Effect of a Diet Enriched in Whole Linseed and Sunflower Oil on Goat Milk Fatty Acid Composition and Conjugated Linoleic Acid Isomer Profile

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ABSTRACT

The aim of the present research was to study changes in milk composition and fatty acid profile, specifically conjugated linoleic acid (CLA) and its isomers, in goat milk as affected by dietary supplementation of sunflower oil and whole linseed (0.81 and 1.84% of dry matter on basal diet, respectively) and to assess the persistency of the response. To achieve this objective, bulk milk from a herd and from 6 individual dairy goats fed a diet supplemented with sunflower oil and whole linseed was monitored for a period of 3 mo. Gas chromatography and silver ion HPLC were used to analyze total CLA content and the isomeric profile of these fatty acids, respectively. The contents of α -linolenic acid increased from 0.35% with the reference diet to 0.62% with the supplemented diet. Similarly, CLA milk content increased from 0.46 to 1.18%. The same pattern was also observed for *trans*-11 C18:1 (1.38 to 4.05%, respectively) in goat milk after 3 mo of lipid supplementation. In contrast, changes in other *trans* C18:1 isomers were less remarkable. There was a strong linear correlation between *cis*-9, *trans*-11 C18:2, the main CLA isomer, and *trans*-11 C18:1 under the conditions assayed and their concentration remained stable throughout the duration of the study. Levels of the minor CLA isomers were also enhanced as a consequence of lipid supplementation. The most remarkable increases were observed for 11-13 (*trans-trans* and *trans-cis* geometric isomers), whereas *trans*-7, *cis*-9 (the second most important CLA isomer from a quantitative point of view) and *trans*-10, *cis*-12 increased only slightly with lipid supplementation.

Key words: goat milk, conjugated linoleic acid, rumenic acid, α -linolenic acid

INTRODUCTION

Goat milk has been identified as an alternative for consumers who are either sensitive or allergic to cow's milk. Lipid composition is one of the most important components of the nutritional quality of goat milk. The nutritional advantage of goat milk fat compared with cow's milk has been attributed to the high content of C6:0 to C10:0 fatty acids, lack of agglutinin, a high percentage of the short- and medium-chain fatty acids esterified on the carbon 3 of the glycerol skeleton, and to a small size of fat globules; hence making the dairy product easily digestible (Chilliard et al., 2006).

Different fatty acids are potentially involved as positive or negative predisposing factors for human health. Rumenic acid (**RA**, *cis*-9, *trans*-11 C18:2), the main natural isomer of conjugated linoleic acid (**CLA**) seems to act positively on the prevention of some forms of cancer (Lee et al., 2005). The content of RA in goat milk fat can vary widely (Chilliard and Ferlay, 2004; Chilliard et al., 2006) and the underlying factors resulting in this variation are related predominantly to differences in the production systems applied (Mir et al., 1999; Kitessa et al., 2001; Sanz-Sampelayo et al., 2004). Chilliard et al. (2003) reviewed studies involving effects of lipid supplements on milk fat synthesis and concluded that there were many similarities among ruminants, but often goats responded differently than cows. Several other minor CLA isomers generated in the rumen or in the ruminant mammary gland could also be found in goat milk fat but few data are published on the influence of feeding on these minor compounds.

Modifications in milk fatty acids composition through nutrition may result in positive or adverse changes in the flavor and nutritional properties of dairy products from goats. The characteristic flavor of goat cheese, for instance, is particularly affected by the content of short- and medium-chain fatty acid (C6 to C10). Increasing the content of potentially healthy fatty acids in goat milk by dietary manipulation may increase its value.

Received June 14, 2007.

Accepted September 14, 2007.

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Table 1. Ingredient and nutrient composition of the experimental rations

Ingredient (% of DM)	Ration	
	Reference	Enriched
Alfalfa hay	16.90	13.79
Oats hay	17.44	14.23
Barley straw	14.85	12.12
Beet pulp	7.50	6.12
Oats grain	4.37	3.56
Barley grain	5.70	10.04
Palm oil	1.06	0.87
Corn grain	7.49	6.11
Soybean meal	16.30	13.30
Wheat middlings	2.01	1.64
Wheat grain	1.71	1.39
Distillers dried grains	1.73	5.06
Sunflower meal	1.25	1.02
Canola meal	0.53	0.44
Sepiolite	0.38	0.31
Calcium carbonate	0.51	0.52
Molasses	0.14	0.11
Magnesium oxide	0.02	0.02
Sodium chloride	0.10	0.11
Monocalcic phosphate	0.04	0.03
Corn gluten feed	—	3.60
Peas	—	2.90
Whole linseed	—	1.85
Sunflower oil	—	0.81
Nutrients		
CP	18.5	18.8
NDF	36.4	34.3
NFC	35.8	34.3
Ether extract	3.62	5.1
ME (Mcal/kg)	2.81	2.98
Palmitic acid	1.84	1.72
Linoleic acid	0.51	1.11
α -Linolenic acid	0.09	0.38

Concentrations of specific health-promoting fatty acids, such α -linolenic acid and RA have been increased in cows milk fat by feeding oilseeds rich in C18:3 and C18:2, respectively. However, the maintenance of these changes over time has been questioned by some authors (Chilliard and Ferlay, 2004).

