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Influence of supplementing hay with grass silage on the fatty acid composition of mountain milk

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Abstract

During winter (from December 2004 to March 2005), bulk-tank milk from cows in the mountains (1130±300 m) fed a basic diet of hay supplemented or not with grass silage and similar amounts of concentrates was collected each month from 5 and 7 dairies, respectively. In total, 20 silage and 28 non-silage milk samples were analyzed for their fatty acid (FA) composition. An average supplementing hay with 27% grass silage combined with 4% whole crop maize silage resulted in significant decreases in the concentrations of saturated fatty acids (SFA) (-1.7%; $P \leq 0.001$) and branched FA (-6.0%; $P \leq 0.001$) as well as increases in monounsaturated FA (MUFA) (+6.5%; $P \leq 0.001$), polyunsaturated FA (PUFA) (+4.1%; $P \leq 0.01$), conjugated linoleic acid (CLA) (+12.0%; $P \leq 0.001$), trans FA (+17.5%; $P \leq 0.001$) and n-6 FA (+4.5%; $P \leq 0.05$). Despite the higher α-linolenic acid intake, the concentration of this important n-3 FA in milk fat decreased significantly (-8.0%) when hay was supplemented with grass silage.

Introduction

Farms in mountainous areas produce milk under more constrained conditions than elsewhere. The constraining factors are the shorter vegetation period, steep hills, less fertile soil, soil and climate only suitable for grassland and long distances from the consumers in the cities. In order that farmers continue to make a living under these conditions it is necessary that they produce milk and food with an added value. In the mountain regions of Switzerland cows were fed on pastures or grass without or with low supplements of concentrate during summer and for the remainder of the year on hay from mountain grass supplemented with grass silage and low amounts of concentrate. During the grazing period in the mountains, markedly low levels of milk fat SFA and high levels of milk fat n-3 FA and CLA were found (Tschager et al. 1994; Bugaud et al. 2001; Collomb et al. 2002a, b, 2004; Kraft et al. 2003; Leiber et al. 2004; Collomb et al. 2008). These effects are essentially due to grass feeding with or without a supplement of low amounts of concentrate but also the specific environmental conditions of the mountains as well as a particularly high botanical diversity of grass species which could specifically affect the FA composition of milk fat. Compared to the lowlands, mountain pastures are 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 (Collomb et al. 1999, 2002a, b; Bugaud et al. 2001).

Only a few experiments have shown the effects of diets rich in hay (Decaen & Adda, 1970; Bartsch et al. 1979; Shingfield et al. 2005; Ferlay et al. 2006; Morel et al. 2006b) or grass silage (Shingfield et al. 2005; Ferlay et al. 2006; Morel et al. 2006a) on the fatty acid composition of milk. Chilliard et al. (2000) reported that hay making strongly decreases the FA concentration and the α-linolenic in grass FA, whereas silage making, when carried out properly, does not. The concentration of α-linolenic acid in silage may nevertheless decrease when undesirable fermentations occur (Lough & Anderson, 1973) or when silage is wilted (Dewhurst & King, 1998). Other factors such as particle size, which affects bacterial colonization and lipolysis of lipids in dried grass or silage (Gerson et al. 1988; Ferlay et al. 2006), and variations in the maturity of grass contributed to the influence of forage conservation method on milk FA composition (Kelly et al. 1998). Hay or silage from specific plant species of mountain areas could also affect the FA composition of milk fat. Recently, Shingfield et al. (2005) reported that milk from cows fed hay contained more α-linolenic and linoleic acids than milk from a silage diet. Incubations in vitro showed that the rate and extent of biohydrogenation in the rumen was higher for ensiled than for dried grass (Boufaïed et al. 2003).

Up to now no study has been carried out to analyze the FA composition of bovine milk during the winter season in the mountain regions of Switzerland. The aim of this study was therefore to study the influence of typical winter fodder from the mountains on the fatty acid composition of bovine milk fat. This study was conducted in natura under the usual conditions of farming, and management of herds in the mountains rather than under strictly defined and controlled experimental conditions.

Materials and methods

Aim and approach

During the winter season (from December 2004 to March 2005), bulk-tank milk from cows fed non-silage or silage fodder in the mountain regions of Switzerland (1130±300 m) was collected each month from seven and five dairies, respectively. A total of 28 non-silage milk and 20 silage milk samples were analyzed. Herds from farms feeding on non-silage and silage mainly consisted of Brown Swiss, Swiss Fleckvieh, Simmental, Red Holstein, Holstein and Jersey breeds (Table 1). Large differences in breeds were found between the regions.

Table 1 Number of dairies, average milk performance per cow and percentages of dairy cow breeds

	Non-silage		Silage	
	$\bar{x}$	s_x	$\bar{x}$	s_x
Number of dairies	7		5	
Number of returned questionnaires per dairy	14	11	19	14
Milk performance (kg/305 days)	6056	428	6097	530
Brown Swiss (%)	55	49	53	35
Swiss Fleckvieh (%)	13	18	24	22
Simmental (%)	4	5	12	23
Red Holstein (%)	18	28	9	12
Holstein (%)	8	10	1	2
Jersey (%)	2	4	3	3
Others (%)	0	1	0	1

Fodder composition

Fodder composition and milk production data are presented in Table 2. Data were obtained from a standardised questionnaire filled out by the farmers. The data were partially complemented by estimations based on the quantity of milk produced. Non-silage and silage fodder did not significantly differ with respect to roughage or total concentrates. Compared to the non-silage diet (81% of hay), silage diet only differed significantly with regard to the lower percentage of hay (53%) combined with a supplement of 27% grass and 4% of whole crop maize silage. Three types of concentrate were fed. These were: cereal concentrate (energy 7.1 MJ NEL (net energy lactation), 10% crude protein), protein compensation concentrate (7.0 MJ NEL, 40% crude protein) and milk performance concentrate (7.1 MJ NEL, 16% crude protein).

With altitude the percentages of hay and concentrates increased significantly and those of grass and maize silage decreased significantly.

Table 2 Mean contents of fodder constituents in both non-silage (n=28, 7 dairies) and silage (n=20, 5 dairies) feeding systems averaged over December to March

Fodder	Non-silage		Silage		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
Returned questionnaires (%)	72	22	64	16	NS				
Quantity of bulk milk (kg/day)	2335	1339	4318	3413	**	NS		NS	
Total ration (kg dry matter/day/cow)	17.54	0.24	17.73	1.55	NS	***	-	*	+
Percentage of feed grasses	81	4	80	5	NS			NS	
Percentage of fresh grass	0	1	0	0	NS	NS		NS	
Percentage of grass silage	0	0	27	6	***	***	+	***	-
Percentage of hay	81	5	53	3	***	***	+	***	+
Percentage of whole crop maize silage	0	0	4	2	***	NS		**	-
Percentage of roughage[a]	87	3	88	4	NS	***	+	***	-
Percentage of concentrates	12.7	2.6	12.1	4.3	NS	***	-	***	+
Cereal concentrate[b] (%)	5.0	1.7	4.2	2.1	NS	**	-	NS	
Protein compensation concentrate[c] (%)	1.1	0.9	1.1	0.8	NS	NS		NS	
Milk performance concentrate[d] (%)	6.5	2.7	6.3	2.3	NS	**	-	***	+
Altitude of preserved feed grasses (m)	1134	295	1137	310	NS	NS		***	+
Altitude of cowshed (m)	1112	276	1169	356	*	**	-	***	+

Group, non-silage versus silage groups; GBF, grass-based feed; AGBF, altitude of GBF; n, number of samples; $\bar{x}$, mean value; s_x, standard deviation; *P*, probability; NS, not significant; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ± , positively or negatively correlated with increasing percentage or altitude of GBF.

