QUICK CHANGES OF MILK FATTY ACIDS AFTER INCLUSION OR SUPPRESSION OF LINSEED OIL IN THE DIET OF GOATS

Running title: Short term changes of milk fatty acids

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ABSTRACT

Background: Lipid supplementation of ruminant diet is an excellent tool to improve the nutritional quality of dairy fat. The purpose of this research was to monitor in detail the goat milk fatty acid (FA) profile during the first 24 h after linseed oil (LO) supplementation or suppression in the diet. Particular emphasis was placed in the changes of FA with bioactive properties. Milk fat was analysed by gas chromatography from milkings at 0, 1, 3, 6, 12 and 24 h after diet shift.

Results: The α-linolenic acid levels increased 12 h after LO incorporation in the diet and decreased 3 h after oil suppression. Most of the milk 10:0 to 16:0 saturated FA decreased 24 h after LO supplementation, whereas oil suppression raised their levels after 6 h. Similarly, raising of mono and polyunsaturated *trans* FA after LO inclusion was delayed in comparison with their decrease after oil suppression.

Conclusion: This study supports that ruminal bacteria and mammary gland would exhibit a fast responsiveness after the inclusion or suppression of LO in ruminant rations. Milk with an improved FA profile could be collected between 12 h after LO supplementation and the last milking before LO suppression in the diet.

Keywords: milk, fatty acids, goats, linseed oil, fat.

INTRODUCTION

Supplementation of dairy cattle diets with oils rich in unsaturated fatty acids (UFA) has been extensively studied, [1-2] as it has proven to be an appropriate strategy to obtain milk fat with a healthier fatty acid (FA) profile. Lipolysis of dietary triglycerides by rumen microorganisms is fast and extensive and releases FA that, if unsaturated, are accessible for the biohydrogenation (BH) processes in the rumen. [3] Several *in vitro* studies with microbial rumen populations have shown a quick disappearance of the UFA after the addition of lipids to the culture media and, at the same time, the emergence of their BH intermediates. [4-6] However, the occurrence of milk FA changes induced by dietary lipids would not be defined only by the activity of rumen bacteria, but should also take into account the time required by FA to leave the rumen, their absorption in the intestine, transportation to the mammary gland and subsequent incorporation to milk triglycerides. In this regard, studies with cannulated animals have demonstrated that the BH process is extremely quick as well as that FA absorbed in the duodenum can be observed in milk fat after 6 h. [7-8]

Changes of milk FA contents after the inclusion of plant oils in the diet have been studied in cows,[9] ewes[10] and goats. [11-12] However, in most of those studies milk sampling began 24 h after dietary supplementation. To our knowledge, only Martínez Marín *et al.* [12] described time-dependent changes of milk FA 1 and 12 h after the inclusion of three differently unsaturated plant oils in the diet, but the number of individual FA reported was limited. Moreover, to our knowledge, there is no available information regarding the evolution of milk fat composition after the removal of the lipid supplement from the ration.

On the other hand, several goat and sheep farmers under intensive production practices milk the animals twice a day (i.e. twelve hours apart) and, in very high producing dairy cows, milking the animals thrice a day is also common. If the rapid change of milk FA profile a few hours after oil supplementation that has been observed *in vitro* and with cannulated animals was to be confirmed *in vivo*, under common farming practices, such results would be useful for farmers, in order to establish suitable collection periods of milk with an improved FA profile after plant oil supplementation and suppression.

The objective of the present work was to provide detailed information of milk FA changes during the first 24 h after the inclusion or suppression of linseed oil (LO) in the diet of dairy goats. Emphasis was put in the evolution of milk FA with a relevant role from a nutritional point of view, such as omega-3 or conjugated linoleic acid (CLA).

EXPERIMENTAL

Animal, diets and experimental design

The present research was carried out at the University of Córdoba facilities in accordance with the EU Directive 2010/63/EU for animal studies. [13] Eight Malagueña goats (initially 118 ± 16 DIM and 52.9 ± 3.7 kg of BW) were allocated to two treatments in a cross-over design with four animals per treatment and two experimental periods of 25 d. The goats were selected from a commercial farm and blocked by body weight and milk production (four animals in each block). Within each block, the animals were randomly assigned to the treatments, so all the blocks were represented in both treatments. During the study, the goats were placed in individual cages of 1.0 × 1.4 m, with slatted floor and individual water and feeding troughs. The goats were machine milked (DeLaval, Madrid, Spain) and stripped out by hand once a day, before morning feeding.

The basal diet consisted of alfalfa hay and a pelleted concentrate (33:67), fed separately, and was supplemented or not with 30 g/d of LO (Table 1). The ration was offered in two equal meals at 09:30 and 17:30 h, and water was provided *ad libitum*. Four goats were fed the basal diet and four goats were fed the LO supplemented diet for 25 d. Then, the former group was abruptly switched to the LO supplemented diet (LO treatment) and the latter group was abruptly switched to the unsupplemented diet (CON treatment). After 25 d, the shift was reversed (Fig. 1). Individual milk samples were obtained from milkings at 0, 1, 3, 6, 12 and 24 h after diet shift. Before each milking, a dose of 2-3 i.u. of oxytocin was administered to the goats. Milkings at 1, 3, 6 and 12 h were stripped out by hand. The milk samples were stored at -20 °C until analysis.

Measurements and chemical analysis

Daily dry matter intake, milk production and diet composition were measured following Martínez Marín *et al.* [14] procedures. Fat, lactose and protein contents in milk were determined with a Milko-Scan FT120 (Foss Electric, Hillerød, Denmark). Milk fat extraction was carried out by double centrifugation and fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of glycerides with KOH in methanol.[14] Gas chromatography with two complementary capillary columns was used to provide a complete FA profile as described by De la Fuente *et al.* [15] Chromatographic analysis were carried out on an Agilent model 6890 N Network Gas Chromatograph (Palo Alto, CA, USA) equipped with auto-injector and FID, fitted with a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., Varian, Middelburg, The Netherlands) and an Agilent gas chromatograph, model 7820A GC System, equipped with auto-injector and FID, fitted

with a SLB-IL111 capillary column (100 m × 0.25 mm i.d., Supelco). In both cases, He was used as carrier gas.

