Differences in CLA Isomer Distribution of Cow's Milk Lipids

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ABSTRACT: The uniqueness of ruminant milk lipids is based on their high concentration of CLA. Maximal CLA concentrations in milk lipids require optimal conditions of ruminal fermentation and substrate availability, conditions like those present in pasture-fed cows. Our previous work showed that farm management (indoor feeding vs. pasture feeding) markedly influenced the CLA concentration. In this study, the objective was to evaluate the influence of the farm management system as dependent on different locations. Milk samples from different locations (Thuringia and the Alps, representing diverse altitudes) were collected during the summer months and analyzed for FA profile and CLA isomer distribution. The proportion of PUFA and total CLA in milk fat was significantly lower in milk from indoor cows compared with the pasture cows in the Alps. The *trans*-11 18:1 in milk fat of Alpine cows was elevated, in contrast to lower values for *trans*-10 18:1. Milk from cows grazing pasture in the Alps was higher in EPA and lower in arachidonic acid than milk from indoor-fed cows. The proportion of *cis,trans/trans,cis* isomers of CLA was 10 times higher from the indoor cows than from the Alpine cows. In addition to the major isomer *cis*-9,*trans*-11, this difference also occurred for the *trans*-11,*cis*-13 isomer, which represented more than a fourth of the total CLA present in milk fat. This is the first report showing a special isomer distribution in the milk fat of cows living under very natural conditions. We hypothesize that the CLA isomer *trans*-11,*cis*-13 is formed in large quantity as a result of grazing mountain pasture, which is rich in α-linolenic acid.

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Cis-9,*trans*-11 octadecadienoic acid (rumenic acid) is the major CLA component in ruminant milk fat and meat fat (1,2). It has been shown that the isomers of CLA in ruminant lipids originate from incomplete biohydrogenation of PUFA (3,4) or that they are synthesized endogenously in the mammary gland from biohydrogenation derivatives (5,6).

The content of CLA in milk fat can vary widely. The underlying factors resulting in this variation are related predominantly to diet and to the farming methods for ruminants (7). Furthermore, the milk fat content of CLA is also related to animal variation (8–10). Our knowledge regarding the variation of isomer distribution in ruminant fat is limited. There are only a few publications dealing with this topic. Normally, after the overwhelmingly predominant CLA isomer *cis*-9,*trans*-11, the *trans*-7,*cis*-9 is the second-most prevalent CLA isomer in ruminant fat (1,11–15). It has been reported that this isomer represents as much as 40% of total CLA under special conditions (16).

Trans-10,*cis*-12 CLA seems to be exclusively rumen derived; and it accumulates under special dietary conditions (17). Its percentage in milk fat is generally very low. Kraft *et al*. (18) found, 5 d after an intraduodenal infusion of a CLA mixture, that milk fat decreased by 40%, indicating that the *trans*-10,*cis*-12 isomer is responsible for inhibition of milk fat synthesis.

In a dose-response experiment Baumgard *et al*. (19,20) confirmed our results when they fed the pure *trans*-10,*cis*-12 isomer. Milk fat from cows supplemented with the highest dose (14 g/d) contained more *trans*-10,*cis*-12 than *cis*-9,*trans*-11, resulting in a dramatically curvilinear reduction in milk fat yield.

The milk fat of cows grazed in the Alps is extraordinarily rich in total CLA, ranging from 1.92 to 2.87 g/100 g fat (21,22). With rising altitude, cows find pasture with a decreased proportion of grasses and an increase in dicotyledonous species, particularly *Compositae, Rosaceae,* and *Plantaginaceae* (23). Collomb *et al*. (24) correlated FA of milk fat with botanical families and individual plant species. The percentages of five plant species [*Leontodon hispidus, Plantago alpina, Aposeris foetida, Lotus corniculatus* (and *alpina*), and *Deschampsia cespitosa*] dominant in the Mountains and Highlands correlated negatively with the concentration of saturated FA (SFA). The percentages of three species [*Leontodon hispidus, Lotus corniculatus* (and *alpina*), and *Trifolium pratense*] correlated positively with the concentration of PUFA and with the concentrations of CLA and monounsaturated *trans* 18:1 FA in milk fat. The fat composition of these herbs must be the reason for the high total CLA content in the milk fat.

The aim of this study was to analyze the CLA-isomer distribution in milk fat from cows feeding at high altitudes in the Alps and compare that with the distribution in milk fat produced under intense farming practices.

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Abbreviations: Ag^+-HPLC, silver-ion HPLC; MUFA, monounsaturated FA; SFA, saturated FA; *t*VA, *trans*-vaccenic acid.

