Effects of different sources of fat (calcium soap of palm oil vs. extruded linseed) in lactating ewes diet on the fatty acid profile of their suckling lambs

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ABSTRACT

The main objective of this study was to evaluate the effects of supplementing lactating ewe diets with extruded linseed on the fatty acid (FA) composition of intramuscular and subcutaneous fat depots of suckling lambs. Twenty-four pregnant Churra ewes were divided into two groups based on the milk production, age, body weight and parity, and assigned to one of two treatments. Each ewe of the control treatment was supplemented with 70 g/day of FAs from a calcium soap of palm oil, while the other treatment group (Lin) was supplemented with 128 g/day of extruded linseed. All lambs were reared exclusively on milk and were slaughtered when they reached 11 kg live weight. FA profiles of ewe milk, lamb meat and subcutaneous adipose tissue were determined by GC. Lamb performance was not affected by the treatments. Muscle fat and adipose tissue from the Lin treatment showed higher proportions of polyunsaturated fatty acids (PUFA). The percentages of α-linolenic acid (C18:3 n-3), docosahexaenoic (C22:6 n-3), vaccenic (*trans*-11 C18:1) and rumenic (*cis*-9, *trans*-11 C18:2) acids in both fat depots were higher in Lin than in Control suckling lambs. Furthermore, meat fat from Lin carcasses displayed a lower n-6/n-3 ratio than control samples. Intramuscular depots clearly showed a greater content of PUFA, including *cis*-9, *trans*-11 C18:2, and a lower n-6/n-3 ratio than subcutaneous fat. The results from this study demonstrate that dietary extruded linseed supplementation of lactating ewes enhances the nutritional quality of suckling lamb fat depots such as intramuscular and subcutaneous fat.

Keywords: Suckling lamb; fatty acid; intramuscular; subcutaneous; meat; extruded linseed

1. Introduction

Suckling lamb meat is widely consumed in some geographical areas of the world such as in Mediterranean countries and is an important commodity in the north of Spain. These lambs, reared exclusively on dam milk, are slaughtered at 30-35 days of age and usually at 10-12 kg of body weight. Lamb producers are trying to adapt their product to consumer preferences in order to enhance sales. In recent years there has been a growing interest in healthy food and more specifically in increasing the n-3 polyunsaturated fatty acid (PUFA) and conjugated linoleic acid (CLA) content in meat (Raes, De Smet & Demeyer, 2004; Schmid, Collomb, Sieber & Bee, 2006; Wood et al., 2008).

It is well documented that the n-3 PUFAs have different beneficial effects on neural function, reduce the risk of cardiovascular events, and manifest anti-inflammatory activity and lipid lowering potential (Simopoulos, 2008; Kaur, Cameron-Smith, Garg & Sinclair., 2011). On the other hand, the most important isomer of CLA in ruminants, *cis*-9, *trans*-11 C18:2 (rumenic acid, RA), is thought to have anticarcinogenic and antiatherosclerotic properties (Lock, Kraft, Rice & Bauman, 2009). Furthermore, *trans*-11 C18:1 (vaccenic acid, VA), the major *trans* fatty acid (FA) in ruminant fats and the precursor of RA in tissues, may also impart additional health benefits to those associated with this CLA isomer (Field, Blewett, Proctor & Vine, 2009).

In humans, ruminant derived foods represent the major dietary source of CLA, with meat accounting for about 25%. Moreover, the highest CLA content in meat has been found in lamb (Bauman et al., 2006; Schmid et al., 2006). Meat FA composition depends on several factors, with diet being one on the most relevant (Raes et al., 2004; Schmid et al., 2006; Wood et al., 2008). Suckling lambs are considered as being functional monogastric from a digestive point of view, as their reticular groove functionality prevents milk from passing into the rumen, so there is no ruminal biohydrogenation of the milk FAs before intestinal absorption. Therefore, changes in milk FA composition due to supplements in the dam diet, can induce important differences in the FA profile of the meat and fat depots of the suckling lamb (Lanza

et al., 2006; Osorio, Zumalacárregui, Figueira & Mateo, 2007; Manso, Bodas, Vieira, Mantecón & Castro, 2011).

Several strategies have been tested in recent years to improve the FA profile of ewe fat, focused on enhancing the content of VA, RA and C18:3 n-3 (α-linolenic acid, ALA) in derived foods. Fresh pasture has been shown to be an excellent source of ALA to increase this FA in milk (Gómez-Cortés et al., 2009a) and subsequently in suckling lamb meat fat (Scerra et al., 2007). When fresh pasture is not available, linseed supplementation (oil or seed) is a reliable alternative feeding strategy to enrich the VA, RA and n-3 PUFA content in milk fat from ewes (Gómez-Cortés et al., 2009b; Bodas et al., 2010; Mele et al., 2011). Nevertheless, dietary fat rich in PUFA, like linseed supplements, may significantly alter the ruminal microbial ecosystem (Palmquist & Jenkins, 1980) and may negatively affect milk production (Palmquist, Lock, Shingfield & Bauman, 2005). Vegetable oils have a more depressing effect on ruminal digestion than oilseeds, and processed oilseeds (extruded, rolled, micronized, roasted…) are more effective at increasing milk CLA content than raw seeds but less efficient than free oil (Doreau, Aurousseay & Martin, 2009a; Doreau, Laverroux, Normand, Chesneau & Glasser, 2009b). Extrusion, the most common technique used, has been proposed in order to decrease ruminal degradability and reduce the negative effects of PUFAs on the ruminal environment (Mughetti et al., 2007).

The information available on the transfer of healthy FAs from ewes milk to suckling lambs when the dam's diet is supplemented with linseed is limited (Manso et al., 2011; Berthelot, Das, Pottier & Normand et al., 2012). It could be hypothesized that feeding diets enriched with ALA to lactating ewes improve the content of this omega-3 FA as well as their long chain metabolites in young sucking lambs. So, the aim of the present work was to investigate whether the supplementation of Churra ewe diet with extruded linseed would be a suitable strategy for improving the intramuscular and subcutaneous FA composition of their suckling lambs, without detrimentally affecting animal performance. Calcium soap of palm oil was used as a control because it is a saturated fat commonly used in sheep feeding (Doreau, Laverroux, Normand, Chesneau & Glasser, 2012).

2. Material and methods

2.1. Animal and experimental diets

Twenty-four pregnant Churra ewes (mean BW 58.56 ± 1.685 kg) were selected before lambing and fed the same control diet that they received during the experimental period but without added fat. Two days after lambing, each ewe based on their milk production, age, BW and parity in randomization was assigned to one of two experimental diets (12 ewes per treatment).

