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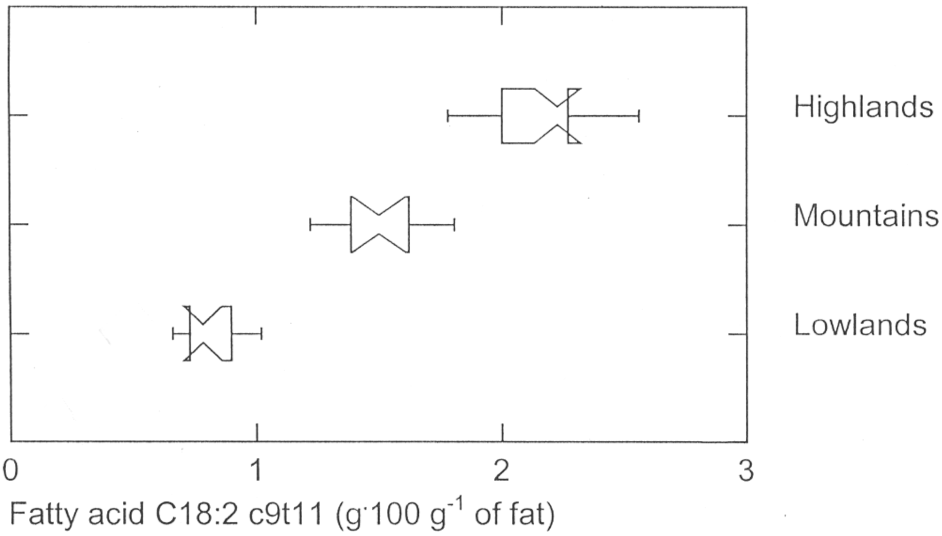
Schematic comparison of the concentration of the conjugated linoleic acid (CLA) C18:2 c9t11 in milk fat from cows fed in Lowlands, Mountains and Highlands



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Conjugated linoleic acid and trans fatty acid composition of cow's milk fat produced in lowlands and highlands

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Conjugated linoleic acids (CLA) have become very significant due to their possible anticarcinogenic as well as their antiatherogenic, immunomodulatory, growthpromoting and lean body mass-enhancing properties (MacDonald, 2000; Parodi, 1999). On the other hand, trans fatty acids are considered a possible potential risk factor for coronary heart disease (Willen *et al.* 1993; Shapiro, 1997). In milk, these two fatty acid groups are intermediate products of the biohydrogenation of linoleic acid (c9,c12-octadecadienoic acid) to stearic acid by the rumen bacterium *Butyrovibrio fibrisodvens* (Kepler *et al.* 1966). There are several isomers of CLA, of which the c9,t11-isomer accounts for more than 82 % of the total CLA concentration (Chin *et al,* 1992). Other isomers in milk fat are, e.g.: c9,c11-; t9,c11-; t9,t11-; c10,c12-; c10,t12-; t10,c12- and t10,t12-octadecadienoic acid.

The CLA concentration of milk can vary within a broad range. In 1756 milk fat samples Precht & Molkentin (1999) found a CLA content from 0·10 g/ 100 g up to 1·89 g/100 g milk fat. CLA in milk can be influenced by several factors such as animal breed (Lawless *et al.* 1999), feeding regimen: feed allowance (Stanton *et al.* 1997a), feeding dietary oils (Stanton *et* al. 1997a ; Kelly *et* al, 1998 ; Dhiman *et* al. 1999, 2000) or feeding full fat rapeseeds or soybeans (Lawless *et* al. 1998). Further determining factors are age of the animals (Stanton *et* al. 1997a; Lal & Narayama, 1984) and season (Parodi, 1977; Stanton *et* al. 1997b; Collomb & Bühler, 2000).

The influence of the botanical composition of grass on milk composition has been studied in the lowlands (altitude 600-650 m), mountains (900-1200 m) and highlands (1275-2120 m) (Project description: Jeangros *et* al. 1997). The lowland grassland is composed only of grasses and legumes and its botanical diversity is distinctly less than that of the highland permanent pastures. With rising altitude, we noticed a decrease in the proportion of grasses and an increase in numerous dicotyledonous species, particularly *Compositae, Rosaceae and Plantaginaceae* (Table 1) (Jeangros et al. 1999).

