Short communication: Feeding linseed oil to dairy goats with competent reticular groove reflex greatly increases n-3 fatty acids in milk fat

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ABSTRACT

A crossover experiment was designed to compare the effects of 2 ways of feeding linseed oil on milk fat fatty acid (FA) composition. Ten lactating goats, trained to keep competent their inborn reticular groove reflex, received a daily dose of linseed oil (38 g/d) either with their solid (concentrate) feed (CON) or emulsified in skim milk and bottle-fed (BOT). Two groups of 5 goats received alternative and successively each of the treatments in two 15-d periods. α -Linolenic acid in milk fat rose up to 13.7% in the BOT versus 1.34% in the CON treatment. The n-6 to n-3 FA ratio was significantly reduced in goats receiving bottle-fed linseed oil (1.49 vs. 0.49). Contents of rumen biohydrogenation intermediates of dietary unsaturated FA were high in milk fat of goats under the CON treatment but low in those in the BOT treatment. These results point to a clear rumen bypass of the bottle-fed linseed oil. This strategy allows obtaining milk fat naturally very rich in n-3 FA and very low in *trans* FA. Translating this approach into practical farm conditions could enable farmers to produce milk enriched in specific FA.

Key words: rumen bypass, goat milk, linseed oil, n-3 fatty acid

Short Communication

Milk fat consumption has been widely discouraged by nutritionists due to its high level of SFA and very low content of n-3 FA, which accounts for less than 1% of total FA (Jensen, 2002). However, recent research has demonstrated that n-3 FA in milk fat can be naturally increased by lipid supplementation in the dairy ruminant diet. The inclusion of linseed in the diet has been used to increase the α -linolenic acid (ALA) contents in milk from cows (Lor et al., 2005), ewes (Gómez-Cortés et al., 2009a), and goats (Martínez Marín et al.,

2011), but, unfortunately, the final contents of ALA in milk were modest and seldom surpassed 2% of total FA. This result is attributable to the high rates of rumen biohydrogenation (BH) of ALA, reducing its absorption in the small intestine and consequently limiting its supply available to the mammary gland for incorporation into milk fat. The use of fat sources protected from rumen BH could be a more practical approach (Scott et al., 1971), but effective procedures of protection of unsaturated fats are proving elusive (Jenkins and Bridges, 2007).

Different researchers have shown interest in knowing the maximal proportions of different unsaturated long-chain FA that milk fat of ruminant species can accommodate. For that purpose, they have used animals cannulated in the abomasum or duodenum to infuse unsaturated FA mixtures past the rumen, avoiding their saturation. Using that procedure, Khas-Erdene et al. (2010) and Yang et al. (2012) increased the content and the yield, respectively, of α -linolenic acid in cow milk fat more than 40 times the values found in non-infused animals. Obviously, this procedure cannot be translated into practical milk production.

Another experimental approach is to foster the reticular groove reflex (RGR) of the newborn ruminants into adulthood and use it to feed emulsified fat sources into the abomasum, bypassing the rumen. Ørskov (1982) highlighted such a possibility, which was used in ruminant lambs (Lawlor et al., 1971). Dobarganes García et al. (2005) used the RGR in adult lactating ewes with emulsified olive oil, showing that it was possible to bypass the rumen effectively according to the observed changes in milk fat FA composition. The aim of the present work was to compare the effects of feeding linseed oil, either mixed in the concentrate or bottle-fed, on milk fat FA composition of goats with competent RGR.

The experiment was carried out in the premises of the Animal Production building of the University of Córdoba (Córdoba, Spain), in accordance with the Spanish regulations on experimental animals. Ten primiparous goats of the Murciano-Granadina breed

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in their third month of lactation were used. They had competent RGR because they were brought up as described by Dobarganes García et al. (2005). Briefly, they were bottle-fed their dams' colostrum and milk up to 7 d old, and then bottle-fed milk replacer plus a solid diet. When the intake of solid feed was enough to wean them, the intake of bottle-fed reconstituted milk replacer (12% by weight) was lowered to 250 mL/d and kept so during the rest of their lives, along with a normal solid diet. They were housed individually in 1.40-m² raised slatted floor cages with troughs for feed and water, in a temperature-controlled room (24 ± 1°C) lit for 16 h daily. Before the experiment, all the animals were vaccinated against contagious agalactia, gangrenous mastitis, *Clostridium* enterotoxemia, and hemorrhagic septicemia (Laboratorios Ovejero, León, Spain).

