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RUNNING HEAD: Linseeds and fish oil on milk fat in goats

Effect of extruded linseeds alone or in combination with fish oil on intake, milk production, plasma metabolite concentrations and milk fatty acid composition in lactating goats

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ABSTRACT

Based on the potential benefits for long-term human health there is interest in developing sustainable nutritional strategies for lowering medium-chain saturated fatty acids (FA) and increasing specific unsaturated FA in ruminant milk. Dietary supplements of extruded linseeds (EL), fish oil (FO) or a mixture of EL and FO increase *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and long-chain n-3 polyunsaturated FA in bovine milk. Supplements of FO cause milk fat depression (MFD) in lactating cows, but information for dairy goats is limited. Fourteen Alpine goats were used in a replicated 3 x 3 Latin square with 28 d periods to examine the effects of EL alone or in combination with FO on animal performance, milk fat synthesis and milk FA composition. Treatments comprised diets based on natural grassland hay supplemented with no additional oil (control), 530 g/d of EL or 340 g/d of EL and 39 g/d of FO (ELFO). Compared with the control, ELFO tended (P = 0.08) to lower milk fat yield, whereas EL increased ($P < 0.01$) milk fat content and secretion (15 and 10%, respectively). Relative to EL, ELFO decreased ($P < 0.01$) the concentration and output of fat in milk (19 and 17%, respectively). Relative to the control and ELFO, EL decreased ($P < 0.05$) milk 10:0-16:0 and odd- and branched-chain FA content and increased 18:0, *cis*-18:1, *trans*-Δ^{13} 18:1 (and their corresponding Δ-9 desaturase products), *trans*-12,*cis*-14 CLA, *cis*-13,*trans*-15 CLA, *cis*-12,*trans*-14 CLA and *trans*-11,*cis*-13 CLA and 18:3n-3 concentrations. ELFO was more effective for enriching ($P<0.05$) milk *cis*-9,*trans*-11 CLA and *trans*-11 18:1 concentrations (up to 5.4- and 7.1-fold compared with the control) than EL (up to 1.7- and 2.5-fold increases). Furthermore, ELFO resulted in a substantial increase in milk *trans*-10 18:1 concentration (5.4% total FA) with considerable variation between individual animals. Relative to the control and EL, milk fat responses to ELFO were

characterized by increases ($P < 0.05$) in milk *trans*-16:1 (Δ9-11), *trans*-18:1 (Δ6-11), *trans*-18:2, CLA (*cis*-9,*trans*-11, *trans*-9,*cis*-11, *trans*-8,*trans*-10 and *trans*-7,*trans*-9) and 20- and 22-carbon FA concentrations. Overall, EL resulted in a relatively high *cis*-9-18:1 concentration and an increase in the 18:3*n*-3/18:2*n*-6 ratio, whereas combining EL and FO resulted in substantial increases in *trans*-FA, marginal enrichment in 20:5n-3 and 22:6n-3, and lower 16:0 concentrations changes associated with a decrease in milk fat content. In conclusion, data provide further evidence of differential mammary lipogenic responses to diet in the goat compared with the cow and sheep.

Key words: goat milk, extruded linseed, fish oil, conjugated linoleic acid, *trans* fatty acid

Implications

The present study reports new data on the effect of supplementing diets based on natural grassland hay with extruded linseeds alone or in combination with fish oil, on the intake and production of Alpine goats and associated changes in milk fatty acid composition and milk fat secretion, with specific emphasis on *trans* 18:1, conjugated and non-conjugated 18:2 isomers. Data generated provides further evidence of differential responses to lipid supplements in goats compared with cows and sheep.

Introduction

Nutrition is the major environmental factor regulating milk fat synthesis and fatty acid (FA) composition in ruminants which is an important determinant of the nutritional quality of milk for human consumers. Specific FA including medium-chain saturated FA and certain *trans*-FA are thought to elicit negative effects when consumed in excess, whilst others (*anteiso*-15:0, *cis*-9 18:1, 18:2n-6, *cis*-9,*trans*-11 conjugated linoleic acid (CLA) and 18:3n-3) may have potentially beneficial effects on human health (Shingfield et al, 2008). For these reasons, the opportunities to enhance the concentration of bioactive FA through dietary supplementation with plant oils or seeds rich in n-3PUFA have been explored. Studies in goats (Nudda et al., 2006; Chilliard et al., 2007; Mele et al., 2008; Martínez Marín et al., 2011) and cows (Chilliard et al., 2007) have demonstrated that plant lipid supplements enriched in 18:3n-3 increase milk 18:3n-3 and *cis*-9,*trans*-11 CLA concentrations, with responses being higher in goats than cows (Chilliard and Ferlay, 2004).

It has been suggested that including lipid in the diet as an oilseed rather than oil would limit the extent of ruminal biohydrogenation of PUFA due to seed hulls restricting the access of bacterial lipases to storage triacylglycerol. However, changes in milk 18:3n-3 concentrations to linseed oil or linseed supplements in goats

and cows suggest that 18:3n-3 in whole unprocessed linseeds is more extensively hydrogenated to 18:0 in the rumen compared with 18:3n-3 in free oils (Chilliard et al., 2003, 2007). However, a detailed assessment of dietary supplements of extruded linseeds on milk FA composition has not been documented for dairy goats.

The potential to increase the concentration of 20:5n-3 and 22:6n-3 in milk by including fish oil (FO) to the diet has been examined in cows (Loor et al., 2005a; Shingfield et al., 2006), ewes (Toral et al., 2010), and goats (Kitessa et al., 2001; Gagliostro et al, 2006; Toral et al., accepted). Dietary FO supplements modify rumen biohydrogenation, leading to several-fold enrichment of milk *cis*-9,*trans*-11 CLA and *trans*-11 18:1 concentrations in goats, cows and sheep, with further increases being reported when diets contain plant oils (Gagliostro et al., 2006; Shingfield et al., 2006; Toral et al., 2010). However, the effect of combining FO and 18:3-rich oilseeds such as linseeds on milk production and milk FA composition is not known in goats.

The influence of dietary FO supplements on milk production varies between ruminant species. In cows and ewes, FO typically decreases milk fat content and yield (Chilliard et al., 2001; Loor et al., 2005a; Shingfield et al., 2006; Toral et al., 2010), but reports in goats are limited (Toral et al., accepted).

The present study was conducted to provide a comprehensive evaluation of the effects of dietary supplements of extruded linseeds alone or in combination with FO on performance and milk FA composition in goats with specific emphasis on *trans* FA.

Materials and methods

Animals, management and experimental design

All experimental procedures were approved by the Animal Care Committee of INRA in accordance with the *Use of Vertebrates for Scientific Purposes Act* 1985. Animals were recruited to experiments and allocated to treatment groups according to milk yield, milk fat and protein content, parity, stage of lactation and genotype score at the $\alpha S1$ casein locus. Goats with medium $\alpha S1$ casein content were used since this polymorphism is associated with effects on milk traits and FA composition (Chilliard et al., 2013). Fourteen multiparous (3.6 ± 0.63) Alpine goats in mid-lactation (85 ± 3.3 d in lactation) were offered three experimental diets according to a replicated 3 x 3 Latin Square design with 28 d experimental periods with 4 or 5 animals per group. Fifteen goats were recruited to the experiment, but due to a high milk somatic cell count associated with sharp decrease in milk production at the beginning of the experiment, one animal was withdrawn from the experiment. Each experimental period comprised 21 d adaptation and 7 d interval for sampling and measurements. Goats were housed in a metabolism unit in individual stalls, with continuous access to water and milked at 08.00 and 16.00 h. Diets were formulated to meet energy and protein requirements (INRA, 1989).

