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ABSTRACT

Twelve lactating Saanen goats were randomly assigned to four experimental diets, which differed in terms of forage:concentrate ratio and soybean oil supplementation. A 4×4 Latin square design was used. On a dry matter (DM) basis, forage:concentrate ratios were 63:37 ('high-forage' diet) and 35:65 ('low-forage' diet/high beet pulp). These diets were given either with oil (100 g·d^{-1}) or without. The inclusion of soybean oil in the diet resulted in a significant increase in milk yield (but with HF diet) and in milk fat yield and concentration (P<0.05). Milk protein content remained unchanged across the diets. Milk protein yield was, however, higher for the high-forage diet containing added oil (forage × oil interaction, P<0.05). Soybean oil in the diet modified the milk fatty acid composition, reducing the levels of medium-chain and saturated fatty acids and increasing the levels of C18:2 n-6 and conjugated linoleic acid (CLA). The addition of soybean oil to the diet resulted in a significant increase in rumenic acid (*cis*-9, *trans*-11 CLA) and vaccenic acid (*trans*-11 C$_{18:1}$) content in the milk fat. Interactions between forage and oil resulted in a significant increase in rumenic acid and vaccenic acid in animals fed a high-forage plus oil diet, and in *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA in animals fed a low-forage plus oil diet, probably due to a shift in the rumen's biohydrogenation of linoleic acid.

Key words: Soybean oil, CLA, *Trans* fatty acid, Dairy goat.

RIASSUNTO

EFFETTI DELLA SUPPLEMENTAZIONE CON OLIO DI SOIA SULLA COMPOSIZIONE DEGLI ACIDI GRASSI DEL LATTE DI CAPRE DI RAZZA SAANEN, ALIMENTATE CON DIETE CONTENENTI DIFFERENTI RAPPORTI FORAGGIO:CONCENTRATO.

Al fine di studiare l'effetto dell'integrazione con olio di soia non protetto in diete a diverso rapporto foraggio/concentrato, 12 capre di razza Saanen, in un disegno sperimentale a quadrato latino del tipo 4x4, sono state alimentate con quattro diete che differivano per il rapporto foraggio/concentrato (alto foraggio

e basso foraggio, rispettivamente pari a 67/37 e 35/65) e per la presenza o meno di olio di soia non protetto (100 g capo/giorno). La produzione giornaliera di latte e di grasso, così come il tenore di grasso del latte, sono risultati positivamente influenzati dall'integrazione della dieta con olio di soia, raggiungendo i valori più elevati nel caso della dieta ad alto foraggio (interazione significativa foraggio x olio, P<0,05). Il tenore di proteina del latte è rimasto invariato fra le diete, mentre la produzione giornaliera di proteina nel latte è risultata significativamente più elevata a seguito dell'integrazione con olio di soia della dieta ad alto foraggio (interazione significativa foraggio x olio, P<0,05). L'introduzione di olio di soia nella dieta ha comportato una diminuzione degli acidi grassi saturi e a media catena nel grasso del latte e un aumento di quelli insaturi, tra cui gli isomeri del C18:1 e quelli coniugati dell'acido linoleico. In particolare l'acido vaccenico (C18:1 trans-11) e l'acido rumenico (C18:2 cis-9, trans-11) sono incrementati rispettivamente del 600% e del 500% a seguito della somministrazione della dieta ad alto foraggio integrata con olio di soia. In questo caso si è verificata anche un'interazione significativa tra l'effetto livello di foraggio e olio nella dieta, che ha comportato una risposta differente in termini di incremento del contenuto di acido vaccenico e di acido rumenico nelle due diete integrate con olio di soia, in funzione del livello di foraggio. Per effetto di tale interazione, l'aumento di questi due acidi grassi nel latte delle capre alimentate con la dieta a basso foraggio e l'aggiunta di olio di soia è stato di entità minore rispetto a quello evidenziato per la dieta ad alto foraggio e la medesima integrazione di olio di soia. Di contro nella dieta a basso foraggio è stato possibile evidenziare un maggior incremento degli isomeri C18:1 trans-10 e C18:2 trans-10, cis-12, probabilmente a causa di uno shift dei processi di bio-idrogenazione ruminale.

Parole chiave: *Olio di soia, CLA, Acidi grassi* trans*, Capra da latte.*

Introduction

The effects of dietary lipid supplementation on dairy goat milk yield and composition have recently been reviewed by Chilliard *et al.* (2003; 2007). In early lactation, lipid supplementation tended to increase milk yield and fat content, while the effects on protein were highly variable. During mid- or late lactation, dietary lipid supplementation did not increase milk yield; however, milk fat content did increase sharply, regardless of the type of fat supplement provided (Chilliard *et al.*, 2003, 2007).

The lipid composition of goat milk has been shown to reflect the composition of fats included in the diet, in spite of the hydrogenation and isomerization of dietary fatty acids that occurs in the rumen (Morand-Fehr *et al.*, 2000a). Different kinds of protected and unprotected lipid supplementation have been tested in dairy goat nutrition, including the use of linseed oil and sunflower oil (Chilliard *et al.*, 2003; Bernard *et al.*, 2005; Nudda *et al.*, 2006). Yeom *et al.* (2003) re-

ported the effects of dietary soybean oil on plasma fatty acids in goats, but did not consider milk yield and composition.