[a] essentially constituted of fresh, dried or ensiled grass and whole crop maize but also fed straw, sugar beet pulp, fodder beets, potatoes and some other fibre rich fresh feed.

[b] energy 7.1 MJ NEL (net energy lactation), 10% crude protein.

[c] 7.0 MJ NEL, 40% crude protein.

[d] 7.1 MJ NEl, 16% crude protein.

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 was added as a preservative and the containers were stored directly at 5° C. The milk samples were centrifuged, and the resulting creams were churned at approximately 5° C. After the resulting molten butter had been filtered through a hydrophobic filter (Schleicher Schuell no. 597 HY1/2), the pure milk fat was collected and stored at -20° C until analysis.

Determination of the fatty acid composition of milk

After dissolution of the pure milk fat in hexane, the glycerides were transesterified to the corresponding methyl esters of fatty acids with a solution of potassium hydroxide in methanol (ISO 1997).

The fatty acid composition was analysed by high resolution gas chromatography with flame ionisation detection according to Collomb and Bühler (2000). The fatty acids were separated on a capillary column CP-Sil 88 (100 m *0.25 mm i.d. *0.20 µm) and quantified using nonanoic acid as an internal standard. The results are expressed in absolute values, as g fatty acids per 100 g fat.

CLA isomers were analyzed by silver-ion (Ag^+)-HPLC on an Agilent LC series 1100 equipped with a photodiode array detector (234 nm) using three ChromSpher Lipids columns in series (stainless steel, 250 x 4.6 mm, 5 µm particle size, Chrompack, Middleburg, Netherlands) according to Collomb et al. (2004). The solvent consisted of UV-grade hexane with 0.1% acetonitrile and 0.5% ethyl ether (flow rate 1 ml/min), prepared fresh daily. The injection volume was 10 µl, corresponding to < 250 µg lipid. The HPLC areas for $C_{18:2}$ t7c9 + t8c10 + c9t11 (c, cis; t, trans) were combined and used for comparison of the peak areas of the three isomers from the GC chromatogram. The results were expressed in absolute values, as mg/g fat.

Statistical analyses

The mean values and standard deviations of FA of non-silage and silage milk fat were calculated. An analysis of covariance was applied to the results of percentages and altitude of feed grasses. Systat for Windows version 11 (Anonymous, 2004) was used for all calculations.

Results and Discussion

Pools of fatty acids

The mean content of each of the FA groups in non-silage and silage milk averaged over the winter season is shown in Table 3. Except for the content of short chain and n-3 FA, the concentrations of all other pools of FA in milk fats from cows supplemented or not with silage were significantly different. A mean supplementation of 27% grass and 4% whole crop maize silage to hay resulted in significant decreases in the concentrations of saturated fatty acids (SFA) (-1.7%; $P \leq 0.001$) and branched FA (-6.0%; $P \leq 0.001$) and increases of monounsaturated FA (MUFA) (+6.4%; $P \leq 0.001$), polyunsaturated FA (PUFA) (+4.1%; $P \leq 0.01$), CLA (+12.0%; $P \leq 0.001$), trans FA (without CLA) (+17.5%; $P \leq 0.001$) and n-6 FA (+4.5%; $P \leq 0.05$).

Ferlay et al. (2006) also reported that the nature of forage had no influence on the proportions of short-chain SFA with 6 or 8 carbon chain lengths because these FA are synthesized in part by metabolic pathways not involving malonyl-CoA and acetyl-CoA carboxylase and thus would not be influenced to a great extent by the nature of the forage. Compared to the hay diet the decrease in the concentration of medium-chain SFA in milk fat from cows fed hay supplemented with silage may be due in part to the more important amounts of dietary PUFA provided by silage than by hay. These PUFA and/or their biohydrogenation products are potent inhibitors of mammary SFA synthesis by directly inhibiting acetyl-CoA carboxylase activity (Bauman & Griinari, 2003).

Significant but relatively low differences in the concentrations of MUFA and PUFA were found between the two groups (Table 3). It is well known that unsaturated FA in the diet are extensively metabolized in the rumen (Harfoot & Hazlewood, 1988) and so only a small proportion escapes to be incorporated into milk triacylglycerides.

The higher contents of branched chain FA found in the current study in milk from cows fed hay than in milk from cows fed hay supplemented with silage confirms that microbial synthesis of these FA is enhanced by diets rich in fiber (Sauvant & Bas, 2001).

Increases of trans FA and CLA in silage milk fat compared to non-silage milk fat found in the current study are consistent with a higher PUFA intake from silage than from hay as well as a higher ruminal biohydrogenation with the diet supplemented with silage. Incubations in vitro showed that the rate and extent of biohydrogenation in the rumen was higher for ensiled than for dried grass (Boufaïed et al. 2003). Nevertheless Shingfield et al. (2005) found that the conservation method (hay and silage) had no clear effect on the milk fat trans C18:1 and CLA concentrations and that differences in milk fat trans C18:1 and total CLA contents between hay and silage diets were much lower than would be expected based on PUFA intakes.

The increasing concentrations of milk fat PUFA, CLA, trans FA, n-3 FA as well as the decreasing concentration of n-6 FA with increasing percentages of GBF in the diet (Table 3, Fig. 1) could be essentially due to increasing percentages of grass silage as well as decreasing percentages of concentrates in the diet (Table 2). The increasing concentrations of milk MUFA, PUFA, CLA, trans FA, n-3 FA and n-6 FA as well as the decreasing concentration of SFA and

Table 3 Mean contents (g/100 g milk fat) of pools of fatty acids in both non-silage (n=28, 7 dairies) and silage (n=20, 5 dairies) milk from December to March