Experimental diets

Diets were based on hay prepared from regrowths of natural grassland pasture offered *ad libitum* and a concentrate mixture containing barley and soyabean meal (Table 1). Treatments comprised the basal diet containing no additional lipid (**Control**), 530 g/d of extruded linseeds (extruded mixture of linseed:wheat, 70:30 wt/wt, Union Invivo, Ets Inzo, Chateau-Thierry, France) (**EL**) or 340 g/d of EL and 39 g/d of anchovy FO (SA Daudruy Van Cauwenberghe and Fils, Dunkerque, F-59 640

139 France) (**ELFO**). EL and ELFO diets were formulated to provide the same amount of
140 FA (Table 1).
141 Concentrates were fed according to milk yield at the start of experiment in order to
142 represent 50-55% of the DMI. Supplements of EL and FO were mixed with
143 concentrate ingredients before feeding. Concentrate were offered as two equal meals
144 at 08.30 and 16.30 h.
145
146 *Measurements and sampling*
147 Individual intakes were recorded daily, but only measurements collected during the
148 last week of each experimental period were used for statistical analysis. During each
149 experimental period, representative samples of hay, ingredients of concentrates
150 (barley and soyabean meal), and EL were collected weekly, composited and used to
151 determine the DM content after 48 h at 103°C. Additional subsamples were stored at
152 -20°C for chemical composition and FA analyses. A representative sample of FO was
153 collected weekly, composited and stored at -20°C. Chemical composition of feed
154 ingredients was determined using standard procedures (AOAC, 1997). Milk yields of
155 individual goats were recorded thrice-weekly, while only measurements collected
156 during the last week of each experimental period were analysed statistically. Samples
157 of milk for the measurement of fat, true protein and lactose were collected from each
158 goat over four consecutive milkings starting at 08.00 h on d 21 of each experimental
159 period and treated with preservative (potassium bichromate, Merck, Fontenay-Sous-
160 Bois, France). Milk fat, protein and lactose content were determined by mid infra-red
161 spectroscopy (AOAC, 1997) calibrated using samples of goat milk for which
162 reference measurements had been made. Unpreserved samples of milk were
163 collected over two consecutive milkings starting at 08.00 h on d 22 of each

experimental period, stored at -20°C, composited according to yield and submitted for FA analysis. A sub-sample of unpreserved milk was submitted for the determination of free FA concentrations measured after storage at 4ºC for 34 h. For the assay of lipoprotein lipase activity, additional samples of unpreserved milk were collected and stored at −20°C until analysis (Bernard et al., 2005). Live weight of experimental animals was measured at the start and end of each experimental period. Blood samples were collected on d 20 of each experimental period at 07.30 h. Samples from the jugular vein were collected into evacuated collection tubes (Venoject; C.M.L., Nemours, France) containing potassium ethylene diamine tetra-acetic acid. Once collected, blood samples were centrifuged (1500 *g* for 15 min at 4°C), stored at -20°C and the plasma recovered analysed for insulin and metabolite concentrations (Bernard et al., 2005).

Lipid analysis

Chemical composition of feed ingredients was determined using standard procedures (AOAC, 1997). Fatty acid methyl esters (FAME) of lipid in feed samples were prepared using a 1-step extraction-transesterification (Sukhija and Palmquist, 1988), with 23:0 (Sigma, Saint-Quentin Fallavier, France) as an internal standard. FAME of milk were obtained by based-catalysed transesterification by the incubation of 2 ml of 0.5 M sodium methoxide in methanol and 1 ml of hexane to 100 mg of lyophilised milk at 50°C for 15 min. After cooling, 1 ml of 5% (vol/vol) methanolic hydrochloric acid was added and the reaction mixture was maintained at 50°C for 15 min. Once cool, 3 ml of 6% (wt/vol) of aqueous potassium carbonate and 1.5 ml of hexane were added. Tubes were shaken vigorously, centrifuged at 1570 *g* for 5 min at 4°C, and the upper organic phase was recovered. Methyl esters were separated and

quantified by gas-liquid chromatography using a gas chromatograph Trace-GC 2000 equipped with a flame-ionization detector (Thermo Finnigan, Les Ullis, France) and 100 m fused silica capillary column (CP-SIL 88; Chrompack 7489, Middelburg, The Netherlands) using hydrogen as the carrier and fuel gas. Total FAME profile was determined in a 0.5 µL sample at a split ratio of 1:50 using a temperature gradient program (Loor et al., 2005b) and isomers of 18:1 were further resolved in a separate analysis under isothermal conditions (Shingfield et al., 2003). Peaks were routinely identified using authentic FAME standards (GLC≠463, Nu-Check Prep Inc, Elysian, MN, USA; iso and ante-iso 13:0, 14:0, 15:0, 16:0, 17:0 and 18:0; Sigma-Aldrich, Saint-Quentin Fallavier, France). Reference butter oil (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors to account for the carbon deficiency in the flame ionization detector response for FAME containing 4 to 10 carbon atoms. Methyl esters not available as commercial standards were identified based on retention time comparisons with milk fat samples for which peaks were identified based on GC-MS analysis of FAME and 4,4-dimethyloxazoline FA derivatives (Shingfield et al., 2006; 2008).

The distribution of CLA isomers in milk fat FAME was determined by HPLC using four silver-impregnated silica columns (ChromSpher 5 lipids, 250 x 4.6 mm; 5 µm particle size, Varian Ltd., Walton-on-Thames, UK) coupled in series and 0.1 % (vol/vol) acetonitrile in heptane as the mobile phase (Shingfield et al., 2003). Concentrations of CLA isomers were calculated from the proportionate peak area responses determined by HPLC and the sum of concentrations of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA and *cis*-9,*trans*-11 CLA weight percentage determined by GC analysis.

Calculations and statistical analysis

Apparent transfer of 20:5n-3, 22:5n-3, and 22:6n-3 from FO into milk was calculated as: [g milk FA yield × (g FA/100 g milk fat – g FA/ 100 g in control milk fat) / (DMI × g FA intake)] × 100.

Experimental data were subjected to Analysis of Variance using the general linear model procedure of Statistical Analysis Systems software package version 8.2 (SAS, SAS Institute, Cary, NC, USA) with a model that included the fixed effects of period and treatment and random effects of goat. Least square means ± SEM are reported and treatment effects were declared significant at $P < 0.05$ and considered a trend towards significance at $P < 0.10$.

Pearson correlation coefficients (r) were generated for the association between individual FA in milk fat and between the abundance of specific milk FA with milk fat content using the CORR procedure of SAS.

Results

Diet composition

Natural grassland hay was of high quality in term of nutritional value and had the following composition (g/kg DM, unless otherwise stated): DM (g/kg fresh weight), 855; organic matter, 907; crude protein, 98; acid-detergent fibre, 281; neutral-detergent fibre, 524; FA content, 17. Concentrations of organic matter and starch were higher for the control than EL and ELFO, whereas acid-detergent fibre, neutral-detergent fibre, crude protein, diethyl ether extract, and total FA content were lower (Table 1).