Statistical analysis

Data of milk fat FA contents were analyzed by repeated measurement analysis [16] using the PROC MIXED of SAS University Edition 3.5 (SAS Institute Inc., Cary, USA). The MIXED procedure was used to analyze milk fat composition data, according to the model: $Y_{ijklm} = \mu + T_i + H_j + P_k + S_l + TH_{ij} + C_m(TPS)_l + e_{ijklm}$, where Y_{ijklm} = dependent variable, μ = overall mean, T_i = fixed effect of treatment (i = 1 to 2), H_i = fixed effect of the sampling time (j = 1 to 6), P_k = fixed effect of the period (k = 1 to 2), S_l = fixed effect of the sequence, TH_{ij} = fixed effect of the interaction treatment x hour, and $C_m(TPS)_{ikl}$ = random effect of animal (i = 1 to 4), nested within the interaction treatment x period x sequence, e_{ijklm} = random residual. The repeated effect was the sampling time and the subject of the repeated measurements was the animal nested within the interaction treatment x period x sequence. A covariance structure appropriate for unequally spaced measures (compound symmetry, ANTE(1) or spatial power) was chosen on the basis of the Schwarz's Bayesian Information model fit criteria. The CONTRAST procedure was used to compare least squares means. Least squares means of each treatment at 1, 3, 6, 12 and 24 h were compared with their respective 0 h. Additionally, the treatments were compared at each sampling time. Significant differences were declared at $P<0.05$.

RESULTS AND DISCUSSION

The average feed intake, milk yield, and milk fat and protein contents were 1714 ± 108 g/d, 1276 ± 373 g/d, 4.60 ± 0.56%, and 3.61 ± 0.16%, respectively. This study was designed to delve into the knowledge of changes in milk fat composition shortly after a

diet shift involving the inclusion or suppression of LO in the diet. The main challenge to discuss these results is the scarcity of previously published research on this matter.

Table 2 shows the temporal changes of several groups of FA. The contents of all the saturated FA (SFA) groups in milk fat were reduced ($P<0.05$) in the CON treatment after 24 h, with concomitant increases ($P<0.05$) of *trans* 18:1, total *trans* monounsaturated FA (MUFA), total MUFA, and all the groups of polyunsaturated FA (PUFA). Most of those changes were significant 12 h after diet shift. Regarding the suppression of LO in the diet, the CON treatment augmented ($P<0.05$) the contents of even linear SFA and total SFA in milk fat, while diminished ($P<0.05$) the levels of *trans* 18:1, total *trans* MUFA, total MUFA, and all the groups of PUFA. Most of those changes were significant 6 h after diet shift.

A total of 110 individual FA were identified and 55 of them showed both significant differences at 0 h and increasing or decreasing trends during the sampling period. The FA that exhibited clear patterns of change with time would be the most important, in order to explain the changes of milk FA after diet shift and to hypothesize about the effects of the ration supplied on ruminal environment and mammary metabolism. Those FA are discussed in detail below.

Saturated fatty acids

The contents of 4:0 and 6:0 in milk fat were not affected ($P>0.05$) by the treatments (Table 3). The contents of even linear medium-chain SFA (10:0-16:0) had a quick increase 6 h after LO suppression, but their reduction with LO supplementation was delayed for 24 h (Table 3). Previous research have reported changes of milk medium-chain SFA contents

between 1 and 4 d after diet changes involving an increase or decrease of the PUFA supplied. For instance, medium-chain SFA decreased after switching to fresh pastures rich in α-linolenic acid [17-19] or including vegetable oils in the diet. [10,12] In contrast, increases of medium-chain SFA were reported when animals were removed from pastures. [20] Those changes have been related to an inhibition of the FA synthesis in the mammary gland due to the competition between dietary UFA or their BH intermediates with the *de novo* synthesized FA for the esterification sites of glycerol. The accumulation of UFA and their ruminal intermediates would promote an inhibitory effect on lipogenic enzymes. [21] Our results would indicate that the mammary gland enzymes involved in the *de novo* FA synthesis respond slower to a rise of PUFA availability than to a decline, but both effects occur soon after a diet shift.

The milk fat contents of most *iso* FA and *anteiso* 15:0 decreased ($P<0.05$) 12 h after diet shift in the LO treatment, while it took 24 h to increase ($P<0.05$) the contents of *iso* 18:0 in the CON treatment (Table 3). Branched-chain SFA found in milk fat are predominantly of ruminal microbial origin, and *iso* FA and *anteiso* 15:0 are mostly related to cellulolytic bacteria. [22] Thus, the observed decrease with LO supplementation would be an evidence of certain negative effects of lipids on the ruminal ecosystem. Furthermore, the poor recovery of branched-chain SFA contents after LO suppression would indicate a slow reversibility of such ruminal changes.

The temporal changes of keto-10 18:0 (Table 3) suggested a quick adaptation of rumen microbial populations to the presence or absence of LO in the diet. Previous *in vitro* tests have reported the production of keto-10 18:0, via hydroxy-10 18:0, after 24 h of incubation of oleic acid (*cis*-9 18:0) with mixed ruminal microorganisms. [23] Our results

