A Study on the Causes for the Elevated n-3 Fatty Acids in Cows' Milk of Alpine Origin

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ABSTRACT: The influence of grass-only diets either from ryegrass-dominated lowland pastures (400 m above sea level) or botanically diverse alpine pastures (2000 m) on the FA profile of milk was investigated using three groups of six Brown Swiss cows each. Two groups were fed grass-only on pasture (P) or freshly harvested in barn (B), both for two experimental periods in the lowlands and, consecutively, two periods on the alp. Group C served as the control, receiving a silage-concentrate diet and permanently staying in the lowlands. Effects of vegetation stage or pasture vs. barn feeding on milk fat composition were negligible. Compared with the control, α-linolenic acid (18:3n-3) consumption was elevated in groups P and B (79%; $P < 0.001$) during the lowland periods but decreased on the alp to the level of C owing to feed intake depression and lower 18:3n-3 concentration in the alpine forage. Average 18:3n-3 contents of milk fat were higher in groups P and B than in C by 33% ($P < 0.01$) at low and by 96% ($P < 0.001$) at high altitude, indicating that 18:3n-3 levels in milk were to some extent independent of 18:3n-3 consumption. The *cis*-9,*trans*-11 CLA content in milk of grass-fed cows was higher compared with C but lower for the alpine vs. lowland periods whereas the *trans*-11,*cis*-13 isomer further increased with altitude. Long-chain n-3 FA and phytanic acid increased while arachidonic acid decreased with grass-only feeding, but none of them responded to altitude. Grass-only feeding increased milk α-tocopherol concentration by 86 and 134% at low and high altitude ($P < 0.001$), respectively. Changes in the ruminal ecosystem due to energy shortage or specific secondary plant metabolites are discussed as possible causes for the high 18:3n-3 concentrations in alpine milk.

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Recently, elevated contents of beneficial functional FA in cows' milk and cheese derived from alpine grazing systems were reported (1–4). Increased contents of n-3 PUFA and an improved ratio of n-3 to n-6 PUFA in milk of cows grazing on alpine pastures were shown (3,5). It was even hypothesized that a relation between high levels of α-linolenic acid (18:3n-3) in alpine cheese and a favorable cardiovascular health status of the alpine population may exist (4). Generally, n-3 PUFA are known to be essential for human health, and problems may arise if they are consumed in too low a proportion relative to n-6 FA (6). Furthermore, the content of CLA (18:2 *cis*-9,*trans*-11 isomer), a FA that also has shown potential to benefit human health (7), was found to be clearly enriched in milk (3) or cheese (1,2) from alpine production systems compared with those from intensive lowland production. Additionally, the proportion of short- and medium-chain saturated FA (SFA), which are supposed to increase the risk of cardiovascular diseases (8), was found to be markedly lower in milk and milk products originating from cows grazing high alpine pastures (2,4,5).

Milk fat derived from cows grazing high alpine pastures therefore seems to have a considerably higher dietetic value than conventional milk, but the reasons for these differences are still unclear. To our knowledge, in previous studies the individual factors of the alpine sojourn of cows potentially affecting milk fat composition were never differentiated, and diets fed in the lowlands for control were either not specified or not restricted to herbage-only, as is usual practice in Swiss alpine dairy systems. Known mechanisms influencing the FA profile in lowland milk are (i) the basic effect of grazing and the related body fat mobilization (9,10) as well as influences of the herbage's botanical composition (10,11), (ii) the degree of usage of concentrates, in particular wheat, barley, and maize in the diets, which mainly contribute SFA, 18:1, and 18:2n-6 but very little 18:3n-3 (10,12,13), and (iii) the effect of herbage conservation (13–15). These factors could also contribute to the differences in composition between common lowland milk and milk from alpine pastures. It is, however, still unclear whether, in addition, specific plants (16), the 18:3n-3 content of the alpine flora (3,17) and even the hypoxic environment *per se* (4) are responsible for the typically high contents of CLA and n-3 PUFA of alpine milk products. Otherwise, the "alpine paradox" (4) would actually be a general phenomenon of feeding grass at any altitude and mainly reflect the difference from mixed, often maize-based, diet types. The objective of the present study was to determine experimentally the factors responsible for the composition of milk fat of cows consuming grass at high altitude. Comparisons included grass-only vs. mixed diets in the lowland, high altitude vs. lowland grazing of the cows, pasture vs. indoor feeding, and young vs. mature swards.

EXPERIMENTAL PROCEDURES

Animals. Eighteen Brown Swiss cows, having been 94 (65 to 127) d into their second lactation at the beginning of the experiment, were blocked by calving date and allocated to three groups (P, pasture group; B, barn-fed group; C, control group) with minimal between-group variance for energy-corrected milk yield, milk protein, and milk fat percentage. Average milk yield, at the start of the experiment, was 21.7, 22.2, and 22.3 kg/d for groups P, B, and C, respectively. The corresponding average contents per kilogram of milk were 43.6, 42.0, and 41.4 g fat; 30.3, 31.1, and 31.8 g protein; and 50.6, 50.4, and 49.1 g lactose, respectively.

Experimental schedule. Six subperiods (S0 to S5) were defined. Each subperiod lasted for 21 d, and intensive sampling was always done during the respective third week. All data and data points in the figures refer to the average values of these intensive sampling weeks within each subperiod. The first part of the experiment took place at the ETH research station Chamau in central Switzerland at 400 m above sea level. During the baseline subperiod (S0) all cows were tethered in a barn and fed a mixed control diet. During the "lowland period," comprising the two subperiods S1 and S2, group P was turned out to pasture for 24 h/d, while groups B and C remained in the barn. Group B was fed only freshly harvested grass *ad libitum*, and group C remained on the control diet. After S2, all cows were kept in the barn again until the alpine vegetation was ready for grazing and harvest. During this time, groups P and B stayed on the grass-only diet at *ad libitum* access. At the end of this transition period, which lasted for 28 d, just before and after the transport to the alpine location, additional milk samples were taken. These samples provided the additional data points in Figures 3 to 6. For the "alpine period," Groups P and B were transported to the alpine ETH research station Alp Weissenstein located at 2000 m above sea level in the Eastern Swiss alps. Half of the group P and B cows were switched to the respective other group in order to counterbalance possible residual effects of the previous treatment. This balanced rearrangement of groups P and B is indicated in the figures by the dotted line. Group C remained in the lowlands and continued to serve as control group. Like the lowland period, the alpine period consisted of two subperiods (S3 and S4). S5 as a final baseline evaluation was carried out with all cows kept in the lowland barn again and fed the control diet.

During the intensive sampling weeks, milk yield was recorded and proportionate milk samples of each cow and each milking were obtained. The samples were frozen at −20°C immediately after sampling. Cows were weighed and blood samples were taken at days 2 and 6 within each sampling week. Two feed samples were drawn every day in each group during the sampling weeks of the subperiods. An aliquot of each feed sample was dried at 60°C for 48 h, and a second was frozen at −20°. Feces were sampled once a day from each animal and frozen. Later, all samples were defrosted, pooled per sampling week (feed) or per animal and sampling week (feces), and then dried and ground through a 0.75-mm sieve for analy-

sis. Feed intake was measured daily for cows kept in barn. On pasture, feed samples were obtained mimicking the selection behavior of the cows for at least 3 h distributed over the whole daytime, and feed intake was calculated using the double-alkane technique (18). As the source for the synthetic marker alkanes (C_{32} and C_{36}), controlled release alkane capsules (CRC; Captec, Auckland, New Zealand) were introduced into the rumen of the cows 8 d prior to each sampling week. Odd-chain alkanes (C_{31} and C_{33}) in feed were used as internal markers. The actual alkane recovery rates in feces were determined with the known feed intake of the group B cows that had also received CRC.