The content of organic matter, neutral-detergent fibre, crude protein, starch, diethyl ether extract, and total FA were similar for EL and ELFO, other than a marginally lower amount (-3%) of acid-detergent fibre for ELFO than EL. By design, the EL and ELFO increased the concentration of specific FA in the diet (Table 1).

Dietary forage:concentrate ratio of the diet (on a DM basis) averaged 52:48, 56:44 and 55:45 for the control, EL and ELFO, respectively.

Animal performance

EL diet supplied the highest amounts of 18:0, *cis*-9 18:1, 18:2n-6 and 18:3n-3 (5.6, 29.9, 28.6, 74.4 g per d, respectively), whereas the ELFO treatment was a source of 20:5n-3 and 22:6n-3 (3.1 and 2.3 g per d, respectively). In ELFO and EL, *cis*-9 18:1, 18:2n-6 and 18:3n-3 were the major FA provided by the diets (26, 24 and 51 g per d, respectively for ELFO, and 30, 29 and 74 g per d, respectively for EL). 18:2n-6 was the major FA provided by the control diet (13.5 g per d), 18:3n-3 was the major FA provided by the 2 lipid supplemented diets and was 31% lower in ELFO diet compared to EL (Table 2).

Compared with the control and EL, ELFO lowered (P = 0.02) DM intake (Table 2). Treatments had no effect (P > 0.05) on the yields of milk, milk protein or lactose. However, compared with the control and ELFO, EL increased (P < 0.01) milk fat yield and milk fat and protein content, whereas these parameters did not differ (P > 0.05) between the control and ELFO (Table 2). Both EL and ELFO enhanced (P < 0.001) lactose concentration relative to the control (Table 2), with the increase being greater for EL than ELFO treatment.

Energy and protein balances (INRA, 1989) were positive for all the dietary treatments. Energy balance was similar among dietary treatments, whereas protein balance was slightly lower for the control compared with EL and ELFO (Table 2).

Plasma metabolite concentrations and milk lipolytic activity

Dietary treatments had no effect ($P > 0.05$) on glucose concentrations while ELFO tended ($P = 0.09$) to decrease plasma insulin concentration compared with the control (Table 3).

Relative to the control, EL and ELFO lowered ($P < 0.001$) plasma acetate and 3-hydroxybutyrate concentrations, with greater decreases ($P < 0.05$) observed for EL than ELFO. Compared with the control and ELFO, EL increased ($P < 0.001$) plasma NEFA concentrations. Even though lipid supplements had no effect ($P > 0.05$) on milk LPL activity, EL decreased ($P < 0.05$) free FA concentration after storage of milk for 34 h at 4°C (Table 3).

Milk fatty acid composition

Both EL and ELFO altered milk FA composition compared the control, changes characterized by decreases ($P < 0.05$) in 8:0 to 16:0, *cis*-9 10:1, *cis*-9 14:1 and *cis*-9 17:1, branched chain FA and an increase in total 18 carbon FA concentration (Table 4).

Relative to the control, only EL increased ($P < 0.05$) milk of 18:0, *cis*-15 18:1 and 18:2n-6 concentrations. Both EL and ELFO enhanced ($P < 0.05$) milk 18:3n-3 concentration, with the response being higher ($P < 0.05$) for EL. Compared with the control and EL, ELFO enriched ($P < 0.05$) milk *cis*-11 20:1, 20:4n-3, 20:5n-3, 22:4n-3, 22:5n-3 and 22:6n-3 concentrations (Table 4).

287 Dietary supplements of EL alone or in combination with FO elevated ($P < 0.001$) milk
288 *trans*-18:1 concentration (Table 3), with the increases compared the control being
289 higher for ELFO (+698%) than EL (+238%).
290 Both EL and ELFO enhanced ($P < 0.05$) milk *trans*-4-9 and 13 + 14 18:1
291 concentrations, while increases ($P < 0.01$) in *trans*-10 18:1 and *trans*-11 18:1 were
292 confined to ELFO diet resulting in concentrations 16- and 7-fold higher than the
293 control diet, respectively (Table 5). Overall, *trans*-10 and *trans*-11 accounted for
294 39.5% and 39.3% of total milk *trans*-18:1 content on the ELFO treatment.
295 Concentrations of total *cis*-18:1 increased ($P < 0.001$) in response to EL (+79%), with
296 enrichment of *cis*-9 18:1 accounting for 90 % of the total increase in *cis*-18:1. Both EL
297 and ELFO enhanced *cis*-13 18:1 and *cis*-14 18:1 content, whereas ELFO resulted in
298 *cis*-11 18:1 enrichment (Table 5).
299 Dietary lipid supplements altered the relative abundance of non-conjugated 18:2
300 isomers (Table 6). Compared with the control, EL and ELFO increased ($P < 0.001$)
301 milk fat *cis*-9, *trans*-12 18:2 and *cis*-9,*trans*-13 18:2 concentrations and ELFO
302 enhanced ($P < 0.001$) the abundance of *trans*-9,*trans*-12 18:2 and *trans*-11,*cis*-15
303 18:2 (Table 6).
304 Apparent transfer of 20:5n-3 and 22:6n-3 from the diet into milk for the ELFO
305 treatment were marginal (2.9 and 3.7% respectively) and much lower than for 22:5n-
306 3 (10.1%; data not presented).
307 Compared with the control and EL, ELFO increased ($P < 0.001$) total CLA
308 concentrations (mean responses +175 and +414%, respectively) with c*is*-9,*trans*-11
309 CLA being the major isomer. Most CLA isomers detected in milk were enhanced ($P <$
310 0.001) by ELFO, and to a lower extent by EL. Both EL and ELFO elevated ($P <$

311 0.001) *trans*-7,*cis*-9 CLA, *cis*-11,*trans*-13 CLA, *trans*-11,*cis*-13 CLA, *trans*-12,*trans*-14
312 CLA and *trans*-13,*trans*-15 CLA.
313 Compared with the control, EL resulted in the specific enrichment (P = 0.001) in order
314 of the relative abundance, *trans*-12,*cis*-14 CLA, *trans*-11,*trans*-13 CLA and *cis*-
315 12,*trans*-14 CLA (Table 6). Relative to EL, ELFO increased (P = 0.001) in order of
316 relative abundance, *cis*-9,*trans*-11 CLA, *trans*-9,*cis*-11 CLA, *trans*-9,*trans*-11 CLA,
317 *trans*-8,*trans*-10 CLA, *trans*-10,*trans*-12 CLA, *trans*-7,*trans*-9 CLA and *trans*-6,*trans*-8
318 CLA.
319
320 **Discussion**
321 Several studies have examined the effect of dietary plant lipid supplements on
322 milk production and milk FA composition in the dairy goat (Chilliard et al., 2007; Mele
323 et al., 2008; Bernard et al., 2009), but few have investigated the interaction of plant
324 oils or oilseeds with FO in this species (Toral et al., accepted). The present study
325 provided a comprehensive assessment of including extruded linseed alone or in
326 combination with FO on the performance and milk FA profile of goats fed diets based
327 on grass hay.
328
329 *Animal performance*
330 Feed consumption was decreased by 6% in goats in response to ELFO as typically
331 observed in cows with FO addition to the diets (Whitlock et al., 2002).
332 Milk production and composition responses to extruded linseeds were consistent with
333 previous studies (Chilliard and Ferlay, 2004; Chilliard et al. 2007, 2013) supporting
334 the view that oilseeds and plant oils typically have no effect on milk yield, increase
335 milk fat concentration and secretion, but have variable effects on milk protein

concentration in goats. In contrast, supplements of EL have been shown to lower milk fat content and yield in lactating cows, with the decrease being more pronounced for diets based on maize silage than grass hay (Ferlay et al., 2013). Inclusion of FO in the diet counteracted the positive effect of EL on milk fat synthesis, highlighting that FO may modify milk fat responses to oilseed supplements in goats. Due to the lack of data, indirect comparison of responses to EL when FO is included in diet between ruminant species is not possible.