The main objective of this research was to produce CLA-enriched goat milk together with increased levels of α -linolenic acid by feeding a diet supplemented with linseed and sunflower oil without impairing animal performance. Special focus was devoted to the evolution CLA isomer profile throughout a 3-mo period.

MATERIALS AND METHODS

Animals and Dietary Treatments

Six multiparous lactating dairy Murciano-Granadina goats (DIM = 32 ± 18 d) were used in this study. Animals were handled following the guidelines of the IRTA (Institut de Recerca i Tecnologia Agroalimentàries) Animal Care Committee. At the beginning of the study goats received a typical lactating ration (Table 1) with

a forage:concentrate ratio of 40:60 (reference diet). After a month consuming this ration, animals were switched to a similar ration but supplemented with sunflower oil and whole linseed (0.81 and 1.84% DM, respectively) for a period of 3 mo (from April to July). The ingredient composition of this ration changed slightly from the reference diet to maintain CP and NDF at levels as close as possible to the reference diet while increasing the fat content and the fatty acid profile of the diet. Milk samples were collected at 0, 15, 30, 60, and 90 d.

Analytical Methods

Fat, protein, lactose, and TS in milk were measured with a MilkoScan FT-6000 (Foss Electric, Barcelona, Spain). Fatty acids of the dietary components were separated and quantified following AOAC (1990) methods. The extracted fat was mixed with 1 mL of 1 M KOH and 1 mL of 14% BF₃ in methanol. The sample was methylated by incubation at 100°C for 60 min and, after cooling to room temperature, was extracted with 5 mL of hexane. The fatty acid methyl esters (FAME) in the hexane layer were analyzed by GC (5890 Series II GC, Hewlett Packard, S.A., Barcelona, Spain). All samples were methylated in duplicate and introduced into a fused-silica capillary column (30 m $\times$ i.d. 0.25 mm, BPX 70, SGE, Austin, TX). Helium was the carrier gas. Column temperature was initially 150°C for 1 min, increased by 4°C/min to 200°C, and was then held at 200°C for 10 min.

Milk fat was extracted following the method of Luna et al. (2005a), and FAME were prepared by base-catalyzed methanolysis of the glycerides (KOH in methanol) according to an ISO-IDF procedure (2002). Analysis of FAME was performed on a GLC (Agilent 6890 N Network System, Palo Alto, CA) with auto injector, fitted with a flame-ionization detector. The FAME profile was determined by split injection (1:100) onto a CP-Sil 88 fused-silica capillary column (100 m $\times$ 0.25 mm, Varian, Middelburg, the Netherlands) using a programmed temperature gradient method. Helium was the carrier gas and the injector and detector were at 250°C. Initial oven temperature was 160°C. After 80 min, oven temperature was then increased at 10°C/min to 210°C and held for 35 min.

A butter oil reference standard (CRM 164; European Community Bureau of Reference, Brussels, Belgium) was used to determine the response factors of the individual fatty acids. To identify different isomers, a mixture (*cis*-9, *trans*-11; *trans*-8, *cis*-10; *cis*-11, *trans*-13; *trans*-10, *cis*-12 C18:2, and small amounts of a variety of all *cis* and *trans* C18:2 isomers) and pure (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 C18:2) CLA methyl esters were

Table 2. Effects of dietary supplementation of whole linseed (1.85% in DM) and sunflower oil (0.81% in DM) on milk yield and milk composition of dairy goats¹