Diets. S1 started at the earliest possible vegetation stage of the first growth; S2 followed 3 wk later on a now more mature sward. The same principle was followed for S3 and S4 at the alpine location. The lowland areas were intensively managed leys with regular manure fertilization. The alpine pasture was native and not fertilized. The botanical composition was visually estimated in the standing sward within each sampling week in eight systematically distributed 9-m^2 plots (four per pasture and harvest paddock, respectively) according to the method of Braun-Blanquet (19). The main species characteristics of the experimental sites are shown in Table 1. The analyzed nutrient and FA composition of the experimental feeds is listed in Table 2. Data for individual vegetation stages are not displayed since vegetation stage effects on milk fat composition were found to be not significant.

Herbage for group B was harvested daily in the early morning from paddocks adjoining those grazed by group P cows; the two were similar in botanical composition. It was then kept tightly packed to avoid any possible losses of FA by wilting of the forage (15). The control diet contained hay, ryegrass silage, and maize silage in proportions of 0.1:0.6:0.3 on a dry-matter (DM) basis, was provided *ad libitum*, and was supplemented by concentrates according to milk yield. The two commercial concentrates used contained rumen-protected fat (Table 2), which made up 410 g/kg of total dietary FA in the control diet. The cows of all groups had *ad libitum* access to NaCl and a nonvitaminized mineral mix (Ca:P = 2:1; Nährkosan, Büron, Switzerland) during the whole experiment.

Analysis. Frozen milk samples were defrosted and pooled per cow and sampling week. After direct transesterification, according to Suter *et al.* (20), the FAME were extracted with *n*-heptane and then separated and quantified with GC using a Sil 88 column (100 m × 0.25 mm, 0.2 µm; Varian Inc, Darmstadt, Germany) on an HP 6890 (Hewlett-Packard, Wilmington, DE). Hydrogen flow was 1.5 mL/min; 0.5 mL of *n*-heptane containing the FAME was injected on column. The temperature program was: 60°C for 5 min; increase by 14°C/min up to 165°C; 2°C/min up to 225°C; isothermal for another 15 min. Temperature program and peak identification were adapted from Collomb and Bühler (21) with some modifications. The peaks for 16:1*trans* and 20:1*trans* may contain some branched-chain FA (22). They were therefore only included in the calculation of total FAME and not separately shown in the tables. The applied chromatographic method also is not able to separate *trans-*

TABLE 1
Frequent Plant Species in the Lowland and the Alpine Pastures Used in the Experiment

	Estimated proportions of total ground covering[a]		
	>10%	4–10%	1–4%
Lowland site (16 plots)			
Monocotyledonae (77%)			
Total species found: 9	*Lolium multiflorum, Poa trivialis, Lolium perenne*	*Poa annua*	*Festuca pratense, Poa pratense*
Leguminosae (6%)			
Total species found: 2		*Trifolium repens*	*Trifolium pratense*
Dicotyledonae (except Leguminosae) (16%)			
Total species found: 8	*Taraxacum officinale*		*Rumex obtusifolius, Ranunculus repens, Bellis perennis*
Alpine site (16 plots)			
Monocotyledonae (36%)			
Total species found: 14	*Festuca rubra*	*Trisetum flavescens, Phleum alpinum, Poa alpina, Festuca pratense*	*Poa trivialis, Nardus stricta, Poa pratense*
Leguminosae (23%)			
Total species found: 11	*Trifolium pratense*	*Trifolium montanum*	*Trifolium repens, Trifolium badii, Lotus* spp., *Trifolium nivale*
Dicotyledonae (except Leguminosae) (40%)			
Total species found: 46		*Rumex arifolius, Alchemilla vulgaris, Taraxacum officinale*	*Peucedaneum osthrutium, Ranunculus montanus, Ranunculus acer, Achillea stricta, Carum carvi, Chaero phyllum cicutaria, Campanula scheuchzeri, Crepis aurea, Veronica chamaedrys, Plantago alpina, Leontodon hispidus*

[a]Visually estimated according to the method of Braun-Blanquet (19).

8,*cis*-10 and *trans*-7,*cis*-9 from the major *cis*-9,*trans*-11 CLA-isomer as well as *trans*-11,*cis*-13 from *cis*-9,*cis*-11 (3,23). However, the concentration of the *trans*-7,*cis*-9 isomer has been described to be about 100-fold lower than the *cis*-9,*trans*-11 isomer in milk, and changes due to (alpine) feeding occurred on a rather low level (3,24). Proportions of and effects on *trans*-8,*cis*-10 are even lower than for *trans*-7,*cis*-9 (24). Thus, we conclude that any distortion of the effect on the *cis*-9,*trans*-11 isomer is small, although it might be slightly biased because both of these minor isomers are likely to increase with altitude of the site where the dairy cows are kept (24). Similarly, the *cis*-9,*cis*-11 isomer, coeluting with the *trans*-11,*cis*-13 isomer, seems to be present only in traces if at all (24; Collomb, M., personal communication); we therefore refer to this peak as *trans*-11,*cis*-13. Response factors were determined using BCR 164 standard milk fat (Certified Reference Material; EC Reference Materials, Brussels, Belgium) as a reference. The milk fat content measured with a standard NIR procedure (Milkoscan 4000; Foss, Höganäs, Sweden) closely correlated with the amount of total FAME obtained with GC ($r^2 = 0.93$). Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) was separated and quantified in the milk samples of S2 and S3 and in the samples drawn directly before and after transfer to the alp, using an

RTX225 column (30 m × 0.32 mm, 0.5 µm; Restek Corporation, Bellefonte, PA). Injection volume was 1 µL in a 30:1 split modus at 240°C. Hydrogen flow was 4.0 mL/min. The temperature program was 140°C for 2 min, ramp 5°C/min up to 215°C, isothermal for 5 min.

Lipids from feeds were extracted by accelerated solvent extraction (ASE 200; Dionex Corp., Sunnyvale CA) using hexane/isopropanol (2:1 vol/vol) and transformed into FAME according to Wettstein *et al.* (25). FAME were dissolved in hexane and analyzed with GC on a Supelcowax™-10 column (30 m × 0.32 mm, 0.25 µm; Supelco Inc., Bellefonte, PA) after split injection (1:30) at 270°C. The injection volume was 2 µL; hydrogen flow was 2.2 mL/min. The temperature program was 160°C for 1 min, ramp 20°C/min up to 190°C, ramp 7°C/min up to 230°C; isotherm at 230°C for 4 min, ramp 20°C/min up to 250°C, isotherm for 4 min.

Tocopherols in milk were analyzed after saponification with KOH with normal-phase HPLC on a Merck-Hitachi system with UV detection (La Chrom; Darmstadt, Germany) according to Rettenmaier and Schüepp (26). The β-, γ-, and δ-tocopherols were always below the detection limit.