Σ Fatty acids	Non-silage		Silage		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
Σ short chain[a]	9.63	1.90	9.67	0.32	NS	NS		NS	
Σ medium chain[b]	47.16	1.92	45.39	2.00	***	NS		***	-
Σ long chain[c]	32.88	2.04	35.45	2.51	***	NS		***	+
Σ saturated[d]	63.86	1.27	62.78	1.51	***	NS		***	-
Σ C12, C14 & C16	41.73	1.62	40.21	1.70	***	NS		***	-
Σ C18:1[e]	18.92	0.95	20.47	1.61	***	NS		***	+
Σ C18:2[f]	3.10	0.28	3.35	0.36	***	*	+	***	+
Σ unsaturated[g]	26.01	1.10	27.59	1.78	***	NS		***	+
Σ monounsaturated[h]	21.57	0.85	22.98	1.51	***	NS		**	+
Σ polyunsaturated[i]	4.40	0.38	4.58	0.42	**	***	+	***	+
Σ branched[k]	2.35	0.11	2.21	0.09	***	NS		*	-
Σ C18:1 t[l]	2.91	0.55	3.46	0.51	***	***	+	***	+
Σ C18:2 t without CLA t[m]	0.68	0.08	0.84	0.07	***	***	+	*	+
Σ C18:2t with CLA[n]	1.52	0.20	1.76	0.19	***	***	+	***	+
Σ CLA[o]	0.92	0.16	1.03	0.15	***	***	+	***	+
Σ trans without CLA[p]	3.73	0.62	4.45	0.57	***	***	+	***	+
Σ trans with CLA[q]	4.58	0.74	5.38	0.70	***	***	+	***	+
Σ n-3 FA[r]	1.36	0.17	1.37	0.16	NS	***	+	*	+
Σ n-6 FA[s]	2.23	0.22	2.33	0.34	*	***	-	***	+
Σ n-3 / Σ n-6 FA	0.62	0.10	0.60	0.13	NS	***	+	***	-

Group, non-silage versus silage group; GBF, grass-based feed; AGBF, altitude of GBF; ±, positively or negatively correlated with increasing percentage or altitude of FG; n, number of samples; Σ, sum of the concentrations; $\bar{x}$, mean value; s_x, standard deviation; *P*, probability; NS, not significant; *,$P \leq 0.05$; **,$P \leq 0.01$; ***,$P \leq 0.001$; t, trans; c, cis; NMID, none methylene interrupted diene; MID, methylene interrupted diene.

[a] $C_{4:0}$, $C_{5:0}$, $C_{6:0}$, $C_{7:0}$, $C_{8:0}$, $C_{10:0}$, $C_{10:1}$.

[b] $C_{12:0}$, $C_{13:0}$ iso, $C_{13:0}$ aiso, $C_{12:1}$ c + $C_{13:0}$, $C_{14:0}$ iso, $C_{14:0}$, $C_{15:0}$ iso, $C_{14:1}$ t, $C_{15:0}$ aiso, $C_{14:1}$ c, $C_{15:0}$, $C_{16:0}$ iso, $C_{16:0}$, $C_{17:0}$ iso, $C_{16:1}$ t, $C_{17:0}$ aiso, $C_{16:1}$ c.

[c] $C_{17:0}$, $C_{18:0}$ iso, $C_{17:1}$ t, $C_{18:0}$ aiso, $C_{18:0}$, Σ $C_{18:1}$, $C_{19:0}$, Σ $C_{18:2}$, $C_{20:0}$, $C_{20:1}$ t, $C_{18:3}$ c6c9c12, $C_{20:1}$ c5, $C_{20:1}$ c9, $C_{20:1}$ c11, $C_{18:3}$ c9c12c15, $C_{20:2}$ c,c (n-6), $C_{22:0}$ $C_{20:3}$ (n-6), $C_{20:3}$ (n-3), $C_{20:4}$ (n-6), $C_{20:5}$ (EPA) (n-3), $C_{22:5}$ (DPA) (n-3), $C_{22:6}$ (DHA) (n-3).

[d] $C_{4:0}$, $C_{5:0}$, $C_{6:0}$, $C_{7:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, Σ branched (iso + aiso), $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{19:0}$, $C_{20:0}$ and $C_{22:0}$.

[e] $C_{18:10}$ -t4, -t5, -t6-8, -t9, -t10-11, -t12, -t13-14 + -c6-8, -c9, -c11, -c12, -c13, -16 + c14.

[f] $C_{18:2}$ -ttNMID, -t9t12, -c9t13 + -t8c12, -c9t12 + -c,c-MID + -t8c13, -t11c15 + -t9c12, -c9c12, -c9c15, -c9t11 + -t8c10 + -t7c9, -t11c13 + -c9c11, -t9t11;

[g] $C_{10:1}$, $C_{14:1}$ ct, $C_{16:1}$ ct, $C_{17:1}$ t, Σ $C_{18:1}$, Σ $C_{18:2}$, $C_{20:1}$ t, $C_{18:3}$ c6c9c12, $C_{20:1}$ c5, $C_{20:1}$ c9, $C_{20:1}$ c11, $C_{18:3}$ c9c12c15, $C_{18:2}$ c9t11 + t8c10 + t7c9, $C_{18:2}$ t11c13 + c9c11, $C_{18:2}$ t9t11, $C_{20:2}$ c,c (n-6), $C_{20:3}$ (n-6), $C_{20:3}$ (n-3), $C_{20:4}$ (n-6), $C_{20:5}$ (EPA) (n-3), $C_{22:5}$ (DPA) (n-3), $C_{22:6}$ (DHA) (n-3).

[h] $C_{10:1}$, $C_{14:1}$ ct, $C_{16:1}$ ct, $C_{17:1}$ ct, Σ$C_{18:1}$, $C_{20:1}$ t, $C_{20:1}$ c5, $C_{20:1}$ c9, $C_{20:1}$ c11.

[i] Σ $C_{18:2}$, $C_{18:3}$ c6c9c12, $C_{18:3}$ c9c12c15, $C_{20:2}$ c,c (n-6), $C_{20:3}$ (n-3), $C_{20:4}$ (n-6), $C_{20:5}$ (EPA) (n-3), $C_{22:5}$ (DPA) (n-3), $C_{22:6}$ (DHA) (n-3).

[k] $C_{13:0}$ iso + aiso, $C_{14:0}$ iso, $C_{15:0}$ iso + aiso, $C_{16:0}$ iso, $C_{17:0}$ iso + aiso, $C_{18:0}$ iso + aiso.

[l] $C_{18:1}$ t4, $C_{18:1}$ t5, $C_{18:1}$ t6-8, $C_{18:1}$ t9, $C_{18:1}$ t10-11, $C_{18:1}$ t12, $C_{18:1}$ t13-14 + c6-8.

[m] $C_{18:2}$ -ttNMID, -t9t12, -c9t13 + -t8c12, -c9t12 + -c,c-MID + -t8c13, -t11,c15 + -t9,c12, -t11c13 + -c9c11.

[n] Σ $C_{18:2}$ trans without CLA + CLA -c9t11 + -t8c10 + -t7c9, -c9c11, -t9t11.

[o] $C_{18:2}$ -t12t14, -t11t13, -t10t12, -t9t11, -t8t10, -t7t9, -t6t8, -ct12 14, -t11c13, -c11t13, -t10c12, -c9t11, -t8c10, t7c9.

