2392	223.4	ns	ns	ns
Fat	123.6 ^{a}	128.1 ^{a}	156.5 ^{ab}	174.9 ^{b}	145.8	145.7	19.02	*	ns	ns
Protein	95.7	100.1	100.7	109.4	101.9	102.0	10.29	ns	ns	ns
Composition, %										
Fat	5.67^{a}	6.16 ^{ab}	6.71 ^{b}	6.74 ^{b}	6.60	6.02	0.385	*	*	ns
Protein	4.54 ^{a}	4.56 ^{a}	4.28 ^{b}	4.39 ^{ab}	4.58	4.31	0.102	*	***	ns

SED: standard error of difference.
[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] Weeks 2 and 3 of lactation
[3]Effects caused by experimental diet (D), time on diet (T), and their interaction (D x T)
[a,b]: Different letters indicate significant differences ($P < 0.05$)
† $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

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 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
Fat	2.79^{ab}	3.55^{a}	2.38^{b}	3.52^{a}	0.755	*	ns	†	ns	*
Ash	1.42^{a}	1.36^{a}	1.29^{a}	0.98^{b}	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.

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

[a-c] Means within a row with different superscripts differ significantly (P<0.05).

Table 4. Milk fatty acid profile (g/100 g of identified fatty acids)

	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^{a}	2.93^{ab}	3.19^{b}	3.01^{ab}	0.263	†	*	ns	ns	ns
C6:0	2.18^{a}	1.88^{ab}	1.89^{ab}	1.64^{b}	0.319	†	*	ns	ns	ns
C8:0	2.27^{a}	1.76^{b}	1.71^{b}	1.40b	0.401	*	**	*	ns	ns
C10:0	7.38^{a}	5.38^{b}	5.26^{b}	4.27^{b}	1.453	*	**	*	ns	ns
C12:0	4.46^{a}	3.41^{b}	3.30^{b}	2.80^{b}	0.855	*	**	*	ns	ns
C14:0	9.74	8.76	9.17	8.40	1.109	ns	†	ns	ns	ns
C15:0	0.88^{a}	0.75^{b}	0.68^{b}	0.70b	0.106	*	**	*	ns	ns
C16:0	24.40^{a}	20.50^{b}	21.53^{b}	20.95^{b}	1.775	**	**	**	ns	ns
C17:0	0.77^{a}	0.57^{b}	0.53^{b}	0.63^{ab}	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)										
cis-9 C14:1	0.11	0.10	0.09	0.10	0.026	ns	ns	ns	ns	ns
trans-6+7+8 C18:1	0.24^{a}	0.80^{b}	0.77^{b}	0.95^{b}	0.270	**	***	**	ns	ns
trans-9 C18:1	0.22^{a}	0.34a^{b}	0.49^{b}	0.31^{ab}	0.194	ns	†	ns	ns	ns
trans-10 C18:1	0.46^{a}	3.52^{b}	2.37^{ab}	3.47^{b}	2.301	ns	*	*	ns	ns
trans-11 C18:1 (VA)	1.16^{a}	3.55^{b}	4.66^{b}	3.28^{b}	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^{a}	1.12^{b}	1.02^{b}	1.21^{b}	0.285	*	**	*	ns	ns
cis-12 C18:1	0.35^{a}	0.84^{b}	0.80^{b}	0.71^{b}	0.177	***	***	***	ns	ns
cis-13 C18:1	0.04^{a}	0.19^{b}	0.17^{b}	0.22^{b}	0.118	†	*	*	ns	ns
cis-15 C18:1	0.13	0.13	0.13	0.13	0.035	ns	ns	ns	ns	ns
Polyunsaturated (PUFA)										
trans-9, trans-12 C18:2	0.09^{a}	0.21^{b}	0.17^{b}	0.20^{b}	0.061	*	**	**	ns	ns
cis-9, trans-11 CLA	0.46^{a}	1.31^{bc}	1.46^{b}	0.97^{c}	0.448	**	**	**	ns	†
trans-9, cis-7 CLA+ C20:0	0.26^{a}	0.27^{ab}	0.29^{b}	0.28^{ab}	0.028	ns	†	ns	ns	ns
trans-10, cis-12 CLA	0.01^{a}	0.05^{bc}	0.07^{b}	0.03^{ac}	0.031	*	**	*	ns	*
cis-11, cis-13 CLA	0.01^{a}	0.15^{b}	0.15^{b}	0.14^{b}	0.028	***	***	***	ns	ns
trans-11, trans-13 CLA	0.01^{a}	0.08^{b}	0.09^{b}	0.10^{b}	0.034	***	***	**	ns	ns
trans-9, trans-11 CLA+C20:1	0.06^{a}	0.15^{b}	0.15^{b}	0.14^{b}	0.037	**	***	***	ns	ns
C18:2 n-6 (LA)	2.79^{a}	4.08^{b}	3.93^{b}	3.82^{b}	0.792	*	**	*	ns	ns
C18:3 n-6 (γ-linolenic acid)	0.08^{a}	0.03^{b}	0.04^{b}	0.01^{b}	0.038	*	**	*	ns	ns
C18:3 n-3 (ALA)	0.52^{a}	1.08^{b}	0.98^{b}	0.89^{b}	0.188	***	***	***	ns	ns
C20:4 n-6 (AA)	0.23^{a}	0.13^{b}	0.12^{b}	0.12^{b}	0.033	***	***	***	ns	ns
C20:5 n-3 (EPA)	0.05	0.04	0.04	0.04	0.008	ns	*	ns	ns	ns
C22:5 n-3 (DPA)	0.12^{a}	0.09^{b}	0.08^{b}	0.10^{ab}	0.023	†	*	*	ns	ns
C22:6 n-3 (DHA)	0.06^{a}	0.04^{b}	0.04^{b}	0.04^{b}	0.011	**	**	*	ns	ns