The type and proportion of forages and concentrates in the diet may also have a significant effect on the yield and composition of the milk given by dairy goats (Morand-Fehr *et al.*, 2000b; Chilliard *et al.*, 2003). Interaction between the basal diet and lipid supplements has been reported in dairy cows, goats and ewes (Chilliard *et al.*, 2002; Collomb *et al.*, 2004; Loor *et al.*, 2005; Shingfield *et al.*, 2005; Mele *et al.*, 2006); however, the effects of different proportions of concentrates in the diet have been well documented for dairy cows (Loor *et al.*, 2005; Shingfield *et al.*, 2005), but few trials have been conducted with dairy ewes and goats (Mele *et al.*, 2006; Chilliard *et al.*, 2007).

The aim of the present work was, therefore, to determine the effect that unprotected soybean oil in the diets of Saanen goats has on their milk fat yield and composition, when varying the forage:concentrate ratio of the diet.

Material and methods

Animals and diets

Twelve Saanen goats (mean body weight±S.D, 62.5±3.5 kg) at their fourth kidding and in the second month of lactation were kept in 12 separate boxes on peat litter. This allowed researchers to monitor the intake and performance of each individual. Four experimental diets were provided, with a low (35:65) or high (67:37) forage to concentrate ratio without added oil (LF/NO, HF/NO) or with soybean oil (LF/O, HF/O)

supplemented at 4% of dry matter. Low forage diets were higher in sugar beet pulp. Details of ingredient and chemical composition of the diets are presented in Table 1. Crude protein, ether extract and ash were determined according to AOAC methods (AOAC, 1990). Fiber fractions were analyzed according to the method described by Van Soest *et al.* (1991). Non structural carbohydrates (NSC) were calculated according to Van Soest *et al.* (1991). Net energy lactation was calculated according to INRA (1989).

With regard to feed samples, fat was ex-

Table 1.   Components and chemical composition of the four ingested diets (on a DM basis).

	Diets[1]			
	HF/NO	HF/O	LF/NO	LF/O
	% of dry matter intake			
Grass hay	63.0	63.0	35.0	35.0
Barley meal	14.5	14.5	21.0	21.0
Soybean meal	7.5	7.5	11.0	11.0
Maize meal	7.6	3.6	11.2	7.2
Sugar beet pulp	7.4	7.4	21.8	21.8
Soybean oil	0.0	4.0	0.0	4.0
DM	89.4	89.7	90.6	90.8
CP	15.9	15.5	16.0	15.7
EE	1.5	5.4	1.4	5.2
NDF	43.1	42.5	37.7	37.2
ADF	26.9	26.6	22.9	22.6
ADL	10.0	9.1	7.9	7.3
NSC	33.0	30.0	39.0	36.5
Ash	6.4	6.3	5.5	5.5
NEL (Mcal·kg^{-1} DM)	1.4	1.5	1.6	1.7
Forage:concentrate	63:37	63:37	35:65	35:65

[1]*Diets: HF/NO=high forage, no oil; HF/O=high forage with oil; LF/NO=low forage, no oil; LF/O=low forage with oil. DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NSC: non structural carbohydrates; NEL: net energy lactation.*

tracted according to the method described by Folch *et al.* (1957). Fatty acids were esterified according to Christie (1982) with C19:0 as the internal standard, and were identified using the same procedure as that described below for medium- and long-chain fatty acids of milk samples. The fatty acid composition of the four experimental diets is presented in Table 2.

Experimental design

A 4×4 Latin square experimental design with factorial arrangement of treatments and three replicates per diet was used. Each experimental period lasted three weeks. The first two weeks of each experimental period were used as a transition between treatments. The whole experiment lasted 12 weeks.

Milk yield and milk sample analyses

The animals were milked twice daily (at 08:00 h and 20:00 h) and on one day per week two samples were taken of each individual goat's milk (one from the morning milking and one from the afternoon milking). The two samples were gathered in a single sample according to the morning and afternoon yield. Milk samples were then analyzed to determine their fat content using official AOAC methods (AOAC, 1990); protein and lactose contents were determined by infrared analysis (Milkoscan 133 B, Italian Foss Electric, Padova, Italy), and somatic cell count (SCC) was performed by means of a Fossomatic 215 cell counter (Foss Electric, DK-3400, Hillerod, Denmark). The SCC data obtained were transformed into a linear score according to the method described by Wiggans and Shook (1987). At the end of each experimental week rennet clotting time (r) and curd firmness after 30 min (A_{30}) were also measured using a Formagraph (Delacroix-Buchet *et al.*, 1994).

Fatty acid analysis

An aliquot of the weekly milk samples was stored at -20°C until fat extraction could be undertaken for fatty acid analysis. Milk fat extraction was performed according to Secchiari *et al.* (2003). Briefly, ammonia (25%, 0.4 mL), ethyl alcohol (95%, 1 mL) and hexane (5 mL) were added to 2 g of a raw milk sample. After being vortexed, the samples were centrifuged at 1,600 x g and 2°C. After phase separation, the upper layer was collected. The extraction was repeated a second time using ethyl alcohol 95% (1 mL) and hexane (5 mL); the samples were centrifuged at 1,600 x g and the upper layer was collected. A

Table 2.   Fatty acid composition of the four experimental diets (g/kg DM).

