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Original article

Seasonal variation in the fatty acid composition of milk supplied to dairies in the mountain regions of Switzerland

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Abstract – The influence of typical feeds from five mountain regions of Switzerland (altitude of grass-based feed (GBF) during the summer and winter seasons: 1247 (± 465) m and 1136 (± 310) m, respectively) on the fatty acid (FA) composition of bovine milk fat was studied over one year (from May 2004 to April 2005). Compared with winter, summer milk had a significantly lower concentration of saturated FA (SFA) (–8.6%) and significantly higher contents of monounsaturated FA (MUFA) (+19.9%), polyunsaturated FA (PUFA) (+21.7%), conjugated linoleic acid (CLA) (+70.1%), and *trans* FA other than CLA (+56.7%). Summer and winter milk from mountains did not significantly differ with respect to the contents of branched, *n*-3 and *n*-6 FA. However, the content of the main *n*-3 FA (α-linolenic acid) was significantly higher in summer than in winter milk and its content was positively correlated with increasing percentages of GBF and altitude.

milk fat / fatty acid / mountain milk / winter milk / summer milk

摘要 – 瑞士山区牛乳脂肪酸组成的季节性变化。本文研究了瑞士 5 个高山地区 (海拔高度分别为 1247 ± 465 m 和 1136 ± 310 m) 夏季和冬季 (2004 年 5 月–2005 年 4 月) 典型的清草基饲料 (不同的海拔高度) 对牛乳脂肪的脂肪酸组成影响。与冬季相比,夏季牛乳脂肪中饱和脂肪酸 (–8.6%) 的含量较低, 但是单不饱和脂肪酸 (+19.9%)、多不饱和脂肪酸 (+21.7%)、共轭亚油酸 (+70.1%) 和反式脂肪酸 (+56.7%) 的含量较高。这些高山地区的牛乳,无论是冬季还是夏季在支链脂肪酸、*n*-3 和 *n*-6 脂肪酸含量上没有显著的差异。但是在主要的 *n*-3 脂肪酸 (α-亚麻酸) 的含量上,夏季牛乳显著地高于冬季牛乳,而且含量与清草基饲料比例的增加和海拔高度呈正相关。

乳脂肪 / 脂肪酸 / 山区牛乳 / 冬季牛乳 / 夏季牛乳

Résumé – Composition en acides gras du lait de montagne suisse. Variations saisonnières. L'influence d'un affouragement typique des régions de montagne suisses (cinq régions ; altitude du fourrage à base d'herbe durant les saisons estivale et hivernale : 1247 (± 465) m et 1136 (± 310) m, respectivement) sur la composition en acides gras du lait de vache a été étudiée pendant

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une année (de mai 2004 à avril 2005). Comparée à l'hiver, la matière grasse du lait d'été avait des concentrations significativement plus basses en acides gras saturés (– 8,6 %) et plus élevées en monoinsaturés (+19,9 %), polyinsaturés (+21,7 %), acides linoléiques conjugués (ALC) (+70,1 %) et en acides gras *trans* (+56,7 %, ALC non inclus). Les concentrations en acides gras ramifiés, *n*-3 et *n*-6 du lait d'été et d'hiver n'étaient pas significativement différentes. Cependant, la concentration de l'acide gras *n*-3 principal de la matière grasse du lait, l'acide α-linolénique, était significativement plus élevée dans les laits d'été que dans ceux d'hiver et était positivement corrélée avec le pourcentage de fourrage à base d'herbe et avec l'altitude.

matière grasse du lait / acide gras / lait de montagne / lait d'hiver / lait d'été

1. INTRODUCTION

The mountain regions of Switzerland are important grassland zones and therefore well suited for milk production and cheesemaking. Milk production and processing are important economic sectors in these mountain areas and have a constructive influence on other sectors such as tourism. Thus, innovative means for maintaining and improving incomes in the dairy sector are crucial for these areas. Better knowledge of the quality of milk fat composition [20] and its influencing factors could lead to the development of products in these areas with a higher added value which should also be communicated to consumers.

Different studies carried out in Austria, Germany, France and Switzerland showed that the FA composition of bovine milk from the highlands differed from that of the lowlands [4, 9, 10, 12, 25, 28, 33] and between seasons [17]. Generally, in milk fat from pasture-fed cows in the mountains, higher concentrations of the main *n*-3 FA (α-linolenic acid) were found, as well as markedly lower concentrations of SFA. Leiber et al. [27, 28] hypothesized that the increase in the α-linolenic acid content of alpine summer milk was mainly due to pasture feeding and the absence or low amounts of concentrates.

The concentration of CLA was also very high (2.51 (± 0.28) g·100 g^{-1} fat) in summer milk fat from the highlands (1275–2120 m) [12]. These high values are essentially due to pasture effects but differences in the botanical composition of grass in the mountains [4, 10] could also modify the FA composition of milk, as reported for the lowlands in the case of grass supplemented with legumes [14, 34]. Mountain pastures were characterized by a higher diversity in the botanical composition of grass than in the lowlands. Environmental conditions (temperature, more walking) which were less favorable for cows in the mountains, as well as low input feeding due essentially to grass-based feeding without or with low amounts of concentrates, could also increase the lipomobilization by cows. All these effects could explain changes in the fatty acid composition of milk fat.

Up to now, most of the studies have been carried out in a single mountain region, and rarely throughout the year [17]. Moreover, the fatty acid composition was often determined using official methods enabling one to quantify a limited number of fatty acids. The scope of this study was therefore to investigate the influence of feeding over one year in five different regions of Switzerland on the fatty acid composition of cow's milk, using a high-performance method. This study was carried out under the usual conditions of farming and management of herds, including the fraction of GBF and altitude of GBF growth.

2. MATERIALS AND METHODS

2.1. Aim and approach

During a 12-month study (from May 2004 to April 2005), bulk-tank milk

samples from 12 dairies were collected monthly. The milk delivered to the 12 dairies was produced by 278 mountain farms with cows fed a typical mountain area diet in Switzerland. In the bulk-tank milk, 220 out of the 278 farms were represented, as in 8 dairies the farmers delivered the milk daily or twice daily and in the 4 other dairies, milk was collected every 48 hours. The dairies were located in the five mountain areas of Grisons-Engadin, Grisons-Rheinwald, Sankt Gallen-Toggenburg, Lucerne-Willisau and Berne-Emmental. The altitude of GBF growth for the summer and winter seasons was 1247 (± 465) m and 1136 (± 310) m, respectively. A total of 71 bulk milk samples from the summer season (May to October) and 48 from the winter season (December to March) were analyzed. Because of changes in the diet during the transition between the two seasons the results obtained for April and November were not taken into account. Herds mainly consisted of Brown Swiss (54 (± 42)%), Swiss Fleckvieh (18 (± 20)%), Simmental (7 (± 15)%), Red Holstein (14 (± 22)%), Holstein (5 (± 8)%) and Jersey breeds (2 (± 4)%). The average milk performance per cow was 6073 (± 450) kg·305 day^{-1}.

