Short term evolution of nutritionally relevant milk fatty acids of goats fed a cereal-based concentrate enriched with linseed oil

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ABSTRACT

Goat diet supplementation with linseed oil (LO) can be a fruitful technological strategy to enhance the nutritional value of milk fat. The objective of the present study was to determine the effect of LO in a basal diet rich in starch on goat milk fatty acid (FA) profile. The FA contents were monitored exhaustively during 24 h after LO supplementation or elimination by gas chromatography. Rumenic acid, the main conjugated linoleic acid isomer and α -linolenic acid, both related with bioactive properties, significantly rose in milk between 12 and 24 h after the inclusion of LO in the diet. On the contrary, medium-chain saturated FA decreased. Changes in other nutritionally relevant milk FA as well as short term effects caused by dietary LO suppression are discussed.

Keywords: fatty acids, goats, linseed oil, starch.

1. Introduction

Goat milk production is economically relevant for semi-arid lands in less favoured areas worldwide. Furthermore, dairy goat farming is an important sector of agri-food industries in developed countries of the Mediterranean region. Caprine milk has been recommended as an ideal substitute for bovine milk because it is more similar to human breast milk, and possesses biologically active properties, such as high digestibility, better buffering capacity as well as therapeutic values in human nutrition (Park, 2009; Zervas and Tsiplakou, 2013). The smaller fat globules of goat milk are better dispersed, being naturally homogenized, and provide a greater fat surface area that would benefit human digestion (Zervas and Tsiplakou, 2013). In addition, goat milk fat contains a high proportion of short-chain and branched-chain fatty acids (FA) (Alonso, Fontecha, Fraga, Lozada and Juárez, 1999), which are positively related to the dairy products flavour and their digestibility.

Information regarding the composition of milk is essential for the successful development of dairy goat industry and for marketing purposes. The variation in milk FA composition is large, with important interactions between the basal diet (types of forages, starchy concentrates, etc) and the supplementation with oils or oilseeds (Chilliard and Ferlay, 2004). Diets enriched with oils can produce dairy fat with an improved nutritional quality (Shingfield, Bonnet and Scollan, 2013).

In dairy goat feeding, different oils as canola (Mir, Goonewardene, Okine, Jaegear, & Scheer, 1999), soybean (Bouattour, Casals, Albanell, Such, & Caja, 2008) and sunflower (Bernard, Leroux, Faulconnier, Durand, Shingfield, & Chilliard, 2009a) have been assayed. Goat diet supplementation with moderate levels of linseed oil (LO) has a negligible effect on animal performance and reduces the milk fat content of medium-chain saturated FA as well as increases the levels of α -linolenic, an omega-3 FA (Martínez Marín et al., 2011, 2012, 2013). The main pathway of α -linolenic acid biohydrogenation in the rumen involves firstly the isomerization of the *cis*-12 double bond to *trans*-11. Then, the obtained *cis*-9 *trans*-11 *cis*-15 18:3 is hydrogenated to *trans*-11 *cis*-15 18:2, and finally to *trans*-11 18:1. *Trans*-11 18:1 (vaccenic acid, VA) is converted by the delta-9 desaturase in the mammary gland (Bernard et al., 2010) to *cis*-9 *trans*-11 18:2 (rumenic acid, RA), an isomer of conjugated linoleic acid with relevant biological properties (Bauman, Lock, Corl, Ip, Salter and Parodi., 2006). A reduced milk fat atherogenicity index and n-6/n-3 FA ratio from LO supplemented rations, suggests that dietary LO could be an excellent technological option to nutritionally improve the FA profile of goat milk fat (Martínez Marín et al., 2012).

Despite the extensive research in this subject, there is few information in the literature describing the extent of the response of FA profile to LO supplementation on a short-term basis (Martínez Marín et al., 2013; Baldin, Rico, Green, & Harvatine, 2018) and very little is known about the quickness of those changes in the contents of FA involved in milk fat nutritive value. To cover this gap, the present research examined in detail the changes in FA composition for 24 h just after LO incorporation in a basal diet rich in cereals. Cereals, due to their high starch content, are commonly used in ruminant feeding to enhance the energy content of the diet and, thus, to increase animal performance. The evolution at short-term of the milk FA profile after dietary LO elimination was also finely monitored and kinetics from both treatments were statistically.

2. Materials and Methods

2.1 Experimental design and animal management.

The present research was carried out at the University of Córdoba facilities in accordance with the EU Directive 2010/63/EU for animal studies (EC, 2010). Eight Malagueña goats in their 2nd and 3rd lactation (118 ± 16 days in milk, 56.7 ± 2.5 kg of body weight, 1.37 ± 0.29 kg/d of milk, and body condition scores of 2.5-3.0 on a scale 1-5) were used. The goats were grouped by body weight and milk production (four animals in each group) and then the animals from each group were randomly were allocated to two treatments in a cross-over design with four animals per treatment and two experimental periods of 25 d. The basal diet consisted of alfalfa hay and a concentrate rich in cereals (Table 1) that was supplemented (LS) or not (CTR) with 30 g/d of LO. Four goats were fed the CTR diet and four goats were fed the LS diet for 25 d. Then, the former group was sharply switched to the LO supplemented diet (CTR-LS treatment) and the latter group was sharply switched to the unsupplemented diet (LS-CTR treatment). After 25 d, the diet was reversed. Individual milk samples were obtained from milkings at 0, 1, 3, 6, 12 and 24 h after each diet shift. Before each milking, a dose of 2-3 i.u. of oxytocin was administered to the goats. Milkings at 1, 3, 6 and 12 h were stripped out by hand, whereas 0 and 24 h were machine milked (DeLaval, Madrid, Spain).

2.2. Measurements and chemical analysis

Daily DMI, body weight, milk production and diet composition were measured following (Martínez Marín *et al.*, 2012). Fat, lactose and protein contents in milk were determined with a Milko-Scan FT120 (Foss Electric, Hillerød, Denmark). Milk fat separation was carried out using the method proposed by Luna *et al.* (2005). Briefly, 30 ml of raw milk were centrifuged at 12.000 rpm for 30 min at 20°C in a Beckman (Fullerton, CA) J2-MC centrifuge. The fat layer was transferred to a microtube and then microcentrifuged at 13.000 rpm for 20 min at room temperature. After this centrifugation, the fat top layer was taken to be analyzed. Fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of glycerides with KOH in methanol (ISO-IDF, 2002). 100 mg of the extracted fat were dissolved in 5 ml of hexane. 0.2 ml of KOH (11.2 g in 100 ml of methanol) were added and the mixture was vigorously stirred. After 5 minutes 0.5 g of solid NaHSO₄H₂O was added mix again and centrifuge at 3 minutes at room temperature. Finally an aliquot from the obtained supernatant was used for gas chromatography.