189 indicate that oleic acid conversion to keto-10 18:0 is quantifiable in milk fat at least 12 h
190 after LO consumption.
191
192 **Monounsaturated Fatty Acids**
193 The quantitatively most important group of MUFA detected in milk fat corresponded to
194 molecules with 18 atoms of carbon (Table 4). The most abundant MUFA was *cis*-9 18:1
195 and its content was not modified by the treatments. The levels of most *trans* 18:1 FA in
196 milk fat were higher ($P<0.05$) in the CON treatment than in the LO treatment at 0 h, while
197 the opposite was true at 24 h. Furthermore, the response to the absence of LO in the ration
198 was faster than the reaction to its incorporation. For instance, the content of vaccenic acid
199 (VA; *trans*-11 18:1), which was the quantitatively most important *trans* 18:1, was reduced
200 ($P<0.05$) 3 h after diet shift in the CON treatment, but 12 h were needed to increase its
201 level in the LO treatment, which agrees with the results of Martínez Marín *et al.*[12] The
202 sharp drop of *trans* 18:1 FA could be related to the lack of UFA that serve as substrates
203 in the rumen BH processes. [20,24-25] Thus, a sustained UFA ingestion would be needed to
204 maintain the BH intermediates above certain levels in milk. On the other hand, the fact
205 that rumen bacteria are able to quickly increase their BH activity, as reflected by the rise
206 of *trans* 18:1 FA levels in milk fat 24 h after diet shift, [10-12] could be explained by their
207 permanent adaptation to biohydrogenate the dietary UFA, in order to overcome their
208 deleterious effects. [26]
209
210 Several 18:1 FA with a double bond in the positions between 13 and 16, (i.e. *cis*-14 18:1,
211 *cis*-15 18:1, *trans*-13/*trans*-14 18:1) exhibited contents in the LO treatment at 24 h very
212 similar to those observed in CON treatment at 0 h and vice versa (Table 4), which would
213 support the idea that those 18:1 FA are actually intermediates of α-linolenic acid BH. [27]

Overall, the rapid changes in the milk fat contents of BH intermediates after the administration or suppression of LO in the diet would be in agreement with the results of Bickerstaffe *et al.* [7]

Almost 30 MUFA with less than 18 atoms of carbon were also quantified, but in limited amounts (< 0.10% of total FAME) (Table 4). Of special interest are *cis*-9 14:1 and *cis*-9 16:1 as well as *trans*-9 16:1, because of their relationship with endogenous metabolism. [28-30] The inclusion of LO in the diet reduced ($P<0.05$) the contents of those *cis*-9 MUFA in milk fat, whereas it augmented ($P<0.05$) the *trans*-9 16:1 levels. *Cis*-9 14:1 and *cis*-9 16:1 rapidly increased 3-6 h after LO suppression, while their decrease was delayed for 24 h after LO supplementation. Former studies have also reported declines of the levels of *cis*-9 14:1 and *cis*-9 16:1 in milk fat 24 h after feeding α-linolenic acid rich diets. [10,17-18] The present study showed that the Δ-9 desaturation ratio of 14:0, calculated as product/(product + substrate), decreased by 10% 24 h after LO supplementation and increased by 14% 24 h after LO suppression, compared with the values observed at 0 h. Those results suggest a fast response of the Δ-9 desaturase activity in the mammary gland to the presence or absence of oil in the diet fed to the animals. Besides, we found that the responses of *trans*-9 16:1 levels were faster after the suppression than the inclusion of LO in the diet. Although the content of *trans*-9 16:1 in milk fat is not specifically related to LO consumption, [28] the results of Kazama *et al.* [31] indicate that LO has to be exposed to rumen microorganisms to rise the *trans*-9 16:1 levels in milk fat.

Polyunsaturated fatty acids

The administration or suppression of LO in the diet triggered quick changes in milk PUFA contents. At 0 h, larger quantities ($P<0.05$) of non-conjugated 18:2 FA were detected in

239 the CON treatment than in the LO treatment, but this result was reversed 24 h after diet
240 shift (Table 5). Most non-conjugated 18:2 FA related to α-linolenic acid BH [27] were
241 reduced (P<0.05) from 6 h onwards in the CON treatment, and increased (P<0.05) from
242 12 h onwards in the LO treatment. For instance, *trans*-11, *cis*-15 18:2 followed a pattern
243 of change similar to its precursor, α-linolenic acid.
244
245 Among the identified CLA isomers (Table 5), rumenic acid (**RA**; *cis*-9, *trans*-11 18:2)
246 was more abundant (P<0.05) at 0 h in the CON treatment than in the LO treatment. Its
247 content was reduced or increased (P<0.05) 6 and 12 h after LO suppression or addition,
248 respectively. Most RA found in the milk fat of goats fed LO derives from Δ-9 desaturation
249 of VA in the mammary gland. [29,32] Feeding rations supplemented with LO was found to
250 increase milk VA and RA contents 12 h after diet shift in goats. [12] In agreement with the
251 pattern of change observed in the present work, milk fat RA levels sharply decreased (~
252 66%) in dairy cows 24 h after switching from pasture to a total mixed ration, while the
253 reverse switch caused a more gradually recovery of RA contents. [24]
254
255 The contents of most 18:3 FA in milk fat were lowered (P<0.05) when LO was eliminated
256 of the ration and increased when LO was added to it (Table 5). The α-linolenic acid
257 content decreased (P<0.05) at 6 h in the CON treatment, whereas its increase (P<0.05)
258 was delayed for 12 h in the LO treatment. Martínez Marín *et al.* [12] formerly reported
259 increases of this FA levels 12 h after LO supplementation in dairy goats. Further
260 investigations have reported increases 24 h after feeding α-linolenic acid rich diets. [10, 18-
261 19] The trends of *cis*-9,*trans*-11,*cis*-15 18:3 contents in milk fat (Table 5**)** reflected those
262 of α-linolenic acid. The presence of *cis*-9,*trans*-11,*cis*-15 18:3 in milk would be mainly
263 ascribed to the BH of α-linolenic acid, [14] and its pattern of change in milk fat would reveal

fast metabolic adaptations of rumen microorganisms to LO presence or absence in the diet. On the other hand, PUFA with 20 or more atoms of carbon were detected in low amounts and without substantial changes over time (Table 5).

In any case more extensive research is also required in order to uncover the underlying mechanisms that are involved in maintaining of lipid homeostasis in ruminant. In the recent years many genes have been identified that are involved in the regulation of lipid metabolism in human and animals. [33-35] However, further studies will be necessary to enlighten our limited understanding of the functional involvement of these genes in ruminant lipid metabolism.