TABLE 1
Short Characterization of Cow Herds

Location	Farming	Altitude (m)	Milk yield (kg/yr)	Number of cows
1. Germany, Thuringia	Indoor-fed cows, silages and high concentrate rations, typical plain situation[a]	~200	>6000	>300
2. Germany, Thuringia	Organic farming, pasturing during the summer, only small amounts of concentrate[b]	~500	4000–5000	120–200
3. Switzerland, Alps	Summer pasturing without concentrate[c]	>1200, different places in Switzerland;	~4500	20–500
4. Switzerland, Alps	Summer pasturing without concentrate[c]	1275–2200, only L´Etivaz	~4500	30–50

[a]Most of the milk in Germany is produced under these conditions.
[b]About 5% of cows in Germany are stocked in organic farms.
[c]Summer pasturing of cows is practiced in the Alps regions of Switzerland, Austria, and Germany.

MATERIALS AND METHODS

Milk samples. Milk samples (n = 16) were collected in four locations during the summer months of the years 2000 and 2001. From each location, four samples of bulk milk from different cow herds were collected. There were significant differences in farm management among the four locations (Table 1).

Lipid extraction and preparation of FAME. Total milk lipids were extracted using a methanol/chloroform/water mixture (1:2:1, by vol) according to Folch *et al.* (25). For the preparation of FAME, $NaOCH_3$ was used. It completely converted esters to FAME, and the base-catalyzed methylation method prevented the isomerization of *cis*/*trans* conjugated bonds to *trans*/*trans* isomers and the formation of artifacts (26).

The resulting FAME were analyzed by two different GC procedures and by silver-ion HPLC (Ag^+-HPLC). The use of both methods was necessary to resolve all FA and CLA isomers (27–29). Additionally, identification of 18:1 isomers was accomplished by Ag^+-TLC before the second GC analysis.

A TAG 23:0 (Larodan Fine Chemicals AB, Malmö, Sweden) was used as an internal standard for quantification (~10% of total FAME). It was added before the methylation stage to ensure a representative sampling. Generally, milk fat contains negligible amounts of 23:0.

Analysis by GC and HPLC. (i) First GC analysis. During the first GC run, the quantification of most of the FA was realized using a gas chromatograph (Shimadzu 17A; Shimadzu, Kyoto, Japan) equipped with FID and automatic injection system (AOC-5000). Analyses were performed using a fused-silica capillary column (DB-225 MS; J&W Scientific, Folsom, CA; 30 m × 0.25 mm, i.d.; 0.2-μm film thickness) and H_2 as carrier gas.

This column was suitable for achieving a successful separation of milk fat FAME ranging from C_4 to C_{22} (including straight and branched structures) in a time-saving manner. Furthermore, it was possible to resolve *cis*-9,*trans*-11/*trans*-7,*cis*-9 CLA from other CLA isomers without interference from other FA. The detailed analysis of minor CLA isomers was achieved by Ag^+-HPLC.

(ii) Second GC analysis. The quantification of *cis* and *trans* isomers of 18:1 resulted from Ag^+-TLC separation followed by GC analysis. A fused-silica capillary column (CP-Sil 88; Chrompack, Middelburg, The Netherlands; 100 m × 0.25 mm, i.d.; 0.2-μm film thickness) was used. The system operated isothermally at 170°C. The results of the short column were combined with those of the better resolution of the 18:1 region using a 100-m CP Sil 88 column.

Ag^+-HPLC analysis. The distribution of CLA-isomers was established using a HPLC system (Shimadzu, LC10A) equipped with a solvent delivery system, an automatic sample injector with a 50-μL injection loop, a UV detector set at 234 nm, and three silver-impregnated ChromSpher 5 Lipids columns in series (each 4.6 mm i.d. × 250 mm stainless steel, 5-μm particle size; Varian-Chrompack). The isocratic mobile phase (0.1% acetonitrile and 0.5% diethylether in hexane) (15) was freshly prepared daily, stirred continuously, and pumped at a flow rate of 1.0 mL/min. Diethyl ether was used to prevent a drift in retention times. The usual injection volumes were 10–20 μL, representing <250 μg lipid. The identification of CLA isomers by Ag^+-HPLC was based on co-injection with commercial reference material (Matreya, Pleasant Gap, PA; Larodan Fine Chemicals AB, Malmö, Sweden) as well as a comparison of the elution order of CLA isomers with the existing literature (12,28).

In the first GC analysis, the main CLA isomer (*cis*-9,*trans*-11) co-eluted with both CLA isomers *trans*-7,*cis*-9 and *trans*-8,*cis*-10 (30). The HPLC areas for *trans*-7,*cis*-9 + *trans*-8,*cis*-10 + *cis*-9,*trans*-11 were added and used for calculation compared with the three isomer peaks from GC chromatogram:

$$\text{main CLA peak area}_{GC} = (t7,c9 + t8,c10 + c9,t11)\ \text{peak areas}_{HPLC} \quad [1]$$

The results were expressed as absolute values in mg/g fat using 23:0 as internal standard in the first GC analysis. The amounts of the other CLA isomers were calculated from their Ag^+-HPLC areas relative to the area of the main isomer *cis*-9,*trans*-11.