Gas chromatography with two different columns was used to provide a complete FAME profile in accordance with the method proposed by De la Fuente, Rodríguez-Pino and Juárez (2015). An Agilent model 6890 N Network Gas Chromatograph (Palo Alto, CA, USA) equipped with auto-injector, fitted with a FID on a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., Varian, Middelburg, The Netherlands) was used. Injector and detector temperature was 250 °C. He was the carrier gas, at an inlet pressure of 193.9 kPa and a split ratio of 1:100. Initial oven temperature was 45 °C. After 4 min, oven temperature was raised at 13 °C min⁻¹ to 165 °C and held for 35 min, then increased to 215 °C at 4 °C min⁻¹ and maintained for 30 min. Another Agilent gas chromatograph, model 7820A GC System also equipped with auto-injector and flame ionization detector, was fitted with a SLB-IL111 capillary column (100 m × 0.25 mm i.d., Supelco, Bellefonte, PA, USA). Injector and detector temperature was 250 °C. The column inlet pressure was set at 241 kPa, resulting in He gas flow rates of 0.8 mL min⁻¹. Milk samples were injected at a split ratio of 1:100. Initial oven temperature was isothermal (168 °C), and after 45 min it was raised at 5 °C min⁻¹ to 210 °C and held for 36.6 min.

Seven FAME mixtures (GLC-403, GLC-409, GLC-411, GLC-423, GLC-461, GLC-481B and GLC-643) were purchased from Nu-Chek Prep. Inc. (Elysian, MN, USA). A mixture of CLA FAME isomers (trans-8 cis-10, cis-9 trans-11, cis-11 trans-13 and 10-trans 12-cis), also from Nu-Chek, was used in the CLA region analysis. In order to form other CLA isomers, this mixture was isomerized using I₂. Additionally, linoleic (Ref. 7792) and linolenic (Ref. 7793) acid methyl ester mixtures covering the entire geometric cis/trans spectrum were purchased from Sigma-Aldrich (Madrid, Spain).

2.3. Statistical analysis

Data of milk fat FA contents were analysed by repeated measurement analysis (Littell, Stroup, Milliken, Wolfinger, & Schabenberger, 2006) using the PROC MIXED of SAS University Edition 3.5 (SAS Institute Inc., Cary, USA). The model included the fixed effects of treatment, sampling time, period, sequence, and the interaction treatment x hour. The random effect was the animal nested within the interaction treatment x period x sequence. The repeated effect was the sampling time and the subject of the repeated measurements was the animal nested within the interaction treatment x period x sequence. A covariance structure appropriate for unequally spaced measures (compound symmetry, ANTE(1) or spatial power) was chosen on the basis of the Schwarz's Bayesian Information model fit criteria. The CONTRAST procedure was used to compare least squares means. Least squares means of each treatment at 1, 3, 6, 12 and 24 h were compared with their respective 0 h. Additionally, both experimental treatments were compared at each sampling time. Significant differences were declared at P<0.05.

3. Results and Discussion

The time elapsed (24 h) was too short to find any effects on animal performance. Neither previous studies have found any negative effects of moderate amounts of LO in similar goat diets supplied for longer times on milk yield and composition (Martínez Marín et al., 2011).

More than 100 individual FA were quantified in milk samples and the most relevant are shown in Tables 2, 3 and 4. Some FA showed both significant differences at 0 h and decreasing or increasing trends during the period monitored after dietary LO suppression or incorporation. However, only those FA that exhibited an unambiguous tendency with time will be discussed below.

Saturated fatty acids (SFA) were the most important group from a quantitatively point of view (Table 2). The response of short-chain (4:0, 6:0 and 8:0) and medium-chain (10:0-16:0) SFA to LO inclusion or suppression in the diet followed different patterns over time. The contents of the former remained unchanged in both treatments, while the latter increased from 6 h onwards after LO suppression and did not change by LO inclusion. Higher contents of medium-chain SFA before LO supplementation, without differences on short-chain FA contents, agrees with the literature (Martínez Marín et al., 2012). The increased availability of long-chain FA in the mammary gland after lipid supplementation has negative effects on the *de novo* synthesis of FA (Chilliard and Ferlay, 2004). The longer the FA chain, the more the *de novo* synthesis is inhibited due to the differential synthesis pathways of both FA groups (Palmquist, 2006). In addition, these results would indicate that the mammary gland enzymes involved in the *de novo* synthesis of SFA respond slower to a rise of long-chain FA availability than to its decline. In this regard, previous research indicated that more than 24 h would be needed in order to induce

changes in medium-chain SFA contents after oil supplementation (Martínez Marín et al., 2013, Soják et al., 2015). The obtained results are positive from both a technological and a nutritive perspective. Caproic (6:0) and caprilic (8:0) FA are positively related to the “goaty” flavor intensity of caprine milk, thus contributing to the organoleptic properties of derived dairy products. Furthermore, milk fat is the only quantitatively relevant source of butyric acid (4:0) in the human diet and its consumption may have positive health effects, while 12:0, 14:0 and 16:0 should be considered detrimental because they can favor cardiovascular diseases (Parodi, 2004).

A total of 13 branched-chain FA (BCFA) were detected in small amounts in milk fat and most of them did not show any trend over time (Table 2). Caprine milk fat presents a wide variety of BCFA other than *iso* and *anteiso* FA (Alonso et al., 1999), which are synthesized when methyl-malonyl-CoA, instead of malonyl-CoA, is incorporated in the *de novo* FA synthesis (Massart-Leën, Roets, Peeters and Verbeke, 1983). These non-terminal monomethyl BCFA (i.e. methyl-4 8:0) lend characteristic flavors to dairy products (Ha and Lindsay, 1991). Our results suggest that a longer sampling period would be needed in order to establish their kinetics over time.

Regarding monounsaturated fatty acids (MUFA) (Table 3), 18:1 FA was the most relevant group and *cis*-9 18:1 (oleic acid) was quantitatively the second FA in milk. It did not experiment any significant trend either under LO inclusion or suppression in the diet, in accordance to Martínez Marín et al. (2013) who reported no changes in oleic acid percentages in milk fat after 21 d of LO supplementation. Other minor *cis* 18:1 isomers (i.e. *cis*-12 18:1 and *cis*-15 18:1), related to α -linolenic acid biohydrogenation (BH) pathways in the rumen (Loor, Ferlay, Ollier, Ueda, Doreau, & Chilliard, 2005; Bell, Griinari, & Kennelly, 2006; Martínez Marín et al., 2015), showed significantly higher contents in the milk fat of the LS-CTR treatment at 0 h than after 24 h, and the opposite behavior in the CTR-LS treatment. It is noteworthy that the effect of LO inclusion on those isomers took 24 h while only 12 h were needed to diminish them in milk after LO suppression. This result would suggest that the response of the rumen microbiota to the presence of LO is very rapid.