Feed contents of DM and nitrogen (N × 6.25 = crude protein) were analyzed with standard methods as described previ-

TABLE 2
Nutrient and FA Contents of Feeds Used in the Experiment[a]

	Origin	Dry matter (DM) (g/kg)	NE$_L$[b] (MJ/kg)	Crude protein (g/kg)	FA (g/kg DM)					
					Total FA	SFA[c]	18:1Δ11	18:1Δ9	18:2n-6	18:3n-3
Green forages[d]										
Pasture	Lowland	190 ± 13	6.12 ± 0.46	142 ± 4	16.2 ± 3.7	2.86 ± 0.60	0.065 ± 0.029	0.470 ± 0.288	2.91 ± 1.16	9.06 ± 1.61
Harvest	Lowland	177 ± 24	6.11 ± 0.54	137 ± 8	14.8 ± 2.7	2.77 ± 0.47	0.057 ± 0.019	0.343 ± 0.177	2.61 ± 1.01	8.27 ± 0.87
Pasture	Alpine	190 ± 4	5.57 ± 0.41	138 ± 8	12.2 ± 2.4	2.77 ± 0.33	0.066 ± 0.012	0.616 ± 0.037	2.35 ± 0.42	5.72 ± 1.45
Harvest	Alpine	186 ± 25	5.63 ± 0.28	138 ± 8	13.4 ± 2.4	2.93 ± 0.37	0.069 ± 0.004	0.646 ± 0.004	2.63 ± 0.34	6.29 ± 1.50
Components of the mixed diet[d]										
Hay	Lowland	770 ± 14	5.62 ± 0.02	178 ± 1	18.4 ± 0.1	4.64 ± 0.09	0.085 ± 0.000	0.439 ± 0.003	2.90 ± 0.03	9.12 ± 0.19
Grass silage	Lowland	514 ± 43	5.32 ± 0.03	168 ± 0	19.0 ± 0.3	4.40 ± 0.19	0.080 ± 0.006	0.669 ± 0.043	3.19 ± 0.11	9.58 ± 0.33
Maize silage	Lowland	334 ± 11	6.33 ± 0.01	62 ± 0	22.8 ± 0.2	4.57 ± 0.06	0.205 ± 0.004	6.103 ± 0.177	11.20 ± 0.20	0.59 ± 0.01
Energy concentrate[e]	Lowland	854 ± 2	7.45 ± 0.05	151 ± 4	61.3 ± 1.6	44.79 ± 1.54	0.335 ± 0.016	5.587 ± 0.324	9.70 ± 0.70	0.78 ± 0.02
Protein concentrate[f]	Lowland	860 ± 0	7.75 ± 0.05	423 ± 6	59.1 ± 0.6	46.23 ± 0.55	0.275 ± 0.006	4.229 ± 0.366	7.68 ± 0.47	0.57 ± 0.09

[a]Means ± SD.
[b]NE$_L$: net energy for lactation, calculated based on formula of RAP (28).
[c]SFA: saturated FA (sum of 10:0 through 24:0).
[d]Average of two subperiods with two samples per subperiod; each sample pooled from 7 d.
[e]g/kg DM: wheat, 300; barley, 190; wheat bran, 150; oats, 120; straw meal, 50; maize, 50; crystalline fat (rumen-protected; Alikon, Bützberg, Switzerland), 40; maize gluten, 30; molasses, 30; canola cake, 20; sugar beet pulp, 20.
[f]g/kg DM: soybean meal, 480; maize gluten, 220; straw meal, 60; wheat, 40; crystalline fat, 40; sugar beet pulp, 40; molasses, 30; wheat bran, 10.

ously (27). Contents of net energy for lactation were calculated based on the equations used in the official Swiss feed evaluation (28). Plasma metabolites and insulin were analyzed with standard assays. Plasma insulin was measured with a radioimmunoassay kit (No. 1064 1401; Pharmacia, Uppsala, Sweden). Plasma concentrations of nonesterified FA (NEFA), β-hydroxybutyrate (BHB), glucose, and urea were analyzed on a COBAS-MIRA chemistry analyzer (Roche, Basel, Switzerland), with the following assays: NEFA with NEFA-C WA 994-75409 (Wako, Neuss, Germany), BHB with product 310-A (Sigma, Buchs, Switzerland), glucose with assay No. 1447513 and urea with assay No. 1489364 (both Roche).

Statistics. Data were evaluated with three mixed models by SAS V8.2e (PROC MIXED; SAS Institute, Cary, NC;). Model 1 included group (P, B, or C) and calving date (classified in three blocks of similar calving date) as fixed factors. Animal was included as random effect, and the individual baseline (S0) values were used as a covariate. The least squares (LS) means and all significances of the group × period interaction, shown in Tables 3 and 4, are based on model 1. The same model, but without the covariate S0, was used to obtain LS means of all subperiods (including S0) for the illustration in the figures. A third model was applied for the calculation of the altitude and the vegetation stage effects, considering vegetation stage (two classes: young or mature) and main period (lowland or alps) as additional fixed effects. Group C, for which feed and altitude remained unchanged, was excluded from the calculations with model 3.

RESULTS

Feed intake and metabolic energy supply. During the lowland period, the DM intake of the grass-only fed cows was lower than in cows of group C [significantly for group P ($P < 0.05$) and as a tendency for group B ($P < 0.1$); Table 3]. Nevertheless, the calculated net energy intake of B and P cows did not differ significantly from C cows during the lowland period (Fig. 1A), and milk yield decreased only slightly (Fig. 2B). However, unlike C cows, P and B cows lost weight from S0 to S1 ($P < 0.05$; Fig. 2A), and all blood plasma metabolites indicated a certain catabolic state (Table 3). In the alpine period (S3 and S4), DM intake in groups P and B decreased ($P < 0.001$) compared with the lowland period and with C cows, and net energy intake was reduced by approximately one-third ($P < 0.01$). Accordingly, milk yield markedly decreased when cows were transferred to the alpine location ($P < 0.001$; Fig. 2). Although body weight did not further decline, plasma levels of BHB ($P < 0.001$) and NEFA ($P < 0.001$) still increased and plasma glucose ($P < 0.05$) and insulin ($P < 0.001$) further decreased during the alpine sojourn of the cows (Table 3).

FA intake. In S1 and S2, individual FA consumption of the grass-only fed cows differed from that of the C cows (Table 3) since lowland grass had clearly higher levels of 18:3n-3 but much lower levels of SFA, monounsaturated FA, and 18:2n-6 than the control diet (*cf.* Table 2). Thus, 18:3n-3 was the main dietary FA for groups P and B, whereas for group C, 16:0 and

TABLE 3
Daily Intake and Metabolic Traits of Cows During the Experimental Periods[a]

Group	Lowland period (avg. of S1 and S2)			Alpine period (avg. of S3 and S4)			SE[b]	Significances		
	Control	Barn	Pasture	Control	Barn	Pasture		Group[c]	Period (altitude)[d]	Group × period[c]
Intake (per cow per day)[e,f]										
DM (kg)	18.2[a]	16.9[a,b]	16.4[b]	18.3[a]	13.8[c]	13.7[c]	0.52	<0.001	<0.001	<0.01
FA (g)										
Total FA	597	238*	254*	580	169*	161*	19.3	<0.001	<0.001	NS[h]
Saturated FA	342.9	45.5*	46.5*	325.3	38.2*	37.5*	14.70	<0.001	<0.001	NS
18:1Δ9	56.3	5.74*	8.05*	54.9	8.54*	8.34*	1.695	<0.001	<0.01	NS
18:1Δ11	3.26	0.97*	1.11*	3.17	0.93*	0.93*	0.112	<0.001	<0.05	NS
18:2n-6	114.0	44.4*	49.0*	112.3	34.7*	32.9*	3.37	<0.001	<0.001	NS
18:3n-3	80.4[b]	138.2[a]	148.5[a]	83.3[b]	82.8[b]	79.8[b]	5.53	<0.001	<0.001	<0.001
Blood plasma concentrations[e,f]										
BHB[g] (mmol/L)	477[d]	667[c]	638[c,d]	510[c,d]	921[b]	1119[a]	35.0	<0.001	<0.001	<0.01
NEFA[g] (mmol/L)	93[c]	64[c]	137[b]	60[c]	94[c]	203[a]	18.0	<0.001	<0.05	<0.05
Glucose (mmol/L)	3.23	3.20	3.02*	3.19	2.92*	2.92*	0.057	<0.05	<0.01	NS
Insulin (pmol/L)	71.2	55.0*	37.1*	66.9	30.2*	24.1*	3.99	<0.001	<0.001	NS