CONCLUSIONS

The contents of several FA (BH intermediates, *de novo* synthesized FA, and mammary gland Δ-9 desaturation products) in goat milk fat showed increasing or decreasing trends within the first 24 h after a diet shift involving LO inclusion or suppression. In general, the suppression of LO in the diet was reflected in the milk FA profile earlier than its inclusion. These results show that both, rumen bacteria and mammary gland would exhibit a fast responsiveness to the inclusion or suppression of moderate amounts of LO in the diet of goats. Milk with an improved FA profile could be collected between 12 h after LO supplementation and the last milking before LO suppression in the diet.

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Table 1. Ingredients and chemical composition of the diets assayed.

	CON[A]	LO[A]
Diet, g/d		
Alfalfa hay	600	600
Pelleted concentrate[B]	1200	1200
Linseed oil[C]	-	30
Chemical composition		
Dry matter (DM), %	89.5	90.0
Ash, % DM	7.3	7.2
Crude protein, % DM	18.1	18.0
Neutral detergent fiber (NDF), % DM	40.7	40.4
Starch, % DM	19.7	18.3
Fat by acid hydrolysis, % DM	2.1	3.9
Fatty acids supplied by the oil[D], g/d		
C16:0	4.6	6.3
C18:0	1.0	2.2
cis-9 C18:1	5.3	10.6
cis-9 *cis*-12 C18:2	13.7	18.4
cis-9 *cis*-12 *cis*-15 C18:3	3.8	17.8

[A]CON: Control diet; LO: Control diet plus linseed oil

[B]Composition (g/kg, as fed): maize, 178.0; barley, 178.0; soybean hulls, 356.0; and soybean meal, 250.0, Concentrate included (g/kg, as fed): vitamin and mineral premix (Trouwmix M-3020, Trouw Nutrition, Madrid, Spain), 30.0; binder (Exal; Tolsa SA, Madrid, Spain), 7.0; and antioxidant (Luctanox; Lucta SA, Barcelona, Spain), 1.0.

[C]The oil was included in the respective concentrate, during mixing prior to pelleting.

[D]Fatty acid profile of linseed oil (g of fatty acid/100 g of fatty acid methyl esters): C16:0 5.5; C18:0: 4.1; *cis*-9 18:1: 17.5; *cis*-9 *cis*-12 C18:2: 15.6; *cis*-9 *cis*-12 *cis*-15 C18:3: 46.6

Table 2. Temporal changes of the main groups of fatty acids (FA) (g/100 g of total FA methyl esters) in milk fat from goats after inclusion (LO treatment) and suppression (CON treatment) of 30 g/d of linseed oil in the diet.

		Hour						
	Treatment	0	1	3	6	12	24	SE
Saturated								
Even linear	LO	70.52^{a}	69.16^{a}	68.98^{a}	71.79^{a}	68.89	68.82^{B}	0.433
	CON	65.31^{b}	64.43^{b}	63.96^{b}	68.76^{Ab}	71.04^{A}	69.47^{A}	
Odd linear	LO	1.80	1.86	1.95	1.98	1.73	1.62^{B}	0.029
	CON	1.69	1.72	1.70	1.74	1.69	1.75	
Iso	LO	0.77^{a}	0.82	0.82	0.73	0.64^{B}	0.70^{B}	0.015
	CON	0.68^{b}	0.74^{A}	0.73	0.65	0.62	0.71	
Anteiso	LO	0.60^{a}	0.62	0.63	0.55	0.51^{B}	0.53^{B}	0.016
	CON	0.52^{b}	0.57	0.56	0.47^{B}	0.46^{B}	0.58	
Other branched-chain	LO	0.10	0.10	0.11	0.12^{A}	0.10	0.08^{B}	0.003
	CON	0.10	0.09	0.10	0.13^{A}	0.10	0.08^{B}	
Total	LO	73.84^{a}	72.56^{a}	72.49^{a}	75.07^{Aa}	72.87	70.59^{B}	0.424
	CON	68.28^{b}	67.55^{b}	67.06^{b}	71.75^{Ab}	73.91^{A}	72.59^{A}	
Monounsaturated								
18:1 *cis*	LO	16.40	17.26	17.56^{A}	15.61	15.10^{B}	16.48	0.364
	CON	16.53	18.54^{A}	19.76^{A}	17.11	15.67	17.42	
18:1 *trans*	LO	2.40^{b}	2.47^{b}	2.31^{b}	1.98^{b}	3.24^{A}	4.60^{Aa}	0.163
	CON	5.68^{a}	5.84^{a}	5.32^{a}	4.19^{Ba}	3.71^{B}	3.43^{Bb}	
Total *cis*	LO	18.52	19.51	19.94^{A}	17.81	17.07^{B}	18.31	0.372

	CON	18.37	20.25^{A}	21.65^{A}	19.01	17.54	19.40	
	LO	2.73^{b}	2.83^{b}	2.67^{b}	2.32^{b}	3.61^{A}	5.00^{Aa}	
Total *trans*								0.171
	CON	6.18^{a}	6.39^{a}	5.88^{a}	4.69^{Ba}	4.20^{B}	3.88^{Bb}	
	LO	21.32^{b}	22.37^{b}	22.64^{Ab}	20.16^{b}	20.71	23.34^{A}	
Total								0.400
	CON	24.56^{a}	26.67^{Aa}	27.56^{Aa}	23.73^{a}	21.77^{B}	23.31^{B}	
Polyunsaturated								
Non-conjugated	LO	2.94^{b}	3.04	2.95	2.65	3.27^{A}	3.35^{A}	
18:2								0.066
	CON	3.72^{a}	3.70	3.58	3.04^{B}	2.91^{B}	2.97^{B}	
	LO	0.69^{b}	0.76^{b}	0.74^{b}	0.66^{b}	0.85^{A}	0.99^{A}	
Conjugated 18:2								0.046
	CON	1.57^{a}	1.60^{a}	1.66^{a}	1.33^{Ba}	1.13^{B}	1.09^{B}	
	LO	0. 62^{b}	0.65^{b}	0.59^{b}	0.60^{b}	1.24^{A}	1.15^{A}	
Total 18:3								0.046
	CON	1.42^{a}	1.39^{a}	1.20^{a}	0.94^{Ba}	0.89^{B}	0.84^{B}	
	LO	4.76^{b}	4.95^{b}	4.80^{b}	4.44	5.83^{A}	5.95^{A}	
Total								0.139
	CON	7.17^{a}	7.13^{a}	6.92^{a}	5.77^{B}	5.37^{B}	5.33^{B}	

^{A,B} Means within rows with “A” or “B” superscripts are significantly ($P < 0.05$) higher or lower, respectively, than values at 0 hour. ^{a,b} Means within columns with “a” or “b” superscripts are significantly ($P < 0.05$) different between treatments at the same time point.