Two groups of 5 goats with competent RGR were used. Each group was allotted to 1 of 2 treatments: 38 g of linseed oil/d added to the concentrate part of the basal diet (**CON** treatment), or the same amount of linseed oil emulsified in skim milk and bottle-fed (**BOT** treatment). After a 15-d period, each treatment switched over to the other group and a new 15-d period ensued. The goats were machine milked and stripped out by hand daily before the morning feeding. Milk samples were taken on d 13, 14, and 15 of each period.

The diets had a solid and a liquid part (Table 1). The solid part of the diet was made of pelleted concentrate, alfalfa hay, and wheat straw. The liquid part of this diet was made of dry skim milk, soybean lecithin, and warm water. Linseed oil was given with the liquid part in the BOT treatment and with the solid part (included in the concentrate) in the CON treatment. Linseed oil used to supplement both diets consisted of 6.3% C16:0, 4.3% C18:0, 18.9% *cis*-9 C18:1, 15.7% *cis*-9,*cis*-12 C18:2, and 46.7% ALA (Gustav Heess SL, Barcelona, Spain). Diets were fed in 2 meals at 0900 and 2000 h. The liquid part of each diet was prepared just before feeding times as described by Dobarganes García et al. (2005).

Milk fats were extracted according to Luna et al. (2005) and FA methyl esters (**FAME**) were prepared by base-catalyzed methanolysis of the glycerides (ISO-IDF, 2002a). Analysis of FAME was performed on a gas chromatograph with flame ionization detector (Agilent 6890 N Network System; Agilent Technologies Inc., Palo Alto, CA) onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm; Varian Inc., Midlandburg, the Netherlands) under similar conditions to those reported by Luna et al. (2005). Individual FAME quantification was performed according to the International Organization for Standardization and International Dairy Federation (ISO-IDF, 2002b) using a milk fat with known composition (CRM 164; European

Community Bureau of Reference, Brussels, Belgium). Individual FA were identified by comparison with standards distributed by Nu-Chek Prep Inc. (Elysian, MN), whereas *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 were previously identified by GC with a covalent adduct chemical ionization tandem mass spectrometry detector (Gómez-Cortés et al., 2009b).

Data were analyzed using PROC MIXED of SAS (version 9.1; SAS Institute Inc., Cary, NC) using period and treatment as fixed effects and the animal as random effect. Statistical significance was declared at $P < 0.05$.

No differences were observed in milk production (733 and 758 g/d; SEM = 76.2) or milk fat content (5.01 and 5.11%; SEM = 0.204) between the CON and BOT treatments, respectively. Results of milk fat FA composition obtained from treatment CON and BOT are shown in Table 2. To compare these results, we included the FA profile of a reference goat milk fat (**RGMF**) obtained with 4 basal diets similar to the solid part of the diets used in the present experiment but without added oil (Martínez Marín et al., 2011, 2012; Table 2). The levels of *cis*-9,*cis*-12 C18:2 and *cis*-9,*cis*-12,*cis*-15 C18:3, both of them necessarily of direct dietary origin, were 3 and 10 times, respectively, higher in milk from the BOT treatment than in milk from the CON treatment. These results would support the effectiveness of the RGR to bypass the rumen with liquid feed in adult animals.

The content of ALA in milk fat of goats fed the BOT treatment was 85 times higher than the average content of this FA in the RGMF (Table 2). The ALA content in the milk fat of BOT goats in the present experiment

Table 1. Experimental diets

Composition	Treatment ¹	
	CON	BOT
Solid part, g/d		
Alfalfa hay	400	400
Wheat straw	100	100
Concentrate ²	800	800
Linseed oil ³	38	—
Liquid part, g/d		
Dried skim milk	60	60
Soybean lecithin	0.4	0.4
Linseed oil	—	38
Water	Up to 0.5 L/d	

¹CON = 38 g of linseed oil/d added to the concentrate; BOT = 38 g of linseed oil/d emulsified in skim milk and bottle fed.

²Composition (g/kg, as fed): maize, 375; barley, 375; soybean meal, 200; binder, antioxidant, and vitamin and mineral premix, 50.

³Linseed oil in the solid part was only given with treatment CON. It was added to the concentrate before pelleting (47.5 g of linseed oil added to 1,000 g of concentrate). Afterward, the mixture was pelleted and 838 g/d of it was given to the goats under the CON treatment.

