Interpretive summary

Time-dependent milk fatty acid changes after plant oil supplementation

Martínez Marín et al.

Individual fatty acid content in milk fat starts to change soon after introducing plant oils into dairy goat diets. Most changes stabilize between one and eight days reaching values found at the usual sampling times of 21 days or more in most experiments. When compared with values from an oil free control diet, typical changes are observed for each of the three oils used: high oleic, regular sunflower or linseed. These changes occur between one hour (α-linolenic acid, with linseed oil) and five days (medium chain saturated fatty acids, with the three oils). Shorter sampling times could be used.

TIME-DEPENDENT CHANGES IN MILK FATTY ACID COMPOSITION AFTER PLANT OIL SUPPLEMENTATION

Time-dependent variations in milk fatty acid content of goats fed with three different plant oils

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ABSTRACT

The effect of sampling time on milk fat fatty acid composition after separately adding three plant oils to an oil free control diet (cereal-soybean meal consisting of concentrate 0.67, alfalfa hay 0.33) was studied in 12 Malagueña goats. Individual animals were randomly allocated to one of the four following treatments: control, or 48 g/d of added high oleic (OSO), regular (RSO) sunflower oil, or linseed oil (LO). Individual milk samples were taken at 0 (covariate), 1, 12, 24, 72, 120, 192, 312 and 504 h after the beginning of the experiment. Milk fat fatty acid contents (g/100 g total fatty acid methyl esters) were analyzed as a completely randomized design with repeated measures using the MIXED procedure of SAS. Comparing results of 15 chosen fatty acids (for example, medium chain saturated fatty acids, *trans*-11 C18:1, *cis*-9, *trans*-11 C18:2, *trans*-10 C18:1 and C18:3n-3) indicated that throughout the duration of the experiment, feeding the control diet had little influence on the concentrations of most fatty acids in milk. Most changes in milk fatty acid composition due to oil supplementation had occurred within 192 h since the beginning of the experiment. However, the concentrations of two fatty acids (*trans*-10 C18:1 in RSO and C18:3n-3 in LO treatments) continued to change until 504 h. By comparing fatty acid values in milk fat from oil treatments with those of the control at the same sampling times, typical value differences for the three supplementary oils found at 504 h (21d) were also observed at 312 h from the beginning of the experiment (13 d), and even earlier in some fatty acids such as: medium chain saturated fatty acids at 120 h in RSO and LO and at 72 h in OSO; *cis*-9,*trans*-11 C18:2 and *trans*-10 C18:1 at 24 h in RSO; *trans*-11 C18:1 at 12 h in RSO and LO; and C18:3n-3 at 1 h in LO. In the conditions assayed in these experiments reliable results of milk fatty acid changes were obtained at sampling times shorter than 21 d. Monitoring early changes in milk fat fatty acids after the addition of plant oils to diets could help in the study of rumen and mammary metabolism of dietary fatty acids.

Key Words: plant oils, fatty acids, goat milk, sampling times

INTRODUCTION

There is a scarcity of information dealing with the kinetics of milk fat fatty acid (**FA**) composition responses to plant oil inclusions in the diet of dairy ruminants. Most information corresponds to work carried out with cows (Dhiman et al., 2000; Roy et al., 2006) and ewes (Gómez-Cortés et al., 2008a, 2008b; Hervás et al., 2008). To our knowledge, only Chilliard et al. (2005) gave data on time-related changes to *cis*-9,*trans*-11 C18:2 content after introducing sunflower or linseed oils into dairy goat diets.

In most published papers the first milk sample was taken two or seven days after the introduction of the plant oil into the diets. However, *in vitro* work done by Mosley et al. (2002) and Jouany et al. (2007) showed changes in the accumulation of biohydrogenation (**BH**) intermediates and end-products as soon as 0.5 h. after oil inclusion in media inoculated with mixed rumen microbes. Fievez et al. (2007) indicated that rumen microorganisms are permanently adapted to biohydrogenate unsaturated FAs from plants because they are always in contact with them in most ruminant diets. Different authors (Moate et al., 2004; Harvatine and Allen, 2006) have used information from *in vivo* experiments to build models describing rumen BH kinetics. These models predict a fast strong,lipolysis-BH reaction, which delivers unsaturated fats into the rumen and provide estimates for FA rumen passage rates. While the model by Moate et al. (2004) practically precludes C18:3n-3 from escaping the rumen unaltered by BH, the model by Harvatine and Allen (2006) allows room for this to happen.