Table 3. Temporal changes of individual saturated fatty acids (FA) (g/100 g of total FA methyl esters) in milk fat from goats after inclusion (LO treatment) and suppression (CON treatment) of 30 g/d of linseed oil in the diet.

		Hour						
	Treatment	0	1	3	6	12	24	SE
Even linear								
4:0	LO	2.66	2.64	2.66	2.70	2.79	2.70	0.025
	CON	2.71	2.70	2.62	2.72	2.78	2.76	
6:0	LO	3.06	3.08	3.13	3.06	3.04	3.02	0.023
	CON	3.12	3.14	3.04	3.11	307	3.06	
8:0	LO	3.42	3.48	3.56	3.38	3.20^{B}	3.26^{B}	0.031
	CON	3.51	3.55	3.46	3.47	3.29^{B}	3.33^{B}	
10:0	LO	11.97^{a}	11.93^{a}	12.05^{a}	12.04	11.63^{B}	11.43^{B}	0.105
	CON	11.40^{b}	11.22^{b}	10.92^{Bb}	11.80^{A}	11.85^{A}	11.68^{A}	
12:0	LO	5.27^{a}	5.41^{a}	5.53^{a}	5.39^{a}	4.98^{B}	4.96^{B}	0.075
	CON	4.36^{b}	4.36^{b}	4.35^{b}	4.63^{Ab}	4.80^{A}	5.18^{A}	
14:0	LO	9.59^{a}	9.50^{a}	9.58^{a}	9.93^{a}	9.33	8.76^{B}	0.100
	CON	8.27^{b}	8.12^{b}	8.19^{b}	9.00^{Ab}	9.19^{A}	9.14^{A}	
16:0	LO	28.19^{a}	26.75^{Ba}	26.22^{Ba}	29.51^{A}	29.38^{A}	26.16^{B}	0.367
	CON	24.86^{b}	23.72^{b}	23.96^{b}	27.49^{A}	29.98^{A}	27.54^{A}	
18:0	LO	6.12	6.13^{b}	6.01^{b}	5.58	5.33^{B}	7.11^{A}	0.159
	CON	6.76	7.30^{a}	7.11^{a}	6.29	5.86^{B}	6.57	
20:0	LO	0.14	0.14	0.14	0.12^{B}	0.12^{B}	0.13^{B}	0.003

	CON	0.13	0.14^{A}	0.14	0.12	0.12^{B}	0.13	
	LO	0.05^{a}	0.05	0.05	0.04^{B}	0.03^{B}	0.04^{B}	
22:0								0.002
	CON	0.04^{b}	0.04	0.04	0.04	0.04^{B}	0.04	
	LO	0.02	0.02	0.02	0.01^{B}	0.01^{B}	0.02^{B}	
24:0								0.001
	CON	0.02	0.02	0.02	0.02	0.01^{B}	0.01^{B}	
	LO	0.03^{b}	0.03^{b}	0.03^{b}	0.03^{b}	0.05^{A}	0.07^{A}	
Keto-10 18:0								0.005
	CON	0.11^{a}	0.12^{a}	0.11^{a}	0.08^{Ba}	0.05^{B}	0.04^{B}	
Odd linear								
	LO	0.02	0.02	0.02	0.03^{A}	0.03^{A}	0.02	
5:0								0.001
	CON	0.02	0.02	0.02	0.03	0.02	0.02	
	LO	0.03	0.02	0.03	0.03	0.03^{A}	0.02	
7:0	CON	0.04	0.03	0.04	0.04^{A}	0.04	0.03	0.002
	LO	0.05	0.05	0.06	0.07^{A}	0.07^{A}	0.05	
9:0								0.003
	CON	0.06	0.05	0.05	0.07^{A}	0.07^{A}	0.05	
	LO	0.08	0.08	0.09	0.10	0.09	0.07	
11:0								0.005
	CON	0.09	0.07	0.08	0.13^{A}	0.10	0.09	
	LO	0.11	0.12	0.14^{Aa}	0.13^{A}	0.11	0.09^{B}	
13:0								0.003
	CON	0.10	0.10	0.10^{b}	0.11^{A}	0.11^{A}	0.11^{A}	
	LO	0.81	0.84	0.87^{A}	0.83	0.74^{B}	0.69^{B}	
15:0								0.015
	CON	0.76	0.79	0.79	0.78	0.77	0.80	
	LO	0.56^{a}	0.58^{a}	0.59^{a}	0.56^{a}	0.53^{B}	0.53^{B}	
17:0								0.005
	CON	0.49^{b}	0.52^{b}	0.51^{b}	0.48^{b}	0.49	0.53^{A}	
19:0	LO	0.07^{a}	0.08	0.08	0.07	0.07	0.07	0.002