Table 2. Milk FA profile (g/100 g of FA methyl esters) in goats with competent reticular groove reflex receiving 38 g of linseed oil/d either in the concentrate (CON) or emulsified in skim milk and bottle fed (BOT)

FA	Treatment		SEM	P-value	RGMF ¹
	CON	BOT			
SFA					
C4:0	2.61	2.01	0.097	<0.001	2.47 ± 0.040
C6:0	3.00	2.45	0.101	0.001	2.76 ± 0.054
C8:0	3.29	2.78	0.109	0.005	3.03 ± 0.078
C10:0	10.33	9.72	0.318	0.19	11.33 ± 0.20
C12:0	4.31	4.82	0.199	0.066	5.22 ± 0.194
<i>iso</i> C13:0	0.02	0.01	0.001	0.001	0.02 ± 0.002
<i>anteiso</i> C13:0	0.05	0.06	0.005	0.88	0.06 ± 0.006
<i>iso</i> C14:0	0.09	0.10	0.018	0.90	0.05 ± 0.002
C14:0	7.10	7.05	0.209	0.89	10.39 ± 0.18
<i>iso</i> C15:0	0.17	0.13	0.010	0.037	0.14 ± 0.004
<i>anteiso</i> C15:0	0.29	0.17	0.018	<0.001	0.28 ± 0.011
C15:0	0.80	0.59	0.044	0.019	0.83 ± 0.047
<i>iso</i> C16:0	0.19	0.14	0.016	0.045	0.15 ± 0.007
C16:0	20.60	18.86	0.657	0.041	32.56 ± 0.58
C17:0	0.38	0.32	0.020	0.12	0.40 ± 0.011
<i>iso</i> C18:0	0.05	0.04	0.003	0.21	0.04 ± 0.003
10- <i>keto</i> C18:0	0.17	0.03	0.557	0.004	0.04 ± 0.004
C18:0	8.32	4.92	0.014	0.001	5.78 ± 0.253
C20:0	0.16	0.13	0.002	0.19	0.11 ± 0.004
MUFA					
<i>cis</i> -9 C10:1	0.41	0.39	0.027	0.62	0.39 ± 0.027
<i>cis</i> -9 C12:1/C13:0	0.23	0.22	0.018	0.84	0.22 ± 0.020
<i>cis</i> -9 C14:1	0.14	0.12	0.010	0.33	0.22 ± 0.021
<i>cis</i> -9 C15:1	0.05	0.03	0.003	0.003	0.05 ± 0.003
<i>trans</i> -9 C16:1/ <i>iso</i> C17:0	0.61	0.18	0.060	<0.001	0.34 ± 0.014
<i>cis</i> -7 C16:1	0.28	0.18	0.017	0.001	0.24 ± 0.007
<i>cis</i> -9 C16:1/ <i>anteiso</i> C17:0	0.91	0.70	0.042	0.001	1.32 ± 0.065
<i>cis</i> -13 C16:1	0.18	0.21	0.016	0.22	0.28 ± 0.022
<i>cis</i> -9 C17:1	0.17	0.15	0.016	0.35	0.21 ± 0.009
<i>trans</i> -6/ <i>trans</i> -7/ <i>trans</i> -8 C18:1	0.27	0.05	0.030	<0.001	0.17 ± 0.010
<i>trans</i> -9 C18:1	0.34	0.07	0.036	<0.001	0.19 ± 0.009
<i>trans</i> -10 C18:1	0.51	0.12	0.077	0.016	0.33 ± 0.034
<i>trans</i> -11 C18:1	3.65	0.23	0.495	<0.001	1.01 ± 0.087
<i>trans</i> -12 C18:1	0.38	0.07	0.039	<0.001	0.18 ± 0.011
<i>cis</i> -9 C18:1	17.45	18.15	0.659	0.59	14.55 ± 0.34
<i>trans</i> -15/ <i>cis</i> -11 C18:1	0.62	0.31	0.043	<0.001	0.31 ± 0.013
<i>cis</i> -12 C18:1	0.74	0.10	0.091	<0.001	0.14 ± 0.010
<i>cis</i> -13 C18:1	0.08	0.02	0.008	<0.001	0.04 ± 0.002
<i>trans</i> -16/ <i>cis</i> -14 C18:1	0.51	0.09	0.050	<0.001	0.18 ± 0.007
<i>cis</i> -15 C18:1	0.52	0.04	0.059	<0.001	0.06 ± 0.002
<i>cis</i> -11 C20:1	0.09	0.07	0.005	0.066	0.05 ± 0.002
Nonconjugated C18:2					
<i>trans</i> -11, <i>trans</i> -15 C18:2	0.12	0.03	0.016	0.001	0.04 ± 0.003
<i>trans</i> -9, <i>trans</i> -12/ <i>cis</i> -9, <i>trans</i> -13/ <i>trans</i> -8, <i>cis</i> -12 C18:2	0.57	0.10	0.062	<0.001	0.17 ± 0.007
<i>trans</i> -8, <i>cis</i> -13 18:2/ <i>cis</i> -9, <i>trans</i> -12 C18:2	0.33	0.11	0.030	<0.001	0.06 ± 0.002
<i>trans</i> -11, <i>cis</i> -15 C18:2	1.40	0.04	0.193	<0.001	0.04 ± 0.002
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.22	6.54	0.512	<0.001	1.72 ± 0.055
Conjugated C18:2					
<i>cis</i> -9, <i>trans</i> -11 C18:2	2.10	0.23	0.290	0.001	0.62 ± 0.050
<i>trans</i> -9, <i>cis</i> -11 C18:2	0.02	0.01	0.002	0.005	0.01 ± 0.001
<i>trans</i> -11, <i>cis</i> -13 C18:2	0.02	Tr ²	0.004	0.010	0.01 ± 0.001
<i>trans</i> -11, <i>trans</i> -13 C18:2	0.02	0.01	0.002	0.13	0.01 ± 0.001
<i>trans</i> -9, <i>trans</i> -11 C18:2	0.03	0.01	0.003	0.023	0.01 ± 0.001
Other PUFA					
C16:2	0.08	Tr	0.010	<0.001	0.01 ± 0.001
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	1.34	13.67	1.465	<0.001	0.16 ± 0.008
<i>cis</i> -9, <i>trans</i> -11, <i>trans</i> -15 C18:3	0.09	0.01	0.013	0.001	0.01 ± 0.001
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 C18:3	0.13	0.03	0.022	0.023	0.04 ± 0.002
C20:3n-3	0.02	0.07	0.008	<0.001	0.01 ± 0.001
C20:4n-6	0.20	0.14	0.020	0.11	0.14 ± 0.005
C20:5n-3	0.07	0.16	0.013	<0.001	0.02 ± 0.001

