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

2

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

6

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26 **ABSTRACT**

27 Based on the potential benefits for long-term human health there is interest in  
28 developing sustainable nutritional strategies for lowering medium-chain saturated  
29 fatty acids (FA) and increasing specific unsaturated FA in ruminant milk. Dietary  
30 supplements of extruded linseeds (EL), fish oil (FO) or a mixture of EL and FO  
31 increase *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and long-chain n-3  
32 polyunsaturated FA in bovine milk. Supplements of FO cause milk fat depression  
33 (MFD) in lactating cows, but information for dairy goats is limited. Fourteen Alpine  
34 goats were used in a replicated 3 x 3 Latin square with 28 d periods to examine the  
35 effects of EL alone or in combination with FO on animal performance, milk fat  
36 synthesis and milk FA composition. Treatments comprised diets based on natural  
37 grassland hay supplemented with no additional oil (control), 530 g/d of EL or 340 g/d  
38 of EL and 39 g/d of FO (ELFO). Compared with the control, ELFO tended ( $P = 0.08$ )  
39 to lower milk fat yield, whereas EL increased ( $P < 0.01$ ) milk fat content and secretion  
40 (15 and 10%, respectively). Relative to EL, ELFO decreased ( $P < 0.01$ ) the  
41 concentration and output of fat in milk (19 and 17%, respectively). Relative to the  
42 control and ELFO, EL decreased ( $P < 0.05$ ) milk 10:0-16:0 and odd- and branched-  
43 chain FA content and increased 18:0, *cis*-18:1, *trans*- $\Delta^{13}$  18:1 (and their  
44 corresponding  $\Delta$ -9 desaturase products), *trans*-12,*cis*-14 CLA, *cis*-13,*trans*-15 CLA,  
45 *cis*-12,*trans*-14 CLA and *trans*-11,*cis*-13 CLA and 18:3n-3 concentrations. ELFO was  
46 more effective for enriching ( $P < 0.05$ ) milk *cis*-9,*trans*-11 CLA and *trans*-11 18:1  
47 concentrations (up to 5.4- and 7.1-fold compared with the control) than EL (up to 1.7-  
48 and 2.5-fold increases). Furthermore, ELFO resulted in a substantial increase in milk  
49 *trans*-10 18:1 concentration (5.4% total FA) with considerable variation between  
50 individual animals. Relative to the control and EL, milk fat responses to ELFO were

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

60

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

63

## 64 **Implications**

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

71

## 72 **Introduction**

73 Nutrition is the major environmental factor regulating milk fat synthesis and  
74 fatty acid (FA) composition in ruminants which is an important determinant of the  
75 nutritional quality of milk for human consumers. Specific FA including medium-chain  
76 saturated FA and certain *trans*-FA are thought to elicit negative effects when  
77 consumed in excess, whilst others (*anteiso*-15:0, *cis*-9 18:1, 18:2n-6, *cis*-9,*trans*-11  
78 conjugated linoleic acid (CLA) and 18:3n-3) may have potentially beneficial effects on  
79 human health (Shingfield et al, 2008). For these reasons, the opportunities to  
80 enhance the concentration of bioactive FA through dietary supplementation with plant  
81 oils or seeds rich in n-3PUFA have been explored. Studies in goats (Nudda et al.,  
82 2006; Chilliard et al., 2007; Mele et al., 2008; Martínez Marín et al., 2011) and cows  
83 (Chilliard et al., 2007) have demonstrated that plant lipid supplements enriched in  
84 18:3n-3 increase milk 18:3n-3 and *cis*-9,*trans*-11 CLA concentrations, with responses  
85 being higher in goats than cows (Chilliard and Ferlay, 2004).

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

90 and cows suggest that 18:3n-3 in whole unprocessed linseeds is more extensively  
91 hydrogenated to 18:0 in the rumen compared with 18:3n-3 in free oils (Chilliard et al.,  
92 2003, 2007). However, a detailed assessment of dietary supplements of extruded  
93 linseeds on milk FA composition has not been documented for dairy goats.

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

103 The influence of dietary FO supplements on milk production varies between  
104 ruminant species. In cows and ewes, FO typically decreases milk fat content and  
105 yield (Chilliard et al., 2001; Loor et al., 2005a; Shingfield et al., 2006; Toral et al.,  
106 2010), but reports in goats are limited (Toral et al., accepted).