The most abundant *trans* 18:1 isomers showed similar patterns (Table 3). At 0 h, milk fat presented higher contents of *trans*-9, *trans*-11 (vaccenic acid, VA), *trans*-12, *trans*-13/*trans*-14 and *trans*-16 in the LS-CTR treatment than in the CTR-LS treatment. At 24 h, these *trans* 18:1 FA turned back and the LS-CTR treatment produced milk fat with significantly lower contents. Vaccenic acid was quantitatively the major *trans* MUFA in milk fat. Its increase after LO addition was expected as VA is the predominant *trans* 18:1 intermediate in the conventional BH route before its complete reduction to stearic acid (Loor et al., 2005; Martínez Marín et al., 2012). On the other hand, milk FA with a *trans* double bond between carbons 13 and 16 are predominantly associated with the presence of LO on diet (Martínez Marín et al., 2015). Overall, the changes in *trans* 18:1 milk levels were slower in response to LO incorporation than to LO suppression.

The behavior of *trans*-10 18:1 partly differed from other *trans* 18:1 isomers (Table 3). The CTR-LS treatment did not modify significantly its percentage in milk fat. This FA is generally linked to the presence of a linoleic acid source on high starch diets. Starch digestion in the rumen can originate environmental conditions that promote microorganisms towards the production of *trans*-10 18:1 from linoleic acid by a shift on ruminal BH pathways. The lack of a *trans*-10 18:1 increase when a starch-rich diet is

fortified with LO would be important from a nutritional perspective because this FA has been associated with the risk of suffering from cardiovascular disease (Bauman et al., 2006; Chardigny et al., 2008).

Linoleic acid (*cis*-9, *cis*-12 18:2) was the predominant polyunsaturated FA in milk fat, followed by rumenic acid (*cis*-9, *trans*-11 18:2, RA), the major conjugated linoleic acid isomer of ruminant origin (Table 4). Linoleic acid was not affected by the experimental treatments, whereas RA contents were higher in the LS-CTR treatment at 0 h and its kinetics mirrored those of VA. Rumenic acid is mainly synthesized in the udder by desaturation of VA which is originated in the rumen and reaches the mammary gland through the bloodstream (Griinari, Corl, Lacy, Chouinard, Nurmela and Bauman, 2000; Bernard et al., 2010; Bichi et al. 2012). It is remarkable that milk VA levels were reduced after 3 h of LO suppression (Table 3), but its consequences on RA contents were not observed until 9 hours later. Emphasis on increasing the RA content in foods has grown substantially in the last decade due to the nutritional benefits associated with the consumption of this FA already demonstrated in animal and *in vitro* studies (Bauman et al., 2006; Shingfield et al., 2013).

Regarding the conversion of VA to RA in the mammary gland due to the activity of the delta-9 desaturase, it was observed that just before the diet shift the desaturase index (calculated as product/product+substrate) was higher in the CTR-LS treatment than in the LS-CTR treatment (0.333 vs. 0.297; $P<0.05$), but the reverse was true after 24 h (0.290 vs. 0.332; $P<0.05$). The kinetics of the desaturase index differed between treatments. In the CTR-LS treatment, the desaturase index decreased to 0.290 after 24 h of LO supplementation ($P<0.05$). In the LS-CTR treatment, the delta-9 desaturase activity was recovered very soon after LO suppression as the desaturase index increased to 0.345 after 3 h ($P<0.05$) and remained with no further significant changes thereafter. The negative response of the desaturase index to lipid supplementation agrees with previous studies in goats (Mele et al., 2008) and could be attributed to an increased availability of polyunsaturated FA in the mammary gland. As a result, the milk fat melting point would tend to decrease and, subsequently, the demand of products of the delta-9 desaturase enzyme would also decrease (Toral et al., 2013).

The highest levels of α -linolenic acid in milk fat as well as its primary polyunsaturated BH intermediates (i.e. *cis*-9, *trans*-11, *cis*-15 18:3, *trans*-11, *cis*-15 18:2 or *trans*-10, *cis*-15 18:2) were detected in the LS-CTR treatment at 0 h as previously reported by other authors (Bernard et al., 2009b; Martínez Marín et al., 2012; Gómez-Cortés et al., 2017) (Table 4). Increases of *cis*-9, *trans*-11, *cis*-15 18:3 and *trans*-11, *cis*-15 18:2 were observed at 6 and 12 h after LO supplementation, respectively. Baldin et al. (2018), in a very recent study, also observed an increase of both intermediates in rumen just few hours after LO addition. On average, the suppression or inclusion of LO in the diet caused changes in the contents of α -linolenic acid BH intermediates after 6 h of dietary shift. In contrast, α -linolenic acid contents decreased after 3 h of LO suppression and increased after 12 h of LO inclusion in the diet, which demonstrates almost instantaneous changes in milk depending on the presence or not of dietary lipids. From a nutritional standpoint, α -linolenic acid exerts well-known biological properties. Although the effects associated with α -linolenic acid BH intermediates are not yet fully understood, some of these omega-3 FA such as *cis*-9, *trans*-11, *cis*-15 18:3 have already been related to anti-obesity properties (Miranda et al., 2011).

4. Conclusion

The application of a technological strategy based on supplementation of goat diet with LO altered the milk fat composition towards a healthier profile. Increases of omega-3 FA and RA were accompanied by decreasing in SFA contents. Most changes were observed in less than 24 h. In general terms, the elimination of LO in the animal diet generated more rapid changes than its incorporation. Our results support a fast responsiveness of milk FA to the inclusion or suppression of LO in regular supplied ruminant's rations.

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6. References

- Alonso, L., Fontecha, J., Lozada, L., Fraga, M.J., & Juarez, M. (1999). Fatty acid composition of caprine milk: Major, branched-chain, and trans fatty acids. *Journal of Dairy Science* 82, 878-884.
- Baldin, M., Rico, D.E., Green, M.H., & Harvatine, K.J. (2018). Technical note: An in vivo method to determine kinetics of unsaturated fatty acid biohydrogenation in the rumen. *Journal of Dairy Science* (in press).
- Bauman, D.E., Lock, A.L., Corl, B.A., Ip, C., Salter, A.M., & Parodi, P.W. (2006). Milk fatty acids and human health: Potential role of conjugated linoleic acid and trans fatty acids. In K. Sjersén, T. Hvelplund, & M.O. Nielsen (Eds.), *Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress*. (pp. 523-555). Wageningen: Wageningen Academic Publishers.
- 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.
- Bernard, L., Leroux, C., Faulconnier, Y., Durand, D., Shingfield, K.J., & Chilliard, Y. (2009a). Effect of sunflower-seed oil or linseed oil on milk fatty acid secretion and lipogenic gene expression in goats fed hay-based diets. *Journal of Dairy Research*, 76, 241-248.
- Bernard, L., Shingfield, K.J., Rouel, J., Ferlay, A., & Chilliard Y. (2009b). Effect of plant oils in the diet on performance and milk fatty acid composition in goats fed diets based on grass hay or maize silage. *British Journal of Nutrition*, 101, 213-224.
- Bernard, L., Mourirot, J., Rouel, J., Glasser, F., Capitan, P., Pujos-Guillot, E., Chardigny, J.M., & Chilliard, Y. (2010). Effects of fish oil and starch added to a diet containing sunflower-seed oil on dairy goat performance, milk fatty acid composition and in vivo delta 9-desaturation of C-13 vaccenic acid. *British Journal of Nutrition*, 104, 346-354.