Statistical analysis. Results are expressed as means and SD. All data were analyzed by a one-way ANOVA followed by the Scheffé test. Differences were considered significant at $P < 0.05$. All analyses were performed with SPSS 10.0 (SPSS, Chicago, IL) software package.

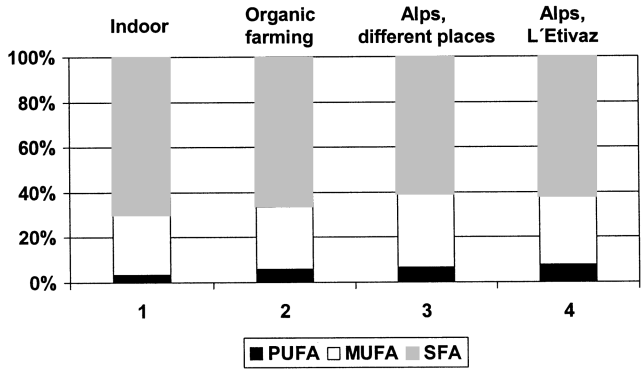


FIG. 1. Distribution of FA groups in milk fat of cows fed at different locations, farming practices, and altitudes.

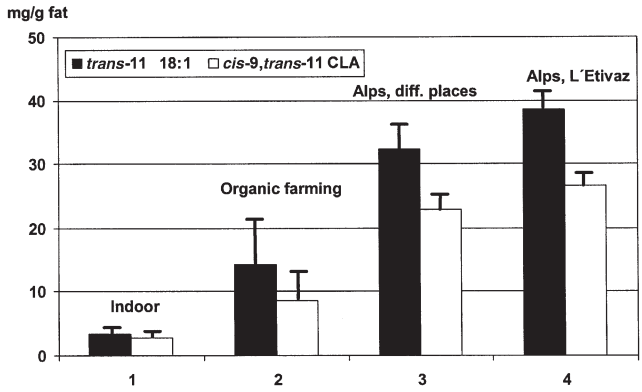


FIG. 2. Content of *cis*-9,*trans*-11 CLA and of *trans*-11 18:1 in milk fat of different origins (all differences between the groups are significant, $P < 0.05$, except between groups 3 and 4).

RESULTS

The results showed greater PUFA content in the milk fat from the pastured cows of the Alps compared with indoor cows (Fig. 1). The milk fat of indoor cows contained the highest proportion of SFA and the lowest of monounsaturated FA (MUFA). The total CLA was significantly lower in indoor cows (group 1) than Alpine cows (group 4) by a factor of 9. Among the *trans* isomers of 18:1, the *trans*-vaccenic acid (*t*VA; *trans*-11) was the most abundant FA (Table 2). Its proportion of total *trans* 18:1 varied significantly from one-fourth in the fat of indoor cows to about two- thirds in Alpine cows. The second-most prevalent *trans*-isomer was the combined *trans*-13/14 18:1 peak. The latter pair was not resolved under the GC conditions. Milk fat of pastured cows contained greater amounts of *t*VA (Fig. 2) and total *trans* 18:1 (Table 2) compared with indoor cows. In contrast, the percentage of the *trans*-10 isomer in pastured cows was significantly lower compared with indoor cows (Table 2). There were only small differences between the analyzed groups relating to the *cis*-isomers of 18:1. The milk fat of group 3 was significantly richer in *cis*-9 18:1. Similar to the *trans* isomers of 18:1, there were significant differences among the four groups mainly in branched-chain FA (*iso*- and *anteiso*-15:0, *iso*- and *anteiso*-17, as well as *iso*-14:0; Table 3). In the same way, more odd-numbered FA were found in the milk fat of Alpine cows (15:0, 17:0).

The content of 20:5 was significantly higher in the groups 2, 3, and 4. In contrast, 20:4 was significantly lower in these groups (Table 4). The most interesting differences were found for the CLA isomers (Table 5). The content of total CLA isomers, mainly the *cis*-9,*trans*-11 CLA, was significantly higher in group 4 than in group 1 by a factor of 10. The portion of *trans*-10,*cis*-12 CLA was very small (0.2%; or 2.3% of total CLA). The *trans*-7,*cis*-9 isomer, identified and quantified on the basis of the Ag^+-HPLC separations (Fig. 3), showed variations depending on the origin of the milk fat (1.1%; or 6.2%

TABLE 2
Isomers of 18:1 in Milk Fat (Values in mg/g fat)