Fatty acids	Diets[1]			
	HF/NO	HF/O	LF/NO	LF/O
C14:0	0.0	0.1	0.0	0.1
C16:0	0.8	4.9	1.3	5.3
C18:0	0.1	1.6	0.2	1.6
C18:1 cis-9	1.1	9.4	1.6	9.8
C18:2 n-6	3.0	23.5	4.7	24.9
C18:3 n-3	0.5	3.5	0.6	3.5
C20:0	0.0	0.1	0.0	0.1

[1]*Diets: HF/NO=high forage, no oil; HF/O=high forage with oil; LF/NO=low forage, no oil; LF/O=low forage with oil.*

third extraction was undertaken using 5 mL of hexane; the samples were centrifuged at 1,600 x g and the upper layer was collected. The fat was obtained after solvent distillation at 35°C using a rotary evaporator; it was then weighed and finally dissolved in hexane. Methyl esters of medium- and long-chain fatty acids were prepared by the alkali-catalyzed trans-methylation described by Christie (1982), using a nonadecanoic acid methyl ester (Sigma Chemical Co., St. Louis, MO, USA) as the internal standard.

Medium- (MCFA, from C10 to C17) and long-chain fatty acid (longer than C17, LCFA) composition was determined by gas chromatography (GC) using a ThermoQuest (Milan, Italy) gas-chromatograph equipped with an FID and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands; 100 m x 0.25 mm internal diameter; film thickness 0.20 μm). Helium was used as the carrier gas at a flow of 1 mL·min⁻¹. The split ratio was 1:80. An aliquot of the sample was injected under the following GC conditions: the oven temperature was taken to 150°C and held at that level for 1 min; it was then increased to 185°C at a rate of 5°C·min⁻¹, and held at that level for 20 min, before being increased to 188°C at 0.3°C·min⁻¹ and held for 1 min, and then to 230°C at a rate of 3°C·min⁻¹, at which temperature it was held for 15 min. The injector temperature was set at 270°C, while the detector temperature was set at 300°C.

Short chain fatty acid composition (SCFA, from C4 to C8) was determined according to Molkentin and Precht (2000) on a second aliquot of the same sample. For this, 25 mg of lipid extract were trans-esterified with 0.2 mL of methanol KOH 2N. Fatty acid methyl esters (FAME) were dissolved in hexane containing methyl valerate as the internal standard. The butyric acid content was calculated using a regression curve based on five response factors obtained by increasing

the C4:0:C5:0 ratio (the ratio varied from 0.15:0.40 mg·mL⁻¹ to 1.20:0.40 mg·mL⁻¹). Fatty acids from C6 to C8 were quantified using methyl valerate as the internal standard. The SCFA content was determined using the gas-chromatograph apparatus described above, initially at a temperature of 40°C for 4 min, which was then increased to 180°C at 5°C·min⁻¹, at which temperature it was held for 35 min.

Individual FAME were identified by comparison with a standard mixture containing 37 FAME (Supelco, Bellefonte PA, USA). The following standards were used to identify polyunsaturated fatty acids: PUFA-2, non conjugated C18:2 isomer mixture, individual cis-5,8,11,14,17 C20:5, cis-4,7,10,13,16,19 C22:6 (Supelco, Bellefonte PA, USA), cis-6,9,12 C18:3 and cis-9,12,15 C18:3 (Matreya Inc., Pleasant Gap, PA, USA). Isomers of C18:1 were identified based on standard commercial mixtures (Supelco, Bellefonte, PA, USA) and published isomeric profiles (Wolff and Bayard, 1995). All those methods that use peak normalization and that express their results in terms of the relative percentage of the area of the analyzed peaks risk overestimation, because the areas of small peaks are not considered. To avoid this problem, nonadecanoic acid was used as the internal standard. All the results obtained with regard to the fatty acid composition of the milk are expressed as g per 100 g of fat.

The different conjugated linoleic acid (CLA) isomers were separated and quantified using a silver ion HPLC column (Chromspher 5 lipids, 250 × 4.6 mm, Varian Inc, Turin, Italy), using the procedure reported by Sehat et al. (1998). CLA isomers were eluted using a fresh mixture of acetonitrile 0.1% (v/v) in hexane at a flow of 1 mL·min⁻¹. The injection loop volume was 50 μL and UV detection was conducted at a wavelength of 233 nm. Since no reliable internal standard for measuring CLA is available yet, quan-

titative measurements were obtained using a calibration curve, using high purity individual *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers (Matreya Inc., Pleasant Gap, PA, USA). A CLA standard mixture (Sigma Chemical Co., St. Louis, MO, USA), and a published isomeric profile (Kramer *et al.*, 2004), were also used to help to identify the CLA isomers in goat milk.