- Bichi, E., Toral, P.G., Hervás, G., Frutos, P., Gómez-Cortés, P., Juárez, M., & De la Fuente, M.A. (2012). Inhibition of $\Delta 9$ -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.
- Bouattour, M.A., Casals, R., Albanell, E., Such, X., & Caja, G. (2008). Feeding soybean oil to dairy goats increases conjugated linoleic acid in milk. *Journal of Dairy Science*, 91, 2399-2407.
- Chardigny, J.M., Destailats, F., Malpuech-Brugere, C., Moulin, Bauman, D.E., Lock, A.L., Barbano, D.M., Mensink, R.P., Bezelgues, J.B., Chaumont, P., Combe, N., Cristiani, I., Joffre, F., German, J.B., Dionisi, F., Boirie, Y., & Sébédio, J.L. (2008). Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the trans Fatty Acids Collaboration (TRANSFACT) study. *American Journal of Clinical Nutrition*, 87, 558-566.
- Chilliard, Y., & Ferlay, A. (2004). Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reproduction Nutrition and Development*, 44, 467-492.
- De la Fuente, M.A., Rodriguez-Pino, V: & Juárez, M. (2015). Use of an extremely polar 100-m column in combination with a cyanoalkyl polysiloxane column to complement the study of milk fats with different fatty acid profiles. *International Dairy Journal*, 47, 52-63.
- European Commission (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the EU*, 276, 33–79.
- Gómez-Cortés, P., Cívico, A., De la Fuente, M.A., Juárez, M., Núñez Sánchez, N., Peña Blanco, F., & Martínez Marín, A.L. (2017). Dietary linseed oil increases trans-10 cis-15:18:2 in caprine milk fat. *Journal of Dairy Science*, 100, 4235-4240.
- Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V., & Bauman, D.E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta-9 desaturase. *Journal of Nutrition*, 130, 2285–2291.
- Ha, J.K., & Lindsay, R.C. (1991). Contributions of cow, sheep, and goat milks to characterizing branched-chain fatty acid and phenolic flavors in varietal cheeses. *Journal of Dairy Science*, 74, 3267-3274.
- ISO-IDF (2002) Milk Fat-Preparation of Fatty Acid Methyl Esters. International Standard ISO 15884-IDF 182:2002.
- Littell, R.C., Stroup, W.W., Milliken, GA, Wolfinger, R.D., & Schabenberger, O. (2006) SAS for mixed models. 2nd ed. SAS Institute Inc., Cary, NC.

- 379 Loor, J.J., Ferlay, A., Ollier, A., Ueda, K., Doreau, M., & Chilliard, Y. (2005). High-
380 concentrate diets and polyunsaturated oils alter trans and conjugated isomers in bovine
381 rumen, blood, and milk. *Journal of Dairy Science*, 88, 3986–3999.
- 382
- 383 Luna, P., Juárez, M., De la Fuente, M.A. (2005). Validation of a rapid milk fat separation
384 method to determine the fatty acid profile by gas chromatography. *Journal of Dairy*
385 *Science*, 88, 3377-3381.
- 386
- 387 Martínez Marín, A.L., Gómez-Cortés, P., Gómez Castro, G., Juárez, M., Pérez Alba, L.,
388 Pérez Hernández, M., & De la Fuente, M.A. (2011). Animal performance and milk fatty
389 acid profile of dairy goats fed diets with different unsaturated plant oils. *Journal of Dairy*
390 *Science*, 94, 5359-5368.
- 391
- 392 Martínez Marín, A.L., Gómez-Cortés, P., Gómez Castro, G., Juárez, M., Pérez Alba, L.,
393 Pérez Hernández, M., & De la Fuente, M.A. (2012). Effects of feeding increasing dietary
394 levels of high oleic or regular sunflower or linseed oil on fatty acid profile of goat milk.
395 *Journal of Dairy Science*, 95, 1942-1955.
- 396
- 397 Martínez Marín, A.L., Gómez-Cortés, P., Gómez Castro, G., Juárez, M., Pérez Alba, L.,
398 Pérez Hernández, M., & De la Fuente, M.A. (2013). Time-dependent variations in milk
399 fatty acid content of goats fed 3 different plant oils. *Journal of Dairy Science*, 96, 3238-
400 3246.
- 401
- 402 Martínez Marín, A.L., Gómez-Cortés, P., Núñez Sánchez, N., Juárez, M., Garzón Sigler,
403 A.I., Peña Blanco, F., & De la Fuente, M.A. (2015). Associations among plant oils fed to
404 dairy goats and C18 isomers in milk fat. *Journal of Dairy Research*, 82, 152-160.
- 405
- 406 Massart-Leëen, A.M., Roets, E., Peeters, G., & Verbeke, R. (1983). Propionate for fatty
407 acid synthesis by the mammary gland of the lactating goat. *Journal of Dairy Science*, 66,
408 1445-1454.
- 409
- 410 Mele, M., Serra, A., Buccioni, A., Conte, G., Pollicardo, A., & Secchiari, P. (2008). Effect
411 of soybean oil supplementation on milk fatty acid composition from Saanen goats fed
412 diets with different forage: concentrate ratios. *Italian Journal of Animal Science*, 7, 297-
413 311.
- 414
- 415 Mir, Z., Goonewardene, L.A., Okine, E., Jaegear, S., & Scheer, H.D. (1999). Effect of
416 feeding canola oil on constituents, conjugated linoleic acid (CLA) and long chain fatty
417 acids in goats milk. *Small Ruminant Research*, 33, 137-143.
- 418
- 419 Miranda, J., Lasa, A., Fernández-Quintela, A., García-Marzo, C., Ayo, J., Dentin, R., &
420 Portillo, M.P. (2011). cis-9, trans-11, cis-15 and cis-9, trans-13, cis-15 CLNA mixture
421 activates PPAR α in HEK293 and reduces triacylglycerols in 3T3-L1 cells. *Lipids*, 46,
422 1005-1012.
- 423
- 424 Palmquist, D.L. (2006). Milk fat: origin fatty acids and influence of nutritional factors
425 thereon. In P.F. Fox, & P.L.H. McSweeney (Eds.), *Advanced Dairy Chemistry*, volume
426 2, 3rd edition (pp. 43-92). Springer: New York, USA.
- 427