Each ewe was individually fed and a total of 2.1 kg DM of the corresponding experimental diet was supplied twice a day, plus 210 g of barley straw/ewe/day and fresh water *ad libitum*. Each ewe consumed the whole amount of TMR supplied daily.

Samples of diets were taken once a week during the whole experimental period for the determination of chemical composition using the AOAC (2003). The ingredients and chemical composition of the experimental diets are given in Table 1.

The newborn lambs (12 per treatment), covered by the protected geographical indication 'Lechazo de Castilla y León', were housed with their respective mothers all day long and were fed exclusively by suckling throughout the experimental period. 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 machine (Alfa-Laval Iberia, S.A., Madrid, Spain) was set to provide 180 pulsations per minute in a 50:50 ratio at a vacuum level of 36 kPa. Once a week, individual ewe milk production was recorded and samples were taken in milk collection jars. One sub-sample of milk was kept at 4ºC until analysed for fat and protein, in accordance with the International Dairy Federation (IDF, 2000), using a MilkoScan-400 analyser (Foss Electric, Hillerød, Denmark). Aliquots from weeks 2 and 4 of the experimental period were stored at –80ºC for FA analysis.

131 *2.3. Slaughter procedure, carcass and meat measurements*

132 Lambs were weighed twice a week until they reached the intended body weight (11 kg).
133 At the conclusion of the trial, 2 or 3 suckling lambs from each group were transported to a
134 commercial EU-licensed abattoir on 4 different days and slaughtered (26,6 ± 4,60 days of
135 age). At the abattoir, the live weight of the suckling lambs was recorded, the lambs were
136 slaughtered and carcasses were immediately transferred to a cooler at 4ºC. After 24 hours,
137 carcasses were weighed again (cold carcass weight, CCW) and chilling losses were calculated
138 as the difference between hot carcass weight (HCW) and CCW expressed as a proportion of
139 the initial HCW. Dressing percentage was calculated as the ratio of CCW to slaughter live
140 weight. Samples tissues of *m. Longissimus dorsi* (dissected from between the 6^{th} and the 13^{th}
141 rib) and subcutaneous dorsal fat (dissected from the rump) were frozen at –80ºC until FA
142 analyses.

143

144 *2.4. Fatty acid analysis*

145 Milk fat was extracted following the method described by Luna, Juárez & De la Fuente
146 (2005) and intramuscular fat using the method described by Bligh & Dyer (1959).
147 Subcutaneous fat was extracted by fusion of individual samples.

148

149 Milk FA composition (individual samples from week 2 and 4 of the suckling period) and
150 fat depots (intramuscular and subcutaneous) were determined by gas-liquid chromatography.
151 Fatty acid methyl esters (FAME) were prepared according to ISO-IDF (2002). Analysis of
152 FAME was performed on a gas-liquid chromatograph (Agilent 6890 N Network System) onto
153 a CP-Sil 88 fused silica capillary column (100 m X 0.25 mm, Varian, Middelburg,
154 Netherlands) under similar conditions to those reported by Luna, Bach, Juárez & De la Fuente
155 (2008). Individual FAME quantification was performed using a milk fat with known
156 composition (CRM 164; European Community Bureau of Reference, Brussels, Belgium).
157 Individual FAs were identified by comparison with standards distributed by Nu-Chek
158 (Elysian, MN, USA), while *trans*-11 *cis*-15 C18:2, *trans*-11 *trans*-15 C18:2, *cis*-9 *trans*-11
159 *cis*-15 C18:3 (Rumelenic acid, CLnA) and *cis*-9 *trans*-11 *trans*-15 C18:3 were identified

using a methodology as described by others (Gómez-Cortés, Tyburczy, Brenna, Juárez & De la Fuente, 2009c).

Desaturase indices were calculated as follows: 14:1 desaturase index = C14:1/ (C14:0 + C14:1), 18:1 desaturase index = C18:1/ (C18:0 + C18:1) and CLA desaturase index = *cis*-9, *trans*-11 C18:2/ (*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1).

2.5. Statistical analysis

Average daily gain was estimated by regression of live weight against time, using the REG procedure of SAS (SAS 9.2., SAS Inst. Inc., Cary, NC, USA). Data regarding milk yield and composition (FAs included) were analysed by repeated-measures analyses using the MIXED procedure and including the fixed effects of the diet (diet, D), week of sampling (time, T) and their interaction (D × T). The rest of the parameters were statistically analysed by one-way analysis of variance using the general linear model (PROC GLM). The CORR procedure was used to calculate the correlation coefficients of the FAs between milk and fat depots. The statistical significance of differences were defined as P values < 0.05 and trends as P values < 0.10.

3. Results

There were no statistical differences in chemical composition between experimental diets.

Average daily milk yield and milk composition of the dams are recorded in Table 2. Milk, fat and protein yields and protein content were not modified (P > 0.10) by dietary treatments. However, supplementation with the extruded linseed tended to decreased fat content (P < 0.10) compared with the Control diet.

Table 3 shows the FA profile of milk fats from ewes fed Control and Lin diets. There were large differences in milk FA profiles due to the type of fat added to the ewe's diet, whereas the effects of time were limited. Dietary inclusion of extruded linseed increased the

188 percentage of C12:0 *(P* < 0.05), C14:0 (*P* < 0.01) and C18:0 (*P* < 0.01) but reduced the C16:0
189 content (*P* < 0.001) by 25%. Oleic acid was the prevailing mono-unsaturated FA observed in
190 milk fat from both treatments and its content was lower (*P* < 0.001) in ewes fed with the Lin
191 diet. Most of the *trans* C18:1 isomers increased in Lin milk samples, mainly VA (3.5-fold; *P*
192 < 0.001).

193

194 The percentage of linoleic acid (*cis*-9, *cis*-12 C18:2) was lower (*P* < 0.001) but the
195 proportion of RA and other CLA isomers, i.e. *trans*-11, *cis*-13 C18:2, *trans*-11, *trans*-13
196 C18:2 and *trans*-12, *trans*-14 C18:2, were higher (*P* < 0.001) in the Lin treatment than in
197 Control. RA milk content followed a similar trend to VA, with a 2.4-fold increase when
198 extruded linseed was supplemented, whereas the content of *trans*-10 *cis*-12 isomer was very
199 low with both diets. Among non-conjugated C18:2 isomers, the highest percentage (*P* <
200 0.001) corresponded to the *trans*-11, *cis*-15 in Lin samples.