Statistical analysis

Data were analyzed as a Latin square with factorial arrangement of treatments using the GLM procedure of SAS (SAS Inst. Inc., 1999). The statistical model included forage level, oil supplementation level, rep-

licate, goat within replicate, period within replicate, forage × oil interaction and residual error. Data are reported as least squares means ± SEM. Overall differences between treatment means and interaction for level of forage and oil were considered to be significant when $P \leq 0.05$.

Results

Average dry matter intake (DMI) did not differ between experimental groups and met the goat's energy requirements in all cases. In fact, the body weights of all the animals remained practically unchanged throughout the whole trial (Table 3).

Table 3. Effect of forage:concentrate ratio and soybean oil supplementation on dry matter intake, body weight, milk fat yield and composition, and variables related to cheese-making potential.

		Diets[1]					Effect		
		HF/NO	HF/O	LF/NO	LF/O	SEM	Forage	Oil	Forage x Oil
DMI	kg·d^{-1}	2.50	2.51	2.54	2.54	0.02	ns	ns	ns
Body weight	kg	64.70	63.99	63.18	63.31	0.58	ns	ns	ns
Milk yield	g·d^{-1}	2160	2390	2280	2260	50.00	ns	*	*
Milk fat content	%	3.19	3.41	3.01	3.23	0.08	ns	*	*
Milk fat yield	g·d^{-1}	69.04	81.39	68.68	73.05	1.30	ns	*	*
Milk protein content	%	3.33	3.30	3.25	3.22	0.13	ns	ns	ns
Milk protein yield	g·d^{-1}	66.15	75.23	70.86	66.12	1.70	ns	*	*
Milk casein content	%	2.44	2.50	2.39	2.44	0.12	ns	ns	ns
Lactose content	"	4.40	4.45	4.38	4.48	0.03	ns	ns	ns
SCC (linear score)		6.36	6.47	6.35	6.55	0.23	ns	ns	ns
R	min.	12:56	13:16	18:09	15:06	01:21	*	ns	ns
A$_{30}$	mm	10.48	9.42	9.77	9.19	1.25	ns	ns	ns

*P<0.05; ns: not significant.
[1]Diets: HF/NO=high forage, no oil; HF/O=high forage with oil; LF/NO=low forage, no oil; LF/O=low forage with oil.
DMI: dry matter intake; SCC: somatic cell count; R: rennet clotting time; A$_{30}$: curd firmness after 30 min.

The oil-supplemented diets resulted in a significant increase in milk yield and in milk fat yield and content. The forage × oil interaction effect was also significant: the highest levels of milk yield, milk fat yield and concentration were, in fact, achieved when the goats were fed the HF/O diet; the goats fed the LF/O diet gave daily milk yields that did not differ from those provided by the goats given the LF/NO diet. Protein content remained unchanged across the diets. Milk protein yield, however, was higher for goats fed the HF/O diet (forage × oil interaction, P< 0.05; Table 3).

As with protein content, the casein content of the milk did not differ significantly from diet to diet. The cheese-making potential of the milk produced was significantly worsened when goats were fed the low-forage diets because rennet clotting time was higher (Table 3).

The composition of the diets had a marked effect on the fatty acid profile of the milk fat produced (Table 4). The oil-supplemented diets depressed both SCFA and MCFA, with the exception of butyric acid alone. The low-forage diets significantly increased the amount of MCFA and branched-chain fatty acids with the exception of C13:0, C15:0, *cis*-9 C16:1, C17:0, and C14:0 *iso*, C16:0 *iso*. All the *cis* and *trans* C18:1 isomers were increased by the inclusion of soybean oil in the diet (Table 4), while the level of forage in the diet was significant only for *trans*-10 C18:1, vaccenic acid (*trans*-11 C18:1) and *cis*-11 C18:1; the effect approached significance for *cis*-12 C18:1 (P=0.07). In two cases a significant forage × oil interaction was also observed: (1) in the LF/O diet, which resulted in slightly higher levels of *trans*-10 C18:1, and (2) in the HF/O diet, which gave the highest levels of vaccenic acid (VA).

Both oil supplement and level of forage affected the concentration of linoleic acid (C18:2 n-6) and α-linolenic acid (C18:3 n-3)

in milk fat. When soybean oil was included in the diet, the level of linoleic acid increased (Table 4). The α-linolenic acid content increased slightly with a high forage:concentrate ratio, and decreased when the soybean oil supplement was provided (Table 4).

In the milk from goats fed diets that did not contain lipid supplements, CLA accounted for less than 1% of total lipids (0.77% and 0.94% in the case of the HF/NO and LF/NO diets, respectively). The levels of CLA were higher when the goats were fed soybean oil (Table 5). In general, all the CLA isomers increased when soybean oil was included in the diet, with the exception of *trans*-11, *trans*-13 CLA, which remained unchanged.

The forage:concentrate ratio had a significant effect on the levels of *trans*-10, *trans*-12 CLA, *trans*-9, *trans*-11 CLA, *trans*-7, *trans*-9 CLA, *trans*-11, *cis*-13 CLA, *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA isomers. The forage × oil interaction was also significant for all the CLA isomers except *trans*-9, *trans*-11 CLA, *trans*-7, *trans*-9 CLA and *cis*-8, *trans*-10 CLA. This interaction resulted in the highest levels of single CLA isomers when the goats were fed with the HF/O diet. The LF/O diet lead to the highest level of *trans*-10, *cis*-12 CLA (Table 5).