[p] $C_{14:1}$ t, $C_{16:1}$ t, $C_{17:1}$ t, $C_{20:1}$t, Σ $C_{18:1}$ trans, Σ $C_{18:2}$ trans without CLA .

[q] Σ trans without CLA + CLA -c9t11 + -t8c10 + -t7c9, -c9c11, -t9t11.

[r] $C_{18:2}$ t11c15 + $C_{18:2}$ c9c15, $C_{18:3}$ c9c12c15, $C_{20:3}$ n-3, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$.

[s] $C_{18:1}$ t12, $C_{18:1}$ c12, $C_{18:2}$ t9t12, $C_{18:2}$ c9t12 + c,c-MID + t8c13, $C_{18:2}$ c9c12, $C_{18:3}$ c6c9c12, $C_{20:2}$ cc, $C_{20:3}$ n-6 and C20:4 n-6.

branched FA with altitude (Table 3, Fig. 2) were surprising because of increasing percentages of hay and concentrates in the diet as well as decreasing percentage of silage with altitude (Table 2). Other factors that changed with altitude which could explain these results were fodder of increasing botanical diversity (Collomb et al. 1999, 2002a, b; Bugaud et al. 2001) specific environmental conditions for cows (i. e. energy shortage) variations in breeds which also affect the FA composition (Ferlay et al. 2006) modifying the ruminal fermentation.

Individual fatty acids

The mean content of each of the individual FA in both non-silage and silage milk averaged over the winter season is shown in Table 4. The concentrations of the main even saturated FA (C6, C8, C10, C12, C14 and C16) and branched FA ($C_{13:0}$ iso, $C_{13:0}$ aiso, $C_{14:0}$ iso, $C_{15:0}$ iso, $C_{15:0}$ aiso, $C_{16:0}$ iso, and $C_{17:0}$ iso) as well as α-linolenic acid ($C_{18:2}$ c9c12c15) were significantly lower in silage milk than in non-silage milk. Conversely, the concentrations of $C_{18:0}$ (stearic acid), $C_{18:1}$ c9 (oleic acid), trans $C_{18:1}$ FA ($C_{18:1}$ -t6-8, -t9, -t10 + t11 (t11, trans vaccenic acid, tVA), -t12, -t13 + 14 + c6-8, -t16 + c14), and $C_{18:2}$ FA ($C_{18:2}$ –ttNMID, -c9t13 + t8c12, -c9t12 + ccMID + t8c13, -t11c15 + t9c12) in silage milk were significantly higher. In both types of milk, the concentrations of linoleic acid ($C_{18:2}$ c9c12) were not significantly different.

The concentrations of the SFA $C_{6:0}$ and $C_{8:0}$ were significantly different between both types of milk but differences were relatively low which confirms that the nature of forage did not have a great influence on the proportion of short-chain SFA with 6 or 8 carbon chain lengths (Ferlay et al. 2006). The lower concentrations of the other even SFA in silage milk compared to non silage milk was consistent with the high inhibitory effect on mammary synthesis of SFA by a higher PUFA contents (Bauman & Griinari, 2003).

The higher concentration of oleic acid (15.1 and 16.1 g/100 g fat) in milk fat from cows fed a supplementation of 27% grass- and 4% whole crop maize silage to hay may be due to the fact that silage contains more PUFA than hay. Underfeeding of the cows and thus enhancing the concentration of this compound in milk fat by body fat mobilization should also be considered.

Conservation of grass by drying rather than ensiling resulted in lower forage linoleic and α-linolenic acids (Chilliard et al. 2001; Shingfield et al. 2005). Shingfield et al. (2005) found higher milk fat linoleic and α-linolenic acid concentrations resulting from hay rather than silage diets supplemented with similar amounts of concentrates (linoleic acid: 1.21 and 0.96; α-linolenic acid: 0.50 and 0.35 g/100 g FAME, respectively). Despite a higher intake of linoleic acid from grass silage and particularly from whole crop maize silage than from hay, no difference appeared between the milk fat concentrations of linoleic acid in the current study. The higher concentration of α-linolenic acid in milk fat from cows fed the hay diet than the hay diet supplemented with silage found in this study confirms the results obtained by other authors (Shingfield et al. 2005; Ferlay et al. 2006), but the differences were relatively low (0.88 and 0.81 g/100 g fat). Moreover, Ferlay et al. (2006) also observed higher values with hay diets than with pasture despite the higher α-linolenic acid levels in grass. Doreau, Ukeda and Poncet (2003) also observed that biohydrogenation was more important with pasture than hay-based diets which is in agreement with the lower estimated transfer efficiency of α-linolenic acid from diet intake into milk with pasture diets.

The higher content of the combined FA $C_{18:2}$ t11c15 + t9c12 in silage milk than in non-silage can be also attributed to fermentation process of feed PUFA, particularly of grass-based α-linolenic acid in the rumen of cows leading to a higher concentration of C18:2 t11c15 FA. It is likely that the biohydrogenation of feed α-linolenic acid was more important when the hay diet was supplemented with grass silage. The pathway for the hydrogenation of feed

Table 4 Mean contents (g/100 g milk fat) of individual fatty acids in both non-silage (n=28, 7 dairies) and silage (n=20, 5 dairies) milk from December to March