2.2. Fodder composition

Fodder composition and milk production data were obtained from a standardized questionnaire completed by the farmers (Tab. I) and were complemented in part by estimations based on the fodder proportions indicated by the farmers and the total daily dry matter consumption of the single herds. The dry matter consumption was calculated starting from daily milk yield and cow weight. Compared with winter, summer feeding was essentially characterized by significantly higher percentages of GBF, fresh grass and roughage, as well as lower fractions of grass silage, hay and milk performance concentrates. In the two seasons, the percentage of maize silage was low (0 to 2% of the diet). Winter feeding was essentially characterized by high percentages of hay and grass silage. Three types of concentrates were fed: cereal concentrate (energy 7.1 MJ net energy lactation (NEL) per kg of fresh matter (FM), 10% crude protein), protein compensation concentrate (7.0 MJ NEL·kg^{-1} FM, 40% crude protein) and milk performance concentrate (7.1 MJ NEL·kg^{-1} FM, 16% crude protein) (Tab. I). In both seasons the main concentrate was cereal concentrate (about 5% of the diet). Milk performance concentrate in summer and winter contributed up to 2 and 6% of the diet, respectively, and protein compensation concentrate 0 and 1%, respectively.

2.3. Sampling and sample treatment

Individual bulk-tank milk samples were collected in 500-mL plastic screw-top containers after stirring the bulk tank for at least 2 min. Bronopol (Merck, Dietikon, Switzerland) was added as a preservative and the containers were stored immediately at 5 °C. The milk samples were centrifuged by 5000× g for 30 min, and the resulting creams were churned at approximately 5 °C. After the resulting molten butter had been filtered through a hydrophobic filter (1PS folded filter, Whatman, Bottmingen, Switzerland), the pure milk fat was collected and stored at –20 °C until analysis.

2.4. Methods of analysis

After dissolution of the pure milk fat in hexane, the glycerides were trans-esterified to the corresponding methyl esters of fatty acids with a solution of potassium hydroxide in methanol [21].

Fatty acid composition was analyzed by high-resolution gas chromatography (Agilent 6890, Santa Clara, CA, USA)

Table I. Mean content of fodder components in summer and winter.

Fodder	Summer		Winter		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
Returned questionnaires (%)	69	24	69	20					
Quantity of bulk milk, kg·day^{-1}	2695	1997	3161	2592					
Total ration, kg dry matter·day^{-1}·cow^{-1}	16.77	1.06	17.62	1.00	***	***	-	*	-
Percentage grass-based feeds (GBF)	92	5	81	4	***			***	+
Percentage of fresh grass	74	23	0	1	***	***	+	*	+
Percentage of grass silage	1	4	11	14	*	ns		ns	
Percentage of hay	16	17	69	14	***	***	-	ns	
Percentage of whole-crop maize silage	0	1	2	2	ns	*	-	ns	
Percentage of roughage[1]	93	4	88	3	**	***	+	ns	
Percentage of concentrates	6.8	4.3	12.5	3.3	**	***	-	ns	
Cereal concentrate (%)[2]	4.8	3.5	4.7	1.9	***	***	-	***	-
Protein compensation concentrate (%)[3]	0.3	0.4	1.1	0.8	ns	***	-	ns	
Milk performance concentrate (%)[4]	1.5	1.9	6.4	2.6	***	***	-	***	-
Altitude of feed grasses growth, m	1247	465	1136	310	***	***	+	***	+
Altitude of hay growth, m	1049	268	1135	298	ns	**	-	***	+
Altitude of cowshed, m	1247	465	1136	310	***	***	+	***	+

Summer = May to October; Winter = December to March; Group = summer versus winter groups; GBF = grass-based feed; AGBF = altitude of GBF; $\bar{x}$ = mean value; s_x = standard deviation; P = probability: *: $P \leq 0.05$; **: $P \leqslant 0.01$; ***: $P \leq 0.001$; ns = non signifiant; ± = positively or negatively correlated with increasing percentage or altitude of GBF.
[1] Essentially constituting fresh, dried or ensiled grass and whole-crop maize but also fed straw, sugar beet pulp, fodder beets, potatoes and some other fiber-rich fresh feed.
[2] Energy 7.1 MJ NEL (net energy lactation), 10% crude protein.
[3] 7.0 MJ NEL, 40% crude protein.
[4] 7.1 MJ NEL, 16% crude protein.

with flame ionization detection according to Collomb and Bühler [8]. The fatty acids were separated on a capillary column, CP-Sil 88 (100 m × 0.25 mm i.d., 0.20 μm; Varian BV, Middleburg, Netherlands), and quantified using nonanoic acid as an internal standard. The results were expressed as g fatty acids per 100 g fat. The pure methylesters of fatty acids, including CLA, were obtained from Matreya Inc., Pleasant Gap, PA, USA.

CLA isomers were analyzed by silver-ion (Ag$^+$)-HPLC (Agilent LC 1100, Santa Clara, CA, USA) equipped with a photodiode array detector (234 nm) using three ChromSpher Lipid columns in series (stainless steel, 250 × 4.6 mm, 5 μm particle size; Chrompack, Middleburg, Netherlands) according to Collomb et al. [12]. The solvent consisted of UV-grade hexane with 0.1% acetonitrile and 0.5% diethyl ether (flow rate 1 mL·min^{-1}), prepared fresh daily. The injection volume was 10 μL, corresponding to < 250 μg lipid. The HPLC areas for $t7c9 + t8c10 + c9t11$ (t = trans, c = cis) were added and used for comparison of the peak area of the three isomers from the GC chromatogram. The results were expressed as absolute values as mg·g^{-1} fat.

2.5. Statistical analyses

The mean values and standard deviations of FA in milk fat from summer and winter were calculated. Analysis of covariance was performed with season as factor, and percentage and altitude of GBF as covariate. Systat for Windows version 11 [1] was used for all calculations.