Location	Germany, Thuringia		Switzerland, Alps	
group	1. Indoor cows	2. Organic farming	3. Different places	4. L'Etivaz
trans-4	0.08 ± 0.02[a]	0.18 ± 0.09[b]	0.12 ± 0.02[a,b]	0.12 ± 0.02[a,b]
trans-5	0.08 ± 0.01[a]	0.15 ± 0.05[b]	0.12 ± 0.02[a,b]	0.12 ± 0.02[a,b]
trans-6-8	1.03 ± 0.06[a]	2.13 ± 0.73[b]	1.95 ± 0.08[b]	2.18 ± 0.12[b]
trans-9	1.01 ± 0.02[a]	1.92 ± 0.45[b]	1.78 ± 0.07[b]	1.97 ± 0.07[b]
trans-10	2.87 ± 0.07[a]	2.04 ± 0.59[b]	1.68 ± 0.25[b]	1.78 ± 0.10[b]
trans-11	3.48 ± 0.08[a]	14.28 ± 6.68[b]	32.31 ± 4.18[c]	38.57 ± 3.41[c]
trans-12	1.27 ± 0.01[a]	3.21 ± 1.37[b]	1.90 ± 0.19[a,b]	2.46 ± 0.33[a,b]
trans-13	2.53 ± 0.16[a]	7.41 ± 3.78[b]	3.69 ± 0.35[a,b]	5.24 ± 0.64[a,b]
trans-15	0.95 ± 0.04[a]	3.15 ± 1.65[b]	1.94 ± 0.14[a,b]	2.33 ± 0.20[a,b]
trans-16	1.56 ± 0.07[a]	4.23 ± 1.86[b]	2.80 ± 0.22[a,b]	3.01 ± 1.53[a,b]
Σ*trans*-18:1	14.84 ± 0.04	38.70 ± 17.23	48.29 ± 4.73	57.77 ± 4.72
cis-9	182.82 ± 8.11[a]	182.61 ± 8.54[a]	205.65 ± 10.17[b]	175.15 ± 8.49[a]
cis-11	6.78 ± 0.17[a]	5.01 ± 0.37[b]	5.37 ± 0.31[b]	5.01 ± 0.28[b]
cis-12	1.19 ± 0.08[a]	2.43 ± 0.86[b]	0.68 ± 0.04[a]	0.86 ± 0.13[a]
cis-13	0.86 ± 0.06[a]	0.81 ± 0.09[a]	0.57 ± 0.07[b]	0.59 ± 0.07[b]
cis-15	0.73 ± 0.17[a]	2.46 ± 1.08[b]	1.45 ± 0.06[a,b]	1.75 ± 0.20[a,b]

[a]Values in a row not sharing a common superscript roman letter differ, $P < 0.05$.

TABLE 3
Branched FA in Milk Fat[a] (values in mg/g fat)

	Germany, Thuringia		Switzerland, Alps	
Group location	1. Indoor cows	2. Organic farming	3. Different places	4. L'Etivaz
iso-13:0	0.77 ± 0.02[a]	0.57 ± 0.17[a,b]	0.52 ± 0.09[b]	0.59 ± 0.09[a,b]
anteiso-13:0	0.25 ± 0.00[a]	0.39 ± 0.05[b]	0.54 ± 0.04[c]	0.54 ± 0.04[c]
iso-14:0	0.80 ± 0.02[a]	1.23 ± 0.10[a,c]	1.64 ± 0.09[b,c]	1.71 ± 0.29[b]
15:0	10.89 ± 0.18[a]	10.83 ± 0.61[a,b]	12.63 ± 0.21[a,b]	13.22 ± 1.48[b]
iso-15:0	2.16 ± 0.03[a]	2.58 ± 0.05[a,c]	3.47 ± 0.22[b,c]	3.32 ± 0.49[b]
anteiso-15:0	4.38 ± 0.02[a]	5.73 ± 0.41[b]	6.88 ± 0.45[c]	7.24 ± 0.59[c]
iso-16:0	2.36 ± 0.08[a]	2.70 ± 0.16[a]	2.87 ± 0.09[a]	2.91 ± 0.27[a]
17:0	5.93 ± 0.07[a]	5.88 ± 0.14[a]	7.49 ± 0.24[b]	6.94 ± 0.42[b]
iso-17:0	3.52 ± 0.07[a]	3.90 ± 0.36[a]	4.76 ± 0.15[b]	4.61 ± 0.37[b]
anteiso-17:0	4.81 ± 0.08[a]	4.89 ± 0.14[a]	5.07 ± 0.12[a]	5.01 ± 0.18[a]

[a]Values in a row not sharing a common superscript roman letter differ, $P < 0.05$.

of total CLA). The *trans*-7,*cis*-9 CLA isomer was identified by Yurawecz *et al*. (12) and reported to be the second-most abundant CLA isomer in normal commercial milk fat. The results of the indoor group (Table 5) were similar, thus confirming the previous report. The higher CLA content was associated mainly with higher levels of *trans*-11,*cis*-13 CLA, whereas the level of *trans*-7,*cis*-9 remained nearly constant (except group 4, Table 5).