Σ Fatty acids	Non-silage		Silage		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
FA concentrations which were significantly different between non-silage and silage milk									
$C_{6:0}$	2.18	0.04	2.11	0.06	***	NS		NS	
$C_{8:0}$	1.23	0.03	1.17	0.05	***	NS		**	-
$C_{10:0}$	2.65	0.17	2.49	0.17	***	NS		***	-
$C_{10:1}$	0.32	0.03	0.29	0.03	***	NS		***	-
$C_{12:0}$	2.92	0.21	2.71	0.21	***	NS		***	-
$C_{13:0}$ iso	0.03	0.00	0.03	0.00	***	NS		NS	
$C_{13:0}$ aiso	0.08	0.01	0.07	0.01	***	NS		***	-
$C_{12:1}$ c + $C_{13:0}$	0.15	0.02	0.14	0.02	**	NS		***	-
$C_{14:0}$ iso	0.15	0.01	0.13	0.01	***	**	+	***	+
$C_{14:0}$	10.34	0.34	9.88	0.44	***	NS		***	-
$C_{15:0}$ iso	0.26	0.01	0.24	0.02	***	NS		NS	
$C_{15:0}$ aiso	0.47	0.02	0.44	0.02	***	**	+	NS	
$C_{14:1}$ c	0.87	0.09	0.80	0.10	***	NS		***	-
$C_{16:0}$ iso	0.28	0.02	0.25	0.02	***	NS		***	+
$C_{16:0}$	28.47	1.23	27.62	1.12	***	*	-	***	-
$C_{17:0}$ iso	0.32	0.02	0.30	0.01	***	NS		NS	
$C_{16:1}$ c	1.17	0.16	1.10	0.11	***	***	-	***	-
$C_{16:1}$ t	0.10	0.02	0.11	0.02	***	**	+	***	+
$C_{17:1}$ t	0.00	0.00	0.01	0.01	***	*	+	NS	
$C_{18:0}$	8.14	0.79	8.99	0.63	***	NS		***	+
$C_{18:1}$ t4	0.01	0.01	0.02	0.01	*	NS		***	+
$C_{18:1}$ t5	0.01	0.01	0.01	0.01	*	NS		***	+
$C_{18:1}$ t6-8	0.13	0.03	0.15	0.04	***	NS		***	+
$C_{18:1}$ t9	0.23	0.03	0.27	0.03	***	NS		***	+
$C_{18:1}$ t10-11	1.97	0.41	2.24	0.38	***	***	+	***	+
$C_{18:1}$ t12	0.16	0.03	0.22	0.04	***	NS		***	+
$C_{18:1}$ t13-14 + c6-8	0.41	0.07	0.55	0.06	***	NS		***	+
$C_{18:1}$ c9	15.12	0.69	16.05	1.17	***	**	-	NS	
$C_{18:1}$ c12	0.14	0.03	0.17	0.04	***	*	-	***	+
$C_{18:1}$ t16 + c14	0.21	0.03	0.28	0.03	***	***	+	***	+
$C_{18:2}$ ttNMID	0.09	0.02	0.11	0.01	***	***	+	NS	
$C_{18:2}$ c9t13 + t8c12	0.14	0.01	0.17	0.02	***	*	+	NS	
$C_{18:2}$ c9t12 + (c,c-MID + t8c13)	0.22	0.01	0.24	0.02	***	NS		***	+
$C_{18:2}$ t11,c15 + t9,c12	0.22	0.04	0.31	0.05	***	***	+	NS	
$C_{20:0}$	0.16	0.02	0.17	0.01	***	NS		***	+
$C_{18:3}$ c9c12c15	0.88	0.12	0.81	0.10	*	***	+	***	+
$C_{20:2}$ c,c (n-6)	0.03	0.00	0.02	0.00	*	NS		**	+
$C_{20:5}$ (EPA) (n-3)	0.08	0.01	0.07	0.01	*	***	+	NS	

Table 4

Σ Fatty acids	Non-silage		Silage		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
FA concentrations which were not significantly different between non-silage and silage milk									
$C_{4:0}$	3.54	0.24	3.56	0.12	NS	NS		***	+
$C_{5:0}$	0.04	0.01	0.03	0.01	NS	NS		NS	
$C_{7:0}$	0.02	0.00	0.02	0.00	NS	NS		**	-
$C_{14:1}$ t	0.00	0.00	0.00	0.01	NS	*	+	NS	
$C_{15:0}$	1.09	0.07	1.10	0.13	NS	***	+	***	-
$C_{17:0}$ aiso	0.48	0.03	0.47	0.04	NS	NS		**	-
$C_{17:0}$	0.58	0.04	0.57	0.04	NS	*	+	NS	
$C_{18:0}$ iso	0.06	0.01	0.06	0.01	NS	NS		NS	
$C_{18:0}$ aiso	0.23	0.03	0.22	0.02	NS	NS		***	-
$C_{18:1}$ c11	0.48	0.03	0.47	0.07	NS	**	-	NS	
$C_{18:1}$ c13	0.05	0.02	0.05	0.01	NS	NS		NS	
$C_{19:0}$	0.09	0.01	0.09	0.01	NS	NS		NS	
$C_{18:2}$ t9t12	0.00	0.01	0.00	0.01	NS	NS		NS	
$C_{18:2}$ c9c12	1.50	0.16	1.49	0.25	NS	***	-	***	+
$C_{18:2}$ c9c15	0.03	0.00	0.04	0.01	NS	NS		***	-
$C_{20:1}$ t	0.03	0.00	0.03	0.00	NS	***	-	NS	
$C_{18:3}$ c6c9c12	0.02	0.00	0.02	0.00	NS	NS		NS	
$C_{20:1}$ c5	0.00	0.00	0.00	0.01	NS	NS		NS	
$C_{20:1}$ c9	0.13	0.01	0.14	0.01	NS	NS		***	+
$C_{20:1}$ c11	0.05	0.01	0.05	0.01	NS	NS		NS	
$C_{22:0}$	0.07	0.01	0.07	0.01	NS	NS		NS	
$C_{20:3}$ (n-6)	0.05	0.01	0.05	0.01	NS	***	-	***	+
$C_{20:3}$ (n-3)	0.03	0.01	0.03	0.01	NS	NS		NS	
$C_{20:4}$ (n-6)	0.10	0.02	0.10	0.01	NS	*	-	NS	
$C_{22:5}$ (DPA) (n-3)	0.10	0.01	0.11	0.01	NS	NS	+	NS	
$C_{22:6}$ (DHA) (n-3)	0.01	0.02	0.01	0.01	NS	NS		NS	

Group, non-silage versus silage 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; $\bar{x}$, mean value; s_x, standard deviation; *P*, probability; NS, not significant; *,$P \le 0.05$; **,$P \le 0.01$; ***,$P \le 0.001$; t = trans; c = cis; NMID: none methylene interrupted diene; MID, methylene interrupted diene; DPA, docosapentenoic acid; DHA, docosahexenoic acid.

α-linolenic acid ($C_{18:3}$ c9c12c15) in the rumen involves an initial isomerization to a conjugated triene ($C_{18:3}$ c9t11c15), followed by reduction of double bounds at carbons 9, 15, and 11 to yield the FA $C_{18:2}$ t11c15, C18:1 t11, and $C_{18:0}$, respectively (Wilde & Dawson, 1966). The significantly higher concentration of stearic acid in milk fat from silage than in milk from hay were in agreement with the results published by Shingfield et al. (2005) and confirmed the higher level of biohydrogenation in the rumen when the hay diet was supplemented with silage.