[a]n = 12 per group and period.
[b]Standard error of groups.
[c]Evaluated by model 1.
[d]Evaluated by model 3 (without control).
[e]Superscript roman letters a–d indicate significant differences ($P < 0.05$) between group × period interaction means according to model 1.
[f]Values marked with asterisks are significantly different ($P < 0.05$) from the respective control group value according to model 1.
[g]BHB, β-hydroxybutyrate; NEFA, nonesterified FA.
[h]NS, not significant; for other abbreviation see Table 2.

18:0, originating from the concentrates, and 18:1 and 18:2n-6, coming from both the concentrates and the maize silage, were the major dietary FA. In the alpine period, the total FA content of the herbage tended to be lower ($P < 0.1$; t-test) than during the lowland period (Table 2). Additionally, the 18:3n-3 proportion of total FA in the herbage declined ($P < 0.01$; t-test). This resulted in a reduced intake of total FA and 18:3n-3 by 23 and 43%, respectively, in the alpine compared with the lowland period for both P and B ($P < 0.001$; Table 3 and Fig. 1B).

Fat content and saturated FA in milk. The fat content in milk was not affected by the grass-only diets in the lowlands but (with concomitantly decreased milk yields) it increased in group P ($P < 0.001$) and in group B ($P < 0.1$) after transport to the alps. With the start of grass feeding in S1, the proportion of the even-chain SFA 6:0 through 14:0 in total milk FAME of groups P and B initially increased ($P < 0.001$). From subperiod S2 on, there was an almost linear decrease in the proportions of these FA in total FAME and in the milk of the P and B cows, with the values finally decreasing below the levels of control group (representatively shown for 10:0 and 12:0 in Fig. 3). Figure 3 illustrates that this was a continuous decline that, to a considerable extent, had already taken place before the transfer to the alpine site. In S5, the concentration of 6:0 through 14:0 returned to the baseline values represented by the C cows. During the lowland period, a decrease in the proportion of 16:0 as part of the total FAME occurred with the herbage-only diets (P cows: $P < 0.01$; B cows: $P < 0.05$) compared with the control diet (C), whereas 17:0 moderately increased ($P < 0.05$ for both groups) and the proportion of 18:0 remained unaffected. With

the alpine sojourn of P and B groups, the 18:0 proportion of total FAME significantly increased whereas those of 16:0 and 17:0 were not affected by the location.

18:1 FA in milk. Among the identified 18:1 *trans* isomers, the 18:1*trans*-10/*trans*-11 proportions of total FAME increased ($P < 0.001$) with lowland grass-only diets by more than 3.3-fold, whereas the 18:1*trans*-4-*trans*-9 isomers were moderately reduced, particularly in group B ($P < 0.01$), compared with C cows (Table 4). During the alpine period, compared with the lowland values in groups P and B, the double-peak comprising 18:1*trans*-11 (*trans*-vaccenic acid) clearly decreased, whereas the concentration of the 18:1*trans*-4 to *trans*-9 in total FAME significantly increased. The proportion of 18:1*cis*-9, as the dominating 18:1, remained unaffected by the grass-only diets in the lowlands but was elevated at high altitude by 32% ($P < 0.001$) compared with the lowland values in P and B cows.

CLA in milk. All identified CLA isomers increased markedly as a proportion of the total FAME when grass-only diets were fed in groups P and B ($P < 0.001$; Table 4). Accordingly, during the lowland periods, the proportion of the main isomer, 18:2 *cis*-9,*trans*-11, was on average 1.9-fold higher in milk fat of P and B cows than in the milk of C cows. From S0 to S1, CLA increased in both P and B cows ($P < 0.001$); from S1 to S2 a further increase occurred only in P cows ($P < 0.05$). This was the reason for the significant difference between P and B cows in the lowland period ($P < 0.01$; Table 4, Fig. 4). During the alpine sojourn, the concentration of the main CLA isomer, 18:2 *cis*-9,*trans*-11, in total FAME decreased ($P < 0.01$), whereas the 18:2 *trans*-11,*cis*-13 isomer significantly increased in the P and B groups

TABLE 4
FA Content of Milk, FA Profile of Milk Fat, and α-Tocopherol Content of Milk[a]