	CON	0.06[b]	0.07	0.06	0.05[B]	0.05[B]	0.06	
	LO	0.04	0.04	0.04	0.03	0.04	0.05[A]	
21:0								0.001
	CON	0.05	0.05	0.04	0.04[B]	0.03[B]	0.04[B]	
	LO	0.03[a]	0.03	0.03	0.03	0.02[B]	0.03	
23:0								0.001
	CON	0.02[b]	0.02	0.01	0.01	0.01	0.02	
Branched								
	LO	0.03	0.03	0.03	0.03[A]	0.03	0.02[B]	
Methyl-4 8:0								0.001
	CON	0.03	0.03	0.03	0.04[A]	0.03	0.02[B]	
	LO	0.01[a]	0.01	0.01	0.01	0.01	0.01	
Dimethyl 4-6								0.001
	CON	0.01[b]	0.01	0.01	0.01	0.01	0.01	
8:0								
	LO	0.05	0.05	0.05	0.06[A]	0.05	0.04[B]	
Methyl 10:0								0.001
	CON	0.05	0.04	0.05	0.06[A]	0.05	0.04[B]	
Methyl-4	LO	0.01	0.01	0.02	0.02[A]	0.01	0.01	
								0.000
12:0	CON	0.01	0.01	0.01	0.02	0.01	0.01	
	LO	0.02[a]	0.02	0.02[A]	0.02	0.01[B]	0.02[B]	
iso 13:0								0.001
	CON	0.01[b]	0.01	0.02	0.01	0.01	0.01	
	LO	0.01	0.01	0.01	0.01	0.01	0.01	
*anteiso*13:0								0.001
	CON	0.01	0.01	0.01	0.01	0.01	0.01	
	LO	0.07	0.08	0.08	0.07	0.06[B]	0.06[Bb]	
iso 14:0								0.002
	CON	0.07	0.08[A]	0.08[A]	0.08	0.07	0.08[a]	
	LO	0.17	0.18	0.19	0.17	0.14[B]	0.15[B]	
iso 15:0	CON	0.15	0.17	0.16	0.15	0.13	0.15	0.005

anteiso 15:0	LO	0.30	0.33	0.33	0.27^{B}	0.24^{B}	0.26^{B}	0.008
	CON	0.28	0.31	0.30	0.26	0.25^{B}	0.30	
iso 16:0	LO	0.19	0.20	0.20	0.17	0.16^{B}	0.18	0.004
	CON	0.17	0.19^{A}	0.18	0.15^{B}	0.15	0.18	
iso 17:0	LO	0.27	0.29	0.28	0.25^{B}	0.23^{B}	0.25^{B}	0.006
	CON	0.24	0.25	0.26	0.23	0.22^{B}	0.25	
anteiso 17:0	LO	0.29^{a}	0.28	0.29	0.27	0.26	0.26	0.009
	CON	0.24^{b}	0.25	0.25	0.20	0.20	0.27	
iso 18:0	LO	0.05^{a}	0.05^{a}	0.05^{a}	0.05^{Ba}	0.04^{B}	0.04^{B}	0.001
	CON	0.03^{b}	0.04^{b}	0.04^{b}	0.03^{b}	0.04	0.04^{A}	

[A,B] Means within rows with “A” or “B” superscripts are significantly ($P < 0.05$) higher or lower, respectively, than values at 0 hour. [a,b] Means within columns with “a” or “b” superscripts are significantly ($P < 0.05$) different between treatments at the same time point.

Table 4. Temporal changes of 18:1 isomers (g/100 g of total FA methyl esters) in milk fat from goats after inclusion (LO treatment) and suppression (CON treatment) of 30 g/d of linseed oil in the diet.

		Hour						
	Treatment	0	1	3	6	12	24	SE
cis-9	LO	15.45	16.28	16.58	14.75	14.02^{B}	15.20	0.357
	CON	15.25	17.17^{A}	18.46^{A}	16.02	14.64	16.41	
cis-11	LO	0.62	0.66	0.68^{A}	0.59	0.62	0.67	0.012
	CON	0.62	0.64	0.65	0.57^{B}	0.54^{B}	0.58	
cis-12	LO	0.24^{b}	0.25^{b}	0.23^{b}	0.20^{b}	0.34^{A}	0.41^{A}	0.015
	CON	0.51^{a}	0.52^{a}	0.47^{a}	0.38^{Ba}	0.37^{B}	0.33^{B}	
cis-13	LO	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	CON	0.01	0.01	0.01	0.01	0.01	0.01	
cis-14	LO	0.02^{b}	0.02^{b}	0.02^{b}	0.02^{b}	0.02^{b}	0.04^{Aa}	0.001
	CON	0.05^{a}	0.05^{a}	0.04^{Ba}	0.03^{Ba}	0.03^{Ba}	0.03^{Bb}	
cis-15	LO	0.02^{b}	0.02^{b}	0.02^{b}	0.02^{b}	0.06^{A}	0.12^{Aa}	0.004
	CON	0.11^{a}	0.11^{a}	0.10^{a}	0.07^{Ba}	0.05^{B}	0.04^{Bb}	
cis-16	LO	0.02^{b}	0.02^{b}	0.02^{b}	0.02^{b}	0.03^{A}	0.03^{A}	0.001
	CON	0.04^{a}	0.04^{a}	0.03^{Ba}	0.03^{Ba}	0.03^{B}	0.02^{B}	
trans-4	LO	0.01^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.02	0.02^{A}	0.001
	CON	0.02^{a}	0.02^{a}	0.02^{Ba}	0.02^{Ba}	0.01^{B}	0.02^{B}	
trans-5	LO	0.01	0.01	0.01	0.01	0.02^{A}	0.02^{A}	0.000
	CON	0.02	0.02	0.02	0.01	0.02	0.01^{B}	
trans-6/*trans*-7	LO	0.07^{b}	0.07^{b}	0.07^{b}	0.05^{B}	0.07	0.08^{A}	0.002
	CON	0.09^{a}	0.09^{a}	0.09^{a}	0.07^{B}	0.07^{B}	0.07^{B}	