CLA isomers

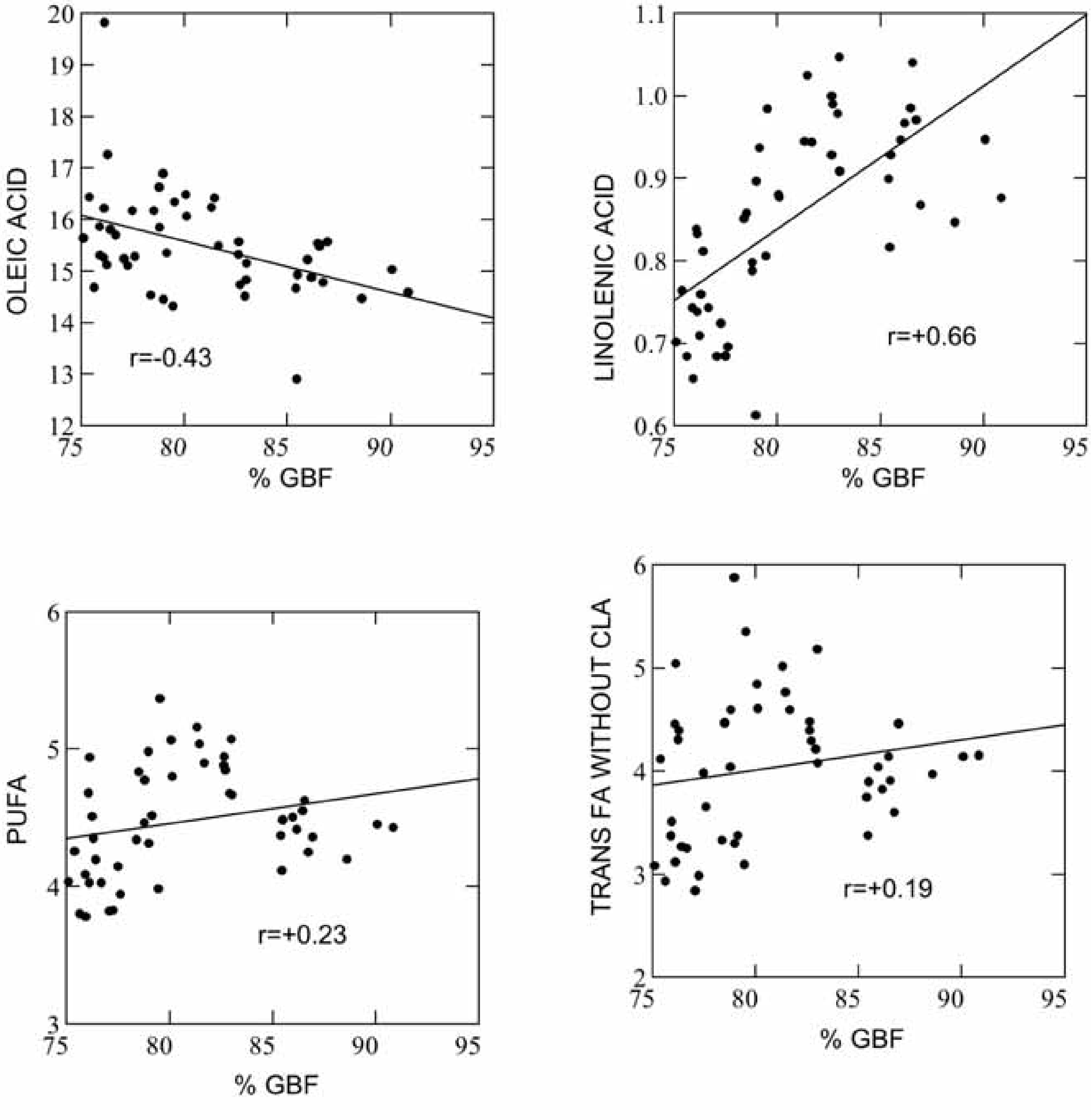
The concentrations of the three most important CLA isomers $C_{18:2}$ c9t11, t11c13 and t7c9 were significantly higher (9.7%, 54.8%, and 14.3%, respectively) in milk fat from hay supplemented with silage than in milk fat from hay (Table 5). These results were consistent with a higher intake of linoleic and α-linolenic acid in the silage diet compared to the hay diet as well as with higher ruminal biohydrogenation of fat from hay supplemented with silage than from hay alone. The CLA-enriching effect of grass silage has been attributed to the effects on biohydrogenation and the provision of α-linolenic acid as a lipid substrate for the formation of tVA in the rumen and its subsequent desaturation to $C_{18:2}$ c9t11 CLA in the mammary gland (Bauman et al. 2003). In the current study the concentration of the CLA isomer $C_{18:2}$ c9t11 was highest, followed by the isomers C18:2 t11c13 and t7c9. Highly significant ($P \leq 0.001$) correlations were found between the daily intake of α-linolenic acid and the concentration of the CLA isomers $C_{18:2}$ t12t14, t11t13, c/t12 14, t11c13 and c11t13 (Collomb et al. 2004). The concentration of the CLA isomer $C_{18:2}$ t11c13 was generally higher than that of CLA t7c9 when grass-based diets rich in α-linolenic acid were fed to cows (Kraft et al. 2003; Collomb et al. 2004). In mountain regions, where essentially grass-based diets are fed to cows, this CLA could be a useful indicator of milk products of alpine origin.

Table 5 Mean contents (mg/g fat) of conjugated linoleic acid isomers in both non-silage (n=28, 7 dairies) and silage (n=20, 5 dairies) milk averaged over December to March

Σ CLA	Non-silage		Silage		Significance (P)				
	$\bar{x}$	s_x	$\bar{x}$	s_x	Group	GBF %/±		AGBF /±	
$C_{18:2}$ t12t14	0.07	0.01	0.10	0.02	***	***	+	NS	
$C_{18:2}$ t11t13	0.15	0.04	0.20	0.04	***	***	+	NS	
$C_{18:2}$ t10t12	0.03	0.01	0.04	0.01	NS	NS		***	+
$C_{18:2}$ t9t11	0.13	0.01	0.13	0.01	NS	***	+	***	+
$C_{18:2}$ t8t10	0.02	0.01	0.02	0.00	NS	NS		***	+
$C_{18:2}$ t7t9	0.09	0.02	0.08	0.01	*	NS		***	+
$C_{18:2}$ t6t8	0.05	0.01	0.04	0.01	***	NS		***	+
$C_{18:2}$ c/t12 14	0.02	0.01	0.03	0.01	***	***	+	NS	
$C_{18:2}$ t11c13	0.42	0.13	0.65	0.17	***	***	+	NS	
$C_{18:2}$ c11t13	0.02	0.01	0.02	0.00	NS	NS		NS	
$C_{18:2}$ t10c12	0.02	0.01	0.02	0.01	NS	NS		***	+
$C_{18:2}$ c9t11	7.74	1.31	8.49	1.30	***	***	+	***	+
$C_{18:2}$ t8c10	0.11	0.02	0.12	0.02	**	*	+	**	+
$C_{18:2}$ t7c9	0.35	0.05	0.40	0.06	***	NS		***	+
CLA t11c13 / CLA t7c9	1.20	0.35	1.68	0.51	***	***	+	**	-
Σ CLA	9.21	1.57	10.34	1.48	***	***	+	***	+

Group, non-silage versus silage 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, none methylene interrupted diene; MID, methylene interrupted diene; $\bar{x}$, mean value; s_x, standard deviation; P, probability; NS, not significant; *,$P \leq 0.05$; **,$P \leq 0.01$; ***,$P \leq 0.001$; t, trans; c, cis.

