Supplementation with Extruded Linseed Cake Affects Concentrations of Conjugated Linoleic Acid and Vaccenic Acid in Goat Milk

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ABSTRACT

The aim of this research was to determine the effect of adding extruded linseed cake to the dry diet of goats on the concentrations of conjugated linoleic acid (CLA) and vaccenic acid (VA) in milk fat. Thirty crossbreed dairy goats were divided into 3 groups. Their diet was supplemented with 0% (control group), 5% (low group), or 10% (high group) of extruded linseed cake (ELC), which supplied 0, 16, and 32 g/d of linseed fat, respectively. The milk fat percentage (overall mean 3.5%) and yield did not differ with the different diets, but fatty acid composition was affected by the ELC supplements. The inclusion of ELC in the diets did not influence the concentration of fatty acids from C6:0 to C12:0. The concentrations of C14:0 and C16:0 decreased as the quantity of ELC supplements increased. The concentrations (mg/100 mg of total fatty acid methyl esters) of VA (0.70, 1.23, and 1.39 in control, low, and high groups respectively) and *cis*-9,*trans*-11 CLA (0.63, 0.96, and 1.05 in control, low, and high groups, respectively) were increased by ELC supplements. The milk fat content of VA and *cis*-9,*trans*-11 CLA were closely correlated ($R^2 = 0.82$). Desaturation of VA in the mammary gland to produce *cis*-9,*trans*-11 CLA was higher in the control group than in the groups with ELC diets. Extruded linseed cake supplementation to lactating goats may enhance the nutritional profile of milk lipids.

Key words: linseed, goat milk, conjugated linoleic acid, vaccenic acid

INTRODUCTION

Dairy products are the main dietary source of conjugated linoleic acid (CLA). Among the individual CLA isomers, the greatest attention has been focused on *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA. The *cis*-9,*trans*-11 CLA is thought to have anticarcinogenic properties and makes up approximately 75 to 85% of the CLA in the

milk fat of cows (Piperova et al., 2004) and 78 to 89% of the CLA in the milk fat of sheep (Antongiovanni et al., 2004). The *trans*-10,*cis*-12 CLA isomer is mainly involved in lipid metabolism and has been shown to reduce milk fat in cows (Baumgard et al., 2000) and body fat in mice (DeLany et al., 1999) and humans (Blankson et al., 2000). Recently, some experiments on laboratory animals appear to suggest that the *trans*-10,*cis*-12 isomer may have detrimental effects on health (Wahle et al., 2004). However, the *trans*-10,*cis*-12 CLA content in dairy products is very low, accounting for less than 1.5% of total CLA (Antongiovanni et al., 2004; Loor et al., 2005). The *cis*-9,*trans*-11 CLA in milk fat is produced either as an intermediate during the rumen biohydrogenation of linoleic acid or in the mammary gland by Δ^9 -desaturase from vaccenic acid (VA; *trans*-11 C18:1), another intermediate in ruminal biohydrogenation (Griinari et al., 2000). The conversion of VA into *cis*-9,*trans*-11 CLA has also been observed in the tissue of humans (Adlof et al., 2000).

Diet is the main factor that influences milk CLA and VA concentrations; the highest content of both fatty acids (FA) was found in the milk of cows and sheep grazing on pasture and in those fed diets supplemented with unsaturated oil. Supplementation with plant oils and seeds such as soybean, sunflower, peanut (high linoleic acid), and linseed (high linolenic acid) produced milk with higher VA and CLA contents (Dhiman et al., 2000; Secchiari et al., 2003). An increase of CLA and VA in milk fat was also obtained in experiments that used olive oil (Secchiari et al., 2003) and Canola oil (Chouinard et al., 2001) as supplements for dairy cow diets.

Mir et al. (1999) reported a marked increase of milk CLA content in goats supplemented with Canola oil. Chilliard et al. (2003) observed a substantial increase in the VA and CLA content in the milk fat of goats given dietary supplements of free oil from linseed or sunflowers. The use of free oil higher than 2% of the diet is not recommended, because it inhibits rumen microbial activity (Jenkins, 1993). The whole crude oilseeds were not as efficient as free linseed or sunflower oils in increasing VA and CLA in goat milk fat (Chilliard et al., 2003). In cows, the use of heat-treated oilseeds

Received January 20, 2005.

Accepted September 20, 2005.