TABLE 4
Distribution of FA in Milk Fat[a] (values in mg/g fat)

Location	Germany, Thuringia		Switzerland, Alps	
group	1. Indoor cows	2. Organic farming	3. Different places	4. L'Etivaz
4:0	37.88 ± 0.50[a]	37.26 ± 1.33[a]	35.29 ± 1.86[a]	35.59 ± 3.27[a]
6:0	26.92 ± 0.21[a]	25.56 ± 1.04[a,c]	21.62 ± 1.25[b]	22.57 ± 2.01[b,c]
8:0	13.93 ± 0.30[a]	13.47 ± 0.86[a]	10.49 ± 0.91[b]	11.25 ± 1.03[b]
10:0	30.50 ± 0.38[a]	27.81 ± 2.23[a,c]	20.93 ± 2.65[b]	23.00 ± 1.91[b,c]
10:1	2.95 ± 0.05[a]	2.76 ± 0.36[a]	2.56 ± 0.40[a]	2.78 ± 0.36[a]
12:0	33.54 ± 0.28[a]	28.23 ± 2.97[b]	22.17 ± 2.78[b,c]	23.81 ± 1.85[b,c]
12:1	0.81 ± 0.22[a]	0.68 ± 0.22[a]	0.60 ± 0.11[a]	0.67 ± 0.11[a]
13:0	1.20 ± 0.01[a]	0.87 ± 0.05[b,c]	0.72 ± 0.06[c]	0.80 ± 0.06[c]
14:0	94.66 ± 1.63[a]	87.75 ± 8.07[a,b]	79.36 ± 6.51[b]	82.80 ± 3.18[a,b]
14:1	10.28 ± 0.13[a]	10.65 ± 2.23[a]	8.05 ± 1.16[a]	8.93 ± 1.12[a]
15:0	10.81 ± 0.18[a]	10.83 ± 0.61[a,c]	12.63 ± 0.21[b,c]	13.22 ± 1.48[b]
16:0	273.46 ± 6.80[a]	226.23 ± 36.79[b]	207.38 ± 10.37[b]	219.44 ± 9.00[b]
16:1	20.57 ± 0.76[a]	14.13 ± 2.73[b]	12.95 ± 1.24[b]	13.48 ± 1.40[b]
17:0	5.93 ± 0.07[a]	5.88 ± 0.14[a]	7.49 ± 0.24[b]	6.94 ± 0.42[b]
17:1	0.05 ± 0.01[a]	0.12 ± 0.05[a]	4.82 ± 0.44[b]	4.71 ± 0.57[b]
18:0	75.34 ± 2.75[a]	106.44 ± 12.60[b]	98.37 ± 11.39[a,b]	86.34 ± 9.24[a,b]
18:2*t*9,*c*12	2.32 ± 0.06[a]	7.38 ± 4.37[b]	5.48 ± 0.51[a,b]	7.02 ± 0.83[b]
18:2n-6	16.33 ± 0.79[a]	15.18 ± 0.82[a]	11.93 ± 0.48[b]	13.05 ± 1.09[b]
18:3n-6	0.45 ± 0.02[a]	1.20 ± 0.69[b]	1.15 ± 0.09[a,b]	1.40 ± 0.13[b]
18:3n-3	3.31 ± 0.22[a]	8.61 ± 2.93[a,b]	11.67 ± 0.47[b]	13.02 ± 1.74[b]
18:4n-3	0.45 ± 0.02[a]	0.48 ± 0.14[a]	0.94 ± 0.38[a,b]	1.20 ± 0.12[b]
20:0	1.08 ± 0.07[a]	1.44 ± 0.09[a,b]	1.60 ± 0.12[b]	1.38 ± 0.18[a]
20:1n-9	0.56 ± 0.03[a,c]	0.63 ± 0.09[a]	0.44± 0.03[b,c]	0.42 ± 0.05[b]
20:3n-6	0.80 ± 0.03[a]	0.63 ± 0.16[a,b]	0.48 ± 0.04[b]	0.63 ± 0.04[a,b]
20:4n-6	1.15 ± 0.05[a]	0.75 ± 0.21[b]	0.56 ± 0.05[b]	0.68 ± 0.04[b]
21:0	0.15 ± 0.04[a]	0.42 ± 0.05[b]	0.39 ± 0.05[b]	0.37 ± 0.07[b]
20:5n-3	0.43 ± 0.04[a]	0.84 ± 0.27[b]	0.91 ± 0.10[b]	1.05 ± 0.12[b]
22:0	0.34 ± 0.05[a]	0.57 ± 0.05[b]	0.91 ± 0.09[c]	0.80 ± 0.09[c]
22:5n-3	0.63 ± 0.04[a]	1.02 ± 0.26[b]	1.01 ± 0.07[b]	1.13 ± 0.08[b]
24:0	0.24 ± 0.03[a]	0.36 ± 0.09[a]	0.69 ± 0.06[b]	0.68 ± 0.07[b]
∑SCFA	79.35 ± 0.94[a]	76.71 ± 2.36[a,b]	67.39 ± 2.45[b]	69.42 ± 6.24[b]
∑MCFA	479.64 ± 7.56[a]	400.98 ± 52.32[b]	367.39 ± 21.47[b]	389.03 ± 16.35[b]
∑LCFA	278.24 ± 6.93[a]	314.04 ± 25.47[a,b]	328.78 ± 20.37[b]	288.96 ± 16.80[a]

[a]Values in a row not sharing a common superscript roman letter differ, $P < 0.05$. SCFA, short-chain FA; MCFA, medium-chain FA; LCFA, long-chain FA.