201

202 The proportion of ALA in milk increased 3-fold with the Lin diet. Moreover, extruded
203 linseed supplementation was accompanied by increases (*P* < 0.001) in CLnA and *cis*-*9, trans*-
204 *11*, *trans*-15 C18:3, two conjugated isomers of C18:3. The eicosapentaenoic acid (C20:5 n-3,
205 EPA), docosapentaenoic acid (C22:5 n-3, DPA) and docosahexaenoic acid (C22:6 n-3, DHA)
206 contents were extremely low, as commonly occurs in ruminant milk. However, milk from
207 ewes fed the Lin diet had increased (*P* < 0.001) concentrations of these n-3 FAs but decreased
208 (*P* < 0.001) concentrations of n-6 PUFA (γ-linolenic acid, C20:3, C20:4 and C22:4).
209 Therefore, the n-6/n-3 ratio was the lowest in milk fat from Lin ewes (*P* < 0.001). Finally, as
210 an indirect measurement of desaturase activity, the 18:1 desaturase index was higher (*P* <
211 0.001) in Control than in the Lin group.

212

213 Lamb performance is shown in Table 4. No differences in suckling lamb carcass yield can
214 be attributed to extruded linseed supplementation (*P* > 0.05), though a trend to increase
215 average daily gain were observed. The FA patterns of suckling lamb meat were similar to
216 those of milk from suckled dams (Tables 5 and 6). C16:0 and *cis*-9 C18:1 were the most

abundant FAs in intramuscular and subcutaneous fats. For both depots Control lambs registered the greatest concentrations of C16:0 ($P < 0.05$). Concerning *cis*-9 C18:1, a significant decrease ($P < 0.01$) and a trend to diminish ($P < 0.10$) were observed in intramuscular and subcutaneous fats respectively, in carcasses from Lin suckling lambs. Lin lambs had the greatest levels of VA, RA, ALA, and DHA in both fat depots ($P < 0.05$). However, Lin supplementation resulted in a lower n-6/n-3 ratio than in the control diet, ($P < 0.001$) this change being lower in intramuscular (2.42 vs. 5.44) than in subcutaneous (3.01 vs. 8.32) depots.

Overall, intramuscular fat was richer in PUFAs than subcutaneous fat, made up of mainly C20:4 n-6 and linoleic acid as well as other n-6 series FAs (Tables 5 and 6). It is remarkable that linoleic acid and its n-6 PUFA metabolites in intramuscular fat were not statistically different between control and Lin diets (Table 5). Furthermore, Lin intramuscular fat displayed the highest concentrations of ALA, RA, EPA, DPA and DHA. 14:1 and CLA desaturase indices were higher in intramuscular than in subcutaneous fat with only one significant reduction ($P < 0.05$) detected for the CLA desaturase index in intramuscular fat of the Lin treatment (Table 5 and 6).

Large significant correlations were observed between VA and RA in milk ($r = 0.97$, $P < 0.001$) and intramuscular fat ($r = 0.97$, $P < 0.001$) while its correlation was less prominent in subcutaneous fat ($r = 0.73$, $P < 0.01$). Significant correlations were also detected in milk fat between ALA vs. EPA ($r = 0.78$, $P < 0.001$), EPA vs. DPA ($r = 0.76$, $P < 0.001$) and DPA vs. DHA ($r = 0.81$, $P < 0.001$). On the other hand, significant correlations were found between linoleic, C20:3 n-6, C20:4 n-6 and C22:4 n-6 in intramuscular ($r = 0.63$, $P < 0.01$; $r = 0.79$, $P < 0.001$ and $r = 0.71$, $P < 0.01$) and subcutaneous fat ($r = 0.64$, $P < 0.01$; $r = 0.79$, $P < 0.001$ and $r = 0.65$, $P < 0.01$).

Positive correlations were observed between milk and intramuscular fat for oleic acid ($r = 0.87$, $P < 0.001$), *trans*-10 C18:1 ($r = 0.90$, $P < 0.001$), VA ($r = 0.79$, $P < 0.001$), RA ($r = 0.81$,

246 P < 0.001), ALA (r = 0.93, P < 0.001) and ClnA (r = 0.67, P < 0.01). Following a similar
247 behaviour, significant correlations were also detected between milk and subcutaneous fat for
248 oleic acid (r = 0.84, P < 0.001), *trans*-10 C18:1 (r = 0.94, P < 0.001), VA (r = 0.84, P <
249 0.001), RA (r = 0,74, P < 0.01), ALA (r = 0.82, P < 0.001) and ClnA (r = 0.68, P < 0.01).
250 However, no significant correlations (P > 0.05) were observed between *trans*-10 C18:1 and
251 *trans*-10 *cis*-12 C18:2 in intramuscular and subcutaneous fat depots.

252

253 **4. Discussion**

254 *4.1. Milk yield and composition*

255 Milk, fat and protein yields, as well as protein percentages were not influenced by the
256 addition of extruded linseed. A similar trend was reported with linseed oil in sheep (Bodas et
257 al., 2010, Manso et al., 2011). In contrast, Gómez-Cortés et al. (2009b) in ewe milk and
258 Hurtaud et al. (2010) in cow milk observed an increase in milk production and milk fat yield
259 when rations were supplemented with extruded linseed. However these increases could be
260 attributed to the greater dry matter intake of ration or to the extra energy supply of the
261 supplemented rations compared with the unsupplemented ones. In addition, Hurtaud et al.
262 (2010) suggested that the changes in milk fat yield with linseed supplementation appear to be
263 fairly random and relatively uncontrollable. As the diets assayed in the present experiment
264 were iso-energetic and iso-nitrogenous, and the amount of feed offered to the animals was the
265 same, no changes in milk yield and milk composition should be expected.

266 *4.2. Milk fatty acid composition*

267 The inclusion of extruded linseed in the diet produced significant increases in most of the
268 C18 FA contents, at the expense of a decrease in C16:0 concentration (Table 3). This decrease
269 had to be attributed to the low amount of palmitic acid in extruded linseed compared to its
270 high content in calcium soap of palm oil (Table 1). Furthermore, because C16:0 is partially
271 derived (about 50%) from *de novo* synthesis in the mammary gland, this decrement could
272 also, in part, be due to the effect of long-chain FAs, which can alter the lipogenic gene
273 networks in mammary epithelial cells. In fact, dietary PUFA are bio-hydrogenated in the
274 rumen to form *trans*-FAs, some of which are recognised as potent inhibitors of lipogenesis in
275 the udder (Kadegowda, Bionaz, Piperova, Erdman, & Loor, 2009).