Fig. 1 Influence of increasing percentages of grass-based feed (GBF) in the diet on the concentration of the most important milk fatty acids (g/100 g milk fat; r=correlation coefficient)



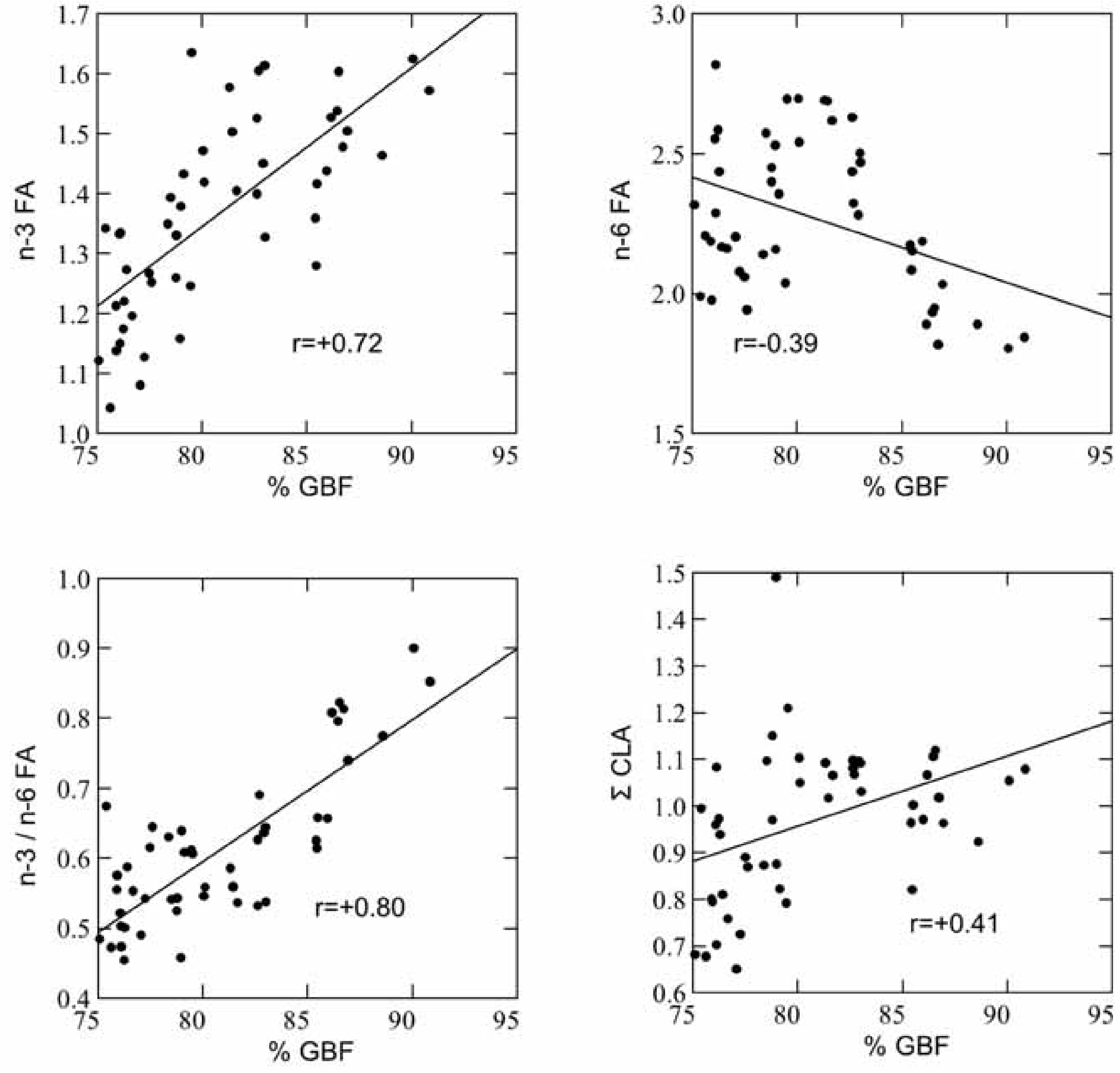
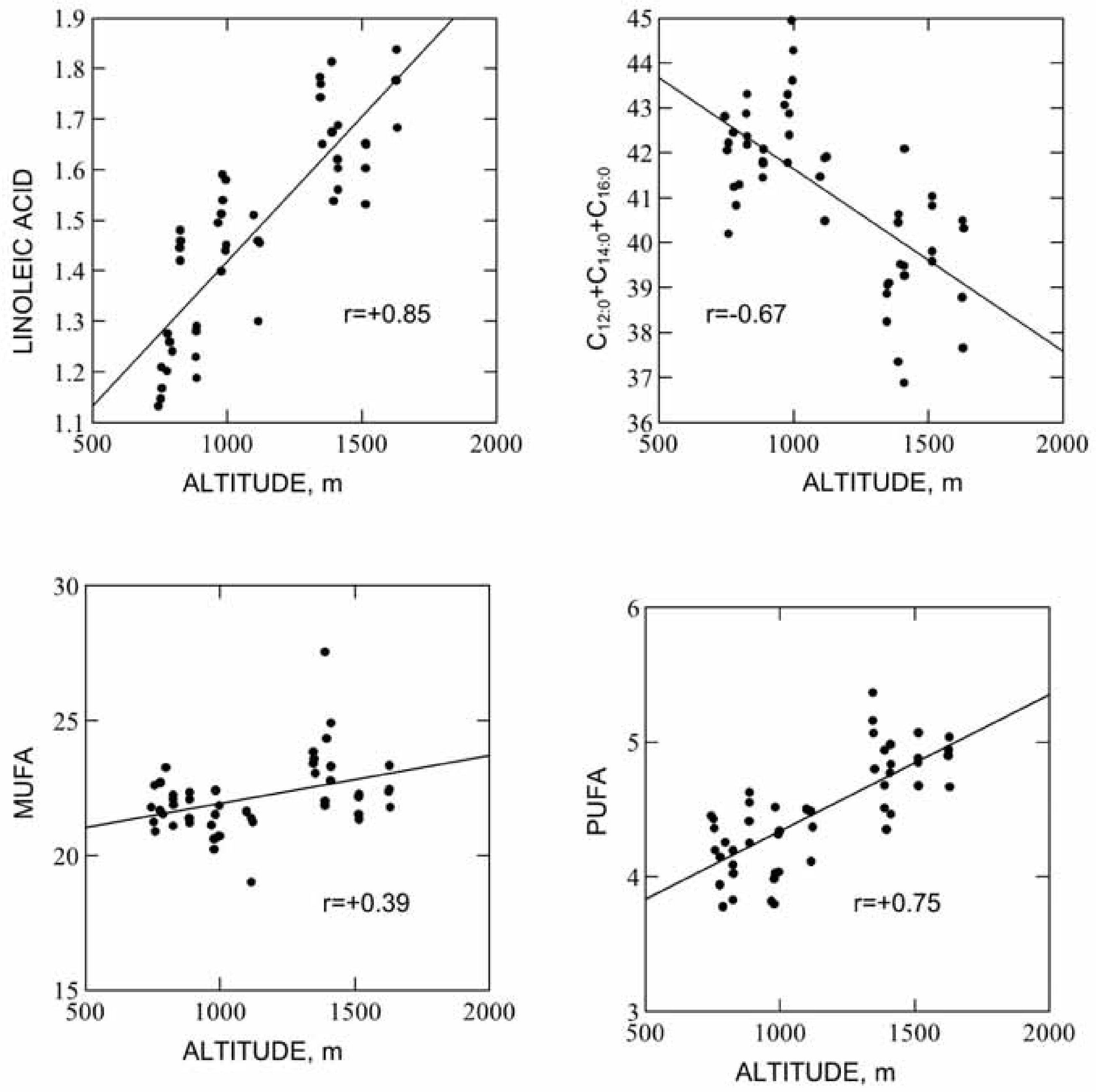
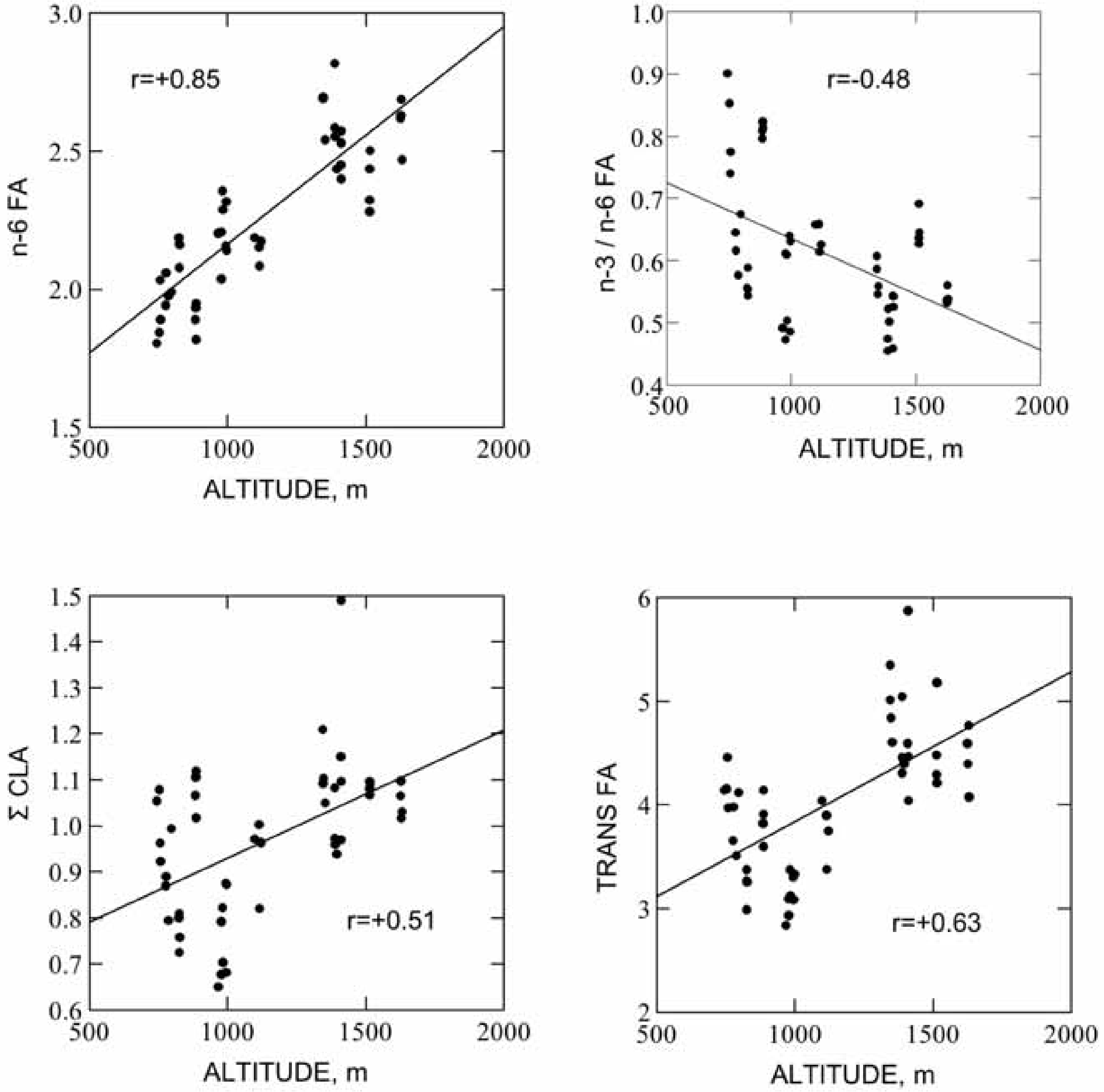