When studying the kinetics of FA responses to the inclusion of plant oils in dairy ruminant diets, one has to bear in mind that the observed effects on milk FA changes also imply the time from leaving the rumen to their secretion as milk triacylglycerols. Furthermore, although several authors have studied the response of milk FA composition to abomasal/duodenal infusions of long chain FAs (Drackley et

al., 2007; Khas-Erdene et al., 2010), their studies show changes occurring in the milk fat FA profile at 5 d from the beginning of the infusions, at the earliest.

The aim of this work was to obtain information about the timing of changes in FA contents in goat milk fat from 1 to 504 h (21 d) after introducing three different plant oils into the diet.

MATERIALS AND METHODS

The experiments were carried out on the premises of the Animal Production Department of Córdoba University. Animals were kept in accordance with Spanish regulations relative to the treatment of experimental animals. Twelve Malagueña goats were used (45±5 DIM, 47±4.2 kg live weight, and 2287±512 g/d milk production at the beginning of the experiment). They were placed in individual cages of 1.0 x 1.4 m with slatted floors equipped with water and feeding troughs. All goats were fed a general purpose diet without added fat (maize, oats, horsebeans and alfalfa hay) from kidding until the beginning of the experiment.

Goats were randomly assigned to one of four treatments (three goats per treatment): a basal control diet (concentrate 0.67, alfalfa hay 0.33) with no added oil, or a basal diet supplemented with 48 g/d of either high oleic sunflower oil (**OSO**), regular sunflower oil **(RSO**) or linseed oil (**LO**) as shown in Table 1. The experiments lasted 21 d. Milk samples were taken from milkings at 0 h (before oil supplementation) and 1, 12, 24, 72, 120, 192, 312 and 504 h after the addition of the corresponding oil. Milkings at 0 (covariate), 1 and 12 h were stripped out by hand after giving an i.v. dose of 2-3 i.u. of oxytocin to the goats. Daily DMI, body weight changes, milk production and sampling, and diet analysis were carried out as in Martínez Marín et al. (2012).

Milk fats were extracted as described by Gómez-Cortés et al. (2008a). Fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of the glycerides (ISO-IDF-200a). Analysis of FAME was performed on a gas-liquid chromatograph (Agilent 6890 N Network System) onto a CP-Sil 88 fused silica capillary column (100 m X 0.25 mm, Varian, Middelburg, Netherlands) under similar conditions to those reported by Luna et al. (2008). Individual FAME quantification was performed according to ISO-IDF (2002b) using a milk fat with known composition (CRM 164; European Community Bureau of Reference, Brussels, Belgium). Individual FAs were identified by comparison with standards distributed by Nu-Chek (Elysian, MN, USA), while *trans*-11 *cis*-15 C18:2, *trans*-11 *trans*-15 C18:2, *cis*-9 *trans*-11 *cis*-15 C18:3 and *cis*-9 *trans*-11 *trans*-15 C18:3 had previously been identified by GC-MS/MS (Gómez-Cortés et al., 2009).

Data of milk fat FA content were analyzed by repeated measurement analysis (Littell et al., 2006) using PROC MIXED of SAS (SAS Institute, 2004). The statistical model included the fixed effects of diet (**D**), time (**T**), their interaction (**D×T**), the covariate, and the random effect of goat nested within treatment. For each FA, the covariance structure of goat nested within treatment (compound symmetry, ANTE(1) or spatial power) was chosen on the basis of the Schwarz Bayesian information model fit criteria. Least square means (adjusted by the covariate) of the DxT interaction were compared using the CONTRAST statement in PROC MIXED. Within treatments the least square means were compared with their respective 504 h values. Least square means from samples of goats fed with added oil were also compared, by sampling times, with the respective control mean. Statistical differences were declared at $P < 0.05$.

RESULTS AND DISCUSSION

To study the effect of time after introducing the oils into the diets, 15 FAs were selected (Table 2) on the basis of their significant differences in DxT interaction and relevance in current knowledge of rumen and mammary metabolism as well as in human nutrition.

144

145 *Comparing respective 504 h values.*

146 In the control treatment, sampling time did not affect the proportion of any FA in Table 2, i.e. for a
147 given FA the values obtained at any sampling time were no different from the corresponding 504 h
148 value (21 d). This provided a proper reference for comparing the effects of sampling time on the three
149 oil treatments.