	Treatment					SE	P-value
	Days of supplemented diet						
	Reference	15	30	60	90		
Yield (g/d)							
Milk	2,917 ^a	2,931 ^a	2,852 ^{ab}	2,770 ^b	2,740 ^b	55.0	<0.001
Fat	121.8 ^a	120.5 ^{ab}	114.3 ^{ac}	102.0 ^{bcd}	117.0 ^{ad}	5.90	0.014
Protein	89.3 ^a	92.4 ^b	88.4 ^a	89.1 ^a	89.2 ^{ab}	2.02	0.017
Lactose	134.3 ^a	131.6 ^a	126.3 ^c	118.9 ^b	118.0 ^b	2.62	<0.001
TS	370.7 ^a	369.2 ^{ab}	355.2 ^{bc}	333.9 ^d	356.3 ^{ac}	5.96	<0.001
Composition (g/100 g of milk)							
Fat	4.21	4.01	3.84	3.63	4.28	0.271	0.080
Protein	3.06 ^b	3.16 ^{ab}	3.10 ^b	3.21 ^{ab}	3.25 ^a	0.054	0.007
Lactose	4.61 ^a	4.43 ^{ab}	4.43 ^{bc}	4.29 ^c	4.31 ^c	0.086	<0.001
TS	12.75 ^{ab}	12.60 ^{ab}	12.48 ^{ab}	12.04 ^b	13.01 ^a	0.285	0.020

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹Data correspond to the analysis of milk from 6 individual goats collected at 0 (reference diet) 15, 30, 60, and 90 d.

purchased from Nu-Chek Prep Inc. (Elysian, MN). To form other CLA isomers, Nu-Chek mixtures were isomerized using I₂ following the method described by Eulitz et al. (1999).

Silver-ion HPLC (Ag⁺-HPLC) separation of CLA methyl esters was conducted using an HPLC apparatus (model SPE-MA10AVP, Shimadzu Kyoto, Japan) equipped with a diode array detector operated at 233 nm at 29°C. Three ChromSpher 5 Lipids analytical silver-impregnated columns (250 mm × 4.6 mm i.d. stainless steel; 5 µm particle size; Varian) were used in series. The mobile phase was 0.1% acetonitrile in hexane and operated isocratically at a flow rate of 1.0 mL/min. The flow was initiated 0.5 h before sample injection, and injection volume was 10 µL. The identification of CLA isomers was based on coinjection with commercial reference material as well as a comparison of the elution order of CLA with existing literature. The chromatographic areas for 7–9, 8–10, and 9–11 were added and used for comparison with the peak area of the 3 isomers from the GC chromatogram. The amounts of the other CLA isomers were calculated from their Ag⁺-HPLC areas relative to the area of the main isomer, 9–11. The results were expressed as absolute values in milligrams per gram of fat.

Statistical Analysis

The data from individual goats were analyzed following an ANOVA using a mixed-effects model that accounted for the random effect of goat and the fixed effects time. Time entered the model as a repeated measure following an autoregressive order 1 variance-covariance structure. The variable time was a categorical

variable with value of zero when goats received the reference ration, and values of 1, 2, 4, and 6 at 15, 30, 60, and 90 d, respectively, following the change of diet and the introduction of the lipid supplementation. Mean comparisons were conducted using the Scheffé adjustment. The data from bulk tank were analyzed similarly, but excluding the random effect of goat. All statistical analyses were conducted with SAS (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

Animal Performance

Milk production and composition for the 3-mo duration of the study are reported in Table 2. Milk yield was not affected during the first month of supplementation but a slight decline in milk production (approximately 6 to 7%) was recorded at 60 and 90 d. This was most likely because of the effect of lactation progression and not due to the change in dietary treatments. However, fat, protein, and TS contents were not altered. Chilliard et al. (2003, 2006) underlined the fact that, unlike the situation in dairy cows, there is no decrease in milk fat content when the diets supplemented with polyunsaturated fatty acids (PUFA) are fed to dairy goats. Chilliard and Ferlay (2004) suggested that the powerful inhibition of acetyl-CoA carboxylase and de novo lipogenesis elicited by long-chain fatty acid (especially with a high level of unsaturation) seems less strong in the goat mammary gland. Therefore, potential inhibitors of lipogenesis present in the rations would be less active in goats than in dairy cows. In regard to milk protein, it has been observed that the addition of

Table 3. Effects of dietary supplementation of whole linseed (1.85% in DM) and sunflower oil (0.81% in DM) on the fatty acid (g/100 g of total fatty acid methyl esters) profile of goat milk¹