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

111

## 112 **Materials and methods**

113

114 *Animals, management and experimental design*

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

131

132 *Experimental diets*

133 Diets were based on hay prepared from regrowths of natural grassland pasture  
134 offered *ad libitum* and a concentrate mixture containing barley and soyabean meal  
135 (Table 1). Treatments comprised the basal diet containing no additional lipid  
136 (**Control**), 530 g/d of extruded linseeds (extruded mixture of linseed:wheat, 70:30  
137 wt/wt, Union Invivo, Ets Inzo, Chateau-Thierry, France) (**EL**) or 340 g/d of EL and 39  
138 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



164 experimental period, stored at -20°C, composited according to yield and submitted  
165 for FA analysis. A sub-sample of unpreserved milk was submitted for the  
166 determination of free FA concentrations measured after storage at 4°C for 34 h. For  
167 the assay of lipoprotein lipase activity, additional samples of unpreserved milk were  
168 collected and stored at -20°C until analysis (Bernard et al., 2005). Live weight of  
169 experimental animals was measured at the start and end of each experimental  
170 period. Blood samples were collected on d 20 of each experimental period at 07.30  
171 h. Samples from the jugular vein were collected into evacuated collection tubes  
172 (Venoject; C.M.L., Nemours, France) containing potassium ethylene diamine tetra-  
173 acetic acid. Once collected, blood samples were centrifuged (1500 g for 15 min at  
174 4°C), stored at -20°C and the plasma recovered analysed for insulin and metabolite  
175 concentrations (Bernard et al., 2005).

176

### 177 *Lipid analysis*

178 Chemical composition of feed ingredients was determined using standard procedures  
179 (AOAC, 1997). Fatty acid methyl esters (FAME) of lipid in feed samples were  
180 prepared using a 1-step extraction-transesterification (Sukhija and Palmquist, 1988),  
181 with 23:0 (Sigma, Saint-Quentin Fallavier, France) as an internal standard. FAME of  
182 milk were obtained by based-catalysed transesterification by the incubation of 2 ml of  
183 0.5 M sodium methoxide in methanol and 1 ml of hexane to 100 mg of lyophilised  
184 milk at 50°C for 15 min. After cooling, 1 ml of 5% (vol/vol) methanolic hydrochloric  
185 acid was added and the reaction mixture was maintained at 50°C for 15 min. Once  
186 cool, 3 ml of 6% (wt/vol) of aqueous potassium carbonate and 1.5 ml of hexane were  
187 added. Tubes were shaken vigorously, centrifuged at 1570 g for 5 min at 4°C, and  
188 the upper organic phase was recovered. Methyl esters were separated and

189 quantified by gas-liquid chromatography using a gas chromatograph Trace-GC 2000  
190 equipped with a flame-ionization detector (Thermo Finnigan, Les Ullis, France) and  
191 100 m fused silica capillary column (CP-SIL 88; Chrompack 7489, Middelburg, The  
192 Netherlands) using hydrogen as the carrier and fuel gas. Total FAME profile was  
193 determined in a 0.5  $\mu$ L sample at a split ratio of 1:50 using a temperature gradient  
194 program (Loor et al., 2005b) and isomers of 18:1 were further resolved in a separate  
195 analysis under isothermal conditions (Shingfield et al., 2003). Peaks were routinely  
196 identified using authentic FAME standards (GLC#463, Nu-Check Prep Inc, Elysian,  
197 MN, USA; iso and ante-iso 13:0, 14:0, 15:0, 16:0, 17:0 and 18:0; Sigma-Aldrich,  
198 Saint-Quentin Fallavier, France). Reference butter oil (CRM 164; Commission of the  
199 European Communities, Community Bureau of Reference, Brussels, Belgium) was  
200 used to estimate correction factors to account for the carbon deficiency in the flame  
201 ionization detector response for FAME containing 4 to 10 carbon atoms. Methyl  
202 esters not available as commercial standards were identified based on retention time  
203 comparisons with milk fat samples for which peaks were identified based on GC-MS  
204 analysis of FAME and 4,4-dimethyloxazoline FA derivatives (Shingfield et al., 2006;  
205 2008).