For all the diets, rumenic acid (RA) was, quantitatively, the major conjugated isomer, accounting for 80%, 91%, 85% and 90% of total CLA in the HF/NO, HF/O, LF/NO and LF/O groups, respectively. Rumenic acid levels in milk fat ranged from 0.23 to 5.54 g/100 g lipids, and were closely associated with variations in the concentration of vaccenic acid in the milk fat: there was strong linear relationship between vaccenic acid and rumenic acid levels (Figure 1).

Normalized ratios [g per 100 g product/(g per 100 g substrate+g per 100 g product)] were estimated in order to assess the extent of Δ9 desaturation of specific fatty acids during milk fat synthesis (Loor and Her-

Table 4. Effect of forage:concentrate ratio and soybean oil supplementation on milk fatty acid composition (g per 100 g total lipids).

	Diets[1]				SEM	Effect		
	HF/NO	HF/O	LF/NO	LF/O		Forage	Oil	Forage x Oil
C4:0	3.83	3.81	3.80	3.83	0.19	ns	ns	ns
C6:0	3.71	3.50	3.79	3.49	0.14	ns	*	ns
C8:0	5.26	4.58	5.04	4.76	0.23	ns	*	ns
C10:0	8.22	6.36	8.69	7.17	0.23	*	**	ns
C11:0	0.07	0.05	0.08	0.06	0.01	*	**	ns
C12:0	3.75	2.65	4.02	3.18	0.16	**	**	ns
C13:0	0.07	0.06	0.07	0.06	0.001	ns	**	ns
C14:0	9.59	7.81	10.03	8.43	0.24	*	**	ns
C14:0 iso	0.09	0.07	0.09	0.07	0.001	ns	**	ns
C14:1 cis-9	0.13	0.09	0.17	0.11	0.01	**	**	ns
C15:0	0.91	0.80	1.00	0.82	0.05	ns	**	ns
C15:0 anteiso	0.41	0.34	0.51	0.39	0.03	*	**	ns
C16:0	28.16	24.01	30.01	24.48	0.81	**	**	ns
C16:0 iso	0.28	0.20	0.28	0.23	0.01	ns	**	ns
C16:1 cis-9	0.35	0.29	0.31	0.29	0.02	ns	*	ns
C17:0	0.57	0.49	0.62	0.50	0.03	ns	**	ns
C17:0 anteiso	0.28	0.07	0.44	0.18	0.06	*	**	ns
C18:0	6.71	8.19	6.70	8.08	0.35	ns	**	ns
C18:1 trans-6 - trans-8	0.13	0.39	0.11	0.34	0.02	ns	**	ns
C18:1 trans-9	0.23	0.54	0.21	0.47	0.02	ns	**	ns
C18:1 trans-10	0.34	0.65	0.50	1.06	0.12	**	**	**
C18:1 trans-11	1.01	7.54	1.09	4.87	0.33	**	**	**
C18:1 trans-12	0.20	0.50	0.16	0.47	0.03	ns	**	ns
Total C18:1 trans	1.97	9.98	2.11	7.15	0.42	**	**	ns
C18:1 cis-9	16.61	18.79	16.62	17.74	0.63	ns	*	ns
C18:1 cis 11	0.36	0.40	0.31	0.36	0.02	*	*	ns
C18:1 cis 12	0.25	0.68	0.19	0.58	0.04	ns	**	ns
C18:1 cis 13	0.07	0.10	0.06	0.10	0.01	ns	**	ns
C18:1 cis 14	0.14	0.26	0.11	0.27	0.02	ns	**	ns
C18:2 n-6	2.77	3.25	2.97	3.32	0.14	*	**	ns
C18:3 n-3	0.49	0.34	0.39	0.32	0.02	**	*	ns
C20:0	0.10	0.17	0.11	0.15	0.01	ns	**	ns
C20:4 n-6	0.21	0.13	0.19	0.14	0.01	ns	**	ns
C20:5 n-3	0.11	0.05	0.12	0.05	0.001	ns	**	ns
C22:6 n-3	0.05	0.02	0.05	0.03	0.001	ns	*	ns
14:1/14:0+14:1	0.014	0.012	0.016	0.013	0.001	*	**	ns
16:1/16:0+16:1	0.013	0.013	0.010	0.012	0.001	ns	ns	ns
18:1/18:0+18:1	0.71	0.70	0.71	0.69	0.12	ns	ns	ns
RA/VA+RA	0.65	0.59	0.77	0.67	0.06	ns	*	ns

*$P<0.05$; **$P<0.01$; ns: not significant.
[1]Diets: HF/NO=high forage, no oil; HF/O=high forage with oil; LF/NO=low forage, no oil; LF/O=low forage with oil.
RA/VA+RA : rumenic acid/vaccenic acid + rumenic acid.