- Park, Y.W. (2009). Bioactive components in goat milk. In Y.W. Park (Ed.), *Bioactive components in milk and dairy products*, (pp. 43-81) Ames: Wiley-Blackwell.
- Parodi, P.W. (2004). Milk fat in human nutrition. *Australian Journal of Dairy Technology*, 59, 3-59
- Shingfield, K.J., Bonnet, M., & Scollan, N.D. (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal*, 7, 132-162.
- Soják, L., Blasko, J., Kubinec, R., Górová, R.L., Szabó, A.H., Máje, P., Margetín, M. (2015). Time-dependent changes in milk fatty acid composition of ewes fed a winter ration supplemented with linseed or sunflower oils. *Czech Journal of Animal Science*, 60, 268-280.
- Toral, P. G., Bernard, L., Chilliard, Y., & Glasser, F. (2013). Short communication: Diet-induced variations in milk fatty acid composition have minor effects on the estimated melting point of milk fat in cows, goats, and ewes: Insights from a meta-analysis. *Journal of Dairy Science*, 96, 1232-1236.
- Zervas, G., & Tsiplakou, E. (2013). Goat milk. In Y.W. Park & G.F.W. Haenlein (Eds.), *Milk and dairy products in Human nutrition*, (pp. 498-518) Chichester: Wiley-Blackwell.

Table 1. Ingredients and chemical composition of control (CTR) and linseed (LS) supplemented diets, and productive characteristics of the goats.

	CTR	LS
Diet, g/kg DM		
Alfalfa hay	330	328
Pelleted concentrate ¹	670	656
Linseed oil ²	-	16
Chemical composition		
Dry matter (DM), %	89.2	89.7
Ash, % DM	7.0	6.8
Crude protein, % DM	18.2	17.8
Neutral detergent fiber (NDF), % DM	27.5	27.7
Non-forage NDF, % DM	10.3	10.5
Starch, % MS	33.6	32.1
Fat by acid hydrolysis, % MS	2.9	4.4
Fatty acids, g/100 g of total fat		
16:0	10.8	9.2
18:0	1.7	2.7
<i>cis</i> -9 18:1	11.8	16.2
<i>cis</i> -9, <i>cis</i> -12 18:2	30.8	26.8
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	7.3	25.4
Productive data		
Body weight, kg	59.5±3.1	57.9±2.2
Milk		
Yield, kg/d	1.55±0.59	1.46±0.68
Fat, %	4.00±0.41	3.83±0.37
Protein, %	3.57±0.43	3.49±0.33
Lactose, %	4.84±0.18	4.98±0.18

¹Composition (g/kg, as fed): maize, 356.0; barley, 356.0; and soybean meal, 250.0. Concentrates included (g/kg, as fed): vitamin and mineral premix (Trouwmix M-3020, Trouw Nutrition, Madrid, Spain), 30.0; binder (Exal; Tolsa SA, Madrid, Spain), 7.0; and antioxidant (Luctanox; Lucta SA, Barcelona, Spain), 1.0.

²The oil was included in the concentrate.

Table 2. Temporal changes of individual saturated fatty acid contents (g/100 g of total fatty acid methyl esters) in milk fat from goats fed a high starch diet after inclusion (CTR-LS) and suppression (LS-CTR) of 30 g/d of linseed oil.

		Hour						SEM
Treatment		0	1	3	6	12	24	
Even linear								
4:0	CTR-LS	2.97	2.94	2.85	2.98	2.92	2.87	0.038
	LS-CTR	3.03	3.16	3.11	3.05	3.05	3.00	
6:0	CTR-LS	3.17	3.10	3.02	3.17	3.06	2.99	0.031
	LS-CTR	3.20	3.25	3.20	3.25	3.30	3.20	
8:0	CTR-LS	3.32	3.25	3.18	3.31	3.12 ^B	3.03 ^B	0.032
	LS-CTR	3.39	3.39	3.36	3.46	3.50	3.40	
10:0	CTR-LS	11.04 ^a	10.63	10.34 ^B	11.42	11.15	10.55 ^{Bb}	0.132
	LS-CTR	10.50 ^b	10.16	10.09	11.42 ^A	12.18 ^A	11.51 ^{Aa}	
12:0	CTR-LS	4.56 ^a	4.54 ^a	4.53 ^a	4.81	4.66	4.40	0.077
	LS-CTR	4.11 ^b	4.07 ^b	4.08 ^b	4.37 ^A	4.81 ^A	4.86 ^A	
14:0	CTR-LS	10.01 ^a	10.07 ^a	9.94 ^a	10.62 ^{Aa}	10.35	9.47 ^B	0.102
	LS-CTR	8.92 ^b	8.68 ^b	8.67 ^b	9.57 ^{Ab}	9.97 ^A	9.43 ^A	
16:0	CTR-LS	28.19 ^a	27.37 ^a	26.85 ^{Ba}	29.76 ^{Aa}	30.58 ^{Aa}	28.24 ^a	0.385
	LS-CTR	23.91 ^b	22.83 ^{Bb}	22.71 ^{Bb}	26.47 ^{Ab}	28.51 ^{Ab}	26.37 ^{Ab}	
18:0	CTR-LS	6.57	6.59	6.62	5.54 ^B	5.40 ^B	7.10	0.210
	LS-CTR	7.85	7.97	7.71	6.89 ^B	6.07 ^B	7.11 ^B	
20:0	CTR-LS	0.12	0.12	0.13	0.11 ^B	0.10 ^B	0.12	0.003
	LS-CTR	0.14	0.15	0.15	0.13 ^B	0.12 ^B	0.14	
22:0	CTR-LS	0.03	0.03	0.03	0.03	0.02 ^B	0.02 ^B	0.001
	LS-CTR	0.03	0.03	0.03	0.03	0.02 ^B	0.03	
24:0	CTR-LS	0.01	0.01 ^B	0.02	0.01	0.01	0.01	0.000
	LS-CTR	0.01	0.02	0.01	0.01	0.01 ^B	0.01	
Keto-10 18:0	CTR-LS	0.05 ^b	0.05	0.05	0.05	0.06 ^A	0.06 ^A	0.003
	LS-CTR	0.08 ^a	0.08	0.08	0.07	0.05 ^B	0.05 ^B	
Odd linear								
5:0	CTR-LS	0.02 ^b	0.02 ^b	0.02 ^b	0.02 ^b	0.02 ^b	0.02 ^b	0.001
	LS-CTR	0.03 ^a	0.03 ^a	0.05 ^a	0.05 ^a	0.05 ^a	0.03 ^a	
7:0	CTR-LS	0.02 ^b	0.01	0.02 ^b	0.02 ^b	0.02 ^b	0.01	0.001
	LS-CTR	0.04 ^a	0.03	0.04 ^a	0.05 ^{Aa}	0.04 ^a	0.03	
9:0	CTR-LS	0.04 ^b	0.04 ^b	0.04 ^b	0.05 ^b	0.05 ^b	0.04 ^b	0.003