n-3 FA
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
75
80
85
90
95
% GBF
r=+0.72
n-6 FA
1.5
2.0
2.5
3.0
75
80
85
90
95
% GBF
r=-0.39
n-3 / n-6 FA
0.4
0.5
0.6
0.7
0.8
0.9
1.0
75
80
85
90
95
% GBF
r=+0.80
Σ CLA
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
75
80
85
90
95
% GBF
r=+0.41

Fig. 2 Influence of increasing altitude on the concentration of the most important milk fatty acids (g/100 g milk fat; r=correlation coefficient)




r=+0.85
n-6 FA
ALTITUDE, m
r=-0.48
n-3 / n-6 FA
ALTITUDE, m
r=+0.51
Σ CLA
ALTITUDE, m
r=+0.63
TRANS FA
ALTITUDE, m
3.0
2.5
2.0
1.5
1.0
0.9
0.8
0.7
0.6
0.5
0.4
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
6
5
4
3
2
500
1000
1500
2000

Conclusions

In mountain areas, many studies have been carried out during the grazing period and have shown that milk and milk products from these regions have an added nutritional value due to markedly lower contents of SFA and higher contents of PUFA including CLA compared to those from the lowlands. These results are essentially due to grass feeding supplemented or not with low amounts of concentrates. The influence of typical plant species from the mountains as well as specific environmental factors for cows can not be excluded. Up to now no study has analyzed the influence of typical winter fodder on the fatty acid composition of milk produced in mountain areas within Switzerland. In the mountain regions cows were essentially fed on hay supplemented with or without grass silage and low amounts of concentrates. Supplementation of hay with grass silage resulted in higher concentrations of the most important milk fat CLA as well as tVA and lower concentrations of most even saturated FA. Supplementation with grass silage is therefore recommended in order to produce milk and also food with an added nutritional value. Despite a higher α-linolenic acid intake, the concentration of this important n-3 FA in milk fat decreased significantly when hay was supplemented with grass silage. This effect has recently been recognized and interpreted as a reduction in ruminal biohydrogenation when essentially hay was fed to cows instead of grass silage.

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