3. RESULTS AND DISCUSSION

3.1. Groups of fatty acids

The concentration of milk SFA was lower in the summer than in the winter season (57.95 and 63.41 g·100 g^{-1} fat, respectively) (Tab. II and Fig. 1). The ratio between roughage and concentrates generally affects the production of acetic acid in the rumen for fatty acid synthesis de novo, but in the current study this ratio is not very different between the two seasons. This synthesis can also be repressed by high levels of *trans* C18:1 FA derived either from the diet or biohydrogenation of PUFA in the rumen [38]. In the current study, the concentration of the sum of the FA C12, C14 and C16 decreased from 41.09 g·100 g^{-1} fat in milk fat in winter to 34.94 g·100 g^{-1} fat in milk fat in summer. This is an important benefit of summer milk for human health, since it is now known that only C12, C14 and C16 adversely affect plasma LDL levels [6, 30]. The concentration of SFA was also negatively correlated with increasing percentages of GBF ($r = 0.76$, $P < 0.001$) and altitude ($r = 0.39$, $P < 0.001$) (Tab. II, Figs. 2 and 3). These results can be explained by the significantly increased percentage of grass with increasing fractions of GBF offered to cows during the summer season (Tab. I), which decreased the concentration of milk SFA [16, 24]. Also, the feeding of grass conservation products (hay or silage) in the winter season essentially increased

the SFA content of milk fat [18]. Other authors [28,39] also observed that the proportion of short- and medium-chain SFA was found to be markedly lower in milk and milk products originating from cows grazing on high alpine pastures. The decreased concentration of SFA at high altitudes confirms the observations of Collomb et al. [9] (600–650 m: 58.90 g·100 g^{-1} fat; 900–1210 m: 54.70 g·100 g^{-1} fat; 1275–2120 m: 52.71 g·100 g^{-1} fat).

Summer milk was richer in MUFA and PUFA than that of winter (Tab. II and Fig. 1), due in part to the higher dietary intake of PUFA from summer feeds (Tab. I). Increasing contents of MUFA and PUFA with increasing percentages of GBF and altitude (Tab. II, Figs. 2 and 3) were also essentially due to increasing percentages of fresh grass (Tab. I) [16, 24]. Bugaud et al. [4] suggested moreover that the higher proportion of PUFA in milk fat from the mountains compared with the lowlands may be related to a lower rate of ruminal biohydrogenation in animals in the mountains. Differences in botanical composition of grass in the mountains compared with the lowlands [4, 10], which modify the bacterial population in the rumen, and lipid mobilization of unsaturated FA, due to energy shortage in the cows, could explain the particularly low content of milk SFA and high content of milk MUFA and PUFA from cows grazing in the highland pastures. In mountain pastures of Switzerland, higher altitudes were associated with a reduction in the *Gramineae* (60 to 25%) and an increase in dicotyledonous species (27 to 43%), particularly *Compositae*, *Rosaceae*, *Cyperaceae* and *Plantaginaceae* [4,9,10].

The concentration of total *trans* FA (other than CLA) in milk was significantly higher during the summer season compared with winter (Tab. II and Fig. 1). In a previous study [9] we found similar mean values at a comparable altitude in the summer season and also increasing

Table II. Mean content (g·100 g^{-1} milk fat) of groups of fatty acids in summer ($n = 71$) and winter ($n = 48$) milk.

Σ Fatty acids	Summer		Winter		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
Σ short chain[1]	8.85	0.62	9.64	1.45	ns	ns		**	-
Σ medium chain[2]	40.39	2.75	46.42	2.12	***	*	-	***	-
Σ long chain[3]	40.87	3.45	33.95	2.56	***	**	+	***	+
Σ saturated[4]	57.95	2.53	63.41	1.46	***	***	-	***	-
Σ C12, C14, C16	34.94	2.72	41.09	1.81	***	***	-	***	-
Σ C18:1[5]	24.07	2.16	19.56	1.47	***	**	+	***	+
Σ C18:2[6]	4.14	0.58	3.20	0.33	***	***	+	***	+
Σ unsaturated[7]	32.04	2.61	26.67	1.61	***	***	+	***	+
Σ monounsaturated[8]	26.56	2.11	22.16	1.35	***	**	+	***	+
Σ polyunsaturated[9]	5.45	0.72	4.48	0.40	**	***	+	***	+
Σ branched[10]	2.54	0.23	2.29	0.12	ns	***	+	*	+
Σ C18:1 t[11]	4.93	0.90	3.14	0.59	***	***	+	***	+
Σ C18:2 t without CLA t[12]	1.13	0.20	0.74	0.11	***	***	+	ns	
Σ C18:2 t with CLA[13]	2.65	0.56	1.62	0.23	***	***	+	ns	
Σ CLA[14]	1.65	0.45	0.97	0.16	***	***	+	ns	
Σ $trans$ without CLA[15)]	6.31	1.13	4.03	0.69	***	***	+	**	+
Σ $trans$ with CLA[16)]	7.83	1.49	4.91	0.82	***	***	+	**	+
Σ n-3[17]	1.65	0.30	1.37	0.16	ns	***	+	**	+
Σ n-6[18]	2.15	0.27	2.27	0.28	ns	***	-	***	+
Σ n-3 / Σ n-6	0.77	0.15	0.61	0.11	ns	***	+	***	+

Summer = May to October; Winter = December to March; Group = summer versus winter group; GBF = grass-based feed; AGBF = altitude of GBF; ± = positively or negatively correlated with increasing percentage or altitude of GBF; n = number of samples; Σ = sum of the concentrations; CLA = conjugated linoleic acid; $\bar{x}$ = mean value; s_x = standard deviation; P = probability: *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ns = non significant; t = $trans$; c = cis; NMID = non-methylene-interrupted diene; MID = methylene-interrupted diene.

[1] C4, C5, C6, C7, C8, C10, C10:1.

[2] C12, C13 iso, C13 $aiso$, C12:1 c+ C13, C14 iso, C14, C15 iso, C14:1 t, C15 $aiso$, C14:1 c, C15, C16 iso, C16, C17 iso, C16:1 t, C17 $aiso$, C16:1 c.

[3] C17, C18 iso, C17:1 t, C18 $aiso$, C18, Σ C18:1, C19, Σ C18:2, C20, C20:1 t, C18:3 $c6c9c12$, C20:1 $c5$, C20:1 $c9$, C20:1 $c11$, C18:3 $c9c12c15$, C20:2 cc (n-6), C22, C20:3 (n-6), C20:3 (n-3), C20:4 (n-6), C20:5 (EPA) (n-3), C22:5 (DPA) (n-3), C22:6 (DHA) (n-3).