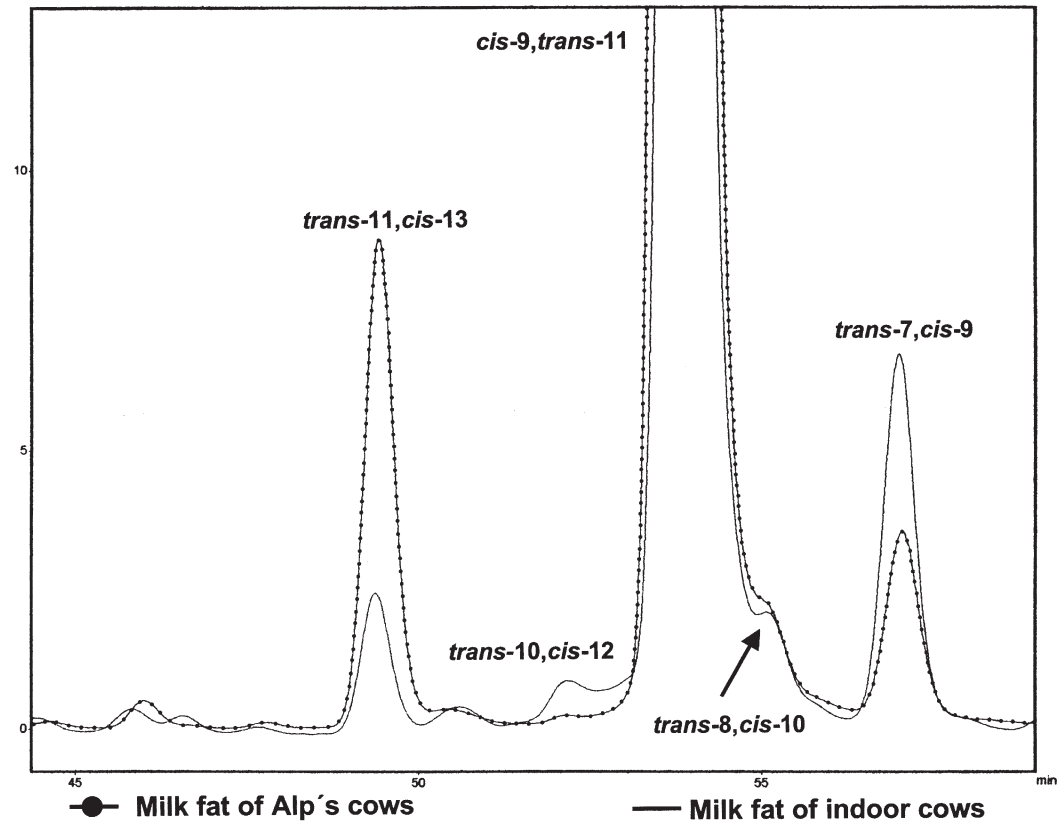


FIG. 3. Partial Ag^+-HPLC chromatogram of a milk fat from cows pastured in the Alps.

Ranking second, after the rumenic acid, was the *trans*-11,*cis*-13 CLA (from 2 to 8% of total CLA; Fig. 3). The amount of this FA per gram of fat was higher in group 4 than group 1 by a factor of 35.

DISCUSSION

FA composition of pasture. The CLA content of milk fat correlates with the FA concentration of the pasture. In a study

TABLE 5
Concentration[a] of Different CLA Isomers of the Milk Fat (values in mg/g fat)