Fatty acid	Treatment					SE	P-value
	Reference	Days of supplemented diet					
		15	30	60	90		
C4:0	3.01 ^b	3.26 ^a	3.07 ^{ab}	3.09 ^{ab}	2.99 ^b	0.056	0.012
C6:0	3.25 ^b	3.61 ^a	3.29 ^b	3.30 ^b	3.15 ^b	0.085	0.003
C8:0	3.47	3.94	3.47	3.51	3.33	0.140	0.095
C10:0	11.53	12.13	11.41	11.27	10.95	0.422	0.140
C10:1	0.28	0.26	0.24	0.25	0.23	0.021	0.265
C12:0	4.32	4.14	4.08	4.06	3.68	0.293	0.340
C13:0	0.18	0.17	0.17	0.17	0.16	0.621	0.434
C14:0 <i>iso</i>	0.07 ^{ab}	0.06 ^b	0.08 ^a	0.07 ^{ab}	0.06 ^b	0.007	0.012
C14:0	8.48 ^a	7.77 ^b	8.16 ^{ab}	7.89 ^b	7.93 ^b	0.218	<0.001
C15:0 <i>iso</i>	0.18 ^a	0.13 ^b	0.14 ^{ab}	0.13 ^{ab}	0.15 ^{ab}	0.007	0.014
C15:0 <i>anteiso</i>	0.26 ^{ab}	0.22 ^b	0.26 ^{ab}	0.25 ^b	0.23 ^b	0.016	0.001
C14:1	0.07	0.06	0.06	0.05	0.06	0.006	0.245
C15:0	0.79	0.71	0.83	0.83	0.74	0.055	0.067
C16:0 <i>iso</i>	0.28 ^{ab}	0.24 ^{ab}	0.30 ^a	0.30 ^{ab}	0.24 ^b	0.020	0.013
C16:0	28.40 ^a	25.91 ^b	28.11 ^a	26.38 ^b	27.14 ^{ab}	0.429	<0.001
C17:0 <i>iso</i> + <i>trans</i> -9 C16:1	0.40 ^c	0.62 ^b	0.56 ^{ab}	0.69 ^{ab}	0.70 ^a	0.118	<0.001
<i>Cis</i> -7 C16:1	0.20	0.21	0.21	0.22	0.22	0.018	0.733
<i>Cis</i> -9 C16:1 + C17:0 <i>anteiso</i>	0.83 ^a	0.66 ^b	0.77 ^{ab}	0.68 ^{ab}	0.70 ^{ab}	0.042	0.015
C17:0	0.58	0.52	0.58	0.57	0.53	0.038	0.058
C17:1	0.09 ^a	0.08 ^{ab}	0.07 ^{ab}	0.07 ^b	0.07 ^{ab}	0.007	0.029
C18:0	11.46	11.03	10.47	10.84	10.25	0.734	0.780
<i>Trans</i> 6+7+8 C18:1	0.30 ^b	0.40 ^a	0.37 ^a	0.40 ^a	0.39 ^a	0.010	<0.001
<i>Trans</i> -9 C18:1	0.28 ^b	0.37 ^a	0.34 ^a	0.36 ^a	0.37 ^a	0.010	<0.001
<i>Trans</i> -10 C18:1	0.41 ^b	0.65 ^a	0.57 ^a	0.66 ^a	0.54 ^{ab}	0.034	<0.001
<i>Trans</i> -11 C18:1	1.38 ^b	3.59 ^a	3.65 ^a	3.68 ^a	4.05 ^a	0.653	<0.001
<i>Trans</i> -12 C18:1	0.50 ^b	0.64 ^a	0.59 ^{ab}	0.63 ^a	0.57 ^{ab}	0.031	0.004
<i>Cis</i> -9 C18:1	13.07 ^a	11.43 ^{ab}	11.31 ^b	11.72 ^{ab}	11.77 ^{ab}	0.747	0.041
<i>Trans</i> -15 + <i>cis</i> -11 C18:1	0.47 ^b	0.55 ^a	0.52 ^{ab}	0.55 ^a	0.50 ^{ab}	0.020	0.013
<i>Cis</i> -12 C18:1	0.36 ^c	0.64 ^{ab}	0.54 ^b	0.69 ^a	0.54 ^b	0.065	<0.001
<i>Cis</i> -13 C18:1	0.05 ^c	0.07 ^a	0.06 ^{ab}	0.07 ^a	0.06 ^{bc}	0.006	0.019
<i>Trans</i> -16 + <i>cis</i> -14 C18:1	0.43	0.48	0.46	0.48	0.44	0.026	0.606
<i>Trans-trans</i> C18:2	0.09 ^b	0.13 ^a	0.13 ^a	0.15 ^a	0.14 ^a	0.005	<0.001
<i>Trans-trans</i> C18:2	0.07 ^a	0.06 ^{ab}	0.06 ^{ab}	0.06 ^a	0.05 ^b	0.003	0.009
<i>Trans</i> -9 <i>trans</i> -12 + other <i>trans-trans</i> C18:2	0.23	0.26	0.25	0.27	0.24	0.020	0.268
<i>Cis</i> -9 <i>trans</i> -12 + other <i>cis-trans</i> C18:2	0.27	0.29	0.26	0.30	0.27	0.017	0.289
<i>Trans</i> -9 <i>cis</i> -12 C18:2	0.04	0.05	0.04	0.04	0.04	0.004	0.700
<i>Trans</i> -11 <i>cis</i> -15 C18:2	0.04 ^b	0.13 ^a	0.10 ^{ab}	0.16 ^a	0.15 ^a	0.018	<0.001
<i>Cis</i> -9 <i>cis</i> -12 C18:2	2.86 ^b	2.94 ^{ab}	2.76 ^b	3.40 ^a	3.04 ^{ab}	0.165	0.006
C20:0	0.16	0.15	0.16	0.14	0.14	0.010	0.170
C18:3	0.35 ^c	0.54 ^{ab}	0.45 ^a	0.62 ^a	0.56 ^a	0.051	<0.001
<i>Cis</i> -9 <i>trans</i> -11 C18:2	0.46 ^b	0.93 ^a	0.96 ^a	1.02 ^a	1.18 ^a	0.145	<0.001