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Table 1. Ingredients, chemical composition, and fatty acid (FA) profiles of the diets

	Diet ¹		
	Control	Low	High
Ingredients, %			
Commercial mix ²	74	74	74
Concentrate ³	16	16	16
ELC ⁴	—	5	10
Peas ⁵	10	5	—
Chemical composition			
DM, %	88.8	88.9	89.3
CP, % of DM	17.5	17.9	18.3
NDF, % of DM	38.5	38.6	38.7
ADF, % of DM	21.8	22.1	22.5
Acid detergent lignin, % of DM	4.1	4.5	4.9
Ash, % of DM	7.7	7.8	8.0
Lipid extract, % of DM	2.4	3.1	3.9
Fatty acid, mg/100 mg of total FA methyl esters			
C14:0			
C16:0	13.5	13.2	12.9
C18:0	3.5	3.5	3.5
C18:1	19.0	18.7	18.5
C18:2 <i>cis</i> -9, <i>cis</i> -12	42.6	41.2	39.8
C18:3 n-3	5.5	7.8	10.1

¹High = 10% extruded linseed cake (ELC, Linopiù-Cortal); Low = 5% ELC; Control = 0% ELC.

²Commercial mix: alfalfa hay, corn meal, soybean hull, beet pulp, sunflower meal, wheat bran, flaked corn, barley, soybean meal, cane molasses, vitamins (A 6,600 IU/kg, D₃ 600 IU/kg, B₃ 6 mg/kg, and E 12 mg/kg) and minerals supplement (Zn 46.8 mg/kg and Mn 24.8 mg/kg).

³Concentrate: corn, barley, and beet pulp.

⁴The percentages of the main FA in ELC fat was: C16:0 = 6.0; C18:0 = 3.5; C18:1 = 19.5; C18:2 = 16.1; and C18:3 = 52.2.

⁵The percentages of the main FA in peas fat was: C16:0 = 12.7; C18:0 = 4.2; C18:1 = 25.8; C18:2 = 44.2; and C18:3 = 7.0.

(e.g., extruded oilseeds) have shown a positive influence on VA and CLA concentration in milk fat compared with raw oilseed (Chouinard et al., 1997; Dhiman et al., 2000). The aim of our work was to investigate whether extruded linseed cake (**ELC**) would be a suitable supplement to enhance CLA and VA contents in milk fat of goats fed a dry diet.

MATERIALS AND METHODS

Animals and Treatments

Thirty lactating crossbreed goats (Alpine × Sarda) milked twice a day were randomly assigned to 1 of 3 experimental groups. The animals (DIM >150) were fed 2 kg/d comprising 1.8 kg of a TMR and 200 g of supplement (Table 1). The TMR (89% of DM) contained a fibrous commercial mix [18% CP, 2.5% ether extract, 41% NDF, 25.5% ADF, 5.4% acid detergent lignin (**ADL**)], and a concentrate composed of corn, barley, and beet pulp (11% CP, 2.2% ether extract, 33.5% NDF, 12.7% ADF, 1.1% ADL). The supplement was 0 g (control), 100 g (low diet), or 200 g (high diet) of ELC (Linopiù-Cortal, Cortal S.p.a., Padova, Italy; 95% DM, 27.8% CP, 16.9% ether extract, 29.3% NDF, 15.5% ADF,

7.3% ADL), which supplied 0 (control), 16 (low), and 32 g/d (high) of linseed fat, respectively.

The ELC was obtained by dry extrusion of whole linseed at 155°C before oil extraction by mechanical press. The CP content of the 3 diets (18% of DM) was kept at similar level by adding 200 and 100 g of peas (89% DM, 25.7% CP, 1.6% ether extract, 28.4% NDF, 8.7% ADF, 0.02% ADL) to the control and low diets, respectively. Goats were fed a diet formulated to meet their energy requirements (INRA, 1989). Samples of dietary ingredients were collected once before the beginning of the experiment to determine DM and for chemical analysis. The trial lasted 21 d, which included 12 d for adaptation and 9 d for data collection. Milk yield was recorded and milk samples were collected twice a day at each milking at 3-d intervals. One aliquot of milk samples was analyzed for milk fat and total protein and another aliquot was stored at -20°C for subsequent FA analysis.

Feed and Milk Analyses

The DM content of the feed was determined by oven-drying at 105°C for 24 h. Dried feed samples were ana-

lyzed for NDF, ADF, and ADL with the procedure of Van Soest et al. (1991) by using the filter bag equipment of Ankom (Ankom Technology Corp., Fairport, NY), ash (AOAC, 2000; method 942.05), CP (AOAC, 2000; method 988.05) and fat (Folch et al., 1957). Chemical analyses were expressed as percentages of DM. The fat and total protein of individual milk samples of a.m. and p.m. milkings were determined using a FIL-IDF procedure (2000), with a Milkoscan-6000 (A.R.A. Sardegna laboratory, Oristano, Italy). The milk samples of a.m. and p.m. milkings were composited in a daily sample to determine milk FA composition, including CLA and VA.

