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Transfer of linoleic and linolenic acid from feed to milk in cows fed isoenergetic diets differing in proportion and origin of concentrates and roughages

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The transfer of ingested α-linolenic acid (ALA) and linoleic acid (LA) determines the nutritional quality of milk, but the factors determining this transfer are unclear. The present experiment investigated the influence of roughage to concentrate proportions and the effect of concentrate types on milk fat composition. Respectively, six lactating dairy cows were fed one of three isoenergetic (5·4±0·05 MJ net energy for lactation/kg dry matter; DM) and isonitrogenous (215±3·5 g crude protein/kg DM) diets, consisting of ryegrass hay only (33 g fatty acids/kg DM; ALA-rich, no concentrate), maize (straw, whole maize pellets and gluten; 36 g fatty acids/kg DM; LA-rich; 560 g concentrate/kg DM), or barley (straw and grain plus soybean meal; 19 g fatty acids/kg DM; LA-rich; 540 g concentrate/kg DM). The fatty acid composition of feeds and resulting milk fat were determined by gas chromatography. The ALA concentration in milk fat was highest ($P<0·001$) with the hay-diet, but the proportionate transfer of ALA from diet to milk was lower ($P<0·001$) than with the maize- or barley-diets. The LA concentration in milk fat was highest with the maize-diet ($P<0·05$, compared with hay) but relative transfer rate was lower ($P=0·01$). The transfer rates of ALA and LA were reciprocal to the intake of individual fatty acids which thus contributed more to milk fat composition than did roughage to concentrate proportions. The amount of *trans*-11 18:1 in milk fat was lowest with the barley-diet ($P<0·001$) and depended on the sum of ALA and LA consumed. The milk fat concentration of *cis*-9, *trans*-11 18:2 (rumenic acid) was more effectively promoted by increasing dietary LA (maize) than ALA (hay). Amounts of 18:0 secreted in milk were four (maize) to seven (hay) times higher than the amounts ingested. This was suggestive of a partial inhibition of biohydrogenation in the maize-diet, possibly caused by the high dietary LA level.

Keywords: Fatty acids, rumenic acid, vaccenic acid, biohydrogenation, maize, barley, grass hay.

Alpha-linolenic acid (ALA; 18:3 *n*-3) is an important factor that contributes to the nutritional quality of milk fat (Barceló-Coblijn & Murphy, 2009). Transfer of this fatty acid from feed to milk is therefore significant for the production of high-quality dairy products. Much of the ALA ingested is biohydrogenated to stearic acid in the rumen (cf. Chilliard et al. 2007), which may explain why the concentrations of ALA in milk fat can be largely independent of the amount of ALA consumed by cows (Leiber et al. 2004, 2005).

Dietary linoleic acid (LA; 18:2 *n*-6) is also subject to intensive ruminal biohydrogenation (Jouany et al. 2007; Moate et al. 2008). However, as LA is a precursor of arachidonic acid (AA; 20:4 *n*-6), it is essential that a portion passes unaltered through the rumen. Biohydrogenation of LA not only decreases the amount that can bypass the rumen, but it also elevates the occurrence of a nutritionally relevant (Benjamin & Spener, 2009) fatty acid (FA), rumenic acid (RA; *cis*-9, *trans*-11 18:2), in milk. This conversion occurs either directly (Jouany et al. 2007) or indirectly via the generation of vaccenic acid (VA; *trans*-11 18:1; Chilliard et al. 2007).

When feeding effects on milk FA patterns are assessed, several external factors, in addition to the FA supply, that may influence the rate of biohydrogenation also need to be considered. Among these are the energy supply (Leiber et al. 2005), the dietary proportion of concentrates (Tsiplakou & Zervas, 2008), and the proportion of fibre (Dewhurst et al. 2006). These are mutually dependent

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factors, since a higher concentrate proportion is usually associated with a higher energy density and lower fibre concentration. The use of concentrate may also change the FA profile of the entire diet, thus causing further direct and indirect effects on milk fat composition.

The aim of the present study was to determine the roles of the roughage to concentrate proportion and the concentrate type on FA profiles of milk fat under exclusion of alterations in dietary energy and protein. For this purpose, milk samples were obtained from an earlier dairy cattle experiment that had employed three different isoenergetic diets (based on ryegrass, barley or maize; Klevenhusen et al. 2010). These diets provided an opportunity to examine the influence of different proportions of concentrate (and fibre), as well as different intakes of LA and ALA from roughages and grains. Additional emphasis was placed on the prevalence of ALA, LA and their biohydrogenation products in milk, in relation to ALA and LA intake.

Materials and Methods

Animals, experimental design and diets

The experiment was approved by the Swiss governmental authority for animal welfare. The experiment was based on a completely randomised design and included 18 medium-to-late lactating dairy cows (Holstein Friesian and Brown Swiss breeds) allocated in a balanced manner for milk yield and stage of lactation to the three isoenergetic and isonitrogenous diets ($n=6$). These diets were fed for 22 d; data and milk samples were collected for the last 8 d (Klevenhusen et al. 2010). The concentrations of net energy for lactation (NEL) and crude protein (CP) were 5·4 ± 0·05 MJ/kg dry matter (DM) and 213 ± 3·5 g/kg DM, respectively (Table 1). One diet consisted of ryegrass hay (diet H). A second diet (diet M) comprised (expressed as g per kg DM) maize stover (444), whole maize pellets (368), maize gluten (151), molasses (20), and urea (14). The third diet (diet B) was composed of barley straw (459), crushed barley grain (266), soybean meal (238), molasses (21), and urea (8). While diet H represents a roughage-only diet, diets M and B represent roughage to concentrate diets with ratios of about 1 : 1. All diets were supplemented with 16 g/kg of a commercial dairy vitamin-mineral premix. Diets M and B additionally contained 2 and 1 g $MgSO_4$/kg respectively. More details on the diets are described in Klevenhusen et al. (2010). The total FA contents and the FA profiles showed that diets H and M represented a moderate lipid supply, with diet H being high in ALA and diet M being high in LA, while diet B represented a low lipid supply with proportionally high LA content.