trans-8	LO	0.08^{b}	0.08^{b}	0.08^{b}	0.07^{b}	0.11	0.16^{Aa}	0.005
	CON	0.15^{a}	0.16^{a}	0.13^{Ba}	0.11^{Ba}	0.10^{B}	0.10^{Bb}	
trans-9	LO	0.20^{b}	0.20^{b}	0.19^{b}	0.17^{Bb}	0.22^{A}	0.27^{A}	0.008
	CON	0.33^{a}	0.34^{a}	0.32^{a}	0.26^{Ba}	0.25^{B}	0.25^{B}	
trans-10	LO	0.32^{b}	0.34^{b}	0.33^{b}	0.26^{b}	0.35	0.47^{A}	0.017
	CON	0.46^{a}	0.53^{a}	0.48^{a}	0.38^{a}	0.37^{B}	0.36^{B}	
trans-11	LO	1.15^{b}	1.17^{b}	1.06^{b}	0.93^{b}	1.70^{A}	2.32^{A}	0.099
	CON	3.26^{a}	3.26^{a}	2.92^{Ba}	2.28^{Ba}	2.00^{B}	1.84^{B}	
trans-12	LO	0.29^{b}	0.30^{b}	0.28^{b}	0.25^{b}	0.41^{A}	0.57^{Aa}	0.018
	CON	0.64^{a}	0.64^{a}	0.58^{Ba}	0.46^{Ba}	0.43^{B}	0.38^{Bb}	
trans-13/*trans*-14	LO	0.16^{b}	0.17^{b}	0.16^{b}	0.13^{b}	0.23	0.47^{Aa}	0.022
	CON	0.49^{a}	0.53^{a}	0.51^{a}	0.40^{Ba}	0.30^{B}	0.24^{Bb}	
trans-16	LO	0.12^{b}	0.12^{b}	0.12^{b}	0.10^{b}	0.11^{b}	0.22^{A}	0.008
	CON	0.22^{a}	0.25^{a}	0.25^{a}	0.20^{a}	0.16^{Ba}	0.16^{B}	

^{A,B} Means within rows with “A” or “B” superscripts are significantly ($P < 0.05$) higher or lower, respectively, than values at 0 hour. ^{a,b} Means within columns with “a” or “b” superscripts are significantly ($P < 0.05$) different between treatments at the same time point.

Table 5. Temporal changes of individual polyunsaturated fatty acids (FA) (g/100 g of total FA methyl esters) in milk fat from goats after inclusion (LO treatment) and suppression (CON treatment) of 30 g/d of linseed oil in the diet.

		Hour						
	Treatment	0	1	3	6	12	24	SE
18:2 non conjugated								
cis-9,*trans*-13 + *trans*-8,*cis*-12	LO	0.23^{b}	0.26^{b}	0.26^{b}	0.21^{b}	0.26^{b}	0.38^{A}	0.014
	CON	0.46^{a}	0.50^{Aa}	0.52^{Aa}	0.42^{Ba}	0.33^{Ba}	0.32^{B}	
trans-8,*cis*-13	LO	0.06^{b}	0.06^{b}	0.06^{b}	0.05^{b}	0.05^{b}	0.09^{A}	0.004
	CON	0.11^{a}	0.12^{a}	0.14^{Aa}	0.11^{a}	0.08^{Ba}	0.08^{B}	
cis-9,*trans*-12	LO	0.03^{b}	0.03^{b}	0.03^{b}	0.03^{b}	0.03	0.04^{A}	0.001
	CON	0.05^{a}	0.05^{a}	0.05^{a}	0.04^{a}	0.04^{B}	0.03^{B}	
trans-9,*cis*-12	LO	0.02^{b}	0.02^{b}	0.02^{b}	0.02	0.03^{A}	0.03^{A}	0.001
	CON	0.03^{a}	0.03^{a}	0.03^{a}	0.02^{B}	0.02^{B}	0.02^{B}	
trans-10,*cis*-15	LO	0.01^{b}	0.02^{b}	0.01^{b}	0.02	0.07^{A}	0.08^{Aa}	0.005
	CON	0.08^{a}	0.08^{a}	0.06^{a}	0.04^{B}	0.03^{B}	0.02^{Bb}	
trans-11,*cis*-15	LO	0.05^{b}	0.05^{b}	0.05^{b}	0.06^{b}	0.29^{A}	0.32^{Aa}	0.027
	CON	0.67^{a}	0.66^{a}	0.53^{a}	0.38^{a}	0.27^{B}	0.20^{Bb}	
cis-9,*cis*-12	LO	2.48	2.54	2.46	2.21^{B}	2.42	2.28	0.052
	CON	2.17	2.11	2.12	1.93^{B}	2.05	2.22	
cis-9,*cis*-15	LO	0.01^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.03^{Aa}	0.04^{Aa}	0.002
	CON	0.04^{a}	0.04^{a}	0.03^{Ba}	0.02^{Ba}	0.02^{Bb}	0.01^{Bb}	
cis-12,*cis*-15	LO	0.01^{b}	0.01^{b}	0.01^{b}	0.01	0.04^{Aa}	0.03^{Aa}	0.002
	CON	0.04^{a}	0.04^{a}	0.03^{Ba}	0.03^{B}	0.02^{Bb}	0.02^{Bb}	