The 3-fold increase with the Lin diet in ALA levels as well as the contents of CLnA, *trans*-11, *cis*-15 C18:2, *cis*-15 C18:1, *cis*-9, *trans*-11, *trans*-15 C18:3, *trans*-11, *trans*-15 C18:2 and *trans*-15 C18:1 corresponded to molecules which escaped completed biohydrogenation of that n-3 PUFA in the dam rumen (Destaillats, Trottier, Gálvez & Angers, 2005; Gómez-Cortés et al., 2009c; Mele et al., 2011). The observed similar effect of extruded linseed supplementation on milk VA is also consistent with the biohydrogenation pathways for ALA in the rumen, which is first isomerized to CLnA and then sequentially reduced to VA.

RA concentration in milk fat increased 2.4 fold with extruded linseed supplementation. The extent of this increase, using extruded linseed, was lower than that observed in ewes by Manso et al. (2011) when free linseed oil was used, but higher than that observed in ewes by Zhang, Mustafa & Zhao (2006) using whole raw linseed. The strong correlation between VA and RA 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 mainly originates from endogenous synthesis from VA via Δ^9-desaturase in the mammary gland (Bichi et al., 2012). The physical form of the linseed supplement could also contribute to an increase in RA levels because the process of extrusion (physical breakdown and heat-processing of linseed) may help to enhance the availability of ALA to rumen microbiota.

According to previous research done with ewes milk (Gómez-Cortés et al., 2009b; Bodas et al., 2010) the levels of *trans*-10, *cis*-12 and *trans*-9, *cis*-11 C18:2 were negligible when a ewe's diet was supplemented with high amounts of ALA. However, significant increases ($P < 0.05$) in other CLA isomers (*trans*-11 *cis*-13, *trans*-12 *trans*-14, *trans*-11 *trans*-13) observed in the Lin diet are in agreement with previously reported studies of ewes fed on pasture (Gómez-Cortés et al., 2009a) or with diets rich in extruded linseed (Gómez-Cortés et al., 2009b; Mele et al., 2011).

The increased EPA, DPA and DHA levels observed in Lin treated milk can be attributed to ALA molecules that avoided rumen biohydrogenation and are transferred to the mammary gland. ALA can be metabolized by desaturation and elongation enzymes to form a series of highly unsaturated n-3 long-chain metabolites, the major products of this pathway being EPA, DPA and DHA (Simopoulos, 2008; Kaur et al., 2011). The significant correlations between ALA vs. EPA, EPA vs. DPA and DPA vs. DHA support this statement. The noticeable

306 decrease of the n-6/n-3 ratio in milk fat when ewes were supplemented with extruded linseed
307 would be positive from a nutritional point of view (Simopoulos, 2008).

308

309 *4.3. Suckling lamb performance*

310 No changes in lamb performance were observed as a result of adding extruded linseed to
311 their dam's diet (Table 4). Awawdeh, Obeidat & Kridli (2009) and Manso et al. (2011) have
312 reported that changes in suckling lamb performance are mainly related to differences in milk
313 yield, milk fat and protein levels. Because the lambs were fed exclusively on maternal milk, a
314 lack of difference in milk yield and composition would explain the lack of effect on lamb
315 performance.

316

317 *4.4. Intramuscular and subcutaneous fatty acid composition*

318 Regardless of fat deposit, the milk FA profile of the suckled dams exerted significant
319 effects on the meat FA content. This relationship has already been described in suckling
320 lambs (Borys, Borys & Pajak, 2005; Scerra et al., 2007; Osorio et al., 2007; Manso et al.,
321 2011). In these young pre-ruminants, the rumen is not functional yet, so there is no ruminal
322 biohydrogenation of the milk FAs before they are absorbed by the intestine. Therefore,
323 changes in dam milk FA composition due to diet can induce significant differences in the FA
324 profile of meat and fat depots of their suckling lambs. Suckling lambs preferentially
325 incorporate essential FAs (linoleic and ALA) into muscle rather than storing them in adipose
326 tissue because of their important metabolic roles. Additionally, intramuscular fat was more
327 abundant in CLA and had a lower n-6/n-3 ratio therefore exhibiting a better FA profile from a
328 nutritional point of view. The major presence of PUFAs in intramuscular fat had previously
329 been observed for suckling lambs (Cañeque et al., 2005; Osorio et al., 2007; Manso et al.,
330 2011) and is based on a higher phospholipid proportion of these depots (Juárez et al., 2010).

331 The difference in palmitic acid content between suckling lamb carcasses from the Control
332 and Lin groups is explained by the different levels of this FA in milk consumed by lambs.
333 Furthermore, it has also been estimated that during the suckling lambs' first weeks of life,
334 FAs absorbed from the intestine contribute to the majority of the total deposited FAs while *de*

335 *novo* synthesized FAs will supply less than 20% (Osorio et al., 2007). The higher levels of
336 oleic acid in intramuscular fat and the trend to increase in subcutaneous adipose tissue
337 observed in control diet could be attributed to milk intake as the 18:1 desaturase indexes in
338 intramuscular and subcutaneous fats were not significantly modified (tables 5 and 6).

339 The fact that RA is less abundant in subcutaneous depot can be explained by the greater
340 CLA desaturase activity in intramuscular tissue in Control and Lin samples (Tables 5 and 6).
341 Besides, the higher correlation coefficient between RA and VA in intramuscular than in
342 subcutaneous fat would also support greater RA endogenous synthesis in intramuscular tissue.
343 These results coincide with those of Palmquist et at. (2004) who reported a higher
344 endogenous synthesis of RA from VA in muscle than in lamb adipose tissue. In contrast to
345 RA, intestinal absorption of the *trans*-10 *cis*-12 C18:2 is the only pathway involved in the
346 presence of this CLA isomer in ruminant products, as animal tissues do not possess the
347 desaturase enzyme capable of inserting a C12-double bond into the *trans*-10 C18:1 molecule.

348 The presence of ALA and ClnA in muscle and adipose tissues in suckling lambs depends
349 on the milk ALA content which, in turn, is related to the dietary composition of their dams.
350 The strong correlation between CLnA and ALA in both depots again emphasizes the
351 considerable influence that dam milk composition has on the FA profile of suckling lambs.
352 Furthermore, other C18:2 and C18:3 PUFA, such as *trans*-11 *cis*-15 C18:2, *trans*-11 *trans*-15
353 C18:2, and *cis*-9 *trans*-11 *trans*-15 C18:3, derived from ALA metabolism in ewes (Gómez-
354 Cortés et al. 2009c; Bichi et al., 2012), also increased significantly in the meat fat of Lin
355 suckling lambs. This pattern too, is consistent with the efficient incorporation of minor CLA
356 isomers such as *trans*-11 *trans*-13, *trans*-11 *cis*-13 or *trans*-12 *trans*-14 in intramuscular and
357 subcutaneous fat (Tables 5 and 6), generally linked to milk FA composition from ewes fed
358 enriched ALA diets (Gómez-Cortés et al., 2009a, 2009b).