(continued)

Table 4. Continued

	Diets[1]						Contrast[2]			
	Control	LO	LO-Syn E	LO-Nat E	RSD	*P* value	1	2	3	4
SFA	69.84[a]	60.54[b]	62.40[b]	60.63[b]	4.091	**	***	***	ns	ns
MUFA	25.08[a]	31.40[b]	29.65[b]	32.18[b]	3.580	*	**	**	ns	ns
PUFA	5.07[a]	8.06[b]	7.95[b]	7.20[b]	1.001	***	***	***	ns	ns
PUFA n-3	0.78[a]	1.33[b]	1.29[b]	1.13[b]	0.193	***	***	***	ns	ns
PUFA n-6	3.26a	4.37[b]	4.20[b]	4.06[b]	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[a]	0.03[b]	0.03[ab]	0.04[c]	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[a]	0.27[ab]	0.24[b]	0.23[b]	0.036	*	*	ns	*	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.
[3]14:1 desaturase index = C14:1/(C14:0 + C14:1); desaturase index = C18:1/ (C18:0 + C18:1); CLA desaturase índex = *cis*-9, *trans*-11 C18:2/ (*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1) ; † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
[a-c] Means within a row with different superscripts differ significantly (P<0.05).

Table 5. FA composition (g/100 g of identified fatty acids) of intramuscular fat of lambs suckling from ewes receiving diets supplemented with or without linseed oil and with 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)										
C12:0	0.74^{a}	0.54^{b}	0.37^{b}	0.52^{b}	0.165	**	**	*	ns	ns
C13:0 *anteiso*	0.02^{a}	0.01^{b}	0.01^{b}	0.01^{b}	0.004	**	**	*	ns	ns
C13:0	0.03^{a}	0.03^{ab}	0.02^{b}	0.02^{b}	0.006	**	**	†	†	ns
C14:0 *iso*	0.04^{a}	0.02^{b}	0.02^{b}	0.02^{b}	0.009	**	***	**	ns	ns
C14:0	6.85^{a}	6.35^{ab}	5.29^{b}	6.12^{ab}	0.891	*	*	ns	ns	ns
C15:0 *iso*	0.09^{a}	0.08^{a}	0.06^{b}	0.07^{ab}	0.018	*	*	ns	†	ns
C15:0 *anteiso*	0.15^{a}	0.11^{b}	0.09^{b}	0.11^{b}	0.025	**	**	*	ns	ns
C15:0	0.48^{a}	0.39^{b}	0.32^{b}	0.37^{b}	0.070	**	**	*	ns	ns
C16:0 *iso*	0.19^{a}	0.15^{b}	0.13^{b}	0.14^{b}	0.029	**	**	*	ns	ns
C16:0	24.02^{a}	22.43^{ab}	20.55^{c}	21.68^{bc}	1.412	**	**	†	†	ns
C17:0 *iso*	0.48^{a}	0.42^{b}	0.37^{c}	0.37^{bc}	0.038	***	***	**	*	ns