Table 1. *Botanical composition of the pastures in the regions considered*

Site	L'Etivaz 1 (highlands)		L'Etivaz 2 (highlands)			Montbovon (mountains)		Posieux (lowlands)
Altitude (m)	1400–1510	1690–1920	1275–1520	1685–1900	1870–2120	900–1210	910–1250	600–650
Exposure	W	W	S	E	E	NW, W	E, SE	± flat
Number of botanical records	7	19	11	12	6	17	14	16
Contribution of *Gramineae* (%)†	50·1 ± 1·8	37·3 ± 2·9	42·6 ± 2·2	37·5 ± 2·7	33·5 ± 3·1	61·1 ± 2·9	66·3 ± 3·0	52·5 ± 3·2
Contribution of *Leguminosae* (%)†	9·4 ± 0·8	5·7 ± 0·7	13·2 ± 0·6	10·9 ± 0·9	7·1 ± 2·0	7·8 ± 0·6	6·9 ± 1·4	47·5 ± 3·2
Contribution of *Compositae* (%)†	12·3 ± 1·7	14·0 ± 1·4	13·8 ± 1·7	17·3 ± 1·4	20·5 ± 2·4	6·4 ± 1·1	6·1 ± 1·4	0
Contribution of *Rosaceae* (%)†	3·1 ± 0·7	8·1 ± 1·0	3·2 ± 0·6	6·7 ± 0·7	7·0 ± 0·7	2·4 ± 0·4	2·6 ± 0·7	0
Contribution of *Plantaginaceae* (%)†	1·9 ± 0·8	5·4 ± 1·0	6·1 ± 0·6	4·5 ± 1·0	8·6 ± 0·9	2·6 ± 0·7	2·3 ± 0·5	0

† Mean per record ± SE.

MATERIALS AND METHODS

Forty-four milk samples were collected an the three sites from the beginning of June to mid-September 1995 (Jeangros *et al.* 1997 ; Collomb *et al.* 1999). CLA and trans fatty acids were determined by GC-FID (Collomb & Bühler, 2000). The method involved a transesteriflcation of milk fat with methanol/potassium hydroxide according to the International Dairy Federation (1999). The Separation was carried out using a 100-m long CP Sil 88 column (column diameter 0·25 mm, layer thickness 0·20µm) and the results were expressed as absolute values (g fatty acids per 100 g fat) using nonanoic acid as internal standard. Statistical evaluation of the results was done by variance analysis comparing the mean values and by the Fisher Least Square Deviation test (SYSTAT).

RESULTS AND DISCUSSION

Table 2 presents the contents of conjugated and trans fatty acids in milk fat from lowlands, mountains and highlands in the regions of Posieux, Montbovon (FR) and L'Etivaz (VD) in Switzerland as well as the corresponding milk yield and fat content. The results show a large increase in CLA and particularly in the isomer c9,t11 as a function of altitude. The milk analysed contained up to 2·87 g CLA/100 g fat, an amount considerably higher than the highest value given by Precht und Molkentin (1999) of 1·89 g/100 g fat. The c9,t11-isomer of CLA, which is regarded as the most effective in cancer prevention (MacDonald, 2000), accounts for more than 92 % of the total CLA concentration.

These results also show that apart from the well-known factors such as animal breed, feeding regimen, age of animals and season, the altitude of the production site can also influence the CLA concentration of the milk fat. This increase with an increase in the grazing altitude of the cows is likely to be related to the fodder composition (Table 1). Thus these elevated CLA values may be correlated with the non leguminous herbal dicotyledones, very often found in the mountains and highlands swards (0·0 % in lowlands, 34·2 % in the mountains and 55·6 % in the highlands) and especially with *Compositae (0·0,* 8·8 and 16·1 %, respectively), *Rosaceae (0·0,* 2·7 and 7·5 %, respectively) as well as with *Plantaginaceae (0·0,* 3·2 and 5·6 %, respectively) (Jeangros *et al.* 1999).

According to Dhiman *et al.* (1999) cows feeding only on pasture had more CLA (2·21 g) than those grazing two-thirds (1·43 g) or one-third pasture with supplemental feed (0·89 g/100 g fatty acids). It was clearly demonstrated by Kelly *et al.* (1998) and Dhiman *et al.* (2000) that the CLA concentration in milk fat can be enhanced by the addition of polyunsaturated fatty acids to the diet, especially oils rich in linoleic acid. The former authors found CLA concentrations of 1·33, 2·44 and 1·67 g/100 g milk fat from cows fed with peanut oil, sunflower oil and linseed oil, respectively. The latter authors indicated average CLA concentrations of 0·39 and 2·10, 1·58 and 1·63 g/100 g total fatty acids in control, 3·6 % soybean oil, 2·2 % linseed oil, and 4·4 % linseed oil treatments, respectively. According to Lawless *et al.* (1998) the feeding of pasture supplemented with unmolassed beet pulp (3·0 kg/d per cow), full fat rapeseeds (3·0 kg/d per cow) and full fat soybeans (3·1 kg/d per cow) resulted in milk fat CLA concentrations of 1·74, 2·23 and 2·49 g/100 g fatty acid methyl esters, respectively.