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

214

### 215 *Calculations and statistical analysis*

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

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

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

228

## 229 **Results**

230

### 231 *Diet composition*

232 Natural grassland hay was of high quality in term of nutritional value and had the  
233 following composition (g/kg DM, unless otherwise stated): DM (g/kg fresh weight),  
234 855; organic matter, 907; crude protein, 98; acid-detergent fibre, 281; neutral-  
235 detergent fibre, 524; FA content, 17. Concentrations of organic matter and starch  
236 were higher for the control than EL and ELFO, whereas acid-detergent fibre, neutral-  
237 detergent fibre, crude protein, diethyl ether extract, and total FA content were lower  
238 (Table 1).

239 The content of organic matter, neutral-detergent fibre, crude protein, starch, diethyl  
240 ether extract, and total FA were similar for EL and ELFO, other than a marginally  
241 lower amount (-3%) of acid-detergent fibre for ELFO than EL. By design, the EL and  
242 ELFO increased the concentration of specific FA in the diet (Table 1).  
243 Dietary forage:concentrate ratio of the diet (on a DM basis) averaged 52:48, 56:44  
244 and 55:45 for the control, EL and ELFO, respectively.

245

#### 246 *Animal performance*

247 EL diet supplied the highest amounts of 18:0, *cis*-9 18:1, 18:2n-6 and 18:3n-3 (5.6,  
248 29.9, 28.6, 74.4 g per d, respectively), whereas the ELFO treatment was a source of  
249 20:5n-3 and 22:6n-3 (3.1 and 2.3 g per d, respectively). In ELFO and EL, *cis*-9 18:1,  
250 18:2n-6 and 18:3n-3 were the major FA provided by the diets (26, 24 and 51 g per d,  
251 respectively for ELFO, and 30, 29 and 74 g per d, respectively for EL). 18:2n-6 was  
252 the major FA provided by the control diet (13.5 g per d), 18:3n-3 was the major FA  
253 provided by the 2 lipid supplemented diets and was 31% lower in ELFO diet  
254 compared to EL (Table 2).

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

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

265

#### 266 *Plasma metabolite concentrations and milk lipolytic activity*

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

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

276

#### 277 *Milk fatty acid composition*

278 Both EL and ELFO altered milk FA composition compared the control, changes  
279 characterized by decreases ( $P < 0.05$ ) in 8:0 to 16:0, *cis*-9 10:1, *cis*-9 14:1 and *cis*-9  
280 17:1, branched chain FA and an increase in total 18 carbon FA concentration (Table  
281 4).

282 Relative to the control, only EL increased ( $P < 0.05$ ) milk of 18:0, *cis*-15 18:1 and  
283 18:2n-6 concentrations. Both EL and ELFO enhanced ( $P < 0.05$ ) milk 18:3n-3  
284 concentration, with the response being higher ( $P < 0.05$ ) for EL. Compared with the  
285 control and EL, ELFO enriched ( $P < 0.05$ ) milk *cis*-11 20:1, 20:4n-3, 20:5n-3, 22:4n-3,  
286 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 *cis*-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

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

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

353

#### 354 *Milk fatty acid composition*

##### 355 *Response to extruded linseeds*

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



361 lower plasma concentrations of acetate and 3-hydroxybutyrate that serve as  
362 precursors for *de novo* FA synthesis in the mammary gland. Supplements of EL  
363 specifically increased *cis*-9 18:1, *cis*-12 18:1, *cis*-15 18:1 and *cis*-9,*trans*-12 CLA.  
364 Indirect comparisons of changes in milk fat composition for cows fed similar diets  
365 (Lerch et al., 2012a,b; Ferlay et al., 2013) indicate the increase in milk *cis*-9 18:1 and  
366 18:0 content to EL supplements can be expected to be higher in goats, consistent  
367 with the findings of earlier studies (Chilliard et al., 2007; 2013). Moreover, milk fat  
368 content was positively correlated with milk 18:0 concentration (Figure 1 and Table 7;  
369  $r=0.61$ ), providing additional support to the hypothesis that this substrate is a major  
370 factor regulating mammary lipogenesis in goats (Chilliard and Ferlay, 2004).