150

151 In the OSO treatment, only four FAs out of the 15 presented in Table 2 showed differences due to
152 sampling time compared with the corresponding value obtained at 504 h: medium chain saturated fatty
153 acids (MCSFA), C18:0, *cis*-9 C18:1 and *trans*-15(+*cis*-11) C18:1. There were no significant
154 differences in the percentages of MCSFA and C18:0 relative to the final samples collected after one
155 and three days, respectively. The effect of OSO on MCSFA content in milk fat may be mediated by
156 two different causes which are not mutually exclusive: one is the negative effect of oil on VFA
157 produced in rumen fermentation, and the other is the direct effect of long chain FAs absorbed in the
158 small intestine on *de novo* FA synthesis in the mammary gland. According to Martínez Marín et al.
159 (2012), who used the same diets in their experiments as those in this work, the likelier cause is the
160 second because the short chain FAs (C4:0 to C8:0) showed no negative or even positive responses to
161 oil supplementation (Table 2). The effect of OSO on C18:0 and *cis*-9 C18:1 milk fat content probably
162 reflected its high level of *cis*-9 C18:1 and the particular rumen BH – mammary desaturation cycle of
163 these FAs.

164

165 Five FAs in the RSO treatment showed values statistically different from their corresponding 504 h
166 values which did not differ significantly at 72 h (MCSFA, *cis*-9,*cis*-12 C18:2), or at 120 h (*trans*-11
167 C18:1 and *cis*-9,*trans*-11 C18:2; Figure 1) or at 192 h (short chain saturated fatty acids –**SCSFA**-), i.e.
168 between 3 and 8 d (Table 4). Roy et al. (2006) and Shingfield et al. (2006) observed in cows and

Gómez-Cortés et al. (2008a, 2011) in ewes, that the contents of *trans*-11 C18:1 and *cis*-9,*trans*-11 C18:2 in milk fat reached maximum values between 6-7 days after introducing linoleic acid rich oils into the diet, but then decreased and leveled off to values higher than those of the control diet. In the current research the highest milk fat content values of these two FAs were reached at 8d, but rather than subsequently dropping, they leveled off at these highest values. On the other hand, the sampling time values of *trans*-10 C18:1 were all different from the corresponding 504 h value which was higher than the apparent plateau reached between 120 and 312 h (Figure 2). This type of response was not observed in cows (Roy et al., 2006 with forage-concentrate ratios of 27:73 and 48:52; or by Shingfield et al., 2006 with a forage concentrate-ratio of 60:40). Both authors reported a plateau of *trans*-10 C18:1 content in milk fat around 10-12 d reaching up to 20 and 28 d, respectively. However, Gómez-Cortés et al. (2008a) found the highest value of *trans*-10 C18:1 on the last sampling day of their experiment (21 d) with a 20:80 forage:concentrate diet supplemented with 6% soybean oil. Gómez-Cortés et al. (2011) also found the same type of result at 28 d when including 2% sunflower oil in a 30:70 forage:concentrate diet. All of this suggests that temporal changes in *trans*-10 C18:1 milk fat content observed in cows could be different from those in ewes and goats. In these species, at least with high concentrate diets, the potential maximum value of *trans*-10 C18:1 content in milk fat doesn't seem to have been reached at 21 or 28 d after introducing the dietary oil.

Nine of the fifteen FAs listed in Table 2 that showed statistically significant differences between their first sampling time values and those of the corresponding 504 h sampling in LO treatment did not differ significantly at 24 h (MCSFA, *trans*-11 C18:1, *trans*-15(+*cis*-11) C18:1, *cis*-9,*cis*-12 C18:2, *cis*-9,*trans*-11 C18:2, *cis*-9,*trans*-11,*cis*-15 C18:3), or at 120 h (*cis*-15 C18:1) or at 192 h (*trans*-11,*cis*-15 C18:2, *cis*-9,*trans*-11,*trans*-15 C18:3). The content of C18:3n-3 showed numerically increasing values with time which were all lower than the 504 h value (Table 2), suggesting that the potential maximum content of C18:3n-3 in milk fat was not reached before or even 504 h (21 d) after the introduction of LO in the dairy goat diets (Figure 2). A similar response was observed by Luna et al. (2005) working with ewes fed linseed for six weeks.

196

197 In the current study, the final values of *trans*-11 C18:1 and *cis*-9,*trans*-11 C18:2 were 58% and 42%
198 higher respectively in RSO than in LO treatment. These results indicate that the response of *trans*-11
199 C18:1 and *cis*-9,*trans*-11 C18:2 to diets supplemented with LO was faster but weaker than with RSO.
200 Bernard et al. (2009) also found higher *trans*-11 C18:1 and *cis*-9,*trans*-11 C18:2 contents in goat milk
201 fat when the basal diet was supplemented with sunflower oil instead of LO.