Location	Germany, Thuringia		Switzerland, Alps	
group	1. Indoor cows	2. Organic farming	3. Different places	4. L'Etivaz
Isomers				
Σ*cis,trans/trans,cis*	3.14 ± 0.12[a]	10.15 ± 4.24[b]	24.97 ± 2.49[c]	29.84 ± 1.50[c]
11,13	0.07 ± 0.00[a]	0.85 ± 0.32[b]	1.49 ± 0.23[b]	2.49 ± 0.42[c]
10,12	0.08 ± 0.02[a]	0.06 ± 0.08[a]	0.05 ± 0.09[a]	0.07 ± 0.07[a]
9,11	2.76 ± 0.12[a]	8.72 ± 3.50[b]	22.94 ± 2.33[c]	26.7 ± 1.08[c]
7,9	0.22 ± 0.01[a]	0.20 ± 0.08[a]	0.29 ± 0.02[a,b]	0.37 ± 0.05[b]
Unidentified peaks	0.01	0.32	0.20	0.21
Σ*trans/trans*	0.35 ± 0.01[a]	1.52 ± 0.59[b]	0.93 ± 0.07[a,b]	1.31 ± 0.07[b]
12,14	0.05 ± 0.01[a]	0.30 ± 0.11[b]	0.22 ± 0.05[b]	0.32 ± 0.04[b]
11,13	0.09 ± 0.01[a]	0.52 ± 0.20[b,c]	0.37 ± 0.03[b]	0.57 ± 0.04[c]
10,12	0.05 ± 0.00[a]	0.11 ± 0.08[a]	0.07 ± 0.01[a]	0.08 ± 0.02[a]
9,11	0.08 ± 0.01[a]	0.40 ± 0.07[b]	0.12 ± 0.01[a,c]	0.17 ± 0.03[c]
8,10	0.03 ± 0.00[a]	0.05 ± 0.04[a]	0.05 ± 0.00[a]	0.06 ± 0.02[a]
7,9	0.03 ± 0.00[a]	0.12 ± 0.10[a,b]	0.07 ± 0.02[b]	0.08 ± 0.02[b]
Unidentified peaks	0.02	0.02	0.03	0.03
Σ*cis/cis*	0.06 ± 0.02[a]	0.21 ± 0.25[a]	0.03 ± 0.00[a]	0.04 ± 0.00[a]

[a]Values in a row not sharing a common superscript roman letter differ, $P < 0.05$.

including three different cow herds over a full year, the highest CLA content of milk fat was found during the summer months in the two herds grazing pasture (31). The primary FA in grass is α-linolenic acid; its content was ~50% of the total FAME (32).

The milk fat of Alpine cows had the highest PUFA content and the lowest SFA content (Fig. 1). The fodder of indoor cows consists mainly of fermented grass (silage: microbially biohydrogenated fat) and concentrates containing more SFA. Concentrates and silage contribute partially to the *de novo* synthesis of 16:0; the remainder originates from the uptake of preformed FA (33). Therefore, the milk fat of indoor cows is rich in SFA.

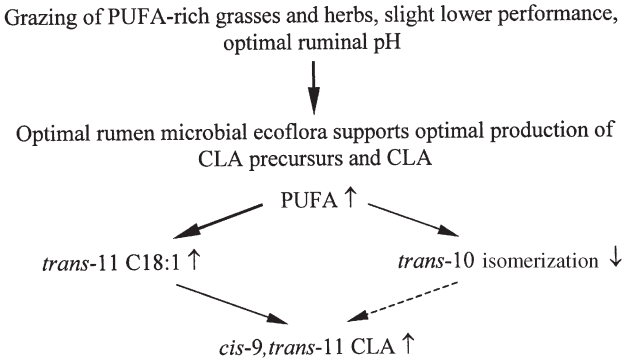
CLA and t*VA in milk fat.* Milk fat is the richest natural source of CLA. Under normal animal husbandry practice, it may contain 2.4 to 37.0 mg CLA/g fat (34). This corresponds exactly with the range for total CLA from our analysis (Fig. 2). Dhiman *et al*. (35) found that cows feeding only on pasture synthesize a CLA-richer milk fat than those grazing two-thirds or one-third pasture. Jeangros *et al*. (23) also observed an elevated CLA content in the milk of Alpine cows, which correlates with dicotyledons such as *Compositae, Rosaceae*, and *Plantaginaceae* occurring in mountain pasture. In connection with the results shown in Figure 2, it is of interest that green leaves of immature pasture plants contain more lipid extract than leaves from mature forage (36). Due to the short vegetation period, the meadows at higher altitude in the Alps are physiologically young. Furthermore, under the lower environmental temperatures typical of the highlands, plant tissues contain a higher percentage of α-linolenic acid (36). It has been suggested that feeding linseed oil (a rich source of α-linolenic acid) results in a large increase in the production of rumen *trans*-11 18:1, which can be used by the mammary gland for rumenic acid synthesis (5,37,38).

The low ruminal pH often found in high-performance cows fed concentrate-rich rations alters the microbial ecosystem to favor synthesis of *trans*-10 monoene or conjugated diene, or both (Table 3). FA with the *trans*-10 double bond inhibit mammary milk fat synthesis as well as the tissue synthesis of CLA from *t*VA by down-regulation of stearoyl-CoA desaturase 1 gene expression (39) and, accordingly, other unknown mechanisms. On the other hand, optimal ruminal fermentation in cows grazing herb-rich pasture (optimal pH, PUFA as substrate for *t*VA) minimizes the formation of *trans*-10 FA. The absence of this depressing agent maximizes the desaturation of *t*VA (40) (Fig. 4). Milk fat synthesized under these conditions is rich in CLA and relatively poor in *t*VA. Some authors described close correlations between *t*VA and *cis*-9,*trans*-11 CLA (40–42). Figure 2 shows a wide correlation between the precursor and the desaturated product. The ratio CLA/*t*VA ranged from 0.25 in group 1 to 0.52 in group 4. Evidently, a higher percentage of *t*VA is converted into *cis*-9,*trans*-11 CLA in the Alpine cows. The Δ9-desaturase acts very effectively in the mammary gland of Alpine cows (Fig. 4). More ineffective conversion ratios were found under experimental conditions using different oil supplements (43). Feeding a combination of fish oil and sunflower oil increased CLA in milk fat to >6% (mean), with individual cows exceeding 8% CLA. This was accompanied by *t*VA concentration >18%.