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹Data correspond to the analysis of milk from 6 individual goats collected at 0 (reference diet) 15, 30, 60, and 90 d.

fat to the goat diets results in no change in milk protein content and may, in fact, lead to an increase (Sanz-Sampelayo et al., 2007).

Fatty Acid Composition of Milk

Table 3 shows the evolution of the fatty acid profile during the 3-mo monitoring period. A secondary effect due to lactation stage on milk fatty acid could not be excluded in the present study, even if dietary effects are likely to be the major effects. Goats entered the study at about 30 DIM, and thus it is possible that

some animals were still under negative energy balance, which might have increased the proportion of saturated long-chain fatty acids in milk. The lactation stage effect is marked and mainly linked to lipid store mobilization in early lactation, but it only lasts a few weeks each year (Chilliard et al., 2003).

Lipid supplementation did not substantially influence the concentration of fatty acids from C4:0 to C12:0, whereas a decline ($P < 0.001$) in the contents of myristic (C14:0) and palmitic (C16:0) acids was observed (Table 3). However, the decreases in C14:0 and C16:0 levels were never greater than 10% compared with the values

obtained with the reference diet (Table 3). In agreement, Nudda et al. (2006) did not detect significant changes in the concentration of fatty acids from C6:0 to C12:0 in goat milk fat after supplementation with different amounts of extruded linseed cake. Furthermore, Chilliard et al. (2003) and Chilliard and Ferlay (2004) reported no changes in C4:0 to C8:0 contents after lipid supplementation with several types of basal diets enriched in PUFA. The lack of changes in the content of short-chain fatty acids (C4:0 to C8:0) could be attributed to the fact that they are partly synthesized by metabolic pathways that are independent of acetyl-CoA carboxylase (Chilliard and Ferlay, 2004).

The stability (C10:0 to C12:0) or the relatively modest decrease (C14:0 to C16:0) observed in the contents of saturated fatty acids could be attributed to the specific characteristics of the metabolism of fatty acids in the goat mammary gland. In contrast to dairy cows, enzymes involved in the pathways of *de novo* lipogenesis in the goat mammary gland seemed less affected by the lipid supplementation with PUFA. A slight decrease of C8 to C16 in response to feeding lipid supplements was partially attributed to a small variation in acetyl-CoA carboxylase activity and other enzymes involved in *de novo* synthesis of saturated fatty acids in the mammary gland (Bernard et al., 2005). Additionally the modest decrease in C16 could also be due, in part, to the lower intake of palm oil with the enriched ration (Table 1).

The incorporation of whole linseed in the diet resulted in significant increases ($P < 0.001$) of α -linolenic acid in milk fat (Table 3). Although this increase was far from reaching the proportions documented by Chilliard et al. (2003) when goats received 4% added fat from either crushed or formaldehyde-treated linseeds, it was similar to the increases reported by Nudda et al. (2006) when supplementing a diet with extruded linseed. In contrast, linoleic acid levels in milk fat did not significantly increase (Table 3) when whole linseed and sunflower oil were supplemented despite the fact that diet linoleic acid concentration was 118% greater in the enriched ration (Table 1). The lack of increase in linoleic acid when supplementing with sunflower oil (rich in linoleic acid) could be partially attributed to fact that the majority of linoleic acid supplied by the supplemented diet was in free form (oil) and thus susceptible to biohydrogenation in the rumen. In any case, previous works compiled by Chilliard et al. (2006) suggest that when rations are supplemented with linoleic acid-rich seeds or oils such as sunflower or soybeans, the linoleic acid proportion in milk fat rarely exceeds that observed with unsupplemented diets by more than 1.5%. Addition of canola oil containing 20% of linoleic acid up to 6% in goat diet did not modify the secretion of this fatty acid in goat milk (Mir et al., 1999).