202

203 In LO treatment the contents of *trans*-11 C18:1 and *cis*-9,*trans*-11 C18:2 were not very different from
204 their respective 504 h values at 24 h (i.e. a plateau was reached up to the final sampling time), but in
205 RSO treatment the plateau started later (120 h) (Figure 1 and Table 2). Work *in vitro* by Jouany et al.
206 (2007) with a mixed rumen microbial population indicated that BH of C18:3n-3 was faster, reaching a
207 maximum at 5 h, whereas in the case of *cis*-9,*cis*-12 C18:2, BH was still important between 5 and 24
208 h. According to these authors the reason for the delayed BH of *cis*-9,*cis*-12 C18:2 could be due to the
209 preferential uptake of this FA by bacteria, or differences in microbial isomerase or saturase affinity
210 between the two FAs. Work with dairy goats by Chilliard et al. (2005) indicated that the *cis*-9,*trans*-11
211 C18:2 increase in milk fat of goats fed sunflower and linseed oils which was observed at 7 d, lasted up
212 to the end of the experiment (35 d). Luna et al. (2008), also working with goats supplemented with
213 whole linseed and sunflower oil, found that the maximum values in milk fat of *trans*-11 C18:1 and
214 *cis*-9,*trans*-11 C18:2 were at 15 d. Unfortunately, no data was reported for the week prior to
215 supplementation in those experiments.

216

217 *Comparing oil treatments with control*

218 Comparison of FA contents from oil treatments with the corresponding values in the control treatment
219 at the different sampling times are shown in Table 2. Of the 45 possible comparisons, i.e. 15 selected
220 FAs and 3 oil treatments, 28 (seven, nine and 12 in OSO, RSO and LO treatments, respectively) had

221 significantly different values from the corresponding values in the control at one or more sampling
222 times. Out of these 28 comparisons, 16 showed differences of the same algebraic sign (positive or
223 negative) to their corresponding 312 and 504 h values in control treatment. All three oils lowered the
224 MCSFA content in milk fat, whereas increases in *trans*-11 C18:1 and *cis*-9,*trans*-11 C18:2 content
225 were common in RSO and LO treatments. Specific for each oil were the increases in C18:0 in OSO
226 treatment, SCSFA, *trans*-10 C18:1 and *cis*-9,*cis*-12 C18:2 in RSO treatment, and *trans*-15(+*cis*-11)
227 C18:1, *cis*-15 C18:1, *trans*-11,*cis*-15 C18:2, C18:3n-3 and *cis*-9,*trans*-11,*cis*-15 C18:3 in LO
228 treatment.

229

230 Some of the changes observed when comparing oil supplemented diets with the control diet at 312 and
231 504 h were also detected for shorter time periods. For example, the MCSFA content in milk fat of
232 goats fed with oil-supplemented diets was seen to respond in the same way at 312 and 504 h,
233 sometimes as soon as 120 h, and even earlier (at 72 h in OSO treatment). Other FAs responded faster:
234 *cis*-9,*trans*-11 C18:2 and *trans*-10 C18:1 contents in milk fat of RSO and *cis*-9,*trans*-11,*cis*-15 C18:3
235 in LO were already different from the control at 24 h. Differences with the control treatment appeared
236 at 12 h for *trans*-11 C18:1 in both RSO and LO, for *cis*-9,*cis*-12 C18:2 in RSO, and for *cis*-15 C18:1,
237 *cis*-9,*trans*-11 C18:2 and *trans*-11,*cis*-15 C18:2 in LO. Furthermore, only 1 h was necessary to obtain
238 a significant increase in C18:3n-3 in LO. All the responses mentioned in the paragraph above were
239 consistent in time (i.e. differences with control values were statistically significant at all sampling
240 times after the first one was observed). In contrast, other FA comparisons failed to give a continuous
241 significant response after the first difference with control was seen (Table 2).

242

243 The fact that the milk FA profile can be changed shortly after dietary lipid introduction had already
244 been observed by Scott et al. (1971) and Gulati et al. (1997). These authors introduced fat into diets
245 and observed clear changes in milk fat FA composition at the first milk sampling at 36 h in cows and
246 96 h in goats. It can be argued that these authors used protected oils or oilseeds, but the relevant fact is

247 the short time required to obtain clear results. This is not surprising in view of the results obtained by
248 Li et al. (2009). These authors observed increased *cis*-9,*trans*-11 C18:2 in rumen fluid of dairy goats at
249 1, 3 and 6 h after being fed daily with a diet supplemented with 4% soybean oil.

250

251 The observed evolution of FA contents determined in the present work could not be necessarily
252 identical for other types of diets (i.e. when lipid supplementation is accompanied by other changes
253 such as the dietary forage to concentrate ratio). However, a shortening of the period for assessing
254 changes in milk fat following the introduction of dietary lipid supplements may be possible. Although
255 the data suggest that longer periods after lipid supplementation would be required for an accurate
256 assessment of some fatty acids (mainly *trans*-10 C18:1), a stabile milk fat general profile could be
257 achieved at shorter times than 21 d.