Group	Lowland period (avg. of S1 and S2)			Alpine period (avg. of S3 and S4)			SE[b]	Significances		
	Control	Barn	Pasture	Control	Barn	Pasture		Group[c]	Period (altitude)[d]	Group × period[c]
Total FAME[e] (g/L)	41.1[b]	43.8[a,b]	41.3[b]	40.1[b]	46.0[a,b]	47.0[a]	1.76	NS[i]	<0.001	<0.05
FA[e,f] (g/100 g FAME)										
4:0	3.48[b]	3.48[b]	3.55[b]	3.26[c]	3.89[a]	3.55[b]	0.071	<0.05	<0.01	<0.001
6:0	2.37[b]	2.55[a]	2.55[a]	2.19[c]	2.17[c]	2.04[d]	0.033	NS	<0.001	<0.01
8:0	1.31[b]	1.52[a]	1.53[a]	1.21[c]	1.06[d]	1.02[d]	0.020	NS	<0.001	<0.001
10:0	2.74[b]	3.46[a]	3.54[a]	2.53[b]	1.91[c]	1.95[c]	0.011	NS	<0.001	<0.001
12:0	3.14[b]	4.04[a]	3.85[a]	3.00[b]	2.23[c]	2.09[c]	0.067	NS	<0.001	<0.001
14:0	10.7[b]	12.1[a]	11.5[a,b]	10.8[b]	9.5[c]	8.5[d]	0.25	<0.05	<0.001	<0.001
16:0	34.0	29.3*	25.3*	33.7	29.5*	25.4*	0.79	<0.001	NS	NS
17:0	0.627[c]	0.875[a]	0.843[a,b]	0.642[c]	0.811[b]	0.843[a,b]	0.0089	<0.001	<0.05	<0.05
18:0	10.2[b,c]	9.3[c]	10.1[b,c]	9.4[c]	10.4[b]	11.8[a]	0.25	<0.01	<0.001	<0.001
18:1*t*4-*t*8	0.249[b]	0.185[c]	0.236[b,c]	0.238[b,c]	0.285[a,b]	0.313[a]	0.0161	NS	<0.001	<0.01
18:1*t*9	0.426[a,b]	0.309[c]	0.328[b,c]	0.422[a,b]	0.450[a]	0.469[a]	0.0256	NS	<0.001	0.05
18:1*t*10-11	0.84[d]	3.34[a]	4.01[a]	0.73[d]	2.37[c]	3.12[b]	0.219	<0.001	<0.001	<0.05
18:1*t*12	0.182	0.191	0.185	0.166	0.187	0.177	0.0090	NS	NS	NS
18:1*c*9	18.8[b,c]	16.4[c]	18.3[c]	20.2[b,c]	21.6[a,b]	24.1[a]	0.88	NS	<0.001	<0.001
18:1*c*11	0.477	0.399	0.460	0.541	0.576	0.653	0.0371	NS	<0.001	NS
18:1*c*12	0.160	0.092*	0.095*	0.174	0.101*	0.117*	0.0042	<0.001	<0.001	NS
18:2*c*9,*t*11[g]	0.529[c]	1.370[b]	1.705[a]	0.551[c]	1.163[b]	1.340[b]	0.098	<0.001	<0.01	<0.05
18:2*t*9,*t*11	0.041	0.082*	0.088*	0.045	0.077*	0.085*	0.0032	<0.001	NS	NS
18:2*t*10,*c*12	0.038[c]	0.046[b]	0.044[b,c]	0.041[b,c]	0.042[b,c]	0.051[a]	0.0020	<0.05	NS	<0.05
18:2*t*11,*c*13[h]	0.020[c]	0.073[b]	0.073[b]	0.020[c]	0.102[a]	0.114[a]	0.0072	<0.001	<0.001	<0.05
18:2n-6	1.38[b]	0.84[c]	0.94[c]	1.31[b]	1.42[b]	1.57[a]	0.040	<0.01	<0.001	<0.001
18:2*c*9,*c*15	0.033[c]	0.049[b]	0.047[b]	0.032[c]	0.050[b]	0.054[a]	0.0011	<0.001	<0.01	<0.05
18:3n-6	0.028[a]	0.021[b]	0.021[b]	0.029[a]	0.017[c]	0.018[c]	0.0009	<0.001	<0.001	<0.05
18:3n-3	0.495[d]	0.619[c]	0.703[c]	0.536[d]	0.950[b]	1.146[a]	0.030	<0.001	<0.001	<0.001
20:0	0.162[b]	0.136[c]	0.139[c]	0.171[b]	0.183[a,b]	0.200[a]	0.0054	NS	<0.001	<0.001
20:4n-6	0.077	0.071	0.066*	0.083	0.073*	0.066*	0.0020	<0.001	NS	NS
20:5n-3	0.060	0.073*	0.083*	0.065	0.083*	0.083*	0.0038	<0.001	NS	NS
22:5n-3	0.078	0.113*	0.109*	0.085	0.118*	0.120*	0.0048	<0.001	NS	NS
22:6n-3	ND	0.009*	0.009*	ND	0.010*	0.009*	0.002	<0.001	NS	NS
Total n-3	0.81[d]	1.32[c]	1.43[c]	0.85[d]	1.64[b]	1.98[a]	0.056	<0.001	<0.001	<0.001
Total n-6	2.18[b]	1.62[c]	1.77[c]	2.11[b]	2.16[b]	2.36[a]	0.056	<0.05	<0.001	<0.001
n-6/n-3[f]	2.71	1.24*	1.24*	2.54	1.32*	1.21*	0.067	<0.001	NS	NS
α-Tocopherol[f] (mg/L)	0.75	1.42*	1.37*	0.67	1.57*	1.57*	0.041	<0.001	<0.05	NS

[a]n = 12 per group and period.
[b]Standard error of groups.
[c]Evaluated by model 1.
[d]Evaluated by model 3 (without control).
[e]Superscript roman letters indicate significant differences (*P* < 0.05) between group × period interaction means according to model 1.
[f]Values marked with asterisks are significantly different (*P* < 0.05) from the respective control group value according to model 1.
[g]Including the *trans*-8,*cis*-10 and *trans*-7,*cis*-9 isomers.
[h]Possibly including traces of the *cis*-9,*cis*-11 isomer.
[i]NS, not significant; ND, not detected.

(*P* < 0.001). In S5, the CLA concentration and secretion returned to the baseline value (Fig. 4).

Linolenic acid in milk. Whereas the γ-linolenic acid (18:3n-6) proportion of total FAME decreased with grass-only diets (*P* < 0.01) in the lowland period, the concentration of α-linolenic acid (18:3n-3) increased by 34% (*P* < 0.001; Table 4). Slightly lower 18:3n-3 proportions were observed for B cows compared with P cows (*P* < 0.01) and for S2 compared with S1 (*P* < 0.05; Fig. 5). After transfer to the alpine site, 18:3n-3 proportions of total FAME of pastured cows (group P) were 0.63-fold higher compared with the respective lowland levels

(*P* < 0.001) and 1.14-fold higher than the value found in the C group (*P* < 0.001). The increase in B cows was less pronounced. The analysis of the milk samples obtained directly before and after the transfer to the alps showed that the increase of 18:3n-3 proportion was a direct response to the alpine sojourn rather than a continuous time effect of grass feeding (Fig. 5). In contrast to the proportionate values, the absolute daily 18:3n-3 secretion with milk remained unaffected by feeding grass to cows kept in barn. The pastured cows still showed a significantly higher daily 18:3n-3 secretion in the lowlands (*P* < 0.01) and at the alpine site (*P* < 0.001) compared with the
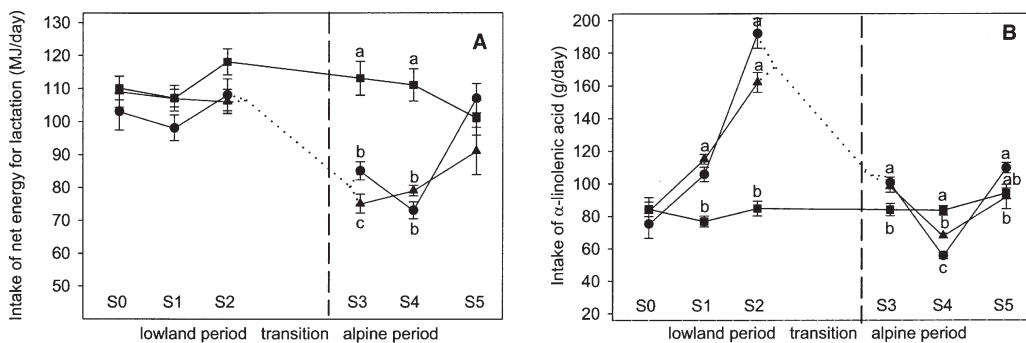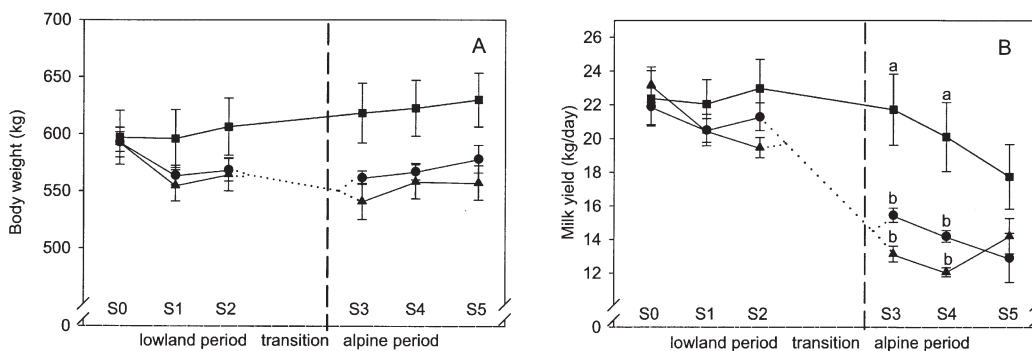
FIG. 1. Average intake of net energy and α-linolenic acid throughout the entire experiment. S0 and S5 baseline subperiods; S1 and S2, lowland subperiods; S3 and S4 alpine subperiods. ● Pasture group; ▲ indoor grass group; ■ control group; n = 6 per group. Dotted lines indicate that half of the cows were switched between pasture and indoor group. Dashed lines mark the day of transport of the grass-fed cows to the alpine location. Error bars represent SE. Different superscripts indicate significant group differences at $P < 0.05$.
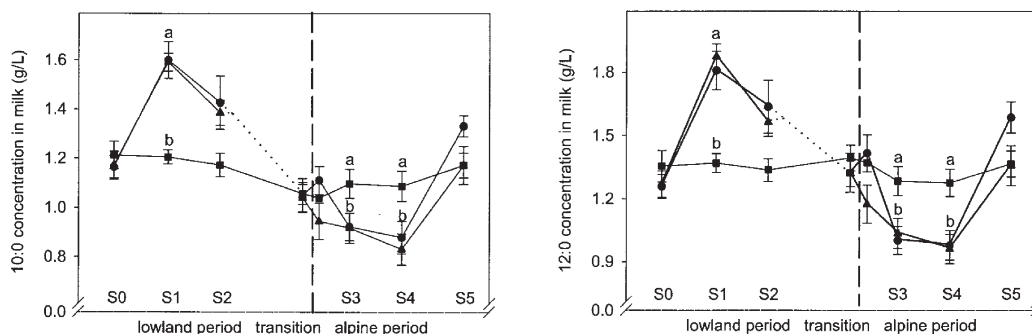