371 The EL treatment (216 g EL/kg DM) increased milk *trans*-11 18:1 (+147%) and *cis*-  
372 9,*trans*-11 CLA (+69%) concentrations, in line with other data in goats with extruded  
373 linseed (Nudda et al., 2006; Chilliard et al., 2007), but in lower magnitude than  
374 recently observed in response to 170 g EL/kg DM in goats offered diets containing  
375 less starch (and using the same extrusion method to extrude linseeds) (Chilliard et  
376 al., 2013), differences that may be associated with the higher starch content of the  
377 basal diet in the present study (hay-barley based diet). However, the enrichment of  
378 milk *trans*-11 18:1 and *cis*-9,*trans*-11 CLA on the EL treatment was higher than the  
379 increases to 50 g EL/kg DM reported for cows fed a similar basal diet (50% hay  
380 containing 114 g starch/kg DM; Ferlay et al., 2013). Conversely, EL had no influence  
381 on milk *trans*-10 18:1 and *trans*-10,*cis*-12 CLA concentrations, the abundance of  
382 which was found to increase in cows (Ferlay et al., 2013). Differential responses  
383 between ruminant species may be associated with a greater stability of the ruminal  
384 *trans*-11 biohydrogenation pathway in the goat compared with the cow, in which a  
385 shift to the *trans*-10 pathway is more frequently observed (Shingfield et al., 2010).

386 As expected, milk 18:3n-3 concentration and the  $\sum n-3/\sum n-6$  ratio were markedly  
387 increased on the EL treatment (Table 4). An enrichment of 18:3n-3 in milk of up to  
388 2.19 g/100 g on a diet supplying 30.6 g 18:3 n-3/kg DM is higher than the abundance  
389 in milk of 1.53 g/100 g from cows fed a similar diet providing 27.2 g 18:3n-3 /kg DM  
390 (Ferlay et al., 2013). This observation is in line with previous reports of a greater  
391 increase in milk 18:3n-3 concentration to linseed based supplements in the goat  
392 compared with the cow (Chilliard et al., 2007) which may be related to less extensive  
393 biohydrogenation of dietary PUFA in the rumen of goats than cows. Other hypothesis  
394 such as differences among goats and cows of 18:3n-3 partitioning among tissues  
395 and/or mammary extraction from circulating 18:3n-3 would also merit to be  
396 investigated.

397

#### 398 *Response to extruded linseeds and fish oil*

399 The ELFO treatment increased the  $\sum n-3/\sum n-6$  ratio in milk due to 18:3n-3 and long-  
400 chain n-3 PUFA enrichment (Table 4). Given the relatively high concentration of  
401 20:5n-3, 22:5n-3 and 22:6n-3 on the control and EL treatment and for non-  
402 supplemented diets in earlier studies with goats (Toral et al., accepted), the apparent  
403 transfer of long-chain n-3PUFA (20:5n-3, 22:5n-3 and 22:6n-3) were calculated  
404 taking into account their secretion on the control. The efficiency of transfer (3.7-  
405 10.1%) was in the same range reported for goats fed FO alone or as a mixture with  
406 plant oils (from 1.4 to 3.4% for 20:5n-3 and 22:6n-3; Toral et al., submitted). Transfer  
407 of 22:5n-3 was higher (~ 10%) as has been reported previously in goats (Toral et al.,  
408 accepted), cows (Lor et al., 2005a) and ewes (Toral et al., 2010). Part of the higher  
409 transfer efficiency may be explained by a lower apparent disappearance of 22:5n-3 in  
410 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

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

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

471 In cows, relatively few biohydrogenation intermediates are known (*trans*-10,*cis*-12  
472 CLA) or putative (*trans*-9,*cis*-11 CLA, *cis*-10,*trans*-12 CLA) inhibitory effects on  
473 mammary lipogenesis (Shingfield et al., 2010), and it is possible that others may be  
474 active in the goat, including *trans*-7,*trans*-9 CLA and *trans*-8, *trans*-10 CLA. The  
475 ELFO treatment increased *trans*-9,*cis*-11 CLA concentrations 14.5-fold (0.058 g/100  
476 g FA). In earlier studies, *trans*-9,*cis*-11 CLA was not detected in milk from goats fed  
477 hay- or maize silage-based diet supplemented with plant oils, whereas *trans*-9,*trans*-  
478 11 CLA was increased (Bernard et al., 2009). An increase in milk *trans*-9,*cis*-11 CLA  
479 content was confined to the same individuals for which concentrations of *trans*-10  
480 18:1 were elevated which explains the close association between these FA in milk  
481 (Table 7). Similarly, a close relationship between *trans*-9,*cis*-11 CLA and *trans*-10  
482 18:1 concentrations have been reported in milk of cows (Shingfield et al., 2006) and  
483 sheep (Toral et al., 2010) fed with a mixture of sunflower oil and marine oils or from  
484 cows fed diets containing oilseeds (Lerch et al., 2012a,b). Collectively, these  
485 observations suggest significant variability in the functioning and diversity of the