258

259 **CONCLUSIONS**

260 In summary, compared with their respective 504 h values, most FAs responding to the addition of any
261 of the three plant oils used in this study reached a value which did not differ greatly between 24 and
262 192 h (i.e. between 1 and 8 d). That means that for most of the FAs studied, the time from introducing
263 the plant oil into the diet up to having a response in milk fat content not different of that obtained at 21
264 d was 8 d. Results obtained under the conditions described in this paper show that typical changes in
265 milk fat FA content by separately adding three different plant oils (high oleic sunflower or regular
266 sunflower or linseed oil) to the diet of dairy goats, compared to a control diet without added fat, can be
267 obtained as soon as 13 d after introducing the oils into the diet, although the maximum attainable
268 values for some FAs may require longer.

269

270 **ACKNOWLEDGMENTS**

271 This work was financed by the Ministerio de Ciencia e Innovación (MICINN; AGL2008-04805 and
272 the Consolider Ingenio 2010 Programme; FUN-C-FOOD CSD2007-063) and the Comunidad
273 Autónoma de Madrid (2009-AGR-1469). The authors wish to thank Mr. A. Jiménez Secilla and Mr. B.
274 Garfia Catalán of Laboratorio Agropecuario Regional of Córdoba for their help with milk composition
275 analysis. We are also grateful to Mrs. I. Andújar-Ramírez (Universidad de Córdoba, Córdoba, Spain)
276 and Mrs. M.V. Rodríguez-Pino (CSIC, Madrid, Spain) for their technical assistance in processing milk
277 samples and Mr. J.J. Pérez-Hernández (Universidad de Córdoba, Córdoba, Spain) for his assistance in
278 animal feeding and management.

279

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364 **Table 1.** Ingredients, chemical composition and nutritive value of the experimental diets

Item	Treatments[1]			
	Control[2]	OSO	RSO	LO
Diet, g/d				
Alfalfa hay	600	600	600	600
Concentrate[3]	1200	1200	1200	1200
High oleic sunflower oil[4]	-	48	-	-
Regular sunflower oil[4]	-	-	48	-
Linseed oil[4]	-	-	-	48
Chemical composition				
DM,%	90.6	90.5	91.0	91.2
CP, % DM	17.0	16.4	16.4	16.5
NDF, % DM	28.2	27.5	27.0	26.9
AHEE[5], % DM	2.4	5.6	5.5	5.8
Ash, % DM	7.6	7.5	7.5	7.5
Nutritive value[6]				
ME, Mcal/kg DM	2.67	2.77	2.77	2.77
MP, g/kg DM	123	119	119	119
Fatty acids from oil, g/d				
C16:0	-	1.8	2.9	2.6
C18:0	-	1.4	2.0	1.8
cis-9 C18:1	-	41.0	14.2	10.0
C18:2n-6	-	2.7	27.9	8.0
C18:3n-3	-	-	-	23.9

365 [1]Control = basal diet with no added oil; OSO, RSFO and LO = diets enriched with 48 g/d of high oleic
366 sunflower oil, regular sunflower oil or linseed oil, respectively.

[2]Control diet supplied 5.1, 0.8, 6.9, 18.9 and 6.5 g/d of C16:0, C18:0, *cis*-9 C18:1, *cis*-9,*cis*-12 C18:2 and C18:3n-3, respectively, calculated according to INRA (2002).

[3]Composition (g/kg, as fed): maize, 375; barley, 374.9; soybean meal, 200; vitamin and mineral premix (Maxi Nutral Ovejas, Nutral, Madrid, Spain), 30; binder (Exal, Tolsa, Madrid, Spain), 20; antioxidant (Luctanox, Lucta, Barcelona, Spain), 0.1.

[4]Included in the respective concentrates. High oleic sunflower oil and regular sunflower oil were purchased from Carrefour S.A. (Madrid, Spain). Linseed oil was supplied by Gustav Heess (Barcelona, Spain).

[5]Acid hydrolysis ether extract.

[6]Calculated from NRC (2007).

Table 2. Fatty acid content (g/100 g of fatty acid methyl esters) at different sampling times in milk fat from goats fed control or oil supplemented diets.