A recent study reported that supplementing the diet with a 31:69% (wt/wt) mixture of FO and linseed oil (5.8 g/kg DM) increased milk fat secretion and content in goats (Toral et al., accepted). The reason for the differences between studies may be due to the form of supplementary lipid, and basal diet composition, in particular the nature and amount of starch (128 g/kg DM from corn (Toral et al., accepted) *vs* 169 g /kg DM from barley present study) which is a major determinant of milk fat responses to plant lipids in lactating cows (Shingfield et al, 2010). However, differences in dietary starch content have been demonstrated to have no effect on milk fat yield and content responses to FO in goats (Toral et al., accepted) or to a mixture of FO and sunflower-seed oil in cows (Shingfield et al., 2005).

Milk fatty acid composition

Response to extruded linseeds

The impact of dietary EL supplementation (equivalent to an additional 52 g oil/kg DM) on the concentrations of the major FA in milk is consistent with earlier studies in lactating goats and cows (Chilliard et al., 2007; 2013), characterized by decreases in medium-chain saturated FA and increases in 18:0, *cis*-9 18:1 and 18:3n-3. The reduction in medium-chain saturated FA to EL, may at least in part, be related to

lower plasma concentrations of acetate and 3-hydroxybutyrate that serve as precursors for *de novo* FA synthesis in the mammary gland. Supplements of EL specifically increased *cis*-9 18:1, *cis*-12 18:1, *cis*-15 18:1 and *cis*-9,*trans*-12 CLA. Indirect comparisons of changes in milk fat composition for cows fed similar diets (Lerch et al., 2012a,b; Ferlay et al., 2013) indicate the increase in milk *cis*-9 18:1 and 18:0 content to EL supplements can be expected to be higher in goats, consistent with the findings of earlier studies (Chilliard et al., 2007; 2013). Moreover, milk fat content was positively correlated with milk 18:0 concentration (Figure 1 and Table 7; r=0.61), providing additional support to the hypothesis that this substrate is a major factor regulating mammary lipogenesis in goats (Chilliard and Ferlay, 2004).

The EL treatment (216 g EL/kg DM) increased milk *trans*-11 18:1 (+147%) and *cis*-9,*trans*-11 CLA (+69%) concentrations, in line with other data in goats with extruded linseed (Nudda et al., 2006; Chilliard et al., 2007), but in lower magnitude than recently observed in response to 170 g EL/kg DM in goats offered diets containing less starch (and using the same extrusion method to extrude linseeds) (Chilliard et al., 2013), differences that may be associated with the higher starch content of the basal diet in the present study (hay-barley based diet). However, the enrichment of milk *trans*-11 18:1 and *cis*-9,*trans*-11 CLA on the EL treatment was higher than the increases to 50 g EL/kg DM reported for cows fed a similar basal diet (50% hay containing 114 g starch/kg DM; Ferlay et al., 2013). Conversely, EL had no influence on milk *trans*-10 18:1 and *trans*-10,*cis*-12 CLA concentrations, the abundance of which was found to increase in cows (Ferlay et al., 2013). Differential responses between ruminant species may be associated with a greater stability of the ruminal *trans*-11 biohydrogenation pathway in the goat compared with the cow, in which a shift to the *trans*-10 pathway is more frequently observed (Shingfield et al., 2010).

As expected, milk 18:3n-3 concentration and the ∑n-3/∑n-6 ratio were markedly increased on the EL treatment (Table 4). An enrichment of 18:3n-3 in milk of up to 2.19 g/100 g on a diet supplying 30.6 g 18:3 n-3/kg DM is higher than the abundance in milk of 1.53 g/100 g from cows fed a similar diet providing 27.2 g 18:3n-3 /kg DM (Ferlay et al., 2013). This observation is in line with previous reports of a greater increase in milk 18:3n-3 concentration to linseed based supplements in the goat compared with the cow (Chilliard et al., 2007) which may be related to less extensive biohydrogenation of dietary PUFA in the rumen of goats than cows. Other hypothesis such as differences among goats and cows of 18:3n-3 partitioning among tissues and/or mammary extraction from circulating 18:3n-3 would also merit to be investigated.

Response to extruded linseeds and fish oil

The ELFO treatment increased the ∑*n*-3/∑*n*-6 ratio in milk due to 18:3n-3 and long-chain n-3 PUFA enrichment (Table 4). Given the relatively high concentration of 20:5n-3, 22:5n-3 and 22:6n-3 on the control and EL treatment and for non-supplemented diets in earlier studies with goats (Toral et al., accepted), the apparent transfer of long-chain n-3PUFA (20:5n-3, 22:5n-3 and 22:6n-3) were calculated taking into account their secretion on the control. The efficiency of transfer (3.7-10.1%) was in the same range reported for goats fed FO alone or as a mixture with plant oils (from 1.4 to 3.4% for 20:5n-3 and 22:6n-3; Toral et al., submitted). Transfer of 22:5n-3 was higher (~ 10%) as has been reported previously in goats (Toral et al., accepted), cows (Loor et al., 2005a) and ewes (Toral et al., 2010). Part of the higher transfer efficiency may be explained by a lower apparent disappearance of 22:5n-3 in the rumen (Lee et al., 2008; Shingfield et al., 2012) and higher extraction and uptake

411 of 22:5n-3 across the mammary gland (Offer et al., 1999; Chilliard et al., 2000; Loor
412 et al., 2005a).
413 Results indicate that supplements of FO and EL is more effective for increasing milk
414 *cis*-9,*trans*-11 CLA and *trans*-11 18:1 concentrations compared with EL alone which
415 is in agreement with previous observations on the interaction between FO and plant
416 oils in cows and the inhibitory effect of certain FA in FO on the reduction of *trans*
417 18:1 isomers to 18:0 in the rumen (AbuGhazaleh et al., 2003; Shingfield et al., 2006;
418 Chilliard et al., 2007).
419 The ELFO caused greater increases in milk *trans*-10 18:1 than EL resulting in an
420 equal abundance of *trans*-10 18:1 and *trans*-11 18:1. A milk *trans*-10 18:1
421 concentration of 5.4 g/100 g FA is much higher than 1.04 g/100 g FA in a previous
422 experiment with goats fed FO and linseed oils (Toral et al., accepted). Differences
423 between studies may be partly due to differences in the form of linseed lipid and
424 dietary starch content. However, increases in the starch content of diets based on
425 lucerne hay had no effect on milk *trans*-10 18:1 in goats offered FO supplements
426 (Toral et al., accepted), suggesting that interactions between the type of linseed
427 supplement and basal diet, in particular the nature of starch (barley present study vs
428 corn and/or barley Toral et al., (accepted)), may, at least in part, explain these
429 differences. Enrichment of *trans*-10 18:1 on the ELFO treatment is notable given that
430 the stability of the *trans*-11 ruminal biohydrogenation pathway is generally greater in
431 the goat than cow and concentrations of *trans*-10 18:1 are typically lower than 3.5
432 g/100 g FA in milk from goats fed high starch diets supplemented with plant oils
433 (Chilliard et al., 2007; Bernard et al., 2009).
434 Milk *trans*-10 18:1 concentration (mean 5.4% SD 6.96) varied considerably between
435 individual animals. Enrichment in milk for four goats ranged between 13 and 19 g/100