Table 5.   Effect of forage:concentrate ratio and soybean-oil supplementation on CLA isomer content of milk (mg per g total lipids).

	Diets[1]					Effect		
	HF/NO	HF/O	LF/NO	LF/O	SEM	Forage	Oil	Forage x Oil
Trans-11, trans-13	0.045	0.057	0.058	0.051	0.011	ns	ns	ns
Trans-10, trans-12	0.028	0.172	0.033	0.098	0.011	*	**	**
Trans-9, trans-11	0.242	0.543	0.121	0.407	0.125	*	**	ns
Trans-8, trans-10	0.043	0.105	0.051	0.089	0.006	ns	**	*
Trans-7, trans-9	0.053	0.122	0.035	0.082	0.012	*	**	ns
Other trans, trans	0.070	0.318	0.072	0.178	0.011	*	**	*
Cis-13, trans-15	0.016	0.064	0.017	0.023	0.018	ns	*	*
Cis-12, trans-14	0.009	0.087	0.007	0.013	0.033	ns	*	*
Trans-11, cis-13	0.053	0.216	0.068	0.100	0.021	*	**	**
Trans-10, cis-12	0.041	0.086	0.047	0.101	0.015	*	**	*
Cis-9, trans-11	6.081	37.922	7.981	26.722	2.150	*	**	**
Trans-9, cis-11	0.380	0.813	0.320	0.760	0.130	ns	**	ns
Trans-7, cis-9	0.478	1.606	0.604	1.450	0.080	ns	**	*
Total CLA	7.615	42.723	9.521	30.964	2.294	*	**	**

*$P<0.05$; ** $P<0.01$; ns: not significant.
[1]Diets: HF/NO=high forage, no oil; HF/O=high forage with oil; LF/NO=low forage, no oil; LF/O=low forage with oil.

bein, 2003). When goats were fed diets without soybean oil, C14:1/(C14:0+C14:1) and RA/(RA+VA) ratios significantly increased, as a consequence of a higher desaturation of C14:0 and VA (Table 4).

Discussion

In our study, we found that milk yield and milk fat content and secretion increased when goats were fed diets supplemented with soybean oil and that the response to lipid supplementation significantly differed between animals fed high- and low-concentrate diets, as a consequence of a significant forage x oil interaction effect (Table 3). Bernard *et al.* (2005) reported that feeding Alpine dairy goats a fat-supplemented diet sharply increased milk fat content and yield,

as a consequence of the net increase in the fatty acids made available to the mammary gland by the lipid supplement in the diet. Similar results have also been obtained when dairy ewes were given diets supplemented with unprotected soybean oil (Mele *et al.*, 2006). Loor *et al.* (2005) reported that feeding dairy cows high-concentrate diets substantially reduced milk fat percentage and yield whether or not they were supplemented with linseed oil. However, reductions in the amount of milk fat yielded were less pronounced in cows fed diets that did not contain oil supplements, as a consequence of a significant oil × concentrate interaction effect. Other authors have reported marked reductions in milk fat percentage and yield in dairy cows fed high-concentrate diets in conjunction with corn or soybean oil

Figure 1.   Relationship between rumenic acid and vaccenic acid concentrations in the milk fat of dairy goats fed the experimental diets.
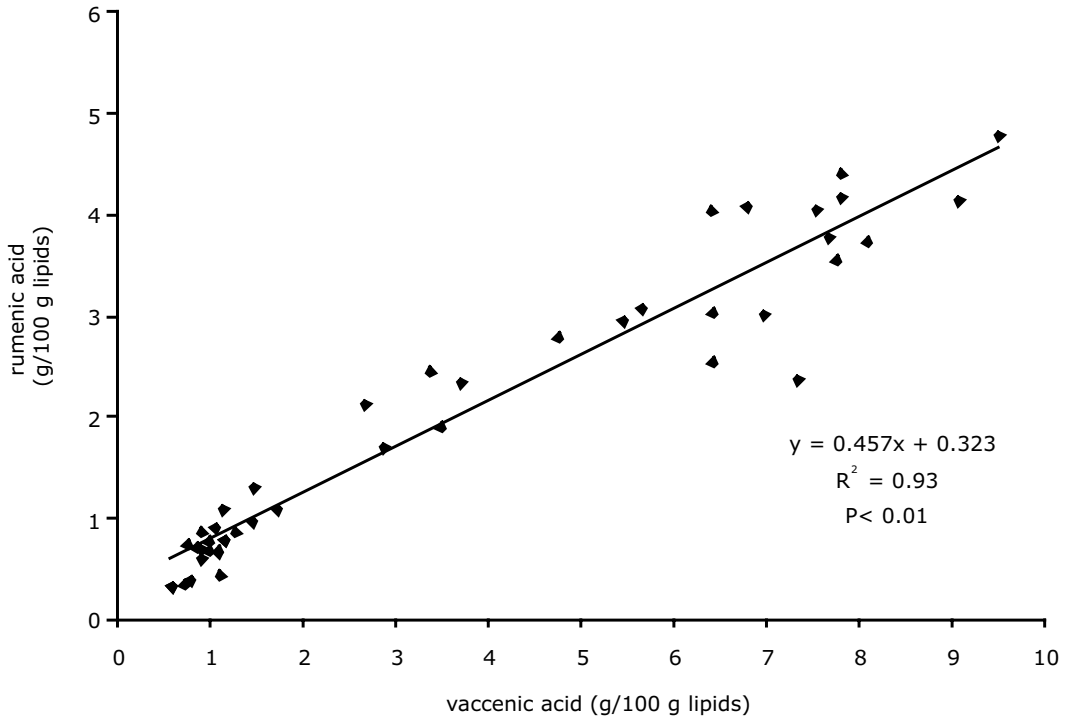
(Griinari *et al.*, 1998; Piperova *et al.*, 2000), whereas Chilliard *et al.* (2007) recently reported that in the goat nearly all types of lipid supplements added to a large variety of basal diets induce a sharp increase in milk fat content, in contrast to the cow. We used the same forage:concentrate ratios as Loor *et al.* (2005) did for dairy cows, though the level of oil supplementation given in our study was 1% higher (4% *vs* 3% on a DM basis), but, unlike dairy cows, our study found that in dairy goats dietary lipid supplementation induces an increase in milk fat secretion, especially when associated to a high-forage diets (forage x oil interaction, P<0.05). In literature, several authors have highlighted that responses to fat supplementation differ considerably according to the species (Chilliard *et al.*, 2003; Pulina *et al.*, 2006; Chilliard *et al.*, 2007). The reasons for this are not easy to identify. Some of these differences may be linked to digestive and metabolic interactions between basal diet (the type and proportion of forages and concentrates in the diet) and the dietary lipid source.