359 EPA, DPA and DHA increased in fat deposits of suckling lambs from ewes supplemented
360 with extruded linseed, mainly in the intramuscular tissue. It is well established that diets rich
361 in ALA result in an increased level of EPA in the fat depots of muscle and subcutaneous
362 adipose tissue of suckling lambs by desaturation and elongation (Raes et al., 2004). Evidence
363 also suggests that ALA would be the preferred substrate for the Δ^5 and Δ^6-desaturase enzymes
364 (Wood et al., 2008). There is less consensus with regard to the evolution of the DHA content.
365 Lanza et al. (2006) doubled the DHA content in *Longissimus dorsi* muscle when lambs were

fed ALA high content milk. However, other studies in suckling lambs (Scerra et al., 2007) or kids (Nudda et al., 2008) did not report any remarkable increases in this n-3 PUFA when dam milk was ALA enriched.

The fact that linoleic acid and its n-6 long chain metabolites were found to be similar in carcass intramuscular deposits from Lin and Control treatments indicates that diet influenced the muscle content of ALA more than that of linoleic acid, in accordance with previous observations in lamb (Palmquist et al., 2004) and kid (Nudda et al., 2008) meat. A similar, or even more pronounced pattern was reported by Lanza et al. (2006) and Osorio et al. (2007). All this information has to be interpreted in the light of the competition between n-6 and n-3 PUFAs for both, incorporation into the intramuscular fat and enzymatic conversion: elongation and desaturation. Although potentially, these enzymes have a preference for the n-3 PUFA (Raes et al., 2004), linoleic acid incorporation into adipose tissue and muscle in relation to the amount in the diet is greater than for other FAs (Wood et al., 2008). On the other hand, linoleic acid is a precursor of C20:4 n-6 and other n-6 PUFAs in the mammalian organism, and thus the amount of these FAs in carcass fat must be reciprocally related. This would also be applicable for ALA and the longer n-3 FA.

In any case, in accordance with the higher levels of n-3 PUFA in lamb meat from ewes fed extruded linseed, the n-6/n-3 ratio was significantly lower in this group in intramuscular and subcutaneous fat. These values (2.42 intramuscular and 3.01 in subcutaneous) of Lin suckling lambs would be in accordance with nutritionist recommendations, which are for a ratio of n-6/n-3 PUFA of less than 5 (Raes et al., 2004; Simopoulos, 2008).

Conclusions

The addition of extruded linseed to the diet of lactating ewes did not affect neither ewe milk yield nor lamb performance. However, this lipid supplement did modify milk FA composition considerably and subsequently suckling lamb meat fat composition. Carcasses of animals from the linseed-fed diet group contained significantly higher levels of RA, VA, ALA, EPA, DPA and DHA and a lower n-6/n-3 ratio, mainly in intramuscular depots. Therefore, from a dietetic point of view, meat from suckling lambs whose dams have been supplemented with the most commonly used fat in sheep feeding (calcium soap of palm oil)

exhibited a less favourable lipid profile in meat than suckling lambs reared by dams fed a linseed supplemented diet. Results from this experiment therefore support the idea that incorporating extruded linseed in the feeding system of lactating ewes would produce lamb meat with a healthier lipid profile.

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

	Control	Lin
Ingredients, % as fed		
Dehydrated alfalfa	39.38	36.95
Soybean meal	13.77	12.92
Corn grain	11.83	11.10
Oat grain	10.38	9.74
Barley grain	7.86	7.37
Beet pulp	7.86	7.37
Molasses	4.95	4.64
Calcium soap of palm oil[a]	3	-
Extruded linseed[b]	-	9
Vitamin mineral premix	1.00	0.91
Chemical composition, % DM		
DM, %	88.87	88.81
Ash	9.07	8.87
NDF	28.34	26.59
ADF	17.56	16.48
Crude Protein	16.86	17.69
Ether extract	5.30	5.16
ME[c]	11,6	11,6

537
538 [a]Calcium soap of palm oil (Magnapac®,Norel Animal Nutrition, Madrid, Spain) contained (%
539 identified fatty acids) C12:0 (0.26), C14:0 (1.20), C16:0 (46.9), C18:0 (40.7) and C18:1
540 (9.70).
541 [b]Extruded linseed (Tradilin®, S.A.S. Valorex, La Messayais, Combourtille, France). Product
542 consisted of 30% wheat middlings and 70% extruded linseed. Fatty acid composition (%
543 identified fatty acids): C12:0 (0.05), C14:0 (0.10), C16:0 (6.40), C18:0 (4.00), C18:1 (15.10),
544 C18:2 (18.20) and C18:3 (54.30).
545 [c] ME: metabolizable energy (MJ/Kg DM) estimated using FEDNA (2003)
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551 **Table 2.** Milk production and chemical composition of milk.

	Diets[a]			P value[b]		
	Control	Lin	SED	D	T	D x T
Yield, g/d						
Milk	1790.9	1746.2	199.54	ns	ns	ns
Fat	90.2	77.1	14.47	ns	ns	ns
Protein	77.5	77.1	8.58	ns	ns	ns
Composition, %						ns
Fat	4.98	4.35	0.497	†	ns	ns
Protein	4.38	4.47	0.122	ns	ns	ns

552 [a] Diets supplemented with calcium soaps of palm oil (Control) and extruded linseed (Lin).

553 [b] Effects caused by dietary treatment (D), time on diet (T), and their interaction (D x T).