FIG. 2. Average body weight and milk yield throughout the entire experiment. S0 and S5 baseline subperiods; S1 and S2, lowland subperiods; S3 and S4 alpine subperiods. ● Pasture group; ▲ indoor grass group; ■ control group; n = 6 per group. Dotted lines indicate that half of the cows were switched between pasture and indoor group. Dashed lines mark the day of transport of the grass-fed cows to the alpine location. Error bars represent SE. Different superscripts indicate significant group differences at $P < 0.05$.
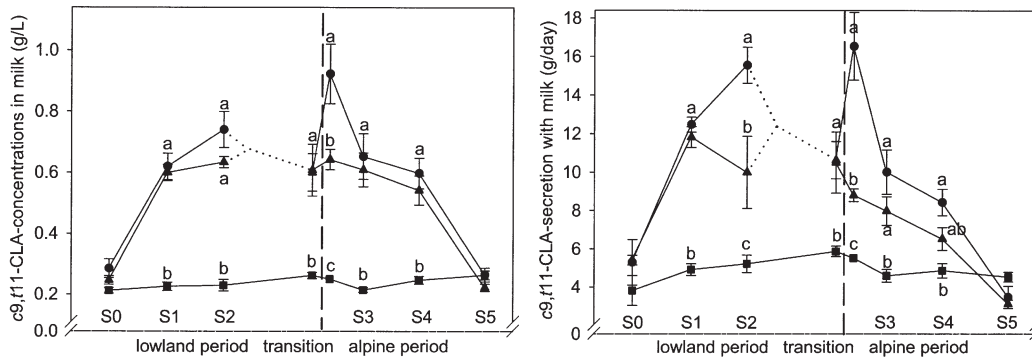

FIG. 3. Concentrations of medium-chain saturated FA in milk throughout the entire experiment. S0 and S5 baseline subperiods; S1 and S2, lowland subperiods; S3 and S4 alpine subperiods. ● Pasture group; ▲ indoor grass group; ■ control group; n = 6 per group. Dotted lines indicate that half of the cows were switched between pasture and indoor group. Dashed lines mark the day of transport of the grass-fed cows to the alpine location. Error bars represent SE. Different superscripts indicate significant group differences at $P < 0.05$.
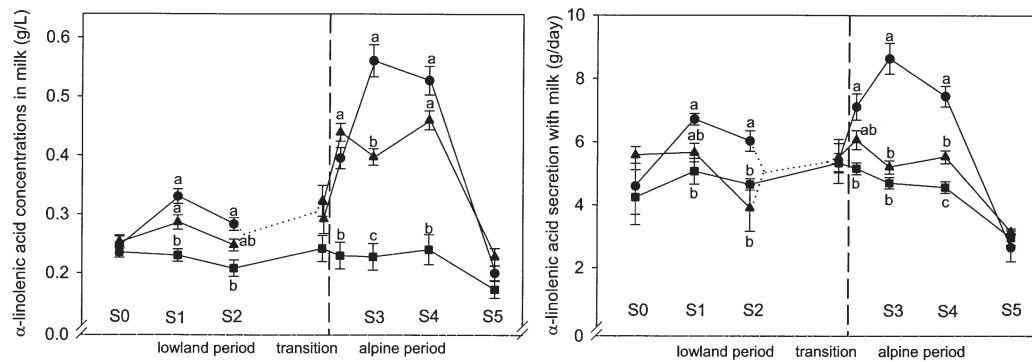
FIG. 4. Milk concentrations and daily excretion of CLA (18:2c9,t11) throughout the entire experiment. S0 and S5 baseline subperiods; S1 and S2, lowland subperiods; S3 and S4 alpine subperiods. ● Pasture group; ▲ indoor grass group; ■ control group; n = 6 per group. Dotted lines indicate that half of the cows were switched between pasture and indoor group. Dashed lines mark the day of transport of the grass-fed cows to the alpine location. Error bars represent SE. Different superscripts indicate significant group differences at *P* < 0.05.

FIG. 5. Milk concentrations and daily excretion of α-linolenic acid (18:3n-3) throughout the entire experiment. S0 and S5 baseline subperiods; S1 and S2, lowland subperiods; S3 and S4 alpine subperiods. ● Pasture group; ▲, indoor grass group; ■ control group; n = 6 per group. Dotted lines indicate that half of the cows were switched between pasture and indoor group. Dashed lines mark the day of transport of the grass-fed cows to the alpine location. Error bars represent SE. Different superscripts indicate significant group differences at *P* < 0.05.

control cows. In S5, 18:3n-3 concentration returned to the baseline value (Fig. 5).

Long-chain PUFA in milk. Concentrations of the long-chain n-3 PUFA (EPA, 20:5n-3; docosapentaenoic acid, 22:5n-3; DHA, 22:6n-3) were higher in milk samples of the grass-only groups than in C cows (*P* < 0.01), whereas 20:4n-6 (arachidonic acid) was somewhat lower in P and B cows (Table 4). The ratio of all n-6/n-3 PUFA was diminished by half in P and B compared with C. The long-chain PUFA responded neither to the alpine sojourn (Table 4) nor to the stage of vegetation (data not shown).

Phytanic acid. Phytanic acid concentration in milk fat, which was analyzed in a smaller subset of samples (S2, transition period, and S3), was markedly higher with grass-only feeding compared with the control diet (*P* < 0.001; Fig. 6). There was a slight, but not significant, additional increase with alpine sojourn.

α-Tocopherol. The α-tocopherol concentration was 0.86- and 1.31-fold higher in milk of P and B cows than that of C

cows during the lowland and the alpine period, respectively (*P* < 0.001; Table 4). In S5, the values of the previously grass-fed groups dropped nearly to the baseline level (from 1.57 mg/L in S4 to 0.95 mg/L in S5; the baseline value in S0 had been 0.79 mg/L for P and B on average).