[4] C4, C5, C6, C7, C8, C10, C12, Σ branched (iso + $aiso$), C14, C15, C16, C17, C18, C19, C20, C22.

[5] C18 :1 $-t4$, $-t5$, $-t6$-8, $-t9$, $-t10$-11, $-t12$, $-t13$-14 + $-c6$-8, $-c9$, $-c11$, $-c12$, $-c13$, $t16$ + $c14$.

[6] C18:2 $-tt$NMID, $-t9t12$, $-c9t13$ + $-t8c12$, $-c9t12$ + $-c,c$-MID + $-t8c13$, $-t11c15$ + $-t9c12$, $-c9c12$, $-c9c15$, $-c9t11$ + $-t8c10$ + $-t7c9$, $-t11c13$ + $-c9c11$, $t9t11$.

[7] C10:1, C14:1 ct, C16:1 ct, C17:1 t, Σ C18:1, Σ C18:2, C20:1 t, C18:3 $c6c9c12$, C20:1 $c5$, C20:1 $c9$, C20:1 $c11$, C18:3 $c9c12c15$, C18:2 $c9t11$ + $t8c10$ + $t7c9$, C18:2 $t11c13$ + $c9,c11$, C18:2 $t9t11$, C20:2 c,c (n-6), C20:3 (n-6), C20:3 (n-3), C20:4 (n-6), C20:5 (EPA) (n-3), C22:5 (DPA) (n-3), C22:6 (DHA) (n-3).

[8] C10:1, C14:1 ct, C16:1 ct, C17:1 ct, Σ C18:1, C20:1 t, C20:1 $c5$, C20:1 $c9$, C20:1 $c11$.

[9] Σ C18:2, C18:3 $c6c9c12$, C18:3 $c9c12c15$, C20:2 c,c (n-6), C20:3 (n-3), C20:4 (n-6), C20:5 (EPA) (n-3), C22:5 (DPA) (n-3), C22:6 (DHA) (n-3).

[10)] C13 iso + $aiso$, C14 iso, C15 iso + $aiso$, C16 iso, C17 iso + $aiso$, C18 iso + $aiso$.

[11] C18:1 $t4$, C18:1 $t5$, C18:1 $t6$-8, C18:1 $t9$, C18:1 $t10$-11, C18:1 $t12$, C18:1 $t13$-14 + $c6$-8.

[12] C18:2 $-tt$NMID, $-t9t12$, $-c9t13$ + $-t8c12$, $-c9t12$ + $-c,c$-MID + $-t8c13$, $-t11c15$ + $-t9c12$, $-t11c13$ + $-c9c11$.

[13] Σ C18:2 t without CLA + CLA $-c9t11$ + $-t8c10$ + $-t7c9$, $-t11c13$ + $-c9c11$, $-t9t11$.

[14] C18:2 $-t12t14$, $-t11t13$, $-t10t12$, $-t9t11$, $-t8t10$, $-t7t9$, $-t6t8$, $-ct$ 12, 14, $-t11c13$, $-c11t13$, $-t10c12$, $-c9t11$, $-t8c10$, $t7c9$.

[15] C14:1 t, C16:1 t, C17:1 t, C20:1 t, Σ C18:1 t, Σ C18:2 t without CLA.

[16] Σ t without CLA + CLA $-c9t11$ + $-t8c10$ + $-t7c9$, $-c9c11$, $-t9t11$.

[17] C18:2 $t11c15$ + C18:2 $c9c15$, C18:3 $c9c12c15$, C20:3 (n-3), C20:5, C22:5 and C22:6.