11:0	LS-CTR	0.07 ^a	0.07 ^a	0.08 ^a	0.11 ^{Aa}	0.09 ^{Aa}	0.07 ^a	0.003
	CTR-LS	0.06 ^b	0.06	0.06	0.07 ^{Ab}	0.07 ^b	0.06 ^b	
13:0	LS-CTR	0.09 ^a	0.08	0.09	0.11 ^{Aa}	0.11 ^{Aa}	0.09 ^a	0.002
	CTR-LS	0.09	0.09	0.09	0.10 ^A	0.09	0.07 ^{Bb}	
15:0	LS-CTR	0.09	0.09	0.10 ^A	0.11 ^A	0.11 ^A	0.10 ^{Aa}	0.013
	CTR-LS	0.62 ^b	0.63	0.66	0.64 ^b	0.59 ^b	0.54 ^{Bb}	
17:0	LS-CTR	0.75 ^a	0.75	0.77	0.80 ^{Aa}	0.77 ^a	0.77 ^a	0.008
	CTR-LS	0.44 ^b	0.46	0.48 ^A	0.41 ^B	0.40 ^B	0.44 ^b	
19:0	LS-CTR	0.50 ^a	0.49	0.50	0.48	0.46 ^B	0.52 ^a	0.002
	CTR-LS	0.07	0.07	0.07	0.06	0.06 ^B	0.06 ^B	
21:0	LS-CTR	0.07	0.08	0.07	0.07 ^B	0.07 ^B	0.08	0.001
	CTR-LS	0.03 ^b	0.03 ^b	0.03	0.02 ^B	0.03	0.03	
23:0	LS-CTR	0.04 ^a	0.04 ^a	0.04	0.04 ^B	0.04 ^B	0.04 ^B	0.001
	CTR-LS	0.02 ^a	0.02	0.02	0.02	0.02	0.02	
	LS-CTR	0.02 ^b	0.02	0.02	0.02	0.02	0.02	
	CTR-LS	0.02 ^b	0.02	0.02	0.03 ^b	0.02	0.02	
Methyl-4 8:0	LS-CTR	0.03 ^a	0.02	0.03	0.04 ^{Aa}	0.03	0.02	0.002
	CTR-LS	0.02	0.02	0.02	0.02	0.02 ^B	0.02 ^B	
Dimethyl 4-6 8:0	LS-CTR	0.02	0.02	0.02	0.02	0.02	0.02	0.001
	CTR-LS	0.04	0.03	0.03	0.04 ^b	0.04	0.03	
Methyl 10:0	LS-CTR	0.05	0.04	0.05	0.08 ^{Aa}	0.06 ^A	0.04	0.002
	CTR-LS	0.01 ^b	0.01	0.01	0.01 ^b	0.01 ^b	0.01	
Methyl-4 12:0	LS-CTR	0.01 ^a	0.01	0.02 ^A	0.02 ^{Aa}	0.02 ^{Aa}	0.01	0.001
	CTR-LS	0.01	0.01	0.01	0.01	0.01 ^B	0.01 ^B	
iso 13:0	LS-CTR	0.01	0.02	0.02 ^A	0.01	0.01	0.01	0.001
	CTR-LS	0.01 ^b	0.01	0.01	0.01	0.01	0.01	
anteiso13:0	LS-CTR	0.01 ^a	0.01	0.01	0.01	0.01	0.01	0.001
	CTR-LS	0.03	0.03	0.03	0.03	0.03 ^B	0.03 ^{Bb}	
iso 14:0	LS-CTR	0.04	0.04 ^A	0.04 ^A	0.03	0.03	0.04 ^{Aa}	0.004
	CTR-LS	0.14	0.15 ^A	0.15 ^A	0.14	0.12 ^B	0.12 ^B	
iso 15:0	LS-CTR	0.15	0.16 ^A	0.16 ^A	0.14	0.13 ^B	0.15	0.005
	CTR-LS	0.22	0.24 ^A	0.25 ^A	0.22	0.19 ^B	0.19 ^{Bb}	
anteiso 15:0	LS-CTR	0.24	0.25	0.26 ^A	0.22 ^B	0.21 ^B	0.26 ^a	
	CTR-LS	0.24	0.25	0.26 ^A	0.22 ^B	0.21 ^B	0.26 ^a	

<i>iso</i> 16:0	CTR-LS	0.15	0.14	0.15	0.13	0.12 ^B	0.13	0.006
	LS-CTR	0.14	0.14	0.15	0.12	0.11 ^B	0.14	
<i>iso</i> 17:0	CTR-LS	0.23 ^a	0.24	0.25	0.22	0.20 ^B	0.21 ^B	0.004
	LS-CTR	0.21 ^b	0.22	0.24 ^A	0.21	0.19	0.23 ^A	
<i>anteiso</i> 17:0	CTR-LS	0.26	0.27	0.28	0.24	0.21 ^B	0.23 ^B	0.006
	LS-CTR	0.27	0.25	0.25	0.21 ^B	0.21 ^B	0.27	
<i>iso</i> 18:0	CTR-LS	0.04	0.04	0.05 ^a	0.04 ^B	0.04 ^B	0.04 ^B	0.001
	LS-CTR	0.03	0.03	0.02 ^b	0.02	0.03	0.03	

^{A,B} Means within rows with “A” or “B” superscripts are significantly ($P < 0.05$) higher or lower, respectively, than the mean at 0 hour.

^{a,b} Means within columns with “a” or “b” superscripts are significantly ($P < 0.05$) different between treatments (CTR-LS vs LS-CTR) at the same time point.

Table 3. Temporal changes of 18:1 isomer contents (g/100 g of total fatty acid methyl esters) in milk fat from goats fed a high starch diet after inclusion (CTR-LS) and suppression (LS-CTR) of 30 g/d of linseed oil.