Milk fat extraction and fatty acid methyl esters (FAME) preparation were performed as detailed by Nudda et al. (2005). The FAME were separated on a capillary column (CB-Fame CP-select; 100 m $\times$ 0.32 mm i.d., 0.25- μ m film thickness, Varian Inc., Palo Alto, CA). The injector and flame-ionization detector temperatures were 255°C. The programmed temperature was 75°C for 1 min. It was then increased to 165°C at a rate of 8°C/min, and maintained for 35 min, increased to 210°C at a rate of 5.5°C/min, and finally increased to 240°C at a rate of 15°C/min. The split ratio was 1:40 with He at a pressure of 2.55 bar as the carrier gas. Individual FAME were identified by comparing the relative retention times of FAME peaks from samples, using the standards mixture (37 Component FAME Mix, Supelco, Bellefonte, PA). The standards PUFA-2, nonconjugated 18:2 isomer mixture, and individual *cis*-5,8,11,14,17 C20:5, *cis*-4,7,10,13,16,19 C22:6 (Supelco), *cis*-6,9,12 C18:3, and *cis*-9,12,15 C18:3 (Matreya Inc., Pleasant Gap, PA) were used to identify polyunsaturated fatty acids. High-purity individual CLA *cis*-9,*trans*-11 and *trans*-10,*cis*-12 (Matreya Inc.) were used. Verification of other CLA peaks was done using a commercially available CLA standard mix (Nu-Chek Prep, Inc., Elysian, MN). Individual *trans*-9 C18:1 and *trans*-11 C18:1 (Supelco) were used to identify *trans* C18:1 isomers. The isomer *trans*-10 C18:1 was not available commercially. Therefore, the *trans* C18:1 isomers reported were identified by order of elution as described by Griinari et al. (1998). The relative amount of each FAME was quantified by integrating the area under the peak and dividing the result by the total area for all FA. The internal standard (C19:0) was used to convert peak area percentage to weight percentage (mg/100 mg of total FAME). Fatty acids of feed lipid extract were esterified using the procedure described by Palmquist and Jenkins (2003) and determined under the same chromatography conditions as those for milk FA. The FA composition of the experimental diets was calculated from the FA composition of the individual dietary ingredients. The ratio [*cis*-9,*trans*-11 CLA]/[*cis*-

9,*trans*-11 CLA + VA], as an indirect measurement of Δ^9 -desaturase activity in the mammary gland, was calculated separately for each experimental group.

Data were analyzed with the following mixed linear model according to a repeated measures design (Littell et al., 1998):

$$Y_{ijkl} = \mu + D_i + P_j + (D \times P)_{ij} + A_k(i) + \varepsilon_{ijkl}$$

where Y_{ijkl} = observation (milk, fat and protein yields, fat and protein contents, FAME), μ = overall mean, D_i = fixed effect of diet i ($i = 3$), P_j = fixed effect of the period j ($j = 3$), $(D \times P)_{ij}$ = interaction between diet and period, $A_k(i)$ = random effect of animal k ($k = 30$) nested within diet i , and ε_{ijkl} = random residual. The effect of the diet was tested by using the variance associated with animal within treatment as error term. Pairwise comparisons among means were performed using the Tukey test. Differences were declared significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Ingredients, chemical composition, and FA profile of the experimental diets are reported in Table 1. Addition of ELC increased the total lipid content in the high and low groups compared with the control group. Because ELC contained 16.9% of lipids, and linolenic acid represented 52.2% of the FA in the extracted lipids, including ELC in the diets caused a linear increase of C18:3 FA.

Milk production traits and the FA profile of milk fat are reported in Table 2. Milk yield, fat and protein content, and fat and protein yield were not influenced by the addition of ELC to the diets. Although fat supplements in the diets of dairy cows (Chilliard et al., 2003) and sheep (Nudda et al., 2004) often decrease milk protein content, this was not the case in this study, which agrees with the results of previous studies on goats (Chilliard et al., 2003).