C17:0 *anteiso*	0.49^{a}	0.39^{b}	0.37^{b}	0.35^{b}	0.054	**	***	**	ns	ns
C17:0	0.96^{a}	0.72^{b}	0.73^{b}	0.74^{b}	0.213	**	***	***	ns	ns
C18:0	13.52	13.4^{7}	13.32	14.31	1.052	ns	ns	ns	ns	ns
C24:0	0.10^{a}	0.06^{b}	0.10^{a}	0.06^{b}	0.029	*	†	*	ns	*
Monounsaturated (MUFA)										
cis-9 C14:1	0.26^{a}	0.23^{ab}	0.20^{b}	0.20^{b}	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^{c}	0.50^{ab}	0.41^{b}	0.59^{a}	0.142	***	***	***	ns	*
trans-9 C18:1	0.19^{b}	0.41a	0.32^{a}	0.42^{a}	0.076	***	***	***	ns	†
trans-10 C18:1	0.30^{b}	1.00^{ab}	1.13^{a}	1.38^{a}	0.686	†	*	†	ns	ns
trans-11 C18:1	0.67^{b}	3.09^{a}	3.10^{a}	3.66^{a}	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^{a}	0.27^{ab}	0.35^{ab}	0.17^{b}	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^{b}	1.08^{a}	0.95^{a}	0.95^{a}	0.180	***	***	***	ns	ns
cis-13 C18:1	0.06^{b}	0.15^{a}	0.13^{a}	0.16^{a}	0.038	**	***	***	ns	ns
cis-15 C18:1	0.18	0.18	0.17	0.16	0.041	ns	ns	ns	ns	ns
Polyunsaturated (PUFA)										
trans-9, trans-12 C18:2	0.09^{c}	0.29^{a}	0.19^{b}	0.23^{b}	0.046	***	***	***	**	ns
cis-9, trans-11 CLA	0.50^{b}	1.62^{a}	1.54^{a}	1.63^{a}	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^{b}	0.05^{a}	0.06^{a}	0.06^{a}	0.021	***	***	***	ns	ns
cis-11, cis-13 CLA	0.02^{b}	0.13^{a}	0.12^{a}	0.15^{a}	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^{b}	0.15^{ab}	0.16^{a}	0.16^{a}	0.025	*	**	†	ns	ns
C18:2 n-6 (LA)	5.97^{b}	5.61^{b}	7.23^{a}	5.74^{b}	0.947	*	ns	ns	†	*
C18:3 n-6 (γ-linolenic acid)	0.07^{ab}	0.05^{b}	0.08^{a}	0.07^{ab}	0.017	*	ns	†	**	ns
C18:3 n-3 (ALA)	0.62^{b}	0.97^{a}	1.21^{a}	1.05^{a}	0.219	**	***	*	ns	ns
C20:4 n-6 (AA)	2.67^{a}	1.41^{b}	2.62^{a}	1.45^{b}	0.677	**	*	**	†	**
C20:5 n-3 (EPA)	0.30^{b}	0.27^{b}	0.57^{a}	0.31^{b}	0.147	**	ns	ns	*	**
C22:5 n-3 (DPA)	0.77^{ab}	0.51^{b}	0.87^{a}	0.56^{b}	0.235	*	ns	†	†	*
C22:6 n-3 (DHA)	0.41^{ab}	0.25^{b}	0.51^{a}	0.26^{b}	0.161	*	ns	ns	†	*