Figure 1. Relationship between rumenic acid (*cis*-9 *trans*-11 C18:2) and vaccenic acid (*trans*-11 C18:1) in goat milk fat from goats fed a reference diet and a diet supplemented with whole linseed (1.85% in DM) and sunflower oil (0.81% in DM). FAME = fatty acid methyl esters.

Feeding whole linseed and sunflower oil greatly increased ($P < 0.001$) the amount of milk fat *trans* C18:1 isomers (Table 3). Increases in *trans* C18:1 fatty acids in milk fat is well documented when goats are supplemented with vegetable oils (Chilliard et al., 2003, 2006; Chilliard and Ferlay, 2004). It is also well established that fatty acids with the *cis*-9, *cis*-12 isomerism such as linoleic acid, are isomerized to the *cis*-9, *trans*-11 conjugated diene before rumen biohydrogenation occurs. Although the predominant biohydrogenation end-product would be stearic acid, several monounsaturated intermediates are often generated (Palmquist et al., 2005; Sanz-Sampelayo et al., 2007) and thereafter found in the milk. In this study, the most important increase (from 1.38 to 4.05% of total FAME) was for *trans*-11 (vaccenic acid, VA). This increase could be attributed to the α -linolenic and linoleic intakes, because both fatty acids are precursors of VA in the rumen.

Supplementation was also an effective means of increasing RA in milk fat (from 0.46 to 1.18%). Although α -linolenic acid is not a direct precursor of RA, feeding whole linseed would result in a large increase in the production of ruminal VA, which can be transported to the mammary gland where it is used for endogenous synthesis of RA by stearoyl-CoA desaturase. Figure 1 shows a strong correlation between RA and VA in the samples of milk fat examined. This correlation is consistent with the predominant origin of this CLA isomer in the goat mammary gland from VA via a desaturase (Chilliard and Ferlay, 2004; Nudda et al., 2006). The ratio of RA:VA in milk fat from goats when fed the lipid supplement (0.25 to 0.29) was lower than that from goats when feeding the reference diet (0.34). These results support the idea that the desaturation ratio in

the mammary gland is decreased by diets that increase the availability of either PUFA or *trans* fatty acids, as these fatty acids are putative inhibitors of the Δ^9 -desaturase (Sessler and Ntambi, 1998). Bernard et al. (2005) observed that lipid supplementation led to a decrease in mammary stearoyl-CoA desaturase mRNA and enzyme activity, suggesting negative regulation by dietary PUFA and long-chain fatty acids or, for the unsaturated fatty acids, by their ruminal biohydrogenation products. Chilliard et al. (2006) summarized results from more than 30 different diets and also reported greater values in the ratio RA:VA in unsupplemented diets compared with diets supplemented with lipids.

It should be stressed that the achievement of greater levels of RA in milk fat with oil supplements was accompanied by an enhancement not only of VA, but also of other nonconjugated C18:2 and *trans* isomers of C18:1 (Table 3). However, maximum concentrations of *trans*-10 C18:1 did not exceed 0.70% and the increase in the VA contents was proportionally greater than the elevation of the levels of the remainder of the *trans* unsaturated fatty acids. Furthermore, the increase of *trans*-10 C18:1 in goat milk fat detected in the present study did not involve a simultaneous reduction in the levels of VA and RA. This fact could be justified considering the basal diet (60% of concentrate in DM) as well as the moderate contents of lipids from sunflower oil and whole linseed (2.8% in DM) added to the diet. The characteristic shift in the biohydrogenation route toward the formation of *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 observed in cows (Palmquist et al., 2005) is related to an altered rumen environment favored by diets with high percentages of concentrate and rich in sources of PUFA. The greatest proportion of each *trans* fatty acid isomer in milk was obtained in goats fed a high-concentrate diet (Andrade and Schmidely, 2006a; Chilliard et al., 2006). Furthermore, in the present study, secretion of C15 and C17 in milk, often resulting from microbial fatty acid metabolism in the rumen (Vlaeminck et al., 2006), was not significantly different between reference and supplemented diets (Table 3), which suggests that the diet assayed (relatively low in concentrate) was efficient in producing sufficient levels of VA without disturbing more rumen metabolism.

Values of RA, VA, and *trans*-10 C18:1 in milk remained stable during the period monitored in individual goats (Table 3). Previous research in cows fed with a high-fiber diet and different plant oils (Bell et al., 2006) showed that the milk VA and RA responses to lipid supplementation were stable for 9 wk together with moderate *trans*-10 C18:1 increases. Chilliard and Ferlay (2004) reported that goats would respond better than cows because in goat milk, the RA response was

stable for at least 2 or 3 mo. With the lipid supplementation levels assayed, our results show that RA was maintained for at least a 3-mo period.