554 SED: standard error of the difference.

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

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558 **Table 3.** Milk fatty acid profile (g/100 g of total fatty acid methyl esters)

	Diets[a]				SED	P value[b]		
	Control		Lin			D	T	D x T
	Week 2	Week 4	Week2	Week4				
Saturated (SFA)								
C4:0	4.61	4.46	4.720	4.56	0.189	ns	ns	ns
C6:0	2.78	2.96	3.39	3.66	0.216	***	ns	ns
C8:0	2.24	2.48	2.84	3.18	0.254	***	ns	ns
C10:0	5.56	6.50	7.04	8.34	0.773	**	*	ns
C12:0	2.95	3.42	3.55	4.05	0.366	*	†	ns
C13:0 *iso*	0.015	0.014	0.021	0.021	0.0017	***	ns	ns
C13:0 *anteiso*	0.020	0.027	0.022	0.028	0.0034	ns	**	ns
C13:0	0.081	0.099	0.087	0.107	0.0130	ns	*	ns
C14:0 *iso*	0.073	0.074	0.074	0.075	0.0057	ns	ns	ns
C14:0	7.08	7.86	8.16	9.07	0.480	**	*	ns
C15:0 *iso*	0.17	0.17	0.20	0.19	0.011	***	ns	ns
C15:0 *anteiso*	0.25	0.27	0.32	0.33	0.019	***	ns	ns
C15:0	0.61	0.65	0.68	0.72	0.039	**	ns	ns
C16:0 *iso*	0.19	0.18	0.21	0.19	0.014	ns	ns	ns
C16:0	26.30	27.89	19.30	21.00	0.992	***	*	ns
C17:0	0.68	0.54	0.60	0.50	0.077	ns	*	ns
C18:0 *iso*	0.089	0.070	0.065	0.050	0.0102	**	*	ns
C18:0	11.81	10.36	13.79	11.89	0.840	**	**	ns
C20:0	0.17	0.19	0.17	0.17	0.008	†	ns	ns
C21:0	0.036	0.039	0.045	0.042	0.0027	**	ns	ns
C22:0	0.058	0.061	0.061	0.065	0.0040	ns	ns	ns
C23:0	0.030	0.034	0.031	0.033	0.0027	ns	ns	ns
C24:0	0.025	0.028	0.030	0.030	0.0021	*	ns	ns
Monounsaturated (MUFA)								
cis-9 C10:1	0.17	0.22	0.18	0.26	0.031	ns	**	ns
cis-9 C14:1	0.073	0.993	0.071	0.099	0.0136	**	**	ns
cis-9 C15:1	0.059	0.066	0.073	0.073	0.0049	**	ns	ns
trans-9 C16:1+ C17:0 *iso*	0.33	0.31	0.52	0.42	0.025	***	**	*
cis-7 C16:1	0.26	0.27	0.25	0.24	0.011	*	ns	ns
cis-9 *C*16:1 + C17:0 *anteiso*	0.96	1.01	0.79	0.80	0.054	***	ns	ns
cis-13 C16:1	0.033	0.045	0.033	0.054	0.0086	ns	*	ns
cis-9 C17:1	0.25	0.20	0.17	0.14	0.035	*	ns	ns
trans-4 C18:1	0.027	0.028	0.025	0.024	0.0025	†	ns	ns
trans-5 C18:1	0.030	0.030	0.026	0.025	0.0024	**	ns	ns
trans-6 +7+8 C18:1	0.32	0.31	0.39	0.37	0.033	**	ns	ns
trans-9 C18:1	0.26	0.25	0.34	0.33	0.024	***	ns	ns
trans-10 C18:1	0.40	0.37	0.55	0.64	0.167	†	ns	ns
trans-11 C18:1	0.85	0.85	3.57	2,50	0.393	***	†	†
trans-12 C18:1	0.32	0.32	0.52	0.57	0.048	***	ns	ns
cis-9 C18:1	24.54	22.03	18.54	16.84	1.977	***	ns	ns
trans-15 + *cis*-11 C18:1	0.52	0.47	0.73	0.73	0.028	***	ns	ns

cis-12 C18:1	0.22	0.21	0.59	0.63	0.042	***	ns	ns
cis-13 C18:1	0.046	0.043	0.065	0.067	0.0058	***	ns	ns
trans-16 + *cis*-14 C18:1	0.30	0.30	0.58	0.60	0.032	***	ns	ns
cis-15 C18:1	0.054	0.054	0.266	0.323	0.0248	***	ns	ns
cis-16 C18:1	0.022	0.021	0.045	0.049	0.0028	ns	ns	ns
cis-11 C20:1	0.055	0.050	0.046	0.039	0.0040	**	*	ns
Non conjugated C18:2								
trans-11, *trans*-15 C18:2	0.012	0.013	0.135	0.102	0.0182	***	ns	ns
trans-11, *cis*-15 C18:2	0.03	0.03	0.85	0.74	0.114	***	ns	ns
C18:2 n-6	2.44	2.36	1.84	1.77	0.099	***	ns	ns
Other non conjugated C18:2	0.38	0.37	0.58	0.69	0.049	***	ns	ns
Conjugated C18:2								
cis-9, *trans*-11 C18:2	0.35	0.39	0.97	0.81	0.114	***	ns	ns
trans-9, *cis*-11 C18:2	0.006	0.006	0.008	0.009	0.0017	†	ns	ns
trans-10, *cis*-12 C18:2	0.006	0.006	0.003	0.005	0.0011	*	ns	ns
trans-11, *cis*-13 C18:2	0.007	0.006	0.058	0.037	0.0084	***	†	ns
trans-12, *trans*-14 C18:2	0.009	0.010	0.059	0.087	0.0062	***	**	**
trans-11, *trans*-13 C18:2	0.014	0.014	0.055	0.063	0.0064	***	ns	ns
Other *trans*-*trans* conjugated C18:2	0.019	0.017	0.012	0.012	0.0029	**	ns	ns
Other PUFA								
C18:3 n-6 (γ-Linolenic acid)	0.038	0.042	0.022	0.021	0.0040	***	ns	ns
C18:3 n-3 (α-Linolenic acid)	0.32	0.30	0.95	0.92	0.075	***	ns	ns
C18:3 (*cis*-9, *trans*-11, *cis*-15)	0.030	0.030	0.116	0.095	0.0126	***	ns	ns
C18:3 (*cis*-9, *trans*-11, *trans*-15)	0.006	0.005	0.042	0.036	0.0058	***	ns	ns
C20:3 n-6	0.018	0.018	0.010	0.010	0.0022	***	ns	ns
C20:4 n-6 AA	0.12	0.12	0.11	0.09	0.011	***	ns	ns
C20:5 n-3 EPA	0.030	0.029	0.060	0.060	0.0055	***	ns	ns
C22:4 n-6	0.019	0.021	0.014	0.011	0.0018	***	ns	*
C22:5 n-3 DPA	0.075	0.069	0.104	0.096	0.0083	***	ns	ns
C22:6 n-3 DHA	0.030	0.029	0.049	0.039	0.0052	***	ns	ns
SFA	65.81	68.40	65.42	68.33	1.881	Ns	*	ns
MUFA	28.63	26.02	26.88	24.35	1.816	Ns	†	ns
PUFA	3.97	3.90	6.05	5.70	0.378	***	ns	ns
TOTAL CLA	0.41	0.45	1.17	1.02	0.130	***	ns	ns
Ratios								
14:1 desaturase index[c]	0.010	0.012	0.008	0.011	0.0012	†	*	ns
18:1 desaturase index[c]	0.70	0.71	0.65	0.66	0.013	***	ns	ns
CLA desaturase index[c]	0.29	0.32	0.21	0.25	0.011	***	***	ns
n-6/n-3	5.84	6.04	1.80	1.75	0.286	***	ns	ns