436 g FA and was not associated with a decrease in milk fat content. In the goat,
437 increases in milk *trans*-10 18:1 concentrations are associated with marginal
438 increases in milk fat yield, whereas the reverse is true in cows (Shingfield et al.,
439 2010). Furthermore, the lack of an association between that milk *trans*-10 18:1
440 concentration and milk fat synthesis in the present study suggests that other
441 biohydrogenation intermediates or other factors may account for the suppression of
442 increases in milk fat yield to EL when FO is included in the diet. Examination of the
443 association between milk fat content and milk FA composition highlighted a negative
444 association with milk *cis*-11 16:1, *cis*-11 18:1, *cis*-11 20:1, *trans*-8,*trans* 10 CLA, and
445 *trans*-7,*trans*-9 CLA concentrations (Figure 1; Table 7). A similar relationship
446 between milk fat and milk *cis*-11 18:1 concentration has been reported for cows fed
447 supplements of FO and sunflower oil (Shingfield et al., 2006).
448 However, the control diet also contained *cis*-11 18:1, while *cis*-11 16:1 and *cis*-11
449 20:1 supplied exclusively on the ELFO treatment originated from FO (Table 4).
450 Previous study suggests that the appearance of *cis*-11 16:1 and *cis*-11 20:1 in milk is
451 related to ruminal escape rather than formation during biohydrogenation of FA in EL
452 or FO (Kairenius et al., 2011). Studies in bovine adipocytes (Burns et al., 2006) have
453 demonstrated that *cis*-11 18:1 can be synthesized endogenously by the elongation of
454 *cis*-9 16:1. It is therefore possible that *cis*-11 16:1 and *cis*-11 20:1 may originate from
455 the elongation of *cis*-9 14:1 and *cis*-9 18:1, respectively, in adipose which during
456 mobilization of body tissue and uptake across the mammary gland could be made
457 available for milk fat synthesis. In vitro, *cis*-11 18:1 has been shown to lower
458 lipogenesis and FASN gene expression in bovine adipocytes (Burns et al., 2006),
459 highlighting that this FA may act as an inhibitor of lipogenesis at least in adipose.
460 Even though the mode of action of this FA on lipogenesis in adipocyte has not been

elucidated, the possibility that *cis*-11 18:1 inhibits mammary lipogenesis cannot be excluded. However, post-ruminal infusion studies of a mixture of 18:1 (30 g/d) isomers containing 12.5% of *cis*-11 18:1 were found to have no effect on milk fat synthesis in cows (Shingfield et al., 2007) suggesting a neutral effect of this isomer at least in cows.

A negative relationship between milk fat content and milk *cis*-11 16:1 or *cis*-11 20:1 concentrations may be related to a co-dependence between these parameters rather than a direct effect on lipogenesis. A negative association was also observed for *trans*-8,*trans*-10 CLA and *trans*-7,*trans*-9 CLA but there is no direct evidence on the role of these isomers in the regulation of mammary lipogenesis in ruminants.

In cows, relatively few biohydrogenation intermediates are known (*trans*-10,*cis*-12 CLA) or putative (*trans*-9,*cis*-11 CLA, *cis*-10,*trans*-12 CLA) inhibitory effects on mammary lipogenesis (Shingfield et al., 2010), and it is possible that others may be active in the goat, including *trans*-7,*trans*-9 CLA and *trans*-8, *trans*-10 CLA. The ELFO treatment increased *trans*-9,*cis*-11 CLA concentrations 14.5-fold (0.058 g/100 g FA). In earlier studies, *trans*-9,*cis*-11 CLA was not detected in milk from goats fed hay- or maize silage-based diet supplemented with plant oils, whereas *trans*-9,*trans*-11 CLA was increased (Bernard et al., 2009). An increase in milk *trans*-9,*cis*-11 CLA content was confined to the same individuals for which concentrations of *trans*-10 18:1 were elevated which explains the close association between these FA in milk (Table 7). Similarly, a close relationship between *trans*-9,*cis*-11 CLA and *trans*-10 18:1 concentrations have been reported in milk of cows (Shingfield et al., 2006) and sheep (Toral et al., 2010) fed with a mixture of sunflower oil and marine oils or from cows fed diets containing oilseeds (Lerch et al., 2012a,b). Collectively, these observations suggest significant variability in the functioning and diversity of the

rumen microbiome and adaptations to changes in diet composition between ruminant species that merits further investigation.

Furthermore, milk fat secretion and FA composition responses to the ELFO treatment demonstrated considerable between-animal variation in the synthesis and secretion of *trans*-9,*cis*-11 CLA, and for *trans*-8,*trans*-10 CLA and *trans*-7,*trans*-9 CLA that may also have an influence on milk fat content and yield in goats. These changes were also accompanied by the prevention of an increase in 18:0 supply which may have suppressed the increase in milk fat synthesis observed on the EL treatment. Earlier studies have provided evidence to suggest that a shortage of 18:0 for endogenous *cis*-9 18:1 synthesis may explain or contribute to FO induced milk fat depression in cows (Loor et al., 2005a; Shingfield et al, 2006) and ewes (Toral et al., 2010) due to compromised milk fat fluidity. Overall, a combination of all these alterations may have contributed to FO suppressing the positive effect of EL on milk fat content.

Milk lipolysis

Dietary supplements of EL had an adverse effect on post-milking milk free FA concentrations consistent with lower levels of spontaneous lipolysis in milk from goats supplemented with 18:3n-3-rich lipids (Chilliard et al., 2003, 2013; Eknæs et al., 2009). Such changes may influence the sensory quality of milk by reducing the development of goat flavour (Chilliard et al., 2003). A numerical, but non-significant difference compared with the control was also detected when FO was fed with EL (Table 3). These observations are in line with previous reports in goats fed a combination of plant oils and FO or FO alone (Toral et al., accepted), which suggests that FO has limited influence or may compensate for the effects of plant lipid supplements on milk fat lipolysis.