DISCUSSION

Impact of feeding grass only on milk FA profile. In the lowland period, turnout to pasture and the concurrent start of feeding only grass in the barn did not markedly change the net energy intake relative to controls but clearly altered the amount and composition of the ingested lipids and the FA composition of the milk lipids. The intake of SFA, 18:1, and 18:2n-6 declined for groups P and B in the lowlands, since the concentrates and the included rumen-protected fat, maize, and cereals were no longer available. In turn, 18:3n-3-intake was nearly doubled (Fig. 1).

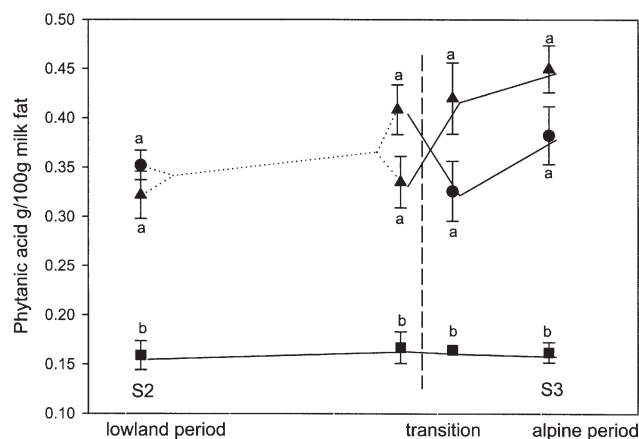Among the unsaturated FA, 18:3n-3 is one of those most

FIG. 6. Phytanic acid concentration in milk fat. S2, lowland period; S3, alpine period. ● Pasture group; ▲ indoor grass group; ■ control group; n = 6 per group. Dotted lines indicate that half of the cows were switched between pasture and indoor group. Dashed line marks the day of transport of grass-fed cows to the alpine location. Error bars represent SE. Different superscripts indicate significant differences at $P < 0.05$.

susceptible to ruminal biohydrogenation (29). Up to 99% of the dietary 18:3n-3 can be partially or completely hydrogenated (30). Main derivatives of 18:3n-3 biohydrogenation are 18:1*trans*-11 and 18:0 (31), which are partly desaturated at the Δ9 position to *cis*-9,*trans*-11 CLA and 18:1*cis*-9, respectively, in the mammary gland (32). As a consequence, under normal ruminal conditions, the high proportion of 18:3n-3 in grass-only diets explains the elevated contents of CLA and 18:1*trans*-11 in milk fat as also found in the current study (Fig. 4, Table 4). However, the increase of *cis*-9,*trans*-11 CLA during lowland grazing in our case was even more pronounced than in other studies (3,10,11,33). We did not find a substantial reduction of CLA in milk fat when harvested grass was fed instead of grazing, as has been previously described by Elgersma *et al.* (34), but this is probably a problem related to the wilting during storage (15), which we had carefully avoided.

The small proportion of 18:3n-3 passing by the rumen may appear in milk in its native form or in the form of endogenous derivatives (20:5n-3, 22:5n-3, and 22:6n-3; 35). Accordingly, all 20- and 22n-3 PUFA were higher in the milk fat of grass-only fed cows compared with the control. However, the moderate increase of these n-3 PUFA proportion found in milk fat in the lowland period of the present study (Table 4) did not reflect the massive increase in 18:3n-3 intake of cows fed only grass (Fig. 1; Table 3). This underlines the high extent and relevance of the biohydrogenation of 18:3n-3 during the lowland period. We assume that the supply of fermentable energy during this period was sufficiently high to support extensive biohydrogenation. Furthermore, mobilized body fat, as indicated by the decrease in body weight (Fig. 2) and the significantly higher NEFA concentration in plasma of the pasture group (Table 3), may have contributed to the 18:3n-3 in milk fat (36). Thus, the actual recovery of ingested 18:3n-3 in the milk can be assumed to be even lower than the apparent recovery.

The relatively sharp increase in the proportion of 6:0 to 14:0

in milk of P and B cows at the beginning of the lowland period (Fig. 3) may be explained by a shift in ruminal production from propionate to acetate, when all concentrates were withdrawn since acetate is the substrate for the FA *de novo* synthesis in the mammary gland. The subsequent continuously decreasing amount of medium-chain SFA might have been a result of a decreased energy intake and mobilization of body fat stores at the expense of *de novo* synthesis. However, as a moderate decrease of these medium-chain SFA also occurred in the control group, which was not exposed to dietary changes, a general effect linked to the progression of lactation may be assumed.

Impact of feeding alpine grass on the milk FA profile. Unlike Kraft *et al.* (3), we found that *cis*-9,*trans*-11 CLA and 18:1*trans*-11 concentrations in milk fat were not further elevated when P and B cows received alpine instead of lowland grass (Fig. 4). These are not necessarily conflicting results, because other influencing factors apart from alpine effects cannot be excluded, since Kraft *et al.* (3) compared milk from different herds. Ferlay *et al.* (12) found a considerable reduction of CLA after 3 wk of alpine grazing and related this to the stage of plant development (17). In the present experiment, no corresponding effect of stage of vegetation occurred at any experimental site (Fig. 4). In contrast to what was indicated by the results of Fievez *et al.* (11), there was also no noticeable effect of forage biodiversity on the CLA concentration. Thus, feeding only grass, irrespective of site and vegetative stage, may *per se* be a main contributor to high *cis*-9,*trans*-11 CLA proportions in milk fat. Whereas the main isomer, *cis*-9,*trans*-11, slightly declined owing to alpine feeding, the *trans*-11,*cis*-13 isomer was further increased, making it an interesting marker and potential target of experiments elucidating its possible physiological effects.

The 18:3n-3 contents of forages were described to decrease with maturation of the grass (37). In the present study, this was also found in the alpine grass (6.81 vs. 4.13 g 18:3n-3/kg DM for young vs. mature grass) but not in the lowlands (6.76 vs. 9.01 g 18:3n-3/kg DM). Furthermore, *Lolium perenne* and *Trifolium repens*, two of the dominant species in the lowland pasture (Table 1), contain far higher contents of 18:3n-3 than many other grasses and legumes (37). The absence of these species and the on-average higher maturity of the alpine forages (27) may at least partly explain the lower 18:3n-3 proportion in total FAME of the alpine grass. Together with alpine-specific anorexia of the cows (27), this drastically decreased 18:3n-3 intake (Fig. 1). In contrast to this, the most surprising and, with respect to human nutrition, most important effect of the alpine sojourn was the sharp increase of 18:3n-3 concentrations in milk fat, which in our case was definitely not related to the 18:3n-3 supply from food as suggested elsewhere (3,17).

Three hypotheses will be discussed here that could explain, alone or in combination, this discrepancy between intake of 18:3n-3 and its concentration in milk fat. One hypothesis (i) is that body fat mobilization with a preference for 18:3n-3 took place as a result of the energy deficiency associated with high altitude. The other hypotheses are that ruminal biohydrogenation activity was reduced by either energy deficiency (ii) or specific

secondary plant ingredients that inhibit ruminal microbes (iii). The fact that less than 10% of 18:3n-3 escapes biohydrogenation in the rumen (30) makes it likely that even small changes in the ruminal ecosystem, influencing hydrogenating microbes, may have large effects on the amount of 18:3n-3 available at the duodenum.