559 [a] Diets supplemented with calcium soaps of palm oil (Control) and extruded linseed (Lin).
560 [b]Effects caused by dietary treatment (D), time on diet (T), and their interaction (D x T).
561 [c] 14:1 desaturase index = C14:1/(C14:0 + C14:1); desaturase index = C18:1/ (C18:0 + C18:1);
562 CLA desaturase índex = *cis*-9, *trans*-11 C18:2/ (*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1)
563 SED: standard error of the difference; AA: arachidonic acid; EPA: eicosapentaenoic acid;
564 DPA: docosapentaenoic acid; DHA: docosahexaenoic acid
565 † P < 0.10, * P < 0.05, **P < 0.01, ***P < 0.001
566 Week 2: samples of the second week of lactation; week 4: samples of the fourth week of
567 lactation

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569 **Table 4.** Suckling lamb performance

	Diets[a]		SED	P value
	Control	Lin		
Birth body weight (kg)	3.91	4.54	0.183	**
Slaughter weight (kg)	10.84	11.30	0.201	ns
Average daily gain (g animal^{-1}day^{-1})	248	279	12.6	†
Hot carcass weight (kg)	5.99	6.21	0.121	ns
Cold carcass weight (kg)	5.84	6.07	0.119	ns
Chilling losses (%)	2.6	2.3	0.17	ns
Dressing percentage (%)	46.1	46.3	0.51	ns

570 [a] Diets supplemented with calcium soaps of palm oil (Control) and extruded linseed (Lin).

571 SED: standard error of the difference

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

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576 **Table 5.** Mean effects of diet supplementation with extruded linseed (Lin) on intramuscular
577 fat fatty acid profile (g/100 g of total fatty acid methyl esters) of suckling lambs.

	Diets[a]		SED	P value
	Control	Lin		
Saturated (SFA)				
C6:0	0.026	0.031	0.0075	ns
C8:0	0.024	0.019	0.0027	ns
C10:0	0.27	0.25	0.020	ns
C12:0	0.37	0.38	0.033	ns
C13:0	0.053	0.060	0.0054	ns
C14:0 *iso*	0.28	0.39	0.044	†
C14:0	4.23	4.07	0.235	ns
C15:0 *iso*	0.087	0.072	0.0058	ns
C15:0 *anteiso*	0.10	0.12	0.007	†
C15:0	0.26	0.29	0.016	ns
C16:0 *iso*	0.13	0.13	0.011	ns
C16:0	22.07	19.48	0.706	*
C17:0	0.66	0.56	0.048	ns
C18:0 *iso*	0.12	0.09	0.011	†
C18:0	12.69	12.57	0.585	ns
C19:0	0.11	0.90	0.012	ns
C20:0	0.13	0.13	0.009	ns
C21:0	0.022	0.024	0.0029	ns
C22:0	0.39	0.45	0.051	ns
C23:0	0.24	0.32	0.032	†
Monounsaturated (MUFA)				
cis-9 C10:1	0.032	0.029	0.0039	ns
cis-9 C14:1	0.18	0.18	0.014	ns
trans-9 C16:1 + C17:0 *iso*	0.43	0.63	0.035	**
cis-7 C16:1	0.057	0.051	0.0051	ns
cis-9 C16:1 + C17:0 *anteiso*	2.32	2.04	0.121	ns
cis-13 C16:1	0.080	0.080	0.0081	ns
cis-9 C17:1	0.40	0.33	0.029	ns
trans-6+7+8 C18:1	0.20	0.20	0.014	ns
trans-9 C18:1	0.22	0.26	0.013	†
trans-10 C18:1	0.27	0.32	0.036	ns
trans-11 C18:1	0.57	2.40	0.281	***
trans-12 C18:1	0.29	0.44	0.018	***
cis-9 C18:1	31.95	25.43	1.392	**
trans-15 + *cis*-11 C18:1	1.35	1.57	0.145	ns
cis-12 C18:1	0.49	1.05	0.082	***
cis-13 C18:1	0.092	0.110	0.0109	ns
trans-16 + *cis*-14 C18:1	0.23	0.38	0.030	**
cis-15 C18:1	0.10	0.21	0.019	**
cis-16 C18:1	0.14	0.18	0.016	ns
cis-11 C20:1	0.14	0.11	0.008	†

Non conjugated C18:2				
trans-11, *trans*-15 C18:2	0.067	0.110	0.0097	**
trans-11, *cis*-15 C18:2	0.076	0.434	0.0279	***
C18:2 n-6	8.41	9.16	0.626	ns
Other non-conjugated C18:2	0.55	0.81	0.053	**
Conjugated C18:2				
cis-9, *trans*-11 C18:2	0.36	1.25	0.129	***
trans-9, *cis*-11 C18:2	0.022	0.023	0.0025	ns
trans-10, *cis*-12 C18:2	0.016	0.015	0.0018	ns
trans-11, *cis*-13 C18:2	0.033	0.069	0.0071	**
trans-12, *trans*-14 CLA	0.027	0.041	0.0042	*
trans-11, *trans*-13 C18:2	0.021	0.046	0.0031	***
Other *trans*-*trans* conjugated C18:2	0.051	0.062	0.0037	†
Other PUFA				
C18:3 n-6 (γ-Linolenic acid)	0.11	0.12	0.009	ns
C18:3 n-3 (α-Linolenic acid)	0.43	1.76	0.147	***
C18:3 (*cis*-9 ,*trans*-11, *cis*-15)	0.54	0.92	0.083	**
C18:3 (*cis*-9, *trans*-11, *trans*-15)	0.025	0.058	0.0053	**
C20:3 n-6	0.077	0.074	0.0062	ns
C20:4 n-6 AA	4.49	4.41	0.497	ns
C20:5 n-3 EPA	0.44	1.42	0.263	*
C22:4 n-6	0.16	0.18	0.024	ns
C22:5 n-3 DPA	1.02	1.60	0.183	*
C22:6 n-3 DHA	0.62	1.25	0.178	*
SFA	42.24	39.53	0.925	†
MUFA	39.54	36.01	1.229	†
PUFA	13.54	23.80	1.628	*
TOTAL CLA	0.48	1.44	0.130	***
Ratios				
14:1 desaturase index[b]	0.039	0.039	0.0024	ns
18:1 desaturase index[b]	0.74	0.72	0.008	ns
CLA desaturase index[b]	0.39	0.34	0.011	*
n-6/n-3	5.44	2.42	0.263	***

[a] Diets supplemented with calcium soaps of palm oil (Control) and extruded linseed (Lin).