Conclusions

Supplementing hay based diets rich in extruded linseed increases milk fat content and yield in goats and alters milk FA composition, characterised by decreases in SFA and increases in 18:0, *cis* 18:1, *trans* 18:1, CLA and 18:3n-3. A strong positive relationship between milk fat content and milk 18:0 concentration reinforce the hypothesis that the supply of 18:0 is involved in the regulation of mammary lipid secretion in goats. In contrast to cows, supplements of FO and EL had no influence on milk fat content and yield in goats. However, when compared with supplements of EL alone, FO prevented the increase in milk fat synthesis to EL and induced larger increases in *trans* 18:1 and CLA isomers in milk fat. Data suggest a specific effect of a mixture of FO and EL on ruminal accumulation and secretion of *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA and *trans*-9,*cis*-11 CLA in milk, isomers that along with a decrease in 18:0 supply may explain the adverse effects on milk fat synthesis. Direct inter-species comparisons are required to define differential responses to dietary FO supplements and their interaction with plant lipids. A more complete understanding of the diversity and functioning of the rumen microbiome may offer an explanation for species specific differences in lipid digestion and metabolism and diet-induced changes in milk fat composition.

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540

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660

Figure caption

Figure 1 Relationships between milk fat content (g/kg) and concentrations of *cis*-11 16:1, *cis*-11 18:1, *cis*-11 20:1, 18:0, *trans*-8,*trans*-10 CLA and *trans*-7,*trans*-9 CLA in milk (g/100g of fatty acids) from goats fed grass hay based diets supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO). Relationships derived using 42 measurements made for 14 animals.

670 **Table 1** *Formulation and chemical composition of experimental diets*
671

	Treatment		
	Control	EL	ELFO
Ingredient (g/kg dry matter)			
Natural grassland hay	518	563	558
Barley	449	208	247
Soya bean meal	32	12	30
Extruded linseeds[1]	0	216	147
Fish oil[2]	0	0	17
Mineral and vitamin premix[3]	4	4	4
Chemical composition (g/kg dry matter)			
Organic matter	936	932	933
Crude protein	116	129	129
Neutral detergent fibre	366	384	376
Acid detergent fibre	171	194	188
Starch	247	159	169
Diethyl ether extract	17	69	69
14:0	0.09	0.11	1.74
15:0	0.03	0.04	0.15
16:0	3.65	6.14	8.54
cis-9 16:1	0.04	0.09	1.58
cis-11 16:1	0.00	0.00	0.10
17:0	0.03	0.12	0.16
18:0	0.33	2.30	2.20
cis-9 18:1	2.17	12.32	11.64
cis-11 18:1	0.14	0.60	0.96
18:2n-6	5.46	11.79	10.39
18:3n-3	2.16	30.64	22.36
20:0	0.09	0.14	0.16
cis-11 20:1	0.00	0.00	0.46
22:0	0.10	0.16	0.14
20:5n-3	0.00	0.00	1.37
24:0	0.09	0.13	0.13
22:5n-3	0.09	0.11	0.38
22:6n-3	0.00	0.00	1.02
Other fatty acids	1.06	1.63	3.96
∑ Fatty acids	16	66	67
Energy (MJ/kg dry matter)[4]	6.62	6.90	6.98
Protein (g PDI/ kg dry matter)[5]	76	87	86

672 [1]Extruded linseeds contained (g/kg DM) 16:0 (16.9), 18:0 (9.6), *cis*-9 18:1 (50.5), 18:2n-6 (40.2),
673 18:3n-3 (131.8) and total fatty acids (256).

[2]Fish oil contained (g/kg FA) 14:0 (98.9), 16:0 (195.8), *cis*-9 16:1 (90.8), *cis*-11 16:1 (2.4), 18:0 (29.9), *cis*-9 18:1 (145.8), *cis*-11 18:1 (30.0), 18:2n-6 (38.2), 18:3n-3 (16.6), *cis*-11 20:1 (27.6), 20:5n-3 (82.7), 22:5n-3 (16.6), 22:6n-3 (61.8) and total fatty acids (992).
[3]Mineral-vitamin premix declared as containing (g/kg): Ca (240), P (60), Mg (50), Na (15), Zn, (7), Mn (6), α-dl-tocopherol (0.3), retinol (0.2) and cholecalciferol (0.002) (Usine d'Ussel, Murat, France).
[4]Net energy for lactation calculated according to INRA (1989).
[5]Digestible protein at the intestine calculated according to INRA (1989).

682 **Table 2** *Effect of dietary supplements of extruded linseeds alone or in combination with fish*
683 *oil on dry matter intake, fatty acid intake, milk yield and milk composition in lactating goats*
684

	Treatment[1]				
	Control	EL	ELFO	SEM [2]	*P*
Dry matter (Kg/day)	2.47[b]	2.45[b]	2.31[a]	0.041	0.013
Fatty acid intake (g/day)					
14:0	0.22[b]	0.27[b]	3.95[a]	0.038	< 0.001
16:0	9.04[c]	14.94[b]	19.47[a]	0.170	< 0.001
cis-9 16:1	0.11[c]	0.21[b]	3.58[a]	0.035	< 0.001
18:0	0.82[c]	5.57[a]	5.00[b]	0.069	< 0.001
cis-9 18:1	5.38[c]	29.91[a]	26.45[b]	0.361	< 0.001
cis-11 18:1	0.36[c]	1.45[b]	2.18[a]	0.021	< 0.001
18:2n-6	13.52[c]	28.64[a]	23.65[b]	0.265	< 0.001
18:3n-3	5.36[c]	74.38[a]	50.88[b]	0.904	< 0.001
cis-11 20:1	0.00[b]	0.00[b]	1.04[a]	0.011	< 0.001
20:5n-3	0.00[b]	0.00[b]	3.11[a]	0.031	< 0.001
22:5n-3	0.22[c]	0.26[b]	0.86[a]	0.009	< 0.001
22:6n-3	0.00[b]	0.00[b]	2.32[a]	0.023	< 0.001
Yield (g/day)					< 0.001
Milk	3019	2895	2938	51	0.111
Fat	91[a]	100[b]	83[a]	3.095	0.008
Protein	89	87	84	1.502	0.225
Lactose	144	148	146	2.558	0.390
Concentration (g/kg)					
Fat	30.0[a]	34.6[b]	27.9[a]	1.053	< 0.001
Protein	29.5[a]	30.8[b]	28.9[a]	0.302	< 0.001
Lactose	47.4[a]	51.1[c]	49.6[b]	0.276	<0.001
Energy balance[3] (MJ/d)	2.91	3.08	3.03	0.285	0.831
Protein balance[4] (g PDI/d)	11[a]	32[b]	29[b]	3.5	<0.001

685 [1]Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds
686 (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).
687 [2]SEM for *n*=14
688 [3]Net energy for lactation, balance calculated according to INRA (1989).
689 [4]PDI = digestible protein at the intestine, balance calculated according to INRA (1989).
690 [a, b, c]Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05**).**
691

Table 3 *Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on plasma insulin and metabolite concentrations, milk lipoprotein lipase activity and free fatty acid concentrations in lactating goats.*

Metabolite	Treatment[1]				
	Control	EL	ELFO	SEM[2]	*P*
Glucose, (m*M*)	3.39	3.25	3.20	0.166	0.160
NEFA (m*M*)	0.201[b]	0.477[a]	0.203[b]	0.046	<0.001
Acetate (m*M*)	0.389[a]	0.187[c]	0.260[b]	0.022	<0.001
3-Hydroxybutyrate (m*M*)	0.298[a]	0.126[c]	0.239[b]	0.020	<0.001
Insulin (µIU/ml)	17.56	16.20	14.19	1.043	0.087
Lipoprotein lipase(nmol/min per ml)	244.9	231.7	242.7	8.544	0.526
Free fatty acids(mmol/100g fat)[3]	2.13[b]	0.99[a]	1.60[ab]	0.284	0.036

[1]Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).
[2]SEM for *n*=14.
[3] Measured after storage at 4°C for 34h post-milking.
[a, b, c]Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).