In our study, in milk produced by goats fed a diet that did not contain soybean oil, the percentage of milk fat was lower than that of milk protein. This phenomenon has been defined as the inversion of percentage syndrome by Morand-Fehr *et al.* (2000b). Providing oil supplements may solve this problem by increasing the milk fat percentage (Table 3). Moreover, lipid supplementation seemed to reduce, in part, the adverse

effects that the high-concentrate diet (LF/NO) had on the rennet clotting time (Table 3), and may be due to the improvement of the fat/protein ratio. Indeed, the milk from goats fed LF/NO diet showed the lowest level of milk fat content (forage x oil interaction was significant, P<0.05) (Table 3). Therefore in dairy goats, as previously suggested by Morand-Fehr *et al.* (2000a), the inclusion of plant oil in a diet low in roughage may contribute to improve the cheese making aptitude of the milk by improving the fat/protein ratio.

With regard to the fatty acid composition of milk, feeding dairy goats a diet that contained added unprotected soybean oil had a similar effect to that reported for dairy cows and sheep (Dhiman *et al.*, 2000; Mele *et al.*, 2006) or goats fed high-linoleic sunflower oil (Chilliard *et al.*, 2007), in that it increased the C18:0 and total C18:1 content of the milk, mainly at the expense of C8:0 to C16:0 (Table 4).

The oil-supplemented diets resulted in a general increase in *trans* C18:1 and CLA isomers; however, the response to soybean oil varied with the concentrate level. Goats fed the HF/O diet produced milk containing the highest amount of *trans* C18:1, while high levels of concentrates in the diet (LF/O) led to lower levels of *trans* C18:1 in milk and caused a shift in the rumen biohydrogenation process toward the formation of *trans*-10 C18:1 at the expense of VA (Table 4). An increase in the ratio of starch to fiber in the diet may decrease the rate of lipolysis of dietary lipids (Shingfield *et al.*, 2005). This rate is an important factor controlling the formation of individual biohydrogenation intermediates in the rumen (Bauman and Griinari, 2001). On the other hand, also the difference in the fiber nature or fiber length between HF and LF diet could have affected to some extent the biohydrogenation process (Dewhurst *et al.*, 2006).

In our study, a high-concentrate diet supplemented with unsaturated oils (LF/O) increased the milk's *trans*-10 C18:1 content, a finding which is in agreement with the literature (Loor *et al.*, 2005). However, this increase in *trans*-10 C18:1 did not result in a decrease in the milk fat content or yield, confirming what was previously reported also by Chilliard *et al.* (2007) for dairy goats fed high concentrate diets supplemented with PUFA-rich oils. In dairy cows, by contrast, a substantial number of published studies have reported that an increase in the percentage of *trans*-10 C18:1 found in milk fat is positively related to milk fat depression (Griinari *et al.*, 1998; Piperova *et al.*, 2000; Peterson *et al.*, 2003; Loor *et al.*, 2005). For example, in response to a high-concentrate diet plus unsaturated oil, *trans*-10 C18:1 accounted for 36% of total *trans* C18:1 in the study of Griinari *et al.* (1998), for 59% in Piperova *et al.* (2000), for 43% in Peterson *et al.* (2003) and for 24% in Loor *et al.* (2005). In our study, however, the level of *trans*-10 C18:1 produced in response to the LF/O diet accounted for only 15% of total *trans* C18:1, which is close to the experimental value obtained in our previous study of dairy sheep (10%, Mele *et al.*, 2006). As a matter of fact, patterns of biohydrogenation appear similar to that of cows, though the low range in NSC variation in the present study or differences in rate of passage across species (Van Soest, 1994) seems to have affected degree of biohydrogenation as well as proportions of end products.