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

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

499

#### 500 *Milk lipolysis*

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

511

## 512 **Conclusions**

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

530

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540



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660

661 **Figure caption**

662

663 **Figure 1** Relationships between milk fat content (g/kg) and concentrations of *cis*-11  
664 16:1, *cis*-11 18:1, *cis*-11 20:1, 18:0, *trans*-8,*trans*-10 CLA and *trans*-7,*trans*-9 CLA in  
665 milk (g/100g of fatty acids) from goats fed grass hay based diets supplemented with  
666 no additional oil (control), 530 g/d of extruded linseeds (EL) or 340 g/d of EL and 39  
667 g/d of fish oil (ELFO). Relationships derived using 42 measurements made for 14  
668 animals.

669

670  
671

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

Ingredient (g/kg dry matter)	Treatment		
	Control	EL	ELFO
Natural grassland hay	518	563	558
Barley	449	208	247
Soya bean meal	32	12	30
Extruded linseeds <sup>1</sup>	0	216	147
Fish oil <sup>2</sup>	0	0	17
Mineral and vitamin premix <sup>3</sup>	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
<i>cis</i> -9 16:1	0.04	0.09	1.58
<i>cis</i> -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
<i>cis</i> -9 18:1	2.17	12.32	11.64
<i>cis</i> -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
<i>cis</i> -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) <sup>4</sup>	6.62	6.90	6.98
Protein (g PDI/ kg dry matter) <sup>5</sup>	76	87	86

672 <sup>1</sup>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).

674 <sup>2</sup>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),  
675 *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),  
676 22:5n-3 (16.6), 22:6n-3 (61.8) and total fatty acids (992).  
677 <sup>3</sup>Mineral-vitamin premix declared as containing (g/kg): Ca (240), P (60), Mg (50), Na (15), Zn, (7), Mn  
678 (6),  $\alpha$ -dl-tocopherol (0.3), retinol (0.2) and cholecalciferol (0.002) (Usine d'Ussel, Murat, France).  
679 <sup>4</sup>Net energy for lactation calculated according to INRA (1989).  
680 <sup>5</sup>Digestible protein at the intestine calculated according to INRA (1989).  
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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 <sup>1</sup>			SEM <sup>2</sup>	P
	Control	EL	ELFO		
Dry matter (Kg/day)	2.47 <sup>b</sup>	2.45 <sup>b</sup>	2.31 <sup>a</sup>	0.041	0.013
Fatty acid intake (g/day)					
14:0	0.22 <sup>b</sup>	0.27 <sup>b</sup>	3.95 <sup>a</sup>	0.038	< 0.001
16:0	9.04 <sup>c</sup>	14.94 <sup>b</sup>	19.47 <sup>a</sup>	0.170	< 0.001
<i>cis</i> -9 16:1	0.11 <sup>c</sup>	0.21 <sup>b</sup>	3.58 <sup>a</sup>	0.035	< 0.001
18:0	0.82 <sup>c</sup>	5.57 <sup>a</sup>	5.00 <sup>b</sup>	0.069	< 0.001
<i>cis</i> -9 18:1	5.38 <sup>c</sup>	29.91 <sup>a</sup>	26.45 <sup>b</sup>	0.361	< 0.001
<i>cis</i> -11 18:1	0.36 <sup>c</sup>	1.45 <sup>b</sup>	2.18 <sup>a</sup>	0.021	< 0.001
18:2n-6	13.52 <sup>c</sup>	28.64 <sup>a</sup>	23.65 <sup>b</sup>	0.265	< 0.001
18:3n-3	5.36 <sup>c</sup>	74.38 <sup>a</sup>	50.88 <sup>b</sup>	0.904	< 0.001
<i>cis</i> -11 20:1	0.00 <sup>b</sup>	0.00 <sup>b</sup>	1.04 <sup>a</sup>	0.011	< 0.001
20:5n-3	0.00 <sup>b</sup>	0.00 <sup>b</sup>	3.11 <sup>a</sup>	0.031	< 0.001
22:5n-3	0.22 <sup>c</sup>	0.26 <sup>b</sup>	0.86 <sup>a</sup>	0.009	< 0.001
22:6n-3	0.00 <sup>b</sup>	0.00 <sup>b</sup>	2.32 <sup>a</sup>	0.023	< 0.001
Yield (g/day)					< 0.001
Milk	3019	2895	2938	51	0.111
Fat	91 <sup>a</sup>	100 <sup>b</sup>	83 <sup>a</sup>	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 <sup>a</sup>	34.6 <sup>b</sup>	27.9 <sup>a</sup>	1.053	< 0.001
Protein	29.5 <sup>a</sup>	30.8 <sup>b</sup>	28.9 <sup>a</sup>	0.302	< 0.001
Lactose	47.4 <sup>a</sup>	51.1 <sup>c</sup>	49.6 <sup>b</sup>	0.276	<0.001
Energy balance <sup>3</sup> (MJ/d)	2.91	3.08	3.03	0.285	0.831
Protein balance <sup>4</sup> (g PDI/d)	11 <sup>a</sup>	32 <sup>b</sup>	29 <sup>b</sup>	3.5	<0.001