Isomers of CLA

Table 4 shows the content of the different CLA isomers determined by Ag⁺-HPLC. From these data, it can be seen that most of the CLA in goat milk corresponds to RA. Given that the *trans*-9, *cis*-11 C18:2 isomer peak is negligible by GC compared with that of *cis*-9, *trans*-11 C18:2 (data not shown), the contents of the peak 9–11 C18:2 obtained from Ag⁺-HPLC analysis should be attributed almost entirely to RA. Thus, at a minimum, 80% of total CLA in goat milk would be assigned to RA (Table 4). The second most prevalent positional isomer in goat milk fat was 7–9 (*cis-trans* plus *trans-cis*) C18:2, representing about 4% of total CLA. According to previous studies in dairy cows (Yurawecz et al., 1998) and dairy ewes (Luna et al., 2005b), most of that peak had to be assigned to the *trans-cis* geometrical configuration.

The milk fat contents of 8–10, 10–12, 11–13, and 12–14 *cis-trans* plus *trans-cis* isomers were clearly lower than 9–11 and 7–9 in the milks studied (below 3% of total CLA), whereas the sum of *trans-trans* was in a range of 3 to 5% of total CLA (Table 4). Individual *trans-trans* isomers were easily identified by Ag⁺-HPLC, and 9–11 was the most abundant. In general terms, these results do not differ substantially from the CLA profile reported for other ruminants (Yurawecz et al., 1998; Luna et al., 2005b).

Most of CLA isomers levels were elevated when the goat's diet was supplemented with vegetable oils (Table 4). The most remarkable increase corresponded to the isomer *trans*-11, *cis*-13 C18:2 (Figure 2). Other CLA isomers (*trans*-12, *trans*-14 and *trans*-11, *trans*-13) also showed sharp enhancements (Figure 2). Few data are reported in the literature on the influence of feeding on goat milk fat CLA profile and, hence, comparisons had to be established with milk from other ruminants. It seems, that dietary supplements high in α -linolenic acid may increase the relative proportion of 11–13 and 12–14 positional isomers in dairy cows (Collomb et al., 2004a) and dairy ewes (Luna et al., 2005c). More recently, Sanz-Sampelayo et al. (2007) reported greater amounts of 11–13 isomers (*trans-cis* and *trans-trans*) in goat milk fat fed with diet supplemented with linseed. Kraft et al. (2003) hypothesized that the CLA isomers *trans*-11, *cis*-13 and *trans*-11, *trans*-13 C18:2 are formed in a large quantity as a result of grazing mountain pasture, which is rich in α -linolenic acid. The pathway for the hydrogenation of this PUFA in the rumen involves an initial isomerization in which the double bond at the carbon-12 position is transferred to the carbon-

Table 4. Effects of dietary supplementation of whole linseed (1.85% in DM) and sunflower oil (0.81% in DM) on the conjugated linoleic acid (CLA, mg/g of fat) isomer profile of goat milk¹

CLA isomer	Treatment					SE	P-value
	Days of supplemented diet						
	Reference	15	30	60	90		
<i>Trans</i> -12, <i>trans</i> -14	0.016 ^a	0.070 ^b	0.045 ^c	0.090 ^d	0.058 ^{bc}	0.0077	<0.001
<i>Trans</i> -11, <i>trans</i> -13	0.060 ^a	0.130 ^{bc}	0.111 ^b	0.140 ^c	0.110 ^b	0.0149	<0.001
<i>Trans</i> -10, <i>trans</i> -12	0.041 ^{ab}	0.038 ^{ab}	0.043 ^{ab}	0.047 ^b	0.035 ^a	0.0063	0.184
<i>Trans</i> -9, <i>trans</i> -11	0.102 ^a	0.139 ^b	0.151 ^b	0.151 ^b	0.152 ^b	0.0103	0.006
<i>Trans</i> -8, <i>trans</i> -10	0.028 ^a	0.040 ^{bd}	0.031 ^{ac}	0.043 ^d	0.036 ^{bc}	0.0035	<0.001
<i>Trans</i> -7, <i>trans</i> -9	0.025 ^a	0.047 ^b	0.037 ^c	0.049 ^b	0.045 ^{bc}	0.0045	<0.001
Σ <i>Trans, trans</i>	0.272 ^a	0.464 ^{bc}	0.418 ^b	0.519 ^c	0.436 ^b	0.0378	<0.001
12,14 (<i>cis/trans</i>) ²	0.027 ^a	0.059 ^b	0.047 ^b	0.072 ^{bc}	0.051 ^{bd}	0.0072	<0.001
<i>Trans</i> -11, <i>cis</i> -13	0.029 ^a	0.122 ^b	0.134 ^b	0.166 ^{bc}	0.197 ^c	0.0213	<0.001
10,12 (<i>cis/trans</i>) ²	0.037 ^a	0.043 ^a	0.042 ^a	0.053 ^{bc}	0.036 ^{ad}	0.0047	0.021
9,11 (<i>cis/trans</i>) ²	4.176 ^a	8.886 ^b	8.945 ^b	9.559 ^b	11.048 ^b	1.3208	0.002
8,10 (<i>cis/trans</i>) ²	0.087 ^a	0.221 ^{bc}	0.176 ^b	0.235 ^c	0.233 ^{bc}	0.0353	<0.001
7,9 (<i>cis/trans</i>) ²	0.353 ^a	0.510 ^b	0.447 ^{ab}	0.523 ^b	0.536 ^b	0.0379	0.015
Σ <i>Cis/trans</i>	4.710 ^a	9.841 ^b	9.790 ^b	10.607 ^b	12.101 ^b	1.3885	0.002
<i>Cis</i> -9, <i>cis</i> -11	0.107 ^a	0.122 ^{ab}	0.143 ^{bc}	0.117 ^{ab}	0.158 ^c	0.0011	0.013