Piperova *et al*. (16) fed a ration supplemented with soybean oil. The cows produced a milk fat consisting of 15.6 g *t*VA/100 g FAME and 0.95 g CLA/100 g FAME, corresponding to a CLA/*t*VA ratio of 0.06. Morales *et al*. (44) confirmed that the apparent desaturation of *t*VA to CLA in the mammary gland is influenced by the oil source. Pasture oil seems to support an optimal conversion.

High rumen microbial activity in Alpine cows. Table 4 also demonstrates higher concentrations of such FA in the milk fat of pastured cows, which are unusual in plant oils. The results showed significant differences among the four groups, mainly in branched-chain FA (*iso*- and *anteiso*-15:0, *iso*- and *anteiso*-17:0, and *iso*-16:0). Furthermore, the results also support the hypothesis of a very active and specific rumen microbial ecoflora (Fig. 4). In organic dairy farming, with rations poorer in energy (starch) and richer in fiber, a more intensive activity of rumen bacteria has been suggested due to pasture feeding, as measured by a higher percentage of branched-chain FA in lipids (31,45).

CLA isomer distribution. The most interesting differences were found for the CLA isomers (Table 2). The content of *cis*,*trans*/*trans*,*cis* isomers showed marked differences from group 1 to 4 by a factor of 10. The proportion of *trans*-10,*cis*-12 CLA was very small (1 to 2% of *ct*/*tc*). Because mammals do not possess Δ12-desaturase, it follows that the *trans*-10,*cis*-12 CLA reported in ruminant milk fat and tissues originates from *trans*-10,*cis*-12 CLA that was absorbed from the intestine. Earlier publications showed that the *trans*-7,*cis*-9 is the second to most prominent CLA isomer *cis*-9,*trans*-11 in ruminant fat (11–14). The CLA isomer ranking depends on



Fatty acid distribution of milk fat	Ratio/Percentage
ratio of *trans*-C18:1/*cis*-9,*trans*-11	≈ 0.5
trans-11,*cis*-13	≈ 5% of total CLA
trans-10,*cis*-12	< 0.1% of total CLA

FIG. 4. Influence of optimal farming management on desaturation of *trans*-11 18:1 to *cis*-9,*trans*-11 CLA (*trans*-10 bonds inhibit Δ9-desaturase). Source: Reference 40.

the feeding regime. The chromatograms in Figure 3 show the impressive difference between indoor cows and Alpine cows in relation to the *trans*-11,*cis*-13 peak. Our results indicate that in the milk fat of pasture-fed cows, the second-most abundant CLA isomer is *trans*-11,*cis*-13 (Table 1). As mentioned above, plants growing under lower environmental temperatures such as in the Alps contain lipids with a higher percentage of α-linolenic acid (36), which could explain this result. α-Linolenic acid has been shown to be converted to *cis*-9,*trans*-11,*cis*-15 conjugated triene in the rumen (46). It is subsequently metabolized to *trans*-11,*cis*-15 18:2, and finally to octadecenoic acid (18:1 containing a *trans*-11, *trans*-15 or *cis*-15 bond, respectively). The pathway from *trans*-11,*cis*-15 (46, see above) to the second-most prominent isomer *trans*-11,*cis*-13 is unclear at this stage. The *trans*-11 double bond seems to be the most stable *trans*-bond found among the isomers of 18:1 and among the CLA isomers in ruminal fermentation. Thus, three different CLA isomers with a *trans*-11 double bond are generally possible: (i) *cis*-9,*trans*-11 (bacterial synthesis in rumen + tissue Δ9 desaturation), (ii) *trans*-11,*cis*-13 (bacterial origin), and (iii) *trans*-11,*trans*-13.

It is clear that *cis*-9,*trans*-11 and *trans*-11,*cis*-13 were the most abundant FA among the *cis,trans/trans,cis*-CLA, and the *trans*-11,*trans*-13 isomer was also the most abundant isomer among the *trans,trans*-CLA (Table 5). Thus, it could be shown by Ag^+-HPLC isomer analysis that milk fat synthesized under natural conditions contained, in addition to the major *cis*-9,*trans*-11 isomer, the *trans*-11,*cis*-13 isomer in a large quantity.

Overall, it can be concluded that the *trans*-11 double bond possesses a high stability during biohydrogenation of PUFA. We hypothesize that linolenic acid is an indirect precursor of *trans*-11,*cis*-13 CLA. More evidence could be provided by an infusion experiment using fistulated cows, infusing linolenic acid into the rumen and taking samples *via* duodenal fistula.

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