685 <sup>1</sup>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 <sup>2</sup>SEM for *n*=14

688 <sup>3</sup>Net energy for lactation, balance calculated according to INRA (1989).

689 <sup>4</sup>PDI = digestible protein at the intestine, balance calculated according to INRA (1989).

690 <sup>a, b, c</sup>Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).

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**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 <sup>1</sup>			SEM <sup>2</sup>	P
	Control	EL	ELFO		
Glucose, (mM)	3.39	3.25	3.20	0.166	0.160
NEFA (mM)	0.201 <sup>b</sup>	0.477 <sup>a</sup>	0.203 <sup>b</sup>	0.046	<0.001
Acetate (mM)	0.389 <sup>a</sup>	0.187 <sup>c</sup>	0.260 <sup>b</sup>	0.022	<0.001
3-Hydroxybutyrate (mM)	0.298 <sup>a</sup>	0.126 <sup>c</sup>	0.239 <sup>b</sup>	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) <sup>3</sup>	2.13 <sup>b</sup>	0.99 <sup>a</sup>	1.60 <sup>ab</sup>	0.284	0.036

696 <sup>1</sup>Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds  
697 (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

698 <sup>2</sup>SEM for n=14.

699 <sup>3</sup> Measured after storage at 4°C for 34h post-milking.

700 <sup>a, b, c</sup>Mean values for each treatment within a row not sharing a common superscript differ (P<0.05).

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

20:0	0.12 <sup>b</sup>	0.14 <sup>a</sup>	0.18 <sup>a</sup>	0.0180	0.080
<i>cis</i> -11 20:1	0.044 <sup>b</sup>	0.039 <sup>b</sup>	0.247 <sup>a</sup>	0.0130	<0.001
20:4n-3	tr <sup>b</sup>	0.001 <sup>b</sup>	0.042 <sup>a</sup>	0.0038	<0.001
20:4n-6	0.101 <sup>a</sup>	0.068 <sup>b</sup>	0.069 <sup>b</sup>	0.0045	<0.001
20:5n-3	0.085 <sup>b</sup>	0.101 <sup>b</sup>	0.146 <sup>a</sup>	0.0065	<0.001
22:0	0.031 <sup>b</sup>	0.033 <sup>b</sup>	0.058 <sup>a</sup>	0.0047	<0.001
<i>cis</i> -11 22:1	tr <sup>b</sup>	tr <sup>b</sup>	0.167 <sup>a</sup>	0.0130	<0.001
22:2n-6	0.022 <sup>a</sup>	0.014 <sup>b</sup>	0.020 <sup>a</sup>	0.0016	0.010
22:4n-3	0.002 <sup>b</sup>	0.013 <sup>b</sup>	0.067 <sup>a</sup>	0.0055	<0.001
22:5n-3	0.121 <sup>b</sup>	0.107 <sup>b</sup>	0.167 <sup>a</sup>	0.0081	<0.001
22:6n-3	0.049 <sup>b</sup>	0.031 <sup>b</sup>	0.098 <sup>a</sup>	0.0068	<0.001
23:0	0.004 <sup>b</sup>	0.001 <sup>b</sup>	0.019 <sup>a</sup>	0.0015	<0.001
Sum of fatty acids					
Σ <i>trans</i> -18:1	1.72 <sup>c</sup>	5.82 <sup>b</sup>	13.72 <sup>a</sup>	1.0366	<0.001
Σ <i>cis</i> -18:1	14.15 <sup>b</sup>	25.35 <sup>a</sup>	12.32 <sup>b</sup>	0.9350	<0.001
Σ 18:2 <sup>6</sup>	2.76 <sup>c</sup>	3.88 <sup>b</sup>	4.80 <sup>a</sup>	0.3014	<0.001
Σ CLA	0.530 <sup>b</sup>	0.991 <sup>b</sup>	2.725 <sup>a</sup>	0.3171	<0.001
Σ saturates	76.15 <sup>a</sup>	58.48 <sup>b</sup>	59.33 <sup>b</sup>	1.0542	<0.001
Σ MUFA	17.93 <sup>c</sup>	32.81 <sup>a</sup>	28.91 <sup>b</sup>	0.7750	<0.001
Σ PUFA	4.31 <sup>c</sup>	7.60 <sup>b</sup>	9.60 <sup>a</sup>	0.5482	<0.001
Ratio of fatty acids					
Σn-3/ Σn-6	0.35 <sup>b</sup>	1.20 <sup>a</sup>	1.18 <sup>a</sup>	0.0336	<0.001
18:3n3/18:2n6	0.26 <sup>c</sup>	1.12 <sup>a</sup>	0.88 <sup>b</sup>	0.0312	<0.001
<i>cis</i> -9 14:1/14:0	0.018 <sup>a</sup>	0.012 <sup>c</sup>	0.014 <sup>b</sup>	0.0007	<0.001
<i>cis</i> -9 16:1/16:0	0.023 <sup>b</sup>	0.022 <sup>b</sup>	0.031 <sup>a</sup>	0.0013	<0.001
<i>cis</i> -9 18:1/18:0	2.517 <sup>a</sup>	1.580 <sup>b</sup>	2.762 <sup>a</sup>	0.1370	<0.001
<i>cis</i> -9, <i>trans</i> -11 18:2/ <i>trans</i> -11 18:1	0.615 <sup>a</sup>	0.437 <sup>b</sup>	0.441 <sup>b</sup>	0.0252	<0.001