	Treatment	Hour						SEM
		0	1	3	6	12	24	
<i>cis</i> -9	CTR-LS	15.88	17.03	18.12 ^A	15.12	14.23 ^B	15.27	0.423
	LS-CTR	16.10	17.01	17.53 ^A	15.37	13.70 ^B	15.30	
<i>cis</i> -11	CTR-LS	0.62	0.65	0.67 ^b	0.55	0.59	0.62	0.024
	LS-CTR	0.70	0.73	0.78 ^{Aa}	0.62 ^B	0.57 ^B	0.69	
<i>cis</i> -12	CTR-LS	0.26 ^b	0.24 ^b	0.23 ^b	0.22 ^b	0.26	0.37 ^A	0.016
	LS-CTR	0.57 ^a	0.56 ^a	0.53 ^a	0.44 ^{Ba}	0.42 ^{Ba}	0.41 ^B	
<i>cis</i> -13	CTR-LS	0.01 ^a	0.02 ^a	0.01	0.01	0.01	0.01	0.000
	LS-CTR	0.01 ^b	0.01 ^b	0.01	0.01	0.01	0.01	
<i>cis</i> -14	CTR-LS	0.03 ^b	0.03 ^b	0.03 ^{Bb}	0.02 ^{Bb}	0.02 ^B	0.03	0.001
	LS-CTR	0.05 ^a	0.05 ^a	0.04 ^a	0.03 ^{Ba}	0.03 ^B	0.03 ^B	
<i>cis</i> -15	CTR-LS	0.03 ^b	0.02 ^b	0.02 ^b	0.03 ^b	0.04	0.09 ^{Aa}	0.005
	LS-CTR	0.15 ^a	0.16 ^a	0.14 ^a	0.10 ^{Ba}	0.07 ^B	0.06 ^{Bb}	
<i>cis</i> -16	CTR-LS	0.02 ^b	0.02 ^b	0.02 ^b	0.02 ^B	0.02	0.03	0.001
	LS-CTR	0.04 ^a	0.03 ^a	0.03 ^{Ba}	0.03 ^B	0.03 ^B	0.03 ^B	
<i>trans</i> -4	CTR-LS	0.02 ^b	0.01 ^B	0.02	0.01	0.02	0.02	0.001
	LS-CTR	0.02 ^a	0.02	0.02 ^B	0.01 ^B	0.01 ^B	0.01 ^B	
<i>trans</i> -5	CTR-LS	0.01	0.02	0.02	0.01	0.02	0.02	0.000
	LS-CTR	0.02	0.02	0.01	0.01	0.01	0.01	
<i>trans</i> -6/ <i>trans</i> -7	CTR-LS	0.08 ^a	0.09	0.08	0.07 ^B	0.08	0.09 ^{Aa}	0.001
	LS-CTR	0.06 ^b	0.08 ^A	0.07 ^A	0.06	0.06	0.06 ^b	
<i>trans</i> -8	CTR-LS	0.13 ^b	0.12	0.12	0.09 ^B	0.11	0.14	0.004
	LS-CTR	0.18 ^a	0.17	0.16 ^B	0.12 ^B	0.12 ^B	0.14 ^B	
<i>trans</i> -9	CTR-LS	0.25 ^b	0.26 ^b	0.25 ^b	0.21 ^B	0.24	0.29 ^{Aa}	0.005
	LS-CTR	0.32 ^a	0.34 ^a	0.32 ^a	0.26 ^B	0.26 ^B	0.26 ^{Bb}	
<i>trans</i> -10	CTR-LS	0.58	0.60	0.56	0.43 ^B	0.45 ^B	0.56	0.037
	LS-CTR	0.55	0.56	0.54	0.42 ^B	0.44 ^B	0.49 ^B	
<i>trans</i> -11	CTR-LS	1.87 ^b	1.83 ^b	1.71 ^b	1.43 ^B	2.06	2.71 ^{Aa}	0.085
	LS-CTR	3.25 ^a	3.27 ^a	2.92 ^{Ba}	2.30 ^B	2.18 ^B	2.19 ^{Bb}	
<i>trans</i> -12	CTR-LS	0.38 ^b	0.37 ^b	0.34 ^b	0.30 ^{Bb}	0.41	0.53 ^{Aa}	0.014
	LS-CTR	0.63 ^a	0.64 ^a	0.57 ^{Ba}	0.48 ^{Ba}	0.46 ^B	0.45 ^{Bb}	
<i>trans</i> -13/ <i>trans</i> -14	CTR-LS	0.17 ^b	0.16 ^b	0.16 ^b	0.14 ^b	0.18	0.38 ^{Aa}	0.016
	LS-CTR	0.43 ^a	0.50 ^a	0.45 ^a	0.35 ^{Ba}	0.28 ^B	0.28 ^{Bb}	
<i>trans</i> -16	CTR-LS	0.13 ^b	0.13 ^b	0.13 ^b	0.11 ^{Bb}	0.10 ^{Bb}	0.17 ^A	0.008
	LS-CTR	0.26 ^a	0.26 ^a	0.26 ^a	0.21 ^{Ba}	0.17 ^{Ba}	0.18 ^B	

^{A,B} Means within rows with “A” or “B” superscripts are significantly ($P < 0.05$) higher or lower, respectively, than the mean at 0 hour.

^{a,b} Means within columns with “a” or “b” superscripts are significantly ($P < 0.05$) different between treatments (CTR-LS vs LS-CTR) at the same time point.

476 **Table 4.** Temporal changes of polyunsaturated fatty acid (PUFA) contents (g/100 g of
477 total fatty acid methyl esters) in milk fat from goats fed a high starch diet after inclusion
478 (CTR-LS) and suppression (LS-CTR) of 30 g/d of linseed oil.