Table 4 *Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on milk fatty acid composition in lactating goats.*

Fatty acid (g/100 g fatty acids)	Treatment[1]				
	Control	EL	ELFO	SEM[2]	*P*
4:0	1.81[b]	1.94[a]	2.03[a]	0.0626	0.049
6:0	2.25[a]	1.98[b]	2.15[ab]	0.0637	0.015
7:0	0.047[b]	0.058[a]	0.066[a]	0.0038	0.006
8:0	2.67[a]	2.11[b]	2.33[b]	0.0970	0.002
9:0	0.10	0.11	0.12	0.0082	0.139
10:0	10.90[a]	6.99[c]	8.02[b]	0.3280	<0.001
cis-9 10:1	0.26	0.14[b]	0.15[b]	0.0103	<0.001
11:0	0.18	0.15	0.19	0.0152	0.293
12:0	6.19[a]	3.24[c]	4.09[b]	0.1812	<0.001
cis-9 12:1	0.18	0.16	0.17	0.0162	0.625
trans-9 12:1	0.022[a]	0.014[b]	0.023[a]	0.0021	0.024
13:0	0.12[a]	0.04[b]	0.07[b]	0.0129	<0.001
13:0 *iso*	0.028[a]	0.011[b]	0.016[b]	0.0020	<0.001
13:0 *anteiso*	0.078[a]	0.033[c]	0.046[b]	0.0029	<0.001
14:0	12.98[a]	7.31[c]	9.55[b]	0.2725	<0.001
14:0 *iso*-	0.11[a]	0.08[b]	0.07[b]	0.0067	<0.001
cis-9 14:1	0.23[a]	0.09[c]	0.13[b]	0.0089	<0.001
trans-9 14:1	0.001[b]	0.010[b]	0.030[a]	0.0030	<0.001
15:0	1.05[a]	0.80[b]	1.02[a]	0.0450	0.002
15:0 *anteiso*	0.46[a]	0.33[c]	0.39[b]	0.0157	<0.001
15:0 *iso*	0.27[a]	0.15[c]	0.22[b]	0.0131	<0.001
trans-5 15:1	0.032[b]	0.021[c]	0.042[a]	0.0027	<0.001
16:0	29.60[a]	16.89[c]	21.79[b]	0.9487	<0.001
16:0 *iso*	0.37[a]	0.24[b]	0.25[b]	0.0180	<0.001
cis-9 16:1	0.69[a]	0.36[b]	0.68[a]	0.0442	<0.001
cis-11 16:1	0.014[a]	0.007[b]	0.018[a]	0.0017	<0.001
cis-9,*cis*-13 16:2	tr [c]	0.023[b]	0.068[a]	0.0073	<0.001
trans-9 16:1	0.09[b]	0.20[b]	0.51[a]	0.0520	<0.001
trans-10 16:1	0.006[c]	0.033[b]	0.094[a]	0.0049	<0.001
trans-11 16:1	0.02[b]	0.14[a]	0.22[a]	0.0394	0.008
trans-12 16:1[3]	0.22[b]	0.28[a]	0.24[b]	0.0106	0.002
17:0	0.56	0.48	0.60	0.0469	0.213
17:0 *iso*[4]	0.53[a]	0.41[b]	0.58[a]	0.0212	<0.001
17:0 *anteiso*	0.60	0.46	0.56	0.0431	0.105
cis-9 17:1	0.25[a]	0.15[b]	0.15[b]	0.0070	<0.001
18:0	5.53[b]	14.76[a]	4.94[b]	0.7494	<0.001
18:0 *iso*	0.046[a]	0.010[b]	0.004[b]	0.0030	<0.001
10-oxo 18:0	0.003[b]	0.074[b]	0.440[a]	0.0424	<0.001
18:3 n-3	0.62[c]	2.19[a]	1.24[b]	0.1006	<0.001
cis-9,*trans*-11,*trans*-15 18:3[5]	0.014[b]	0.036[b]	0.091[a]	0.0107	<0.001
cis-9,*trans*-11,*cis*-15 18:3	0.008[c]	0.150[a]	0.068[b]	0.0129	<0.001
19:0	0.039	0.042	0.044	0.0073	0.893

20:0	0.12[b]	0.14[a]	0.18[a]	0.0180	0.080
cis-11 20:1	0.044[b]	0.039[b]	0.247[a]	0.0130	<0.001
20:4n-3	tr[b]	0.001[b]	0.042[a]	0.0038	<0.001
20:4n-6	0.101[a]	0.068[b]	0.069[b]	0.0045	<0.001
20:5n-3	0.085[b]	0.101[b]	0.146[a]	0.0065	<0.001
22:0	0.031[b]	0.033[b]	0.058[a]	0.0047	<0.001
cis-11 22:1	tr[b]	tr [b]	0.167[a]	0.0130	<0.001
22:2n-6	0.022[a]	0.014[b]	0.020[a]	0.0016	0.010
22:4n-3	0.002[b]	0.013[b]	0.067[a]	0.0055	<0.001
22:5n-3	0.121[b]	0.107[b]	0.167[a]	0.0081	<0.001
22:6n-3	0.049[b]	0.031[b]	0.098[a]	0.0068	<0.001
23:0	0.004[b]	0.001[b]	0.019[a]	0.0015	<0.001
Sum of fatty acids					
Σ *trans*-18:1	1.72[c]	5.82[b]	13.72[a]	1.0366	<0.001
Σ *cis*-18:1	14.15[b]	25.35[a]	12.32[b]	0.9350	<0.001
Σ 18:2[6]	2.76[c]	3.88[b]	4.80[a]	0.3014	<0.001
Σ CLA	0.530[b]	0.991[b]	2.725[a]	0.3171	<0.001
Σ saturates	76.15[a]	58.48[b]	59.33[b]	1.0542	<0.001
Σ MUFA	17.93[c]	32.81[a]	28.91[b]	0.7750	<0.001
Σ PUFA	4.31[c]	7.60[b]	9.60[a]	0.5482	<0.001
Ratio of fatty acids					
Σn-3/ Σn-6	0.35[b]	1.20[a]	1.18[a]	0.0336	<0.001
18:3*n*3/18:2*n*6	0.26[c]	1.12[a]	0.88[b]	0.0312	<0.001
cis-9 14:1/14:0	0.018[a]	0.012[c]	0.014[b]	0.0007	<0.001
cis-9 16:1/16:0	0.023[b]	0.022[b]	0.031[a]	0.0013	<0.001
cis-9 18:1/18:0	2.517[a]	1.580[b]	2.762[a]	0.1370	<0.001
cis-9,*trans*-11 18:2/ *trans*-11 18:1	0.615[a]	0.437[b]	0.441[b]	0.0252	<0.001

[1]Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).
[2]SEM for *n*=14.
[3]Contains *trans*-6,+ -7 + -8 16:1 as a minor component
[4]Contains *cis*-7 16:1 as a minor component.
[5]Contains *trans*-13,*cis*-17 20:2 as a minor component.
[6]Sum of 18:2 fatty acids excluding isomers of CLA.
[a, b, c] Mean values for each treatment within a row not sharing a common superscript differ ($P<0.05$).
CLA, conjugated linoleic acid.
MUFA, monounsaturated fatty acids.
PUFA, polyunsaturated fatty acids.
tr: indicates concentrations below 0.001 g/100 g fatty acids.