Continued

Table 2 (Continued). Milk FA profile (g/100 g of FA methyl esters) in goats with competent reticular groove reflex receiving 38 g of linseed oil/d either in the concentrate (CON) or emulsified in skim milk and bottle fed (BOT)

FA	Treatment		SEM	P-value	RGMF ¹
	CON	BOT			
C22:5n-3	0.12	0.11	0.009	0.47	0.04 ± 0.002
n-6:n-3	1.49	0.49	0.132	<0.001	8.15 ± 0.305
Total SFA	61.97	54.38	1.404	<0.001	76.09 ± 0.431
Total MUFA	28.13	21.51	0.989	<0.001	20.57 ± 0.391
Total PUFA	9.42	23.21	1.711	<0.001	3.33 ± 0.093
Total <i>trans</i> FA ³	9.31	1.40	1.051	<0.001	3.01 ± 0.162

¹Fatty acid profile of reference goat milk fat (RGMF); mean values of milk obtained with 4 control, no oil-added diets from Martínez Marín et al. (2011, 2012). The values are means ± SEM.

²Tr = concentrations below 0.01 mg/100 g of FA methyl esters.

³Includes the sum of all *trans* MUFA plus all nonconjugated C18:2 with at least a *trans* bond.

accounted for 13.7% of total FAME. To our knowledge, no other experiment on the effects of supplementing RGR-competent dairy ruminants with PUFA-rich fats has been published. However, published infusion experiments could be useful to compare these results. Khas-Erdene et al. (2010) used continuous duodenal infusions of emulsified ALA-rich FA mixtures, reporting very high ALA content in milk fat (25.4%) with daily doses of 132 g of ALA. Moallem et al. (2012) also infused dairy cows 0 (control treatment), 110, or 220 g of linseed oil/d into the abomasum in 2 equal doses. The resulting values in ALA content in milk fat were 9 and 16 times, respectively, higher than the value of the control treatment.