(continued)

Table 5. Continued

	Diets[1]						Contrast[2]			
	Control	LO	LO-Syn E	LO-Nat E	RSD	*P* value	1	2	3	4
SFA	48.60[a]	45.51[ab]	41.97[c]	45.20[bc]	2.802	**	**	†	ns	†
MUFA	38.62[b]	42.30[a]	41.60[a]	42.22[a]	2.456	†	**	*	ns	ns
PUFA	12.78[b]	12.19[b]	16.43[a]	12.58[b]	2.330	*	ns	ns	†	**
PUFA n-3	2.17[b]	2.06[b]	3.24[a]	2.25[b]	0.631	*	ns	ns	*	*
PUFA n-6	9.24[ab]	7.38[b]	10.41[a]	7.58[b]	1.599	*	ns	†	†	**
Ratios										
n-6 / n-3	4.32[a]	3.65[b]	3.31[b]	3.40[b]	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[a]	0.35[b]	0.34[b]	0.31[b]	0.037	***	***	**	ns	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
[3]14:1 desaturase index = C14:1/(C14:0 + C14:1); 16:1 desaturase index = C16:1/ (C16:0 + C16:1); 18:1 desaturase index = C18:1/ (C18:0 + C18:1); CLA desaturase índex = *cis-9, trans-11* C18:2/ (*cis-9, trans-11* C18:2 + *trans-11* C18:1) ; † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
[a-c] Means within a row with different superscripts differ significantly (P<0.05).

Table 6. Vitamin E concentrations in ewe milk and in intramuscular fat of suckling lambs.

	Diets[1]						Contrast[2]			
	Control	LO	LO-Syn E	LO-Nat E	RSD	*P* value	1	2	3	4
Milk, μg/ml	0.08 [c]	0.09 [c]	0.53 [b]	1.45 [a]	0.253	***	***	ns	***	***
m. Longissimus lumborum μg/g	0.88 [b]	0.91 [b]	1.30 [ab]	1.53 [a]	0.373	*	†	ns	*	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; † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a-c] Means within a row with different superscripts differ significantly (P<0.05).