[18] C18:1 $t12$, C18:1 $c12$, C18:2 $t9t12$, C18:2 $c9t12$ + c,c-MID + $t8c13$, C18:2 $c9c12$, C18:3 $c6c9c12$, C20:2 cc, C20:3 (n-6) and C20:4 (n-6).
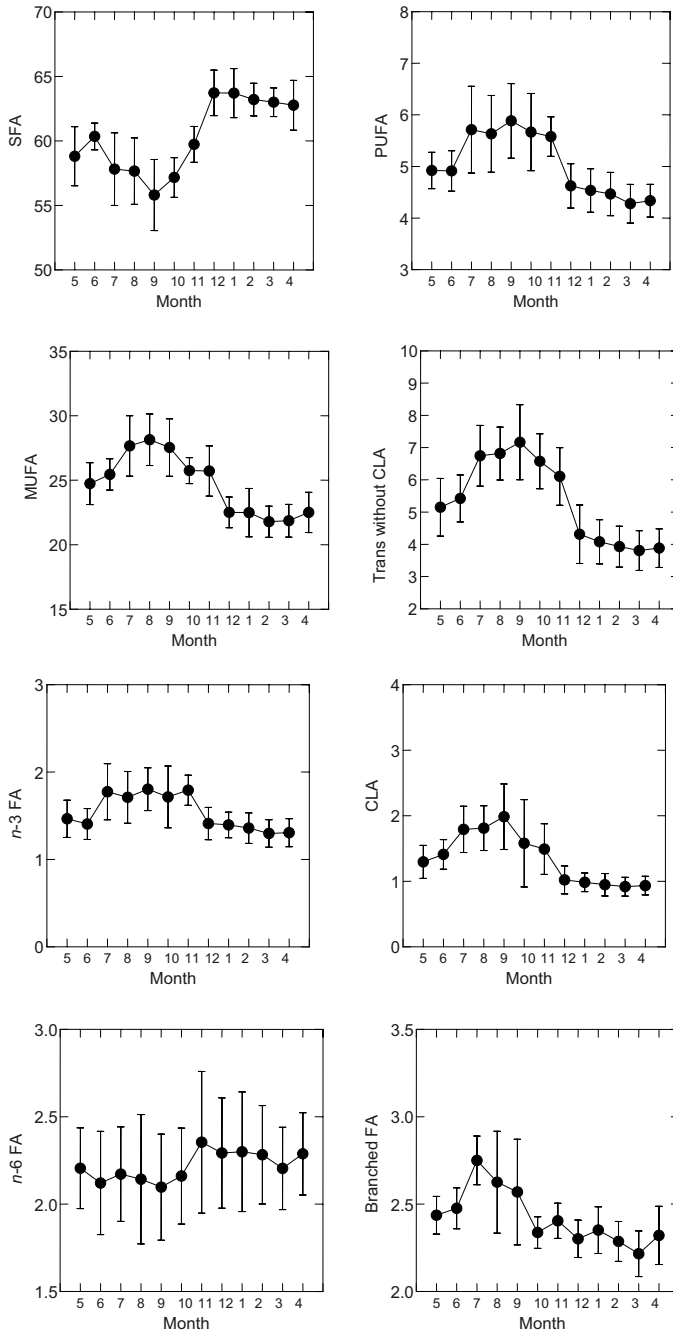
Figure 1. Concentrations (g·100 g^{-1} milk fat) of different groups of FA in milk fat over one year (I = standard deviation).
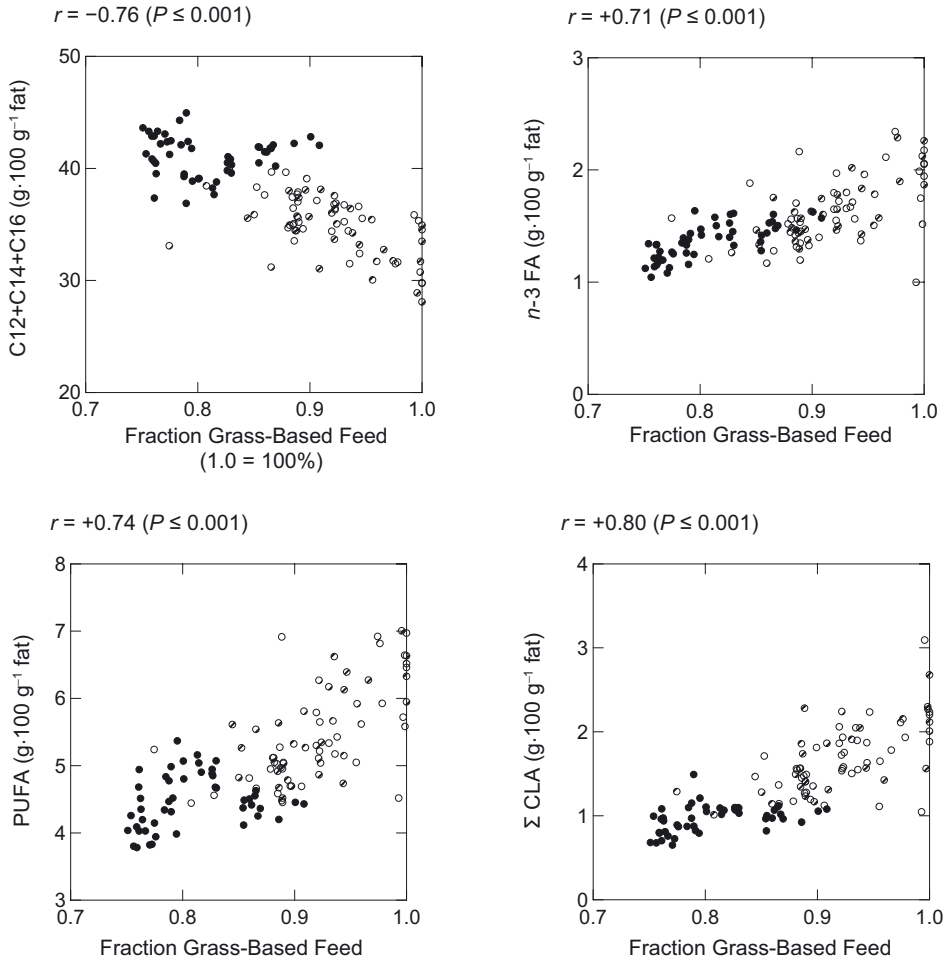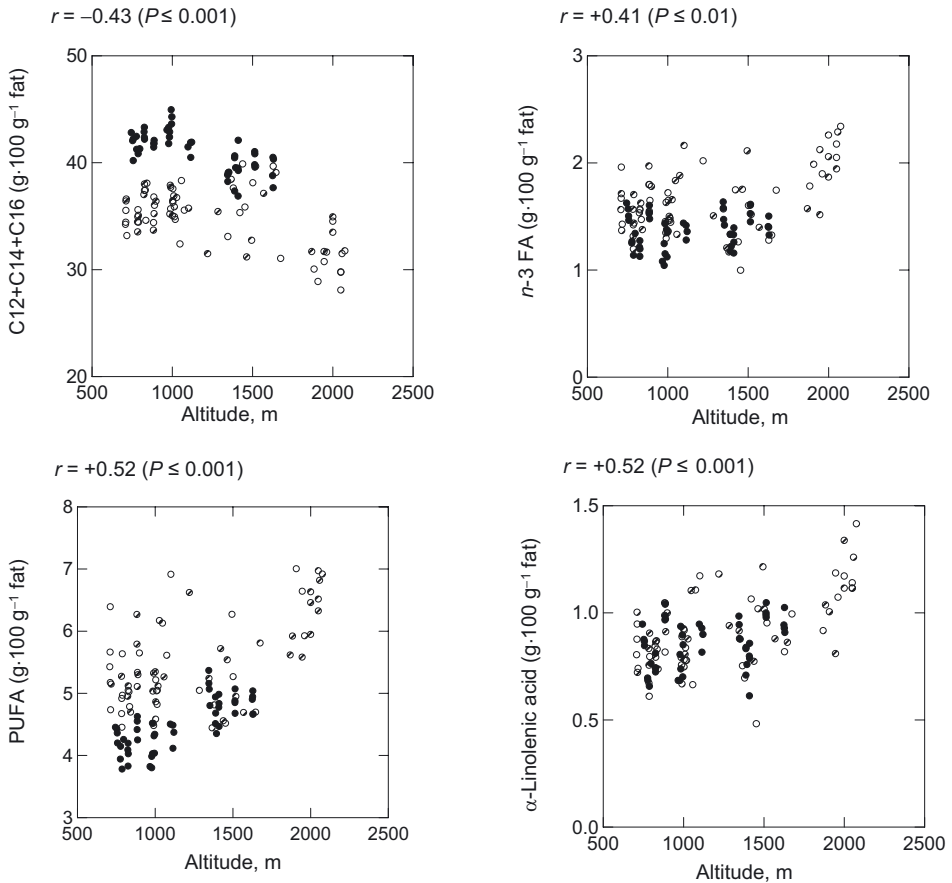
Figure 2. Influence of percentage of grass-based feed (GBF) on the content (g·100 g^{-1} milk fat) of the most important health-beneficial fatty acid groups of mountain milk (r = correlation coefficient) (○ summer; ● winter).