18:2 conjugated								
trans-7,*cis*-9	LO	0.04^{b}	0.04^{b}	0.04^{b}	0.04^{b}	0.04	0.05^{A}	0.001
	CON	0.06^{a}	0.06^{a}	0.06^{a}	0.05^{Ba}	0.05^{B}	0.05^{B}	
cis-9,*trans*-11	LO	0.63^{b}	0.68^{b}	0.66^{b}	0.58^{b}	0.77^{A}	0.90^{A}	0.045
	CON	1.48^{a}	1.49^{a}	1.55^{a}	1.23^{Ba}	1.04^{B}	1.00^{B}	
trans-9,*cis*-11	LO	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	CON	0.01	0.01	0.01	0.01	0.01	0.01	
trans-10,*cis*-12	LO	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	CON	0.01	0.01	0.01	0.01	0.01	0.01	
trans-11,*cis*-13	LO	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	CON	0.01	0.01	0.01	0.01	0.01	0.01	
trans-11,*trans*-13	LO	0.01^{b}	0.01^{b}	0.01^{b}	0.01	0.01	0.01	0.001
	CON	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{Ba}	0.01^{B}	0.01^{B}	
Other PUFA								
16:2	LO	0.01^{b}	0.01^{b}	0.01^{b}	0.01	0.02^{A}	0.02^{A}	0.001
	CON	0.03^{a}	0.03^{a}	0.03^{a}	0.02^{B}	0.02^{B}	0.01^{B}	
18:3 isomers								
cis-6,*cis*-9,*cis*-12	LO	0.03^{b}	0.03^{b}	0.03	0.03	0.05^{Aa}	0.05^{Aa}	0.001
	CON	0.05^{a}	0.05^{a}	0.04	0.04^{B}	0.03^{Bb}	0.03^{Bb}	
cis-9,*cis*-12,*cis*-15	LO	0.48^{b}	0.51^{b}	0.46^{b}	0.45	0.86^{Aa}	0.80^{Aa}	0.032
	CON	0.97^{a}	0.94^{a}	0.81^{a}	0.64^{B}	0.64^{Bb}	0.63^{Bb}	
cis-9,*trans*-12,*cis*-15	LO	0.01	0.02	0.02	0.01	0.02^{a}	0.02	0.003
	CON	0.02	0.02	0.02	0.01^{B}	0.01^{Bb}	0.01^{b}	
trans-9,*cis*-12,*cis*-15	LO	0.02^{b}	0.02^{b}	0.02^{b}	0.03^{b}	0.12^{Aa}	0.11^{Aa}	0.006
	CON	0.15^{a}	0.14^{a}	0.12^{Ba}	0.09^{Ba}	0.07^{Bb}	0.05^{Bb}	

cis-9,*trans*-11,*trans*-15	LO	0.01	0.01	0.01	0.01	0.01	0.01	0.001
	CON	0.02	0.02	0.02	0.01	0.01	0.02	
cis-9,*trans*-11,*cis*-15	LO	0.04^{b}	0.04^{b}	0.04^{b}	0.04	0.07^{Aa}	0.06^{Aa}	0.006
	CON	0.08^{a}	0.08^{a}	0.07^{a}	0.05	0.05^{Bb}	0.04^{Bb}	
Other 18:3	LO	0.02^{b}	0.02^{b}	0.02^{b}	0.03^{b}	0.11^{A}	0.10^{Aa}	0.001
	CON	0.15^{a}	0.14^{a}	0.12^{Ba}	0.10^{Ba}	0.08^{B}	0.06^{Bb}	
20:3 n-6	LO	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{Ba}	0.02^{B}	0.001
	CON	0.01^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.02^{A}	
20:4 n-6	LO	0.23^{a}	0.24^{a}	0.25^{Aa}	0.25	0.23	0.21^{B}	0.006
	CON	0.17^{b}	0.16^{b}	0.18^{b}	0.19^{A}	0.18	0.17	
20:5 n-3	LO	0.05^{b}	0.05	0.05^{b}	0.05	0.05	0.05	0.001
	CON	0.06^{a}	0.06	0.06^{a}	0.06	0.06	0.06	
22:4 n-6	LO	0.04^{a}	0.04^{a}	0.03^{a}	0.04^{a}	0.03^{a}	0.03	0.001
	CON	0.02^{b}	0.02^{b}	0.02^{b}	0.01^{b}	0.01^{b}	0.01	
22:5 n-3	LO	0.09^{b}	0.09	0.10^{A}	0.10^{A}	0.08	0.08	0.002
	CON	0.11^{a}	0.11	0.13^{A}	0.12	0.11	0.11	
22:6 n-3	LO	0.02^{a}	0.02	0.02	0.03^{Aa}	0.02	0.02	0.001
	CON	0.02^{b}	0.02	0.02	0.02^{b}	0.02	0.02	

^{A,B} Means within rows with “A” or “B” superscripts are significantly ($P < 0.05$) higher or lower, respectively, than values at 0 hour. ^{a,b} Means within columns with “a” or “b” superscripts are significantly ($P < 0.05$) different between at the same time point.

Figure captions

Figure 1. Schematic diagram of the experimental design of the current study.

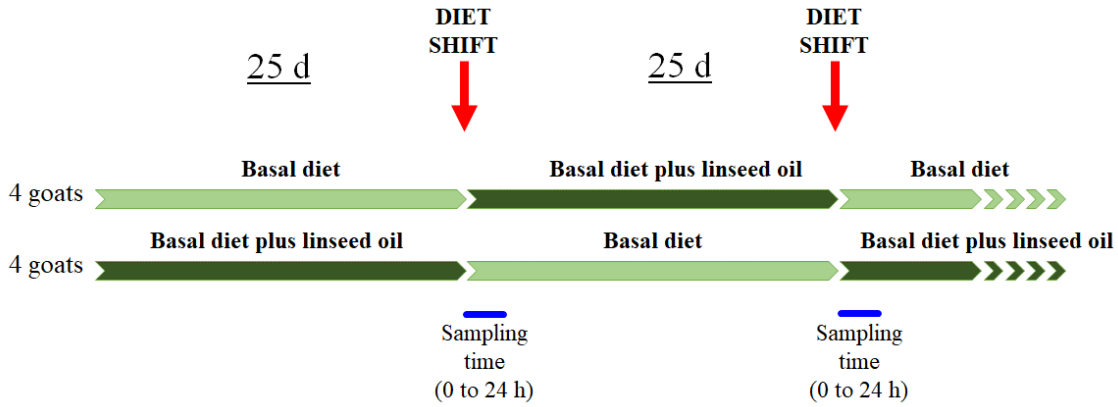
25 d
DIET SHIFT
25 d
DIET SHIFT
4 goats
Basal diet
Basal diet plus linseed oil
Basal diet
4 goats
Basal diet plus linseed oil
Basal diet
Basal diet plus linseed oil
Sampling time (0 to 24 h)
Sampling time (0 to 24 h)

Figure 1