	Treatment	Hour						SEM
		0	1	3	6	12	24	
18:2 non-conjugated								
<i>cis</i> -9, <i>trans</i> -13 + <i>trans</i> -8, <i>cis</i> -12	CTR-LS	0.25 ^b	0.26 ^b	0.27 ^b	0.22 ^b	0.24 ^b	0.32 ^A	0.011
	LS-CTR	0.44 ^a	0.49 ^{Aa}	0.51 ^{Aa}	0.40 ^{Ba}	0.34 ^{Ba}	0.35 ^B	
<i>trans</i> -8, <i>cis</i> -13	CTR-LS	0.06 ^b	0.07 ^b	0.07 ^b	0.06 ^b	0.06 ^b	0.08 ^A	0.003
	LS-CTR	0.11 ^a	0.12 ^a	0.13 ^{Aa}	0.10 ^a	0.08 ^{Ba}	0.08 ^B	
<i>cis</i> -9, <i>trans</i> -12	CTR-LS	0.03 ^b	0.03 ^b	0.03 ^b	0.03 ^b	0.03	0.03	0.001
	LS-CTR	0.04 ^a	0.04 ^a	0.05 ^{Aa}	0.04 ^a	0.03 ^B	0.03 ^B	
<i>trans</i> -9, <i>cis</i> -12	CTR-LS	0.02 ^b	0.02	0.02	0.02	0.02	0.02	0.001
	LS-CTR	0.03 ^a	0.03	0.03	0.03 ^B	0.03 ^B	0.03 ^B	
<i>trans</i> -10, <i>cis</i> -15	CTR-LS	0.01 ^b	0.01 ^b	0.01 ^b	0.02 ^A	0.03 ^A	0.04 ^A	0.004
	LS-CTR	0.08 ^a	0.07 ^a	0.06 ^a	0.04 ^B	0.03 ^B	0.03 ^B	
<i>trans</i> -11, <i>cis</i> -15	CTR-LS	0.09 ^b	0.06 ^b	0.05 ^b	0.11 ^b	0.28 ^A	0.33 ^{Aa}	0.020
	LS-CTR	0.55 ^a	0.50 ^a	0.45 ^a	0.31 ^{Ba}	0.24 ^B	0.19 ^{Bb}	
<i>cis</i> -9, <i>cis</i> -12	CTR-LS	2.11	2.11	2.16	2.02	2.06	2.01	0.038
	LS-CTR	2.07	2.00	2.04	1.90 ^B	1.91 ^B	1.96	
<i>cis</i> -9, <i>cis</i> -15	CTR-LS	0.01 ^b	0.01 ^b	0.01 ^b	0.01 ^b	0.02 ^A	0.03 ^A	0.001
	LS-CTR	0.05 ^a	0.05 ^a	0.04 ^{Ba}	0.03 ^{Ba}	0.03 ^B	0.03 ^B	
<i>cis</i> -12, <i>cis</i> -15	CTR-LS	0.01 ^b	0.01 ^b	0.01 ^b	0.02	0.02 ^B	0.04 ^{Aa}	0.002
	LS-CTR	0.04 ^a	0.04 ^a	0.03 ^a	0.03	0.02 ^B	0.02 ^{Bb}	
18:2 conjugated								
<i>trans</i> -7, <i>cis</i> -9	CTR-LS	0.05	0.05	0.05	0.04	0.04	0.05	0.001
	LS-CTR	0.05	0.06 ^A	0.06 ^A	0.05	0.05	0.06 ^A	
<i>cis</i> -9, <i>trans</i> -11	CTR-LS	0.92 ^b	0.97 ^b	0.97 ^b	0.81 ^b	1.01	1.09 ^A	0.031
	LS-CTR	1.36 ^a	1.47 ^a	1.49 ^a	1.14 ^a	1.05 ^B	1.03 ^B	
<i>trans</i> -9, <i>cis</i> -11	CTR-LS	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	LS-CTR	0.01	0.01	0.01	0.01	0.01	0.01	
<i>trans</i> -10, <i>cis</i> -12	CTR-LS	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	LS-CTR	0.01	0.01	0.01	0.01	0.01	0.01	
<i>trans</i> -11, <i>cis</i> -13	CTR-LS	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	LS-CTR	0.01	0.01	0.01	0.01	0.01	0.01	
<i>trans</i> -11, <i>trans</i> -13	CTR-LS	0.01	0.02	0.02	0.01	0.01	0.01	0.001
	LS-CTR	0.02	0.02	0.02	0.01	0.01	0.01	
Other PUFA								
16:2	CTR-LS	0.01 ^b	0.01 ^b	0.01 ^b	0.01	0.02	0.02 ^A	0.001
	LS-CTR	0.03 ^a	0.03 ^a	0.03 ^a	0.02 ^B	0.01 ^B	0.01 ^B	
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12	CTR-LS	0.03 ^b	0.03 ^b	0.03 ^b	0.03	0.04 ^{Aa}	0.04 ^{Aa}	0.001
	LS-CTR	0.04 ^a	0.04 ^a	0.04 ^a	0.03 ^B	0.03 ^{Bb}	0.03 ^{Bb}	
	CTR-LS	0.35 ^b	0.34 ^b	0.32 ^b	0.36	0.45 ^A	0.50 ^A	0.014

<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	LS-CTR	0.66 ^a	0.63 ^a	0.56 ^{Ba}	0.44 ^B	0.46 ^B	0.50 ^B	
<i>cis</i> -9, <i>trans</i> -12, <i>cis</i> -15	CTR-LS	0.01 ^b	0.02	0.02 ^A	0.02	0.01	0.01	0.001
	LS-CTR	0.02 ^a	0.02	0.02	0.01 ^B	0.01 ^B	0.01 ^B	
<i>trans</i> -9, <i>cis</i> -12, <i>cis</i> -15	CTR-LS	0.02 ^b	0.01 ^b	0.01 ^b	0.03	0.06 ^A	0.07 ^A	0.003
	LS-CTR	0.11 ^a	0.11 ^a	0.10 ^a	0.06 ^B	0.05 ^B	0.05 ^B	
<i>cis</i> -9, <i>trans</i> -11, <i>trans</i> -15	CTR-LS	0.01	0.02 ^{Ab}	0.02 ^A	0.02 ^{Aa}	0.01	0.01	0.001
	LS-CTR	0.01	0.02 ^a	0.01	0.01 ^b	0.02	0.02	
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15	CTR-LS	0.04 ^b	0.04 ^b	0.04 ^b	0.05 ^A	0.06 ^{Aa}	0.05 ^{Aa}	0.004
	LS-CTR	0.06 ^a	0.06 ^a	0.05 ^a	0.04 ^B	0.04 ^{Bb}	0.04 ^{Bb}	
Other 18:3 isomers	CTR-LS	0.01 ^b	0.01 ^b	0.01 ^b	0.02 ^b	0.04 ^A	0.05 ^A	0.000
	LS-CTR	0.09 ^a	0.09 ^a	0.08 ^a	0.06 ^{Ba}	0.06 ^B	0.05 ^B	
C20:3 n-6	CTR-LS	0.02 ^a	0.02 ^{Aa}	0.02 ^{Aa}	0.02	0.02	0.02	0.001
	LS-CTR	0.01 ^b	0.01 ^b	0.02 ^b	0.02 ^A	0.02 ^A	0.02 ^A	
C20:4 n-6	CTR-LS	0.19	0.19	0.21 ^A	0.22 ^A	0.22 ^A	0.18	0.005
	LS-CTR	0.15	0.15	0.16	0.18 ^A	0.18 ^A	0.16	
C20:5 n-3	CTR-LS	0.03 ^b	0.04	0.04	0.04 ^b	0.04 ^b	0.03 ^b	0.001
	LS-CTR	0.04 ^a	0.04	0.05	0.05 ^a	0.05 ^a	0.05 ^a	
C22:4 n-6	CTR-LS	0.04	0.04 ^a	0.04	0.04	0.03	0.03	0.001
	LS-CTR	0.03	0.03 ^b	0.03	0.03	0.03	0.02	
C22:5 n-3	CTR-LS	0.09	0.10	0.10 ^A	0.09	0.08 ^b	0.08 ^b	0.001
	LS-CTR	0.09	0.10	0.11 ^A	0.09	0.09	0.09	
C22:6 n-3	CTR-LS	0.02	0.02	0.02	0.02	0.02	0.02	0.001
	LS-CTR	0.02	0.02	0.02	0.02	0.02	0.02	

^{A,B} Means within rows with “A” or “B” superscripts are significantly ($P < 0.05$) higher or lower, respectively, than the mean at 0 hour.

^{a,b} Means within columns with “a” or “b” superscripts are significantly ($P < 0.05$) different between treatments (CTR-LS vs LS-CTR) at the same time point.