The inclusion of ELC in the diets did not influence the concentration of FA from C6:0 to C12:0, whereas it reduced the concentrations of C14:0, C16:0, and C16:1. The content of C16:0 in milk originates both from *de novo* synthesis in the mammary gland and from uptake from arterial blood. In the ELC groups, the reduction of C16:0 is probably due to decreased synthesis in the mammary gland, because the dietary levels of C16:0 were similar in all diets. A similar trend has been observed with other oil sources (Chilliard et al., 2003). In terms of human health, the decrease of C14:0 and C16:0 represents an improvement in the FA profile of milk because of the potential effects of these FA in cholesterol-raising activity (Kris-Etherton and Yu, 1997). The ELC supplements increased significantly the concentrations of C18:0, *cis*-9 C18:1, and C18:3 n-3 in milk

Table 2. Milk yield, composition, and fatty acids (FA) profile of milk from goats fed diets containing extruded linseed cake (ELC)

	Diet ¹			SE
	Control	Low	High	
Milk, g/d	1,188	1,039	1,138	111.4
Fat, %	3.37	3.50	3.55	0.167
Fat, g/d	39.90	35.45	38.72	3.138
Total protein, %	2.86	2.84	3.00	0.130
Total protein, g/d	33.79	28.98	33.32	2.806
Fatty acid, mg/100 mg total FA methyl esters				
C6:0	1.58	1.46	1.59	0.068
C8:0	2.01	1.87	2.05	0.104
C10:0	8.23	7.40	7.77	0.387
C12:0	3.93	3.48	3.67	0.281
C14:0	10.68 ^a	9.81 ^b	9.46 ^b	0.323
C14:1	0.22	0.18	0.17	0.017
C16:0	35.88 ^a	32.40 ^b	27.50 ^b	1.482
C16:1	0.93 ^a	0.77 ^b	0.70 ^b	0.047
C18:0	6.91 ^b	8.74 ^a	10.01 ^a	0.535
C18:1 <i>trans</i> -6-8	0.18 ^b	0.25 ^a	0.28 ^a	0.016
C18:1 <i>trans</i> -9	0.19 ^b	0.26 ^a	0.26 ^a	0.012
C18:1 <i>trans</i> -10	0.26	0.30	0.29	0.032
C18:1 <i>trans</i> -11 (vaccenic acid)	0.70 ^b	1.23 ^a	1.39 ^a	0.130
C18:1 <i>trans</i> -12	0.21	0.39	0.23	0.048
C18:1 <i>cis</i> -9	18.54 ^b	20.72 ^a	23.50 ^a	1.091
C18:2 <i>cis</i> -9, <i>cis</i> -12	2.36	2.44	2.44	0.124
C18:3 n-6	0.09	0.08	0.08	0.004
C18:3 n-3	0.45 ^c	0.80 ^b	0.99 ^a	0.050
Conjugated linoleic acid (CLA) <i>cis</i> -9, <i>trans</i> -11	0.63 ^b	0.96 ^a	1.05 ^a	0.040
CLA <i>trans</i> -10, <i>cis</i> -12	0.0004 ^b	0.0026 ^a	0.0029 ^a	0.001
CLA total ²	0.90 ^b	1.26 ^a	1.33 ^a	0.042
C20:5 n-3 EPA ³	0.063 ^b	0.077 ^a	0.078 ^a	0.006
C22:6 n-3 DHA ⁴	0.039	0.045	0.041	0.004
CLA desaturase index ⁵	0.48 ^a	0.45 ^b	0.44 ^b	0.007

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

¹High = 10% ELC (Linopiù-Cortal), Low = 5% ELC; Control = 0% ELC.

²CLA total includes C20:0 and C20:1 that eluted in CLA region. The isomers have been summed because the chromatographic conditions were not adequate to allow accurate individual quantification.

³EPA = Eicosapentaenoic acid.

⁴DHA = Docosahexaenoic acid.

⁵Desaturase index calculated as [*cis*-9,*trans*-11 CLA]/[*cis*-9,*trans*-11 CLA + VA] ratio.

fat, but did not influence the *cis*-9,*cis*-12 C18:2 content. The content of C18:3 n-3, which contributes to preventing atherosclerosis in humans (Kris-Etherton and Yu, 1997), increased by 78% in the low group and 120% in the high group ($P < 0.01$) compared with the control group.

The total CLA concentration (Table 2) was significantly affected by ELC supplements and the isomer *cis*-9,*trans*-11 accounted for 77% of total CLA in the milk fat of the ELC groups and 70% in the control group. However, the proportion could be an underestimate due to the elution of C20:0 and C20:1 in the CLA region under these chromatographic conditions (Nudda et al., 2005).