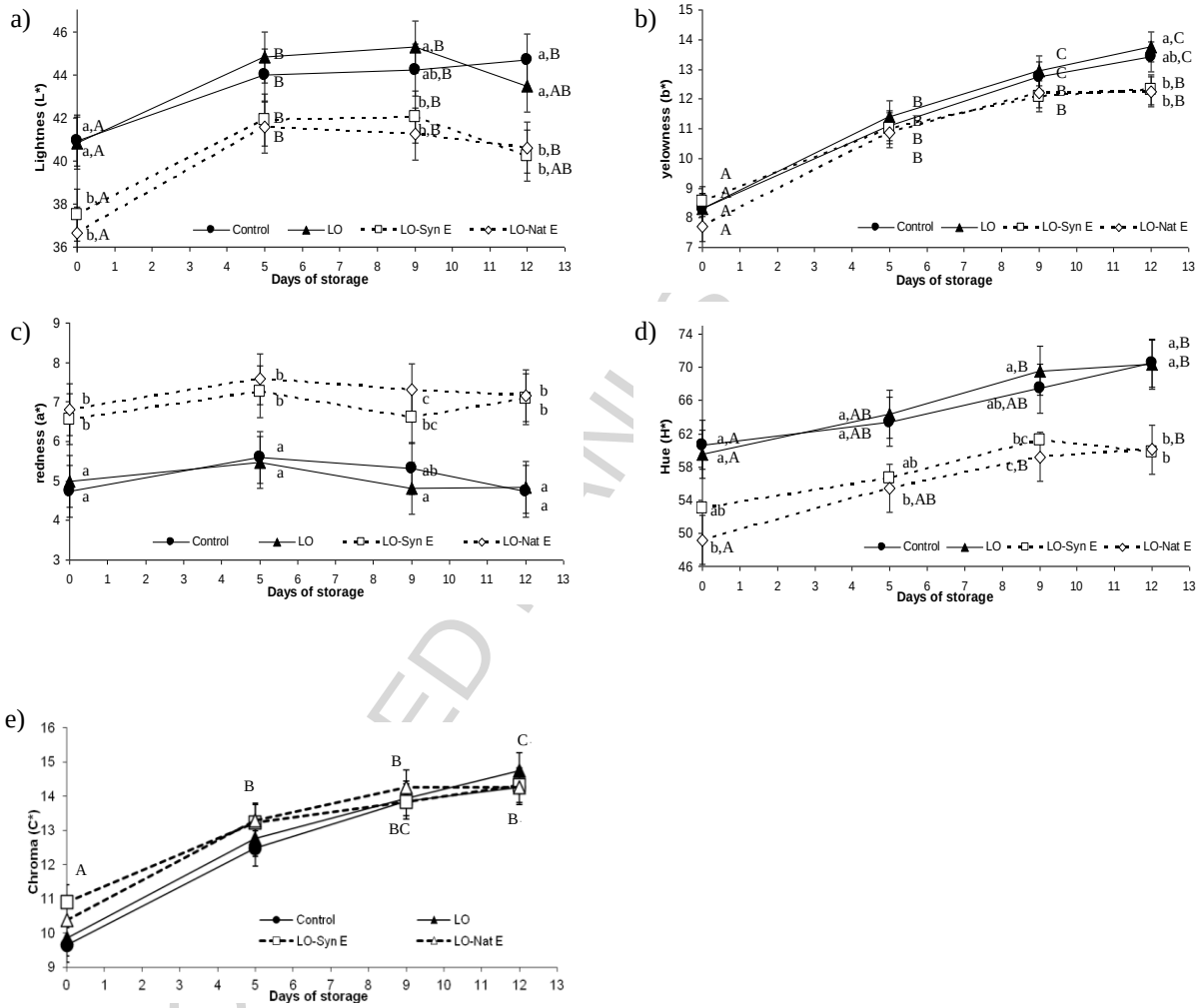

Figure 1. Effect of ewe treatments on the development of lightness (a), yellowness (b), redness (c), hue (d) and chroma in suckling lamb m. *Longissimus thoracis et lumborum* samples stored at refrigerated display conditions for 12 days in polyethylene trays by an oxygen-permeable PVC film. Different 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.

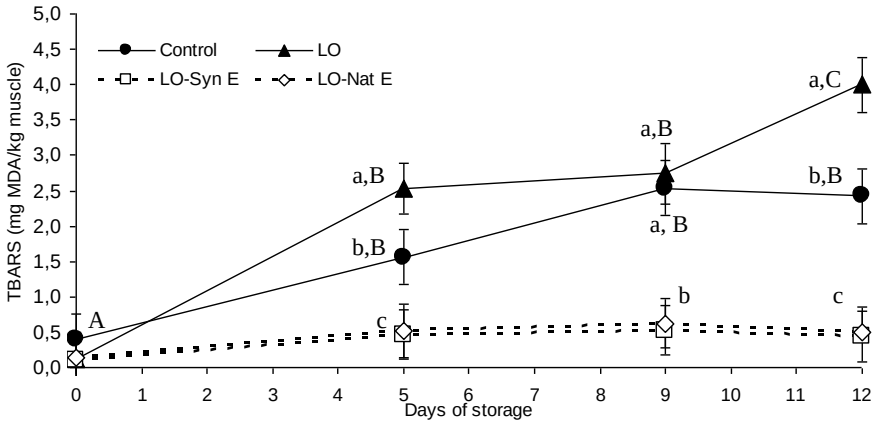

Figure 2. Effect of ewe treatments on the evolution of lipid oxidation during suckling lamb meat display time (TBARS). Different minuscule letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between time within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

Highlights

Linseed oil and natural or synthetic vitamin E fed to lactating ewes

Linseed oil increased the content of healthy fatty acids in meat of suckling lambs

Natural or synthetic vitamin E had a limited effect on milk and meat fatty acid profile

Vitamin E affected the lipid and colour stability of suckling lambs meat