Fatty acids[1]	Treatments[2]	Hour								SEM
		1	12	24	72	120	192	312	504	
SCSFA	Control	10.00	9.62	9.84	9.44	9.62	9.53	8.98	8.95	0.118
	OSO	9.95	9.31	9.54	7.66[a]	8.67	9.00	9.43	8.99	0.191
	RSO	9.64[A]	9.57[A]	9.51[A]	9.37[A]	9.50[A]	10.32	10.13[a]	10.97[a]	0.169
	LO	9.62	9.43	10.02	10.50	10.65	10.50	10.52[a]	9.81	0.163
	SEM	0.172	0.146	0.119	0.391	0.301	0.256	0.246	0.384	
MCSFA	Control	44.84	45.00	43.03	41.73	43.27	45.47	46.83	46.47	0.672
	OSO	45.42[A]	45.50[A]	40.14	32.11[a]	37.63[a]	38.04[a]	39.24[a]	36.73[a]	1.272
	RSO	45.08[A]	45.69[A]	40.06[A]	36.76	34.75[a]	34.70[a]	34.96[a]	33.40[a]	1.015

	LO	45.85^{Aa}	45.48^{A}	40.46	36.04	34.68^{a}	33.34^{a}	35.66^{a}	36.71^{a}	1.336
	SEM	1.030	1.160	0.974	1.161	1.392	2.011	1.892	2.180	
C18:0	Control	7.88	8.57	8.67	9.11	8.23	7.95	7.83	7.178	0.143
	OSO	7.58^{A}	8.22^{A}	10.47^{Aa}	13.72^{a}	10.54	10.91	11.00^{a}	13.88^{a}	0.583
	RSO	7.99	8.61	10.67^{a}	11.40	10.29	9.09	9.76	8.78	0.391
	LO	7.63	7.59	8.75	11.10	11.06	11.83^{A}	10.14	8.220	0.499
	SEM	0.288	0.273	0.380	0.673	0.724	0.696	0.531	0.953	
cis-9 C18:1	Control	15.20	16.30	17.23	19.35	18.27	16.10	15.86	15.31	0.516
	OSO	14.82^{Aa}	15.12^{A}	18.45^{A}	27.29	21.44	20.28	18.07^{A}	21.94^{a}	1.139
	RSO	15.15	14.19	17.15	18.67	18.66	16.35	17.14	16.24	0.488
	LO	14.79^{a}	13.68	14.23	16.88	17.20	17.90	15.33	14.98	0.510
	SEM	0.474	0.690	0.634	1.710	0.864	1.216	0.522	0.840	
trans-10 C18:1	Control	0.19	0.13	0.15	0.13	0.15	0.16	0.20	0.36	0.027
	OSO	0.19	0.24	0.32	0.30	0.29	0.29	0.28	0.22	0.018
	RSO	0.18^{A}	0.24^{A}	0.40^{Aa}	0.44^{Aa}	0.69^{Aa}	0.78^{Aa}	0.72^{Aa}	1.33^{a}	0.088
	LO	0.17^{a}	0.26	0.43^{a}	0.25	0.19	0.20	0.24	0.33	0.035
	SEM	0.040	0.035	0.049	0.032	0.076	0.078	0.063	0.162	
trans-11 C18:1	Control	0.78	0.61	0.69	0.72	0.73	0.69	0.64	0.89	0.050
	OSO	0.81	0.87	1.16	1.39	1.39	1.34	1.29	1.46	0.129
	RSO	0.81^{A}	1.14^{Aa}	1.87^{Aa}	3.18^{Aa}	4.32^{a}	5.15^{a}	4.95^{a}	5.51^{a}	0.467
	LO	0.78^{A}	1.65^{Aa}	3.06^{a}	3.28^{a}	3.65^{a}	3.64^{a}	3.46^{a}	3.49^{a}	0.312
	SEM	0.129	0.147	0.299	0.429	0.584	0.654	0.550	0.680	
trans-15 (+*cis*-11) C18:1	Control	0.30	0.30	0.31	0.32	0.27	0.24	0.27	0.25	0.012
	OSO	0.31	0.32	0.34	0.40^{A}	0.34	0.34	0.27	0.27	0.015