704 <sup>1</sup>Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds  
705 (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

706 <sup>2</sup>SEM for *n*=14.

707 <sup>3</sup>Contains *trans*-6,+ -7 + -8 16:1 as a minor component

708 <sup>4</sup>Contains *cis*-7 16:1 as a minor component.

709 <sup>5</sup>Contains *trans*-13,*cis*-17 20:2 as a minor component.

710 <sup>6</sup>Sum of 18:2 fatty acids excluding isomers of CLA.

711 <sup>a, b, c</sup> Mean values for each treatment within a row not sharing a common superscript differ (*P*<0.05).

712 CLA, conjugated linoleic acid.

713 MUFA, monounsaturated fatty acids.

714 PUFA, polyunsaturated fatty acids.

715 tr: indicates concentrations below 0.001 g/100 g fatty acids.

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

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

739 <sup>1</sup>Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds  
 740 (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

741 <sup>2</sup>SEM for n=14

742 <sup>3</sup>Contains *cis*-10-18:1, and *trans*-15 18:1 as minor components.

743 <sup>4</sup>Contains *trans*-16 18:1 as a minor component.

744 <sup>5</sup>Contains *trans*-17 18:1 as a minor component.

745 <sup>6</sup>Contains *cis*-9, *trans*-12 18:2 as a minor component.

746 <sup>7</sup>Contains *cis*-6 + -7 +-8 18:1 as a minor component.

747 <sup>a, b, c</sup>Mean values for each treatment within a row not sharing a common superscript differ ( $P < 0.05$ ).

748 tr: indicates concentrations below 0.001 g/100 g fatty acids

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

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

752 <sup>1</sup>Diets based on grass hay supplemented with no additional oil (control), 530 g/d of extruded linseeds  
 753 (EL) or 340 g/d of EL and 39 g/d of fish oil (ELFO).

754 <sup>2</sup>SEM for *n* = 14.

755 <sup>3</sup>Contains *cis*-9,*trans*-14 18:2 as a minor component.

756 <sup>4</sup>Contains *cis*-13,*trans*-15 CLA as a minor component.

757 <sup>a, b, c</sup> Mean values for each treatment within a row not sharing a common superscript differ (*P* < 0.05).  
 758 CLA, conjugated linoleic acid.

759

**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<sup>1</sup>

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

<sup>1</sup>Signs indicate the effect of the variable on the predictor

CLA: conjugated linoleic acid

<sup>\*</sup>, <sup>\*\*</sup>, <sup>\*\*\*</sup>  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively.

Figure 1