mean values with increasing altitudes (600–650 m: 4.55 g·100 g^{-1} fat; 900–1210 m: 6.44 g·100 g^{-1} fat; 1275–2120 m: 8.44 g·100 g^{-1} fat). According to Precht and Molkentin [31], the mean contents of *trans*-C18:2 FA other than CLA and *trans*-C18:1 FA amounted to 0.63 and 3.62 g·100 g^{-1} fatty acid methyl ester (FAME) (n = 1756), respectively. Furthermore, three feeding phases, barn (n = 927),

transition (n = 236) and pasture (n = 593), exhibited *trans*-C18:2 and *trans*-C18:1 contents of 0.46, 0.66 and 0.87 g·100 g^{-1} FAME, and 2.65, 3.80 and 5.08 g·100 g^{-1} FAME, respectively. Our results are in accordance with these reported values. Higher GBF and altitude also gave higher values of *trans* FA. Increased intake of substrates (linoleic or α-linolenic acids) due to higher percentages of grass with

Figure 3. Influence of altitude on the content (g·100 g^{-1} milk fat) of the most important health-beneficial fatty acids or groups of fatty acids in mountain milk (r = correlation coefficient) (○ summer; ● winter).

increasing fractions of GBF, as well as altitude (Tab. I), could explain the higher content of *trans* FA in the summer period [15]. It is moreover possible that cows receiving grass from mountainous areas have different bacterial populations inhabiting the rumen. Hence, such changes may encourage the growth of bacteria which do not undertake the final step of biohydrogenation. The work of Latham et al. [26], who reported changes in the amount of *Butyrivibrio fibrosolvens* inhabiting the rumen from 25% of total isolates in a high roughage

diet to 2.3% in a low roughage diet, supports this latest proposal.

The concentration of milk fat CLA in summer was higher than in the winter season (Tab. II and Fig. 1). These values are similar to previously reported values [7, 16, 18, 24, 29]. With an increased fraction of GBF (Tab. II and Fig. 2), the CLA content also increased. According to Stanton et al. [32], higher intakes of fresh grass increased the CLA content of milk, a finding also reported by Kelly et al. [23, 24] and Dhiman et al. [16], who also found that

the CLA content of milk increased in cows when moving from preserved to fresh grass diets. The concentration of CLA was not positively correlated with altitude. This result is not in accordance with our previous results [9] but supports the work of Leiber et al. [27,28], who found no increase in the CLA content when the same cows received alpine instead of lowland feeds.

The concentrations of branched FA found in the current study did not significantly differ between summer and winter or with increasing percentages of GBF or with altitude (Tab. II and Fig. 1). The branched FA are mostly synthesized by rumen bacteria [26] and then transferred to milk. Increasing forage-to-concentrate ratios in the diet was likely to enhance dietary neutral detergent fiber (NDF) content and thus to result in a higher branched FA content [13,35]. In the current study the decreased percentage of hay and concentrates combined with the increased percentages of fresh grass with increasing fractions of GBF, as well as decreasing percentages of cereal concentrates and increasing portions of fresh grass with altitude (Tab. I), could result in a higher content of NDF in the diet.

Although the content of *n*-3 FA in milk fat was higher in summer than in winter, it did not differ significantly (Tab. II and Fig. 1). Hauswirth et al. [20] found higher concentrations of *n*-3 FA in alpine cheeses than in Emmental or Cheddar, and spoke of an alpine paradox. The values obtained by these authors in mountain areas (1.5 g·100 g^{-1} FAME) were similar to our reported values. In the current study, the *n*-3/*n*-6 ratio in milk fat was optimal in both seasons (between 1:1 and 1:2, quotient 0.61 to 0.77), a recommended ratio of < 1:5 being considered highly valuable from a nutritional point of view. The increasing concentration of milk *n*-3 FA with increasing percentages of GBF and altitude (Tab. II, Figs. 2 and 3) found in the current study is essentially in agreement with

increasing percentages of grass in the diet (Tab. I) [16, 24].

3.2. Individual fatty acids

Compared with winter, summer milk had significantly lower contents of the C6 to C16 SFA, higher contents of stearic acid (C18:0), most C18:1 *trans* FA including *trans* vaccenic acid (*t*VA: C18:1 *t*11), oleic acid (C18:1 *c*9), most C18:2 *trans* FA, and α-linolenic acid (C18:3 *c*9*c*12*c*15) (Tab. III). The concentrations of linoleic acid (C18:2 *c*9*c*12) and of the main long-chain *n*-3 FA (C20:5 (EPA), C22:5 (DPA) and C22:6) did not differ significantly between the seasons.

A decrease in the concentrations of endogenous individual SFA in milk fat from cows on pastures compared with winter feeding has already been observed [16, 18, 29]. Conversely, the use of cereal grain concentrates generally resulted in significant increases in endogenous SFA [37]. The significantly higher concentration of stearic acid in milk fat from summer compared with winter found in this study (Tab. III) is due to extensive biohydrogenation of feed unsaturated FA in the rumen [19].

The higher content of oleic acid in milk from summer compared with winter (Tab. III) could be the result of extensive partial biohydrogenation of PUFA from feeds in the rumen, from the use of concentrates rich in oleic acid as well as from lipid mobilization due to energy shortage. The observed increase in the content of oleic acid with altitude (Tab. III) was also found by Bugaud et al. [4], who hypothesized that the decrease in temperature with altitude or greater degree of free movement of the cows on mountain pastures may induce an increase in the concentration of oleic acid in milk due to increased lipid mobilization.

The non-significant differences in linoleic acid with season support the work

of Lock and Garnsworthy [29], who found that linoleic acid fluctuated monthly but did not vary with season. The higher content of α-linolenic acid in milk during the summer season compared with winter (Tab. III) had been already observed [5]. The small number of results obtained with diets rich in hay [2] showed levels of milk fat α-linolenic acid above 1.3 g·100 g^{-1} FAME. According to Leiber et al. [27, 28], increased α-linolenic acid content in alpine summer milk could be mainly due to pasture feeding and the absence or low fractions of concentrates. The positive correlations found between the fractions of GBF as well as altitude (Tab. III and Fig. 3) and the content of α-linolenic acid in milk confirm this hypothesis. These effects could be amplified by specific body fat mobilization in cows with alpine-specific hypoxia as well as reduced ruminal biohydrogenation due to energy shortage or secondary plant ingredients such as polyphenols and terpenoids that inhibit hydrogenating microorganisms in the rumen. Very few experiments [15] have shown the effect of grass silage on milk fat composition. Results from a review [5] suggested that milk FA produced from diets based on more than 58% silage contained more myristic (10 to 15%) and palmitic (34 to 40%) acids, and less oleic (18 to 25%), linoleic (0.6 to 2.0%) and α-linolenic (0.3 to 0.7%) acids than milk produced on pastures.