(i) Metabolic energy deficiency related to altitude. One key issue might be the decrease in energy intake of the cows transferred to the alpine site. Although cows partially reduced the energy deficit by a sharp decline in milk yield, as found in previous studies (27,38), there were several indications of a persisting metabolic energy deficit such as decreased plasma glucose and insulin levels and increased body fat mobilization (as indicated by elevated plasma NEFA and BHB levels). Mobilization of body fat depots is typical for cows kept in high alpine regions (27,39), and a preferential release of 18-PUFA from body fat was observed in undernourished ruminants (36). This would suggest that the elevated 18:3n-3 concentrations in the milk fat obtained in the alpine period (Fig. 5) could originate at least partially from body fat. This hypothesis is underlined by the fact that 18:3n-3 was even more highly concentrated in the P cows because grazing activity is assumed to cause additional energy expenditure in this hypoxic and uneven environment (38). The differences in plasma BHB and NEFA concentrations between the P and the B group during the alpine period further support this explanation. The higher proportion of 18:1*cis*-9 in the alpine milk vs. control and of the milk from P vs. B cows also fits this hypothesis, because 18:1*cis*-9, the major FA in depot fat, is likely to be released from body stores.

(ii) Ruminal energy deficiency. Kelly *et al.* (33) considered ruminal ecosystem changes to be responsible for alterations in PUFA proportions of milk fat. In our study, in response to the nutritional energy deficiency during the alpine period in groups P and B (here: lack of fermentable organic matter), the rumen microbial capacity for biohydrogenation may have been reduced and more 18:3n-3 could have remained available for absorption. This would explain the negative response of *cis*-9,*trans*-11 CLA and 18:1*trans*-11 to alpine sojourn on the one hand and the positive response of 18:3n-3 on the other hand, which was found when comparing lowland and alpine milk samples derived from the grass-fed cows in our experiment (Table 4; Figs. 4 and 5). Milk of cows having restricted access to pasture was reported to have lower 18:3n-3 and increased 18:1*trans*-11 and *cis*-9,*trans*-11 CLA proportions when the animals were supplemented with grain (10). Also, Sasaki *et al.* (30) reported an increase of ruminal biohydrogenation of 18:3n-3 in sheep from 93 to 99% when a roughage-only diet was supplemented with concentrate at 400 g/kg. This supports the assumption of fermentable organic matter being one key factor for milk FA profile.

Both the mobilization hypothesis and the hypothesis of a reduced ruminal biohydrogenation are based on the presence of a serious energy deficiency during high-altitude grazing, which is underlined by many variables concerning performance and metabolic profile. Data are consistent with those of Agenäs *et al.* (9) where, at turnout to pasture, an impaired energy balance

of the cows was associated with elevated 18:3n-3 concentrations in milk.

(iii) Inhibition of ruminal biohydrogenation by dietary components. Another explanation, which would rely on a specific high-alpine phenomenon, is the assumption that typically high concentrations of secondary plant ingredients, such as polyphenols and terpenoids (40,41) as well as others, may inhibit hydrogenating microorganisms in the rumen. Tannins were shown to inhibit several strains of *Butyrivibrio fibrisolvens* (42), one of the most important biohydrogenating ruminal bacteria species (31). On the other hand, the findings of Dewhurst *et al.* (43) that 18:3n-3 biohydrogenation was reduced when feeding *Trifolium pratense* instead of *T. repens* suggest that plant constituents other than tannins also may influence biohydrogenation. In the present study, *T. pratense* was the most abundant legume in the alpine pasture but made up only 1.6% of the lowland forage. We assume that a complex interaction of different factors determines CLA and 18:3n-3 concentrations in milk rather than a monofactorial alpine mechanism. This implies that CLA and 18:3n-3 may be variable across milks from different alpine origins, which also may explain part of the differences between studies.

All hypotheses discussed to explain the higher 18:3n-3 concentrations in alpine pasture-derived milk fat would include the expectation of enhanced 20:5n-3 (EPA) and 22:6n-3 (DHA) content too, as 18:3n-3 can be converted to some extent into these FA in mammals (35). Although all 20- and 22n-3 PUFA were significantly higher in milk of groups P and B compared with group C and thus showed a diet effect, no additional effect of the alpine sojourn was found. It can be speculated that the maximal transfer rate was already reached at the 18:3n-3 concentration met in the lowlands by groups P and B. Furthermore, the transfer of PUFA from blood into milk also has to be considered: In particular, 20 and 22n-3 PUFA are reported to be preferentially bound to phospholipids in lipoproteins and therefore are hardly cleaved by lipoprotein lipases in the mammary gland. This could explain the low transfer rate into milk (44). The answer to the question—Which are the limiting factors of EPA and DHA synthesis and secretion?—is not yet clear (44), but it seems relevant to examine this question, since these n-3 PUFA are of an even higher specificity and importance for human health than 18:3n-3 (35).

Phytanic acid. This branched-chain 20:0 FA, being present in the plasma lipids of cows in proportions as high as 130 g/kg total FA (45), was strongly discriminated for by the mammary gland and was found in milk fat in concentrations between 10 and 500 mg/kg (46). The markedly increased values that were reached on pasture in the present study are at the upper limit of the literature values for butter as reviewed by Brown *et al.* (46). Similar to CLA, phytanic acid is supposed to act as a gene transcription factor and is assumed to exert antidiabetic effects (47). However, in this context a general risk of consuming higher amounts of phytanic acid for Refsum's disease patients, who suffer from inherited disorders of peroxisomal α-oxidation (48), must be considered.

α-Tocopherol. A reduced oxidative stability may be a prob-

lem in PUFA-rich milk lipids (49), including technological, flavor-related, and human health aspects. Our results indicate that pasture in any form obviously provides high amounts of α-tocopherol for milk synthesis and that its concentration is even higher in milk derived from alpine pasture. The α-tocopherol concentrations in pasture-derived milk were much higher than the minima suggested by Al-Mabruk *et al.* (49) to ensure a favorable oxidative stability of milk. According to Jensen *et al.* (50), α-tocopherol is actively secreted into the milk, and its daily amounts excreted with milk remain constant at levels between 20 and 30 mg/d. Indeed, in the grass-only fed cows, the absolute excretion of α-tocopherol per day with milk was on average higher during the lowland than during the alpine period. Thus, the significantly higher concentrations in milk produced during the alpine period were mainly a consequence of the reduced milk yield. On the other hand, the control cows on average did not reach a level of 20 mg α-tocopherol excretion per day, suggesting that the supply with this diet was too low to reach the naturally determined range.

General implications. Concerning high CLA and n-3 PUFA contents, a low n-6/n-3 ratio, and low concentrations of SFA, the alpine milk in this study, as in previous studies, constituted a more favorable FA pattern than conventionally produced lowland milk (2,3,8). However, levels of *cis*-9,*trans*-11-CLA were not different from milk obtained with lowland grazing, even though this pasture was intensively managed and poor in species diversity. We therefore conclude that the effects on this CLA isomer and on 18:1*trans*-FA are linked to grazing in general rather than being the result of a specific alpine pasture effect. In agreement with Kraft *et al.* (3), the same seems to hold true for the long-chain PUFA (20:5, 22:5, and 22:6 n-3). The situation is obviously different for 18:3n-3, which was markedly elevated by the alpine conditions, thus confirming previous findings (3,4). However, the real nature of the alpine grazing effect on 18:3n-3 concentration in milk still has to be elucidated. Where specific plant constituents are one of the major reasons affecting CLA and n-3 PUFA contents, strategies to increase 18:3n-3 further would be applicable also for lowland milk production systems. Where energy deficiency is the major driving factor, there would be only a very limited potential since a conflict would arise between product quality and animal welfare.

ACKNOWLEDGMENTS

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