	RSO	0.32	0.32	0.40[a]	0.38	0.37	0.38	0.33	0.34	0.014
	LO	0.30[A]	0.33[A]	0.49[a]	0.53[a]	0.55[a]	0.55[a]	0.54[Aa]	0.61[a]	0.048
	SEM	0.028	0.026	0.033	0.034	0.038	0.041	0.045	0.061	
cis-15 C18:1	Control	0.05	0.06	0.07	0.04	0.05	0.04	0.04	0.05	0.002
	OSO	0.04	0.03[a]	0.05	0.05	0.05	0.04	0.05	0.03	0.002
	RSO	0.04[A]	0.04[A]	0.07	0.06[A]	0.07	0.07	0.07[A]	0.10	0.005
	LO	0.05[A]	0.10[Aa]	0.21[Aa]	0.23[Aa]	0.31[a]	0.29[a]	0.30[a]	0.30[a]	0.034
	SEM	0.003	0.008	0.019	0.024	0.036	0.036	0.035	0.038	
trans-11,*trans*-15 C18:2	Control	0.01	0.01	0.03	0.01	0.01	0.01	0.010	0.01	0.001
	OSO	0.01[a]	0.01	0.01[a]	0.02	0.02[a]	0.02	0.01	0.02	0.001
	RSO	0.01[a]	0.01	0.02	0.01	0.01	0.02[a]	0.02[a]	0.02	0.001
	LO	0.01	0.01[A]	0.01[a]	0.01	0.01	0.01	0.01	0.03	0.002
	SEM	0.001	0.001	0.003	0.001	0.001	0.001	0.001	0.004	
*trans*11,*cis*-15 C18:2	Control	0.03	0.02	0.04	0.04	0.03	0.03	0.03	0.03	0.002
	OSO	0.03	0.03	0.04	0.05	0.05	0.04	0.04	0.03	0.003
	RSO	0.02	0.02	0.04	0.06[A]	0.08	0.08	0.07	0.13	0.009
	LO	0.04[A]	0.34[Aa]	0.57[Aa]	0.54[Aa]	0.66[Aa]	0.76[a]	0.73[a]	0.87[a]	0.094
	SEM	0.003	0.037	0.064	0.067	0.084	0.103	0.101	0.120	
cis-9,*cis*-12 C18:2	Control	1.42	1.46	1.55	1.76	1.85	1.80	1.85	1.72	0.047
	OSO	1.33[a]	1.28	1.36	1.58	1.60	1.42	1.32	1.35	0.036
	RSO	1.40[A]	1.51[Aa]	1.97[Aa]	2.27	2.62[a]	2.71[a]	2.58[a]	2.82[a]	0.129
	LO	1.38[A]	1.50[A]	1.68	1.80	1.82	2.02	2.06	2.33	0.119
	SEM	0.051	0.062	0.110	0.132	0.172	0.195	0.194	0.244	
cis-9,*trans*-11	Control	0.39	0.30	0.36	0.34	0.39	0.33	0.31	0.46	0.027

C18:2	OSO	0.38	0.37	0.43	0.49	0.51	0.47	0.43	0.60	0.034
	RSO	0.42^{A}	0.44^{A}	0.79^{Aa}	1.19^{Aa}	1.71^{a}	2.09^{a}	1.99^{a}	2.27^{a}	0.201
	LO	0.39^{A}	0.60^{Aa}	0.97^{a}	0.94^{a}	1.20^{a}	1.21^{a}	1.24^{a}	1.60^{a}	0.115
	SEM	0.055	0.058	0.103	0.161	0.229	0.267	0.231	0.294	
C18:3n-3	Control	0.13	0.13	0.15	0.17	0.18	0.16	0.17	0.15	0.007
	OSO	0.12	0.12	0.13	0.17	0.17	0.14	0.13	0.16	0.008
	RSO	0.15	0.12	0.18	0.15	0.17	0.14	0.14^{A}	0.18	0.006
	LO	0.18^{Aa}	0.34^{Aa}	0.50^{Aa}	0.54^{Aa}	0.61^{Aa}	0.65^{Aa}	0.73^{Aa}	0.83^{a}	0.068
	SEM	0.007	0.025	0.041	0.044	0.051	0.062	0.078	0.112	
cis-9,*trans*-11,*trans*-15 C18:3	Control	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.000
	OSO	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.000
	RSO	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.000
	LO	0.01^{A}	0.01^{A}	0.02^{A}	0.03^{A}	0.03^{A}	0.04	0.04	0.04	0.004
	SEM	0.000	0.001	0.002	0.003	0.004	0.005	0.004	0.006	
cis-9,*trans*-11,*cis*-15 C18:3	Control	0.05	0.06^{A}	0.05^{A}	0.05^{A}	0.04	0.04^{A}	0.04	0.03	0.005
	OSO	0.04	0.05	0.05	0.04	0.04	0.04	0.03	0.03	0.002
	RSO	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.001
	LO	0.03^{A}	0.07^{A}	0.09^{a}	0.09^{a}	0.09^{a}	0.11^{a}	0.14^{Aa}	0.10^{a}	0.012
	SEM	0.005	0.007	0.008	0.008	0.008	0.011	0.017	0.013	

380 ^{A}In a fatty acid, the means in a treatment with superscript "A" differ significantly ($P < 0.05$) from the
381 504 h mean in the same treatment. ^{a}In a fatty acid the means in OSO, RSO or LO treatments with
382 superscript "a" differ significantly ($P < 0.05$) from the same mean in the control treatment.

383 ^{1}SCSFA = sum of C4:0, C6:0 and C8:0, MCSFA = sum of C12:0, C14:0 and C16:0.

384 ^{2}Control = basal diet with no added oil; OSO, RSFO and LO = diets enriched with 48 g/d of high oleic
385 sunflower oil, regular sunflower oil or linseed oil, respectively.