Apart from the rise in CLA concentration, the total trans fatty acid values and especially the octadecenoic acids t10 and t11 also increased as a function of altitude (Table 2). The elevated concentration of the sum of the t10 and t11 fatty acids is likely to be mainly due to that of the trans vaccenic acid (C18:1 t11) from which CLA is endogenously synthesised (Griinari et al. 2000). According to Precht and Molkentin (1996) trans vaccenic

Table 2. *Milk yield (kg/lactation), fat (g/kg) and conjugated and trans fatty acids in summer milk fat from lowlands, mountains and highlands (g fatty acids/100 g fat)*

	Lowlands 600–650 m ($n = 11$)		Mountains 900–1210 m ($n = 12$)		Highlands 1275–2120 m ($n = 21$)	
Compounds	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
Milk yield	7500		4500		4500	
fat	332 ± 8		328 ± 15		337 ± 10	
CLA C18:2 c9t11	$0{\cdot}81^{C}$	0·66 – 1·02	$1{\cdot}50^{B}$	1·22 – 1·74	$2{\cdot}18^{A}$	1·78 – 2·56
Σ CLA†	$0{\cdot}87^{C}$	0·71 – 1·08	$1{\cdot}61^{B}$	1·32 – 1·86	$2{\cdot}36^{A}$	1·92 – 2·87
C18:1 t10 + t11‡	$2{\cdot}11^{C}$	1·70 – 2·46	$3{\cdot}66^{B}$	3·08 – 4·05	$5{\cdot}10^{A}$	4·12 – 5·67
Σ trans fatty acids§	$4{\cdot}55^{C}$	3·68 – 5·24	$6{\cdot}44^{B}$	5·55 – 7·11	$8{\cdot}44^{A}$	7·07 – 9·66

CLA = conjugated linoleic acid.
† C18:2 c9t11, c9c11, t9t11.
‡ peaks not resolved.
§ C14:1 t, C16:1 t, C17:1 t, C20:1 t, C18:1 t4, t5, t6 – t8, t9, t10 + t11, t12, t13 + t14, C18:2-trans trans non-methylene interrupted diene, – t9t12, – c9t13, – c9t12, – t11c15 + t9c12.
n = number of samples analyzed.
$\bar{x}$ = mean value, A > B > C = significantly different contents, $P > 0{\cdot}05$.
Range = minimum – maximum.

acid normally represents approximately 90 % of the total of the two acids t10 and t11, which cannot be separated using our chromatographic conditions. These authors showed in feeding tests -where cows stayed in the barn or on the pasture or were fed with rape oil, rapeseed wholemeal or rapeseed pellets - that the increase in the CLA concentration in milk fat usually correlates with the concentration of the trans fatty acids.
The differences between the content in total polyunsaturated fatty acids in milk fat (4·2, 5·4 and 6·9 g/100 g) as a function of altitude prove that they are essentially due to those between the CLA (Table 2). The high value of the trans vaccenic acid in the highlands indicates an intense biohydrogenation in the rumen because the plants on which the cows are feeding are likely to be rich in polyunsaturated fatty acids. The latter are converted to trans isomers which are thermodynamically more stable than their cis isomers. In general, all methods using peak normalisation and expressing results in relative percent of the area of the analysed peaks are subjected to an overvaluation because areas of small peaks are not considered. In the current investigation, we avoided this problem by using nonanoic acid as internal standard.
In conclusion, the composition of fatty acids from milk fat in lowland and highland regions is very different due to big differences in botanical composition. The main characteristic increases concern the conjugated linoleic acid c9,t11 and the t11 vaccenic acid. The high content of t11 vaccenic acid indicates that endogenous synthesis is likely to represent an important source of CLA in milk fat of lactating cows. The plant species on which the animals graze can be considered as a medium for naturally modifying the composition of milk fat.

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