The concentration of *cis*-9,*trans*-11 CLA increased by 52 and 67% ($P < 0.01$), respectively, in the low and high groups. The extent of increase in *cis*-9,*trans*-11 CLA by

using ELC was higher than that observed in goats by Chilliard et al. (2003) when they used whole crude linseed supplements, but lower than their results using free linseed oil. Studies on cows found that adding heat-processed oilseed (i.e., soybean and cottonseed) to the diet increases the CLA content in milk to a greater extent than raw oilseed supplements (Chouinard et al., 1998). This is probably due to less interaction with rumen bacteria. It is worth pointing out that the amount of *trans*-10,*cis*-12, which is thought to be detrimental to human health (Wahle et al., 2004), was about 0.3% of total CLA and was not detected in 60% of individual samples. The content of *trans*-10,*cis*-12 isomer in total CLA was the same as that found in sheep milk when different diets were used (Antongiovanni et al., 2004).

The *trans* C18:1 isomers were significantly affected by the supplements, with the exception of *trans*-10 and

Figure 1. Relationship between *cis-9,trans-11* conjugated linoleic acid (CLA) and vaccenic acid (VA) in milk from goats fed diets containing extruded linseed cake. FA = Fatty acids.

trans-12 C18:1. The relative quantity of VA increased by 76 and 99% ($P < 0.01$) in low and high groups, respectively, compared with the control group. Chilliard et al. (2003) did not observe increases in the VA of the milk fat of goats given whole linseed supplements, but found an increase of 123% when free linseed oil was used.

A close relationship between *cis-9,trans-11* CLA and VA was found in milk (Figure 1). This is consistent with the predominant origin of this CLA isomer in the mammary gland from VA via Δ^9 -desaturase. The linolenic acid supplied by ELC is a direct precursor of the VA produced in the rumen. This is transported to the mammary gland where is used for endogenous synthesis of CLA by Δ^9 -desaturase (Griinari et al., 2000; Piperova et al., 2002). The amount of desaturation, estimated from the ratio $[cis-9,trans-11 \text{ CLA}]/[cis-9,trans-11 \text{ CLA} + \text{VA}]$ as an indirect index of Δ^9 -desaturase activity, was higher in the control group than in the groups given ELC supplements. This indicates that less of the substrate FA is desaturated when goats were fed ELC diets. The polyunsaturated fatty acids content in various tissues has been found to reduce the expression of Δ^9 -desaturase (Sessler and Ntambi, 1998). The higher concentration of C18:3 in the mammary glands of goats fed ELC may have decreased the Δ^9 -desaturase activity. In addition, C18:0 is the preferred substrate for Δ^9 -desaturase in the mammary gland (Chilliard et al., 2000; Mosley and McGuire, 2003). Indeed, a close negative correlation was found between the C18:0 concentration in milk and the CLA desaturase index ($r = -0.83$). This suggests that there is fierce competition for desaturase activity at substrate level.

The eicosapentaenoic acid (C20:5 n-3) and docosahexaenoic acid (C22:6 n-3) contents were extremely low, as is typical in the milk of ruminant species. This may

be explained in part by the high amount of biohydrogenation of these FA in the rumen (Chikunya et al., 2004), and partly by the low elongation and desaturation of C18 and C20 FA in the mammary gland of ruminants (Gulati et al., 2003).

The concentration of almost all FA was not significantly affected by the amount of ELC supplement. Whether the linseed oil supplements were 16 or 32 g/d, the milk fat CLA and VA of the goats used in this trial did not change. The lack of differences between the 2 doses of ELC on CLA and VA concentrations could be due to a threshold effect for uptake of these FA by the mammary gland. This is supported by the results of Schmidely and Morand-Fehr (2004), who observed no change in CLA content in goat's milk after intravenous infusion of CLA.

CONCLUSIONS

The supplement of ELC in the ration of dairy goats did not change the milk fat percentage and yield, but markedly influenced the fatty acid profile of the milk fat. The ELC increased the VA, *cis-9,trans-11* CLA, and C18:3 n-3 contents. The ELC supplements also decreased the content of C14:0 and C16:0, which are known to be hypercholesterolemic in humans. The study showed that the nutritional quality of lipids in goat might be enhanced by dietary manipulation. Under our experimental conditions, the addition of 16 g/d of linseed fat in the form of extruded cake was effective in changing the fatty acid profile of milk fat in goats from a dietary and functional point of view.

ACKNOWLEDGMENTS

This research was funded by the FISIR Project of the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MIUR, Italy; Italian Ministry of Education, University and Research). We wish to thank Salvatore Mulas and Roberto Rubattu for their assistance.

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