Tr, concentrations below 0.001 mg/100 g of fatty acid methyl esters.

405 **Figure 1**. Time-dependent changes of *trans*-11 C18:1 and *cis*-9,*trans*-11 C18:2 in milk fat of goats
406 fed an unsupplemented diet (control) or diets enriched with high oleic sunflower oil (OSO) or regular
407 sunflower oil (RSO) or linseed oil (LO). FAME: Fatty acid methyl esters

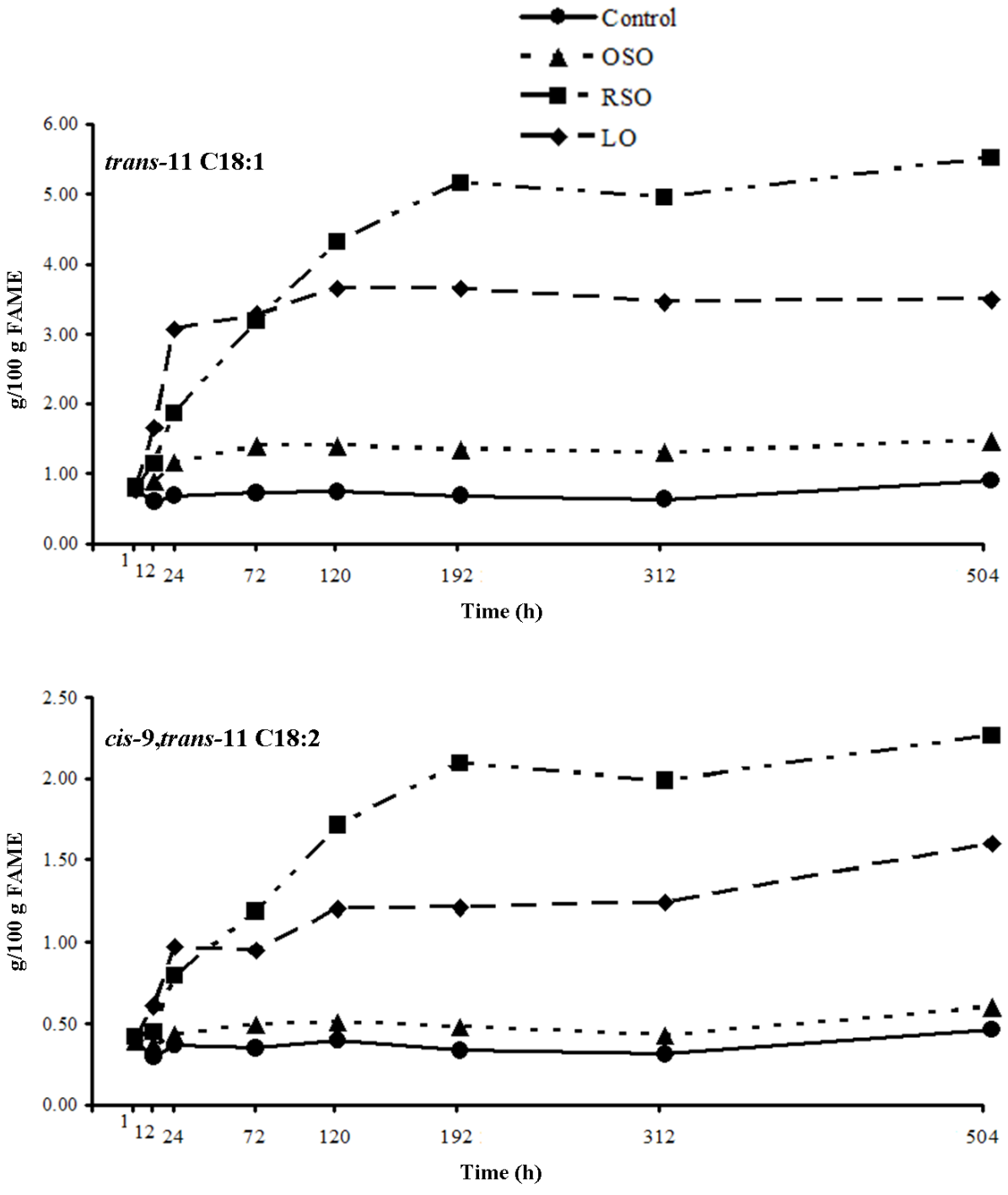

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Figure 2. Time-dependent changes of *trans*-10 18:1 and 18:3n-3 in milk fat of goats fed an unsupplemented diet (control) or diets enriched with high oleic sunflower oil (OSO) or regular sunflower oil (RSO) or linseed oil (LO). FAME: Fatty acid methyl esters