The higher content of the combined FA C18:2 *t*11*c*15 and *t*9*c*12 in summer milk fat than in winter could be attributed to the fermentation process in the rumen of the cow, leading to a higher concentration of *t*11*c*15 FA. It is well known that the pathway for the biohydrogenation of α-linolenic acid (C18:3 *c*9*c*12*c*15) in the rumen involves an initial isomerization into a conjugated triene (C18:3 *c*9*t*11*c*15), followed by a reduction of double bonds at carbons 9, 15 and 11 to yield the FA C18:2

*t*11*c*15, C18:1 *t*11 and C18:0, respectively [36].

The C14:1/C14:0 quotient is the best indicator of Δ^9-desaturase activity because all the C14:0 in milk fat is produced via de novo synthesis in the mammary gland; consequently, desaturation is the only source of C14:1. Increased values indicate higher Δ^9-desaturase activity. Our values averaged over the seasons (C14:1/C14:0 FA) did not differ with the season (in summer: 0.084 (± 0.007); in winter: 0.083 (± 0.007)). Nevertheless, the quotient was positively correlated with altitude ($P \leqslant 0.001$) but not with increasing percentages of GBF in the diet. However, the variation in the Δ^9-desaturase activity between herds and animals was very large.

3.3. CLA isomers

Except for the *t*8*t*10, *t*7*t*9, *t*6*t*8 and *t*11*c*13 isomers, the concentrations of all other CLA isomers were significantly higher in summer milk than in milk from the winter season (Tab. IV). Also, a trend towards higher contents of the isomer *t*11*c*13 in summer milk than in winter milk was shown. In both types of milk the concentration of the CLA isomer *c*9*t*11 was highest, followed by the *t*11*c*13 and *t*7*c*9 isomers. The concentration of the *c*9*t*11 isomer in summer and winter milk fat corresponded to 85.2 and 83.2% of all CLA isomers, respectively.

As in the current study, higher concentrations of the most important CLA isomers were generally observed in summer [11]. The contents of the CLA isomers in summer milk fat found in the current study were similar to those found by Collomb et al. [12] at a similar altitude. The trend was that the content was highest when fresh pasture was plentiful, and decreased throughout the grazing season [29]. On the contrary, in this study the CLA content increased during the pasture feeding season

Table III. Mean content (g·100 g^{-1} milk fat) of individual fatty acids in summer ($n = 71$) and winter ($n = 48$) milk.

Σ Fatty acids	Summer		Winter		Significance (P)				
	$\bar{x}$	s$_x$	$\bar{x}$	s$_x$	Group	GBF %/±		AGBF /±	
FA concentrations which changed significantly between summer and winter									
C6	1.93	0.13	2.15	0.06	***	***	-	***	-
C7	0.01	0.01	0.02	0.00	*	ns		***	-
C8	1.07	0.11	1.21	0.05	***	***	-	***	-
C10	2.19	0.29	2.58	0.19	***	*	-	***	-
C10:1	0.26	0.03	0.31	0.03	***	ns		***	-
C12	2.41	0.31	2.83	0.24	***	*	-	***	-
C12:1 c + C13	0.13	0.02	0.14	0.02	***	ns		***	-
C13 *iso*	0.04	0.01	0.03	0.00	***	***	+	*	+
C13 *aiso*	0.07	0.01	0.08	0.01	**	ns		***	-
C14 *iso*	0.13	0.02	0.14	0.02	***	ns		***	+
C14	8.89	0.77	10.15	0.44	***	*	-	***	-
C14:1 c	0.75	0.09	0.84	0.10	**	ns		***	-
C15	1.08	0.08	1.09	0.10	***	***	+	***	-
C16	23.64	1.76	28.12	1.25	***	***	-	***	-
C16:1 t	0.20	0.05	0.10	0.02	***	***	+	ns	
C16:1 c	1.06	0.08	1.14	0.14	*	ns		***	-
C17 *iso*	0.38	0.03	0.31	0.02	***	***	+	ns	
C17:1 t	0.02	0.01	0.00	0.01	*	***	+	ns	
C18	9.84	0.88	8.50	0.84	***	ns		***	+
C18:1 t9	0.27	0.03	0.24	0.04	***	ns		***	+
C18:1 t10 + t11(tVA)	3.66	0.82	2.08	0.42	***	***	+	**	+
C18:1 t13-14 + c6-8	0.60	0.08	0.47	0.09	***	ns		ns	
C18:1 c9	18.15	1.59	15.51	1.02	***	ns		***	+
C18:1 t16 + c14	0.31	0.04	0.24	0.04	***	*	+	*	+
C18:2 ttNMID	0.16	0.03	0.10	0.02	***	***	+	ns	
C18:2 t9t12	0.01	0.01	0.00	0.01	*	ns		ns	
C18:2 c9t13 + (t8c12)	0.23	0.03	0.15	0.02	***	***	+	ns	
C18:2 c9t12 + (cc-MID + t8c13)	0.26	0.02	0.23	0.02	***	ns		***	+
C18:2 t11c15 + t9c12	0.47	0.13	0.26	0.06	**	***	+	ns	
C18:3 c6c9c12	0.02	0.01	0.02	0.00	*	*	+	ns	
C18:3 c9c12c15	0.92	0.18	0.85	0.11	**	***	+	***	+
C20	0.17	0.02	0.16	0.02	*	**	+	***	+
C20:1 t	0.03	0.01	0.03	0.00	***	**	+	*	+
C20:1 c9	0.15	0.02	0.13	0.01	**	ns		***	+
C20:2 cc (*n*-6)	0.02	0.01	0.03	0.00	*	ns		*	+
C20:3 (*n*-6)	0.05	0.01	0.05	0.01	**	***	-	***	+
C20:3 (*n*-3)	0.02	0.01	0.03	0.01	***	ns		ns	

Table III. Continued.