Table 5 *Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on milk 18:1 composition in lactating goats.*

Fatty acid (g/100 g fatty acids)	Treatment[1]				
	Control	EL	ELFO	SEM[2]	*P*
cis-9 18:1[3]	13.43[b]	22.82[a]	10.80[b]	0.9145	<0.001
cis-11 18:1	0.35[b]	0.45[b]	0.73[a]	0.0444	<0.001
cis-12 18:1	0.13[b]	0.33[a]	0.11[b]	0.0195	<0.001
cis-13 18:1	0.028[b]	0.094[a]	0.066[a]	0.0099	<0.001
cis-14 18:1[4]	0.12[c]	0.88[a]	0.35[b]	0.0552	<0.001
cis-15 18:1[5]	0.08[b]	0.79[a]	0.26[b]	0.0767	<0.001
cis-16 18:1[6]	0.04[c]	0.19[a]	0.12[b]	0.0152	<0.001
trans-4 18:1	0.001[b]	0.022[a]	0.015[a]	0.0037	0.002
trans-5 18:1	0.002[b]	0.020[a]	0.017[a]	0.0036	0.005
trans-6 +-7 +-8 18:1	0.08[c]	0.32[b]	0.48[a]	0.0487	<0.001
trans-9 18:1	0.14[c]	0.31[b]	0.55[a]	0.0393	<0.001
trans-10 18:1	0.33[b]	0.81[b]	5.42[a]	0.9912	0.002
trans-11 18:1	0.76[b]	1.88[b]	5.39[a]	0.5772	<0.001
trans-12 18:1[7]	0.14[b]	0.62[a]	0.77[a]	0.0546	<0.001
trans-13 +-14 18:1	0.26[c]	1.83[a]	1.07[b]	0.1601	<0.001

[1]Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).
[2]SEM for *n*=14
[3]Contains *cis*-10-18:1, and *trans*-15 18:1 as minor components.
[4]Contains *trans*-16 18:1 as a minor component.
[5]Contains *trans*-17 18:1 as a minor component.
[6]Contains *cis*-9,*trans*12 18:2 as a minor component.
[7]Contains *cis*-6 + -7 +-8 18:1 as a minor component.
[a, b, c] Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).
tr: indicates concentrations below 0.001 g/100 g fatty acids

Table 6 *Effect of dietary supplements of extruded linseeds alone or in combination with fish oil on milk 18:2 composition (mg/100g total fatty acids) in lactating goats.*

Isomer	Treatment[1]				
(mg/100 g fatty acids)	Control	EL	ELFO	SEM[2]	*P*
cis-9,*trans*-12 18:2[3]	59[c]	338[a]	169[b]	24.48	<0.001
cis-9,*trans*-13 18:2	151[b]	682[a]	563[a]	41.45	<0.001
trans-9,*trans*-12 18:2	11[b]	35[b]	325[a]	64.36	0.002
trans-11,*cis*-15 18:2	134[b]	722[b]	2208[a]	239.97	<0.001
cis-9,*cis*-12 18:2	2340[a]	1900[b]	1390[c]	76.44	<0.001
cis-9,*trans*-11 CLA	467[b]	787[b]	2502[a]	313.79	<0.001
cis-11,*trans*-13 CLA	1[c]	2[a]	1[b]	0.23	<0.001
cis-12,*trans*-14 CLA	1[b]	2[a]	1[b]	0.18	<0.001
trans-7,*cis*-9 CLA	23[b]	57[a]	70[a]	7.03	<0.001
trans-8,*cis*-10 CLA	7[a]	5[a]	2[b]	1.36	0.055
trans-9,*cis*-11 CLA	4[b]	10[b]	58[a]	9.22	<0.001
trans-10,*cis*-12 CLA	2	3	2	0.56	0.584
trans-11,*cis*-13 CLA	6[b]	34[a]	26[a]	5.43	0.004
trans-12,*cis*-14 CLA[4]	2[b]	23[a]	5[b]	2.81	<0.001
trans-6,*trans*-8 CLA	tr[b]	tr[b]	1[a]	0.24	0.012
trans-7,*trans*-9 CLA	1[b]	1[b]	4[a]	0.30	<0.001
trans-8,*trans*-10 CLA	2[b]	2[b]	9[a]	0.65	<0.001
trans-9,*trans*-11 CLA	7[b]	11[b]	19[a]	1.67	<0.001
trans-10,*trans*-12 CLA	2[b]	3[b]	6[a]	0.77	<0.001
trans-11,*trans*-13 CLA	4[b]	26[a]	7[b]	2.21	<0.001
trans-12,*trans*-14 CLA	2[c]	23[a]	11[b]	2.05	<0.001
trans-13,*trans*-15 CLA	1[c]	2[a]	1[b]	0.19	<0.001

[1]Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).
[2]SEM for n =14.
[3] Contains *cis*-9,*trans*-14 18:2 as a minor component.
[4] Contains *cis*-13,*trans*-15 CLA as a minor component.
[a, b, c] Mean values for each treatment within a row not sharing a common superscript differ (P<0.05).
CLA, conjugated linoleic acid.

Table 7 *Pearson correlation coefficients between milk fat content (g/kg) and concentrations of specific fatty acids in milk (g/100g of fatty acids) in goats fed grass hay based diets containing no additional lipid, extruded linseeds alone or in combination with fish oil. Relationships derived using 42 measurements made for 14 animals*[1]

	Fat content	*cis*-11 16:1	*cis*-11 18:1	*cis*-11 20:1	*trans*-10 18:1	*trans*-9,*cis*-11 CLA	*trans*-8,*trans*-10 CLA	*trans*-7,*trans*-9 CLA	*trans*-10,*trans*-12 CLA
cis-11 16:1	-0.682***								
cis-11 18:1	-0.581***	+0.585***							
cis-11 20:1	-0.533***	+0.626***	+0.864***						
trans-10 18:1	-0.476**	+0.532***	+0.781***	+0.756***					
trans-9,*cis*-11 CLA	-0.503***	+0.573***	+0.834***	+0.511***	+0.964***				
trans-8,*trans*-10 CLA	-0.597***	+0.510***	+0.875***	+0.602***	+0.630***	+0.670***			
trans-7,*trans*-9 CLA	-0.607***	+0.531***	+0.778***	+0.862***	+0.599***	+0.643***	+0.922***		
trans-10,*trans*-12 CLA	-0.460**	+0.490***	+0.794***	+0.728***	+0.923***	+0.884***	+0.679***	+0.613***	
18:0	+0.606***	-0.698***	-0.358*	-0.551***	-0.387*	-0.402**	-0.486***	-0.608***	-0.294

[1]Signs indicate the effect of the variable on the predictor
CLA: conjugated linoleic acid
*, **, *** $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Figure
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Figure 1