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹Data correspond to the analysis of milk from 6 individual goats collected at 0 (reference diet) 15, 30, 60, and 90 d.

²*Cis/trans* = *cis-trans* + *trans-cis*.

11 position forming *cis*-9, *trans*-11, *cis*-15 C18:3, which is then reduced at both *cis* bonds to produce VA through *trans*-11, *cis*-15 C18:2 as an intermediate. Significant increases ($P < 0.001$) of this C18:2 isomer were mea-

sured in samples when goats received the lipid supplement (Table 3). Nevertheless, the pathways from *trans*-11, *cis*-15 C18:2 to *trans*-11, *cis*-13 and *trans*-11, *trans*-13 CLA are still unclear at this stage.

Figure 2. Silver-ion HPLC chromatograms with 3 columns in series and detection at 233 nm of bulk milk fat from goats fed a reference diet (solid line) and a diet supplemented (dotted line) with whole linseed (1.85% in DM) and sunflower oil (0.81% in DM) for 3 mo. AU = arbitrary unit; *c* = *cis*; *t* = *trans*.

Regarding the minor CLA isomers, amounts of *trans*-10, *cis*-12 C18:2 in the milk samples studied were very low, less than 1% of total CLA (Figure 2, Table 4), and values did not change throughout the 3-mo period monitored. This could be explained because in the rumen *trans*-10, *cis*-12 C18:2 formed from linoleic acid is converted to *trans*-10 C18:1. Furthermore, because *cis*-12 desaturase activity has not been detected in the mammary gland or other ruminant tissues, the *trans*-10 C18:1 formed in the rumen by biohydrogenation of *trans*-10, *cis*-12 C18:2 and absorbed from the intestine would not be transformed endogenously to this CLA isomer. Andrade and Schmidely (2006b) also reported that *trans*-10, *cis*-12 C18:2 always remained at trace levels in milk fat from goats, except when this fatty acid was infused postruminally.

CONCLUSIONS

Feeding both whole linseed and sunflower oil to lactating goats changed fatty acid composition with a slight decrease of the secretion of medium-chain fatty acids and a noticeable increase in the secretion of α -linolenic acid, RA, and VA; thereby, giving the lipid fraction a potentially healthier profile. This supplementation was demonstrated to be an effective means of increasing other CLA isomers, mainly 11–13 (*trans-trans* and *trans-cis* geometric isomers), whereas an increase of *trans*-7, *cis*-9 (the second most important CLA isomer from a quantitative point of view) was less remarkable. Additionally, these changes were maintained across lactation. Furthermore, the improvements in the fatty acid profile of milk can be achieved without detrimental effects on animal performance.

ACKNOWLEDGMENTS

The authors are grateful to the Ministerio de Educación y Ciencia (project AGL2005-04727 CO2-01), the Comunidad Autónoma de Madrid (Madrid, Spain; project S-0505/AGR/000153) and Lodyn (Cindad Real, Spain) for partially financing this project. This study was also possible thanks to the supply of livestock feeding supplements by Lodyn. We thank Jesús Romero at Laboratorio de Lactología y Sanidad Animal (Talavera de la Reina, Castilla-La Mancha, Spain) for conducting the milk composition determinations. Also, we would like to thank M. V. Rodríguez-Pino (Madrid, Spain) for her valuable technical assistance.

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