Results of contents in the milk fat of FA originated in the rumen by BH of dietary FA indicated that the BOT treatment had consistently and significantly lower values than the CON treatment (Table 2). Accordingly, *trans*-11,*trans*-15 C18:2, *cis*-9,*trans*-11,*cis*-15 C18:3, and *cis*-9,*trans*-11,*trans*-15 C18:3, previously identified as intermediates of the BH of ALA (Gómez-Cortés et al., 2009b), were found in very low amounts in the BOT treatment. Furthermore, milk from the BOT treatment presented a significant reduction of all *trans* FA (Table 2) isomers, confirming an effective rumen bypass of the linseed oil when bottle-fed to goats. Comparing the contents of FA originated in rumen metabolism (most unsaturated FA) in the BOT and RGMF treatments (Table 2), the effectiveness of the RGR for bypassing the rumen in adult animals was strongly supported.

Short-chain FA (C4:0, C6:0, and C8:0), C16:0, and C18:0 all decreased with the BOT compared with the CON treatment (Table 2), whereas medium-chain SFA were not different between treatments (C10:0, C12:0, and C14:0). The low levels of C18:0 in BOT milk fat (half of the CON treatment) supports again the efficiency of the bypass in RGR-competent animals because this FA is the BH final product of ALA in the

rumen. The decrement of short-chain FA in milk fat was already reported in response to increasing amounts of ALA infused into the duodenum of lactating cows (Khas-Erdene et al., 2010) and could be related to changes of the lipogenic gene network in the mammary epithelial cells due to the increased supply of long-chain FA to the mammary gland (LaCount et al., 1994). The FA synthesized by the mammary gland decreased with mammary uptake of ALA after its duodenal infusion (Yang et al., 2012), which suggests an inhibition of FA synthesis when the amount of ALA taken up by the mammary gland increased. Barbano and Sherbon (1980) hypothesized that a large diglyceride pool of high molecular weight resulting from incorporation of exogenous long-chain FA taken up in the plasma might result in a larger output of C4:0 to maintain fluidity of milk fat at body temperature. Such a compensatory effect was confirmed more important with C16:0 and C18:0 than with *cis*-9 C18:1, which has a low melting point, similar to short-chain FA (Enjalbert et al., 1998). As ALA has an even lower melting point than *cis*-9 C18:1, biosynthesis of short-chain FA likely did not need to be enhanced when ALA was infused into the duodenum. On the contrary, a negative correlation trend between ALA mammary gland uptake and balance of C4:0 was observed (Yang et al., 2012).

Concerning C16:0, its lower level in treatment BOT was in accordance with results of Khas-Erdene et al. (2010), who reported increasing amounts of ALA when infused duodenally. Furthermore, strong negative correlations between ALA and C16:0 have been documented in cow milk fat generated after infusion of flaxseed oil (Moallem et al., 2012) and ALA (Yang et al., 2012) in the digestive tract. Moallem et al. (2012) attributed this decrease to replacement of the portion derived from the circulating C16:0 source (dietary and mobilized) by ALA, but Yang et al. (2012) confirmed a lower synthesis of C16:0 when higher amounts of ALA were taken up

by the mammary gland. Although the decrease in the short-chain FA content in RGR-competent goat milk fat (Table 2) suggests that the decrease in C16:0 would be caused by a suppression of de novo FA synthesis, more research would be needed to clarify this point.

Apart from infusion experiments in the digestive tract, the percentage of n-3 FA measured in milk fat from goats in the BOT treatment is the greatest reported by a large margin in dairy ruminants. According to the European Union Regulations (European Union, 2006, 2010) this milk may qualify as food enriched in n-3 FA. Even more, consumption of this milk may greatly contribute to diminish the n-6:n-3 ratio and the total *trans* FA content compared with milk from the CON treatment (Table 2), which could add to its healthy properties (Lock and Bauman, 2004; Simopoulos, 2008).

In conclusion, using intact, healthy, RGR-competent dairy goats allowed us to produce milk rich in ALA. Other FA could be fed that way to obtain milk rich in, for example, eicosapentaenoic acid and docosahexaenoic acid, among other FA. The possibility of using the RGR in the farm to produce milk naturally enriched in chosen unsaturated FA arises from the results of this work. More research efforts should be made to verify this possibility.

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