[b] 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)

SED: standard error of the difference; AA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid

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

586 **Table 6.** Mean effects of diet supplementation with extruded linseed (Lin) on subcutaneous
587 fatty acid profile (g/100 g of total fatty acid methyl esters) of suckling lambs.

	Diets[a]		SED	P value
	Control	Lin		
Saturated (SFA)				
C5:0	0.047	0.041	0.0062	ns
C6:0	0.042	0.055	0.0073	ns
C7:0	0.037	0.044	0.0060	ns
C8:0	0.069	0.071	0.0074	ns
C10:0	0.88	0.90	0.083	ns
C12:0	1.22	1.26	0.147	ns
C13:0 *iso*	0.023	0.022	0.0019	ns
C13:0 *anteiso*	0.014	0.016	0.0013	ns
C13:0	0.08	0.09	0.007	ns
C14:0 *iso*	0.066	0.068	0.0033	ns
C14:0	9.00	10.29	0.420	*
C15:0 *iso*	0.16	0.18	0.004	***
C15:0 *anteiso*	0.18	0.23	0.010	**
C15:0	0.63	0.68	0.034	ns
C16:0 *iso*	0.19	0.21	0.009	ns
C16:0	31.15	28.00	0.886	*
C17:0	0.92	0.89	0.033	ns
C18:0 *iso*	0.11	0.10	0.008	ns
C18:0	11.81	12.14	0.486	ns
C19:0	0.078	0.079	0.0041	ns
C20:0	0.11	0.11	0.006	ns
C21:0	0.012	0.013	0.0007	ns
C22:0	0.010	0.009	0.0009	ns
C23:0	0.015	0.022	0.0029	†
C24:0	0.017	0.019	0.0017	ns
Monounsaturated (MUFA)				
cis-9 C10:1	0.058	0.062	0.0065	ns
cis-9 C14:1	0.25	0.31	0.017	*
cis-9 C15:1	0.048	0.056	0.0032	†
trans-9 C16:1+ C17:0 *iso*	0.35	0.46	0.012	***
cis-7 C16:1	0.38	0.37	0.012	ns
cis-9 C16:1+ C17:0 *anteiso*	2.40	2.43	0.108	ns
cis-9 C17:1	0.46	0.43	0.029	ns
trans-4 C18:1	0.015	0.016	0.0011	ns
trans-5 C18:1	0.012	0.038	0.0028	***
trans 6+7+8 C18:1	0.29	0.33	0.016	*
trans-9 C18:1	0.30	0.38	0.012	***
trans-10 C18:1	0.37	0.47	0.044	ns
trans-11 C18:1	0.79	2.83	0.271	***
trans-12 C18:1	0.20	0.39	0.028	***
cis-9 C18:1	32.74	29.24	1.276	†
trans-15+*cis*-11 C18:1	0.58	0.65	0.024	***

cis-12 C18:1	0.23	0.49	0.028	***
cis-13 C18:1	0.057	0.085	0.0034	***
trans-16 + *cis*-14 C18:1	0.27	0.49	0.026	***
cis-15 C18:1	0.066	0.251	0.0119	***
cis-11 C20:1	0.077	0.071	0.0067	ns
Non conjugated C18:2				
trans-11, *trans*-15 C18:2	0.007	0.068	0.0054	***
trans-11, *cis*-15 C18:2	0.035	0.639	0.0514	***
C18:2 n-6	1.48	1.10	0.081	**
Other non-conjugated C18:2	0.50	0.95	0.044	***
Conjugated C18:2				
cis-9, *trans*-11 C18:2	0.18	0.41	0.038	***
trans-9, *cis*-11 C18:2	0.008	0.007	0.0005	ns
trans-10, *cis*-12 C18:2	0.004	0.004	0.0003	ns
trans-11, *cis*-13 C18:2	0.005	0.016	0.0012	***
trans-12, *trans*-14 C18:2	0.009	0.028	0.0019	***
trans-11, *trans*-13 C18:2	0.005	0.013	0.0011	***
Other *trans*-*trans* conjugated C18:2	0.040	0.020	0.0032	***
Other PUFA				
C18:3 n-6 (γ-linolenic acid)	0.017	0.016	0.0013	ns
C18:3 n-3 (α-linolenic acid)	0.13	0.32	0.025	***
cis-9, *trans*-11, *cis*-15 C18:3	0.016	0.050	0.0044	***
cis-9, *trans*-11, *trans*-15 C18:3	0.003	0.019	0.0016	***
C20:3 n-6	0.007	0.006	0.0008	ns
C20:4 n-6 AA	0.035	0.034	0.0064	ns
C20:5 n-3 EPA	0.019	0.028	0.0069	ns
C22:4 n-6	0.014	0.014	0.0016	ns
C22:5 n-3 DPA	0.027	0.039	0.0066	ns
C22:6 n-3 DHA	0.015	0.022	0.0020	*
SFA	56.87	55.55	1.116	ns
MUFA	37.11	36.94	1.106	ns
PUFA	2.55	3.80	0.151	***
TOTAL CLA	0.24	0.50	0.040	***
Ratios				
14:1 desaturase index[b]	0.027	0.029	0.0013	ns
18:1 desaturase index[b]	0.75	0.75	0.007	ns
CLA desaturase index[b]	0.18	0.15	0.021	ns
n-6/n-3	8.32	3.01	0.206	***

588 [a] Diets supplemented with calcium soaps of palm oil (Control) and extruded linseed (Lin).
589 [b] 14:1 desaturase index = C14:1/(C14:0 + C14:1); desaturase index = C18:1/ (C18:0 + C18:1);
590 CLA desaturase índex = *cis*-9, *trans*-11 C18:2/ (*cis*-9, *trans*-11 C18:2 + *trans*-11 C18:1)
591 SED: standard error of the difference; AA: arachidonic acid; EPA: eicosapentaenoic acid;
592 DPA: docosapentaenoic acid; DHA: docosahexaenoic acid
593 † $P < 0.10$, * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$