The increase we saw in the amount of VA in the milk of those animals given the HF/O diet was consistent with the results of Chilliard *et al.* (2003) and Chilliard and Ferlay (2004), who investigated the interaction between the type of forage and the provision of vegetable-oil supplements in relation to the composition of goat's milk. In particular, our results are comparable to those obtained when an alfalfa hay diet was supplemented

with 5% linseed oil on a DM basis (i.e. the 8.80 g/100 FA *vs* the 7.54 g/100 g lipids obtained by Chilliard and Ferlay (2004) and the present study, respectively).

The total amount of CLA isomers contained in the milk produced by goats fed the soybean-oil-supplemented diets exceeded the values reported for grazing goats or goats whose diets were supplemented with a free vegetable oil other than soybean oil (Chilliard *et al.*, 2003; Nudda *et al.*, 2003), although high values of rumenic acid (3.5-5.1% of total fatty acids) were also observed in some goat studies reviewed by Chilliard *et al.* (2007). Increases in the level of concentrate in the soybean-oil-supplemented diet led to a decrease in both the total amount of CLA in the milk produced (42.11 mg/g lipids *vs* 30.07 mg/g lipids for HF/O and LF/O diet, respectively) and the rumenic acid content (Table 5). They also led to increases in *trans*-10, *cis*-12 CLA content (Table 5). The total content and distribution of individual CLA isomers seen in response to HF/O and LF/O diets may be due to a shift in ruminal biohydrogenation that resulted in an increase in *trans*-10, *cis*-12 CLA and *trans*-10 C18:1 at the expense of rumenic and vaccenic acid. Because the rumenic acid content of the milk was closely associated with variations in the vaccenic acid content of the milk fat (Figure 1), the reduction seen in the rumenic acid content of the milk fat when the amount of concentrates in the diet was increased could be the result of a decrease in the supply of vaccenic acid.

The increase in *trans*-10, *cis*-12 CLA in response to a high-concentrate diet (LF/O) was significant; however, the total amount of this CLA isomer in milk fat remained negligible in comparison to the amounts of rumenic acid and *trans*-7, *cis*-9 CLA, the two major CLA isomers (Table 5). Although in dairy cows small increases in *trans*-10, *cis*-12 CLA have been associated with milk fat

depression when diets contain low levels of fiber and vegetable oil (Piperova *et al.*, 2000), in our study milk fat and yield were not reduced. This may be a confirmation that, in dairy goats, both *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA are not correlated with milk fat content (Chilliard and Ferlay, 2004). An alternative explanation may be that some threshold concentration of *trans*-10, *cis*-12 CLA, sufficient to impact milk fat depression, was not met. Indeed, in the dairy goat, mammary lipogenesis seems much less responsive to post-ruminally infused *trans*-10, *cis*-12 CLA (De Andrade *et al.*, 2006). However, Shingfield *et al.* (2006) have recently remarked that other ruminal biohydrogenation intermediates may also play a role in milk fat synthesis. Recently, Perfield *et al.* (2006) demonstrated that abomasal infusion of *trans*-10, *trans*-12 CLA reduces the Δ9-desaturase index in dairy cows, but does not affect milk fat yield. In our study, soybean-oil supplementation significantly enhanced the amount of *trans*-10, *trans*-12 CLA in milk fat, relative to unsupplemented diets (Table 5); however, the highest level of this fatty acid (which was seen in the milk of animals fed the HF/O diet) was tenfold lower than that reported by Perfield *et al.* (2006). The desaturase index comprises four ratios of fatty acids that represent a proxy for the Δ9-desaturase enzyme in the mammary gland (Bauman and Griinari, 2003), and studies in lactating mice and goats have demonstrated a positive correlation between these fatty acid pairs and the activity and the mRNA abundance of the enzyme (Singh *et al.*, 2004; Bernard *et al.*, 2005; Bernard *et al.*, 2008). Compared with unsupplemented diets, C14:1/C14:0 and RA/VA ratios were significantly decreased when goats were fed oil-supplemented diets (Table 4). A reduction in Δ9-desaturase was also observed in Alpine goats fed diet supplemented with linseed or sunflower oil (Bernard *et al.*, 2005;

Bernard *et al.*, 2008), or in dairy ewes when soybean oil was added to their diet (Mele *et al.*, 2006).

Conclusions

The inclusion of soybean oil in the diet of dairy goats increased milk yield and milk fat yield and concentration and induced an increase in the amount of unsaturated fatty acids (including CLA and *trans* fatty acids) and a decrease in saturated fatty acids. Nevertheless, the effect of oil supplementation varied according to the forage: concentrate ratio. Since encouraging results have recently come from human studies using dairy products modified by changing ruminant nutrition (Shingfield *et al.*, 2008),

plant oil supplementation appears to be a natural way for farmers to rapidly modulate the milk fatty acid composition. Moreover, results from this study and from literature put in evidence that the goat is a very good responder to dietary lipid supplementation, either in terms of milk fat content or in terms of milk fatty acid composition.

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