Σ Fatty acids	Summer		Winter		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
FA concentrations which did not change between summer and winter									
C4	3.36	0.22	3.55	0.20	ns	*	-	*	+
C5	0.04	0.01	0.04	0.01	ns	ns		ns	
C14:1 t	0.00	0.01	0.00	0.00	ns	ns		ns	
C15 iso	0.28	0.04	0.25	0.02	ns	***	+	**	+
C15 aiso	0.52	0.06	0.46	0.03	ns	***	+	**	+
C16 iso	0.26	0.03	0.26	0.02	ns	ns		***	+
C17	0.61	0.05	0.58	0.04	ns	***	+	***	+
C17 aiso	0.55	0.09	0.48	0.03	ns	***	+	ns	
C18 iso	0.07	0.01	0.06	0.01	ns	ns		*	+
C18 aiso	0.25	0.03	0.22	0.03	ns	***	+	ns	
C18:1 t4	0.02	0.01	0.01	0.01	ns	ns		***	+
C18:1 t5	0.01	0.01	0.01	0.01	ns	ns		***	+
C18:1 t6-8	0.16	0.04	0.14	0.04	ns	ns		***	+
C18:1 t12	0.20	0.04	0.19	0.05	ns	ns		**	+
C18:1 c11	0.51	0.07	0.48	0.05	ns	ns		*	+
C18:1 c12	0.12	0.02	0.15	0.04	ns	***	-	***	+
C18:1 c13	0.05	0.01	0.05	0.01	ns	ns		ns	
C18:2 c9c12	1.38	0.22	1.50	0.20	ns	***	-	***	+
C18:2 c9c15	0.03	0.01	0.04	0.01	ns	ns		**	-
C18:2 t11c13 + c9c11	0.08	0.03	0.05	0.02	ns	***	+	*	+
C18:2 t9t11	0.02	0.01	0.03	0.01	ns	ns		ns	
C19	0.10	0.02	0.09	0.01	ns	ns		ns	
C20:1 c5	0.01	0.01	0.00	0.01	ns	ns		ns	
C20:1 c11	0.04	0.01	0.05	0.01	ns	*	-	*	+
C20:4 (n-6)	0.08	0.02	0.10	0.01	ns	***	-	ns	
C20:5 (EPA) (n-3)	0.08	0.01	0.08	0.01	ns	*	+	**	-
C22	0.07	0.01	0.07	0.01	ns	ns		***	+
C22:5 (DPA) (n-3)	0.11	0.01	0.10	0.01	ns	*	+	ns	
C22:6 (DHA) (n-3)	0.01	0.01	0.01	0.01	ns	ns		ns	
Quotient C14:1/C14	0.084	0.007	0.083	0.007	ns	ns		***	+

Summer = May to October; Winter = December to March; Group = summer versus winter group; GBF = grass-based feed; AGBF = altitude of GBF; ± = positively or negatively correlated with increasing percentage or altitude of GBF; n = number of samples; Σ = sum of the concentrations; CLA = conjugated linoleic acid; t = trans; c = cis; tVA = trans vaccenic acid; NMID = non-methylene-interrupted diene; MID = methylene-interrupted diene; $\bar{x}$ = mean value; s_x = standard deviation; P = probability: *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ns = non significant.

Table IV. Mean content (mg·g^{-1} fat) of conjugated linoleic acid (CLA) isomers in summer ($n = 71$) and winter ($n = 48$) milk.

CLA	Summer		Winter		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
C18:2 $t12t14$	0.16	0.04	0.08	0.02	***	***	+	***	+
C18:2 $t11t13$	0.33	0.09	0.17	0.05	***	***	+	***	-
C18:2 $t10t12$	0.03	0.01	0.03	0.01	**	***	-	***	+
C18:2 $t9t11$	0.15	0.03	0.13	0.01	*	**	+	ns	
C18:2 $t8t10$	0.02	0.01	0.02	0.01	ns	*	-	***	+
C18:2 $t7t9$	0.09	0.02	0.09	0.02	ns	ns		***	+
C18:2 $t6t8$	0.03	0.02	0.05	0.01	ns	**	-	***	+
C18:2 c/t 12, 14	0.05	0.01	0.03	0.01	***	***	+	***	-
C18:2 $t11c13$	0.84	0.33	0.51	0.19	ns	***	+	**	+
C18:2 $c11t13$	0.03	0.01	0.02	0.01	**	***	+	ns	
C18:2 $t10c12$	0.02	0.01	0.02	0.01	***	***	-	***	+
C18:2 $c9t11$	14.03	4.02	8.05	1.34	***	***	+	ns	
C18:2 $t8c10$	0.24	0.06	0.12	0.02	***	***	+	*	+
C18:2 $t7c9$	0.43	0.08	0.37	0.06	***	ns		***	+
CLA $t11c13$ / CLA $t7c9$	1.90	0.60	1.40	0.48	ns	***	+	ns	
Σ CLA	16.46	4.54	9.68	1.62	***	***	+	ns	

Summer = May to October; Winter = December to March; Group = summer versus winter group; GBF = grass-based feed; AGBF = altitude of GBF; ± = positively or negatively correlated with increasing percentage or altitude of GBF; n = number of samples; Σ = sum of the concentrations; t = *trans*; c = *cis*; NMID = non-methylene-interrupted diene; MID = methylene-interrupted diene; $\bar{x}$ = mean value; s_x = standard deviation; P = probability: *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ns = non significant.

from May to September (Fig. 1). Normally, the $t7c9$ isomer is the second most predominant CLA isomer in ruminant fat [3]. The results found in the current study showed that the concentration of the CLA isomer $t11c13$ was the second most predominant isomer in milk fat from cows fed both winter and summer feeds in the mountains. According to a hypothesis by Kraft et al. [25], α-linolenic acid was the indirect precursor of the $t11c13$ isomer. The increased content of this isomer with increasing percentage of GBF and with altitude confirmed the observation of Collomb et al. [9, 12] and Leiber et al. [27] that this CLA isomer could be a useful indicator of

grass feeding as well as of milk products of alpine origin.

4. CONCLUSION

The results from the current study show that compared with winter the concentration of endogenous SFA in milk fat was lowest in summer and MUFA, PUFA, CLA and *trans* FA highest. Generally, grass supplemented with concentrates in the summer season, or high intakes of hay combined with concentrates in winter led to an increase in the SFA content and a decrease in the PUFA content, including

CLA in milk fat. Milk fat from the mountains appears particularly interesting from the nutritional point of view in the summer season because of the marked reduction of the SFA content, particularly of the SFA C12, C14 and C16, which adversely affect plasma LDL levels. The higher content of the main *n*-3 FA (α-linolenic acid) in milk fat from the mountains in the summer season than in milk fat from the lowlands is now generally recognized and confirmed by the increasing values with altitude in this study. In the winter season, the concentration of milk α-linolenic acid was not very different from that found in the summer season despite lower intake due to loss during grass drying. The relatively higher content of tVA in the summer season should be considered as positive since evidence [22] indicates that it may be transformed endogenously into health-promoting CLA. The increased content of the CLA *t*11*c*13 with increasing percentage of GBF and with altitude indicated that this isomer could be a useful indicator of grass feeding as well as of milk products of alpine origin.

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