Prior to the experiment, the cows had been fed hay *ad libitum* plus 3 kg barley grain/d. They were gradually switched, over 6 d, to the respective experimental diets. Milk samples were collected at the first day of this pre-experimental time. During the 22 d of the experiment (14 d of adaptation and 8 d of data collection), the cows were offered the roughages, i.e. ryegrass hay, maize stover or barley straw, *ad libitum*. The concentrates in diets M and B were supplemented in five equal portions over the day. Concentrate amounts were adjusted daily to the amount of roughage consumed on the day before, to maintain roughage to concentrate ratios at the desired NEL and CP proportions. Feed intake and milk yield were measured daily and cows were milked twice per day. During the 8 d data collection period, morning and evening milk samples from each cow were pooled daily and stored at −20 °C. After thawing, milk samples from the 8 d were pooled to one sample per cow and refrozen at −20 °C. Samples of the individual diet ingredients, except urea and molasses, were collected six times over the 8 d and frozen.

Table 1. Ratio of dietary roughage : concentrate, nutrient content and energy content as well as fatty acid profile and intake of experimental dairy cow diets

Diet type	Hay (H)	Maize (M)	Barley (B)
Roughage : concentrate ratio	100 : 0	44 : 56	46 : 54
Net energy lactation, MJ/kg DM	5·37	5·46	5·46
Crude protein, g/kg DM	211	211	217
Neutral detergent fibre, g/kg DM	529	463	465
Acid detergent fibre, g/kg DM	256	274	263
Fatty acids, g/kg DM	33·2	36·0	18·6
Fatty acids, g/kg total fatty acids			
16 : 0	198	131	214
16 : 1	4·6	3·1	1·7
18 : 0	22·5	26·6	26·8
18 : 1 *n*-9	26	256	144
18 : 2 *n*-6	153	492	492
18 : 3 *n*-3	494	48	60
Fatty acid intake, g/d	442	468	281
12 : 0	0·77	0·65	0·29
14 : 0	2·17	1·13	2·92
16 : 0	87·6	61·5	60·4
16 : 1	2·04	1·45	0·47
17 : 0	1·13	0·77	0·43
18 : 0	9·94	12·43	7·54
18 : 1 *n*-9	11·7	119·8	40·5
18 : 2 *n*-6	67·9	230·4	138·3
18 : 3 *n*-3	218·7	22·7	16·9
20 : 0	2·99	3·80	2·01
20 : 1 *n*-9	0·47	1·16	1·62
20 : 2	0·29	0·20	0·26
21 : 0	0·42	0·25	0·27
22 : 0	5·54	2·53	1·98
22 : 1 *n*-9	0·11	0·07	0·29

Nutrient composition of feeds

Feeds were analysed for DM (TGA-500, Leco Corporation, St. Joseph, Michigan, USA) and CP (N × 6·25; C/N-analyser (Leco-Analysator Type FP-2000, Leco Instrumente GmbH, Kircheim, Germany). Neutral (NDF) and acid detergent fibre (ADF) were quantified according to Van

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Soest et al. (1991), correcting for ash content and using α-amylase and sodium sulphite for the NDF analysis. Contents of NEL were calculated from tabulated values (ALP, 2008).

Fatty acid analysis

Lipids were extracted from the feed samples by accelerated solvent extraction (ASE 200, Dionex Corporation. Sunnyvale, CA, USA) with hexane : 2-propanol (3 : 2; v/v). An internal standard (19 : 0) was added and the solvent was evaporated under a stream of N_2 gas. The residue was dissolved in dichloromethane and dried again under N_2. The dried samples were then methylated using 2 M-methanolic sodium hydroxide and 1·3 M-boron trifluoride in methanol. Dyes and non-FA-methyl esters were removed by thin layer chromatography (Khiaosa-Ard et al. 2009). The FA composition was determined by gas chromatography (GC; model HP 6890 equipped with a FID detector, Hewlett-Packard, Palo Alto CA, USA) on a 30 m × 0·32 mm Supelcowax-10^{IM} capillary column (Supelco Inc., Bellefonte PA, USA). A mixed FA methyl ester (FAME) standard (Supelco 37 Component, Supelco Inc., Bellefonte PA, USA) was used for the identification of individual FAs and to quantify total FAs.

Frozen milk samples were thawed at 37 °C in a water bath, then subjected to a base-catalysed trans-esterification, as described by Suter et al. (1997). Briefly, 0·5 ml milk was added to 5 ml mixed internal standard of trivalerin (5 : 0), nonanoic acid methyl ester (9 : 0), triundecanin (11 : 0), and 1-tetradecene (14 : 1) dissolved in 1,4 dioxane. Trans-esterification was carried out by adding 5 ml sodium methylate solution and vortexing for 3 s. The mixture was allowed to react for exactly 60 s, then 4 ml heptane and 10 ml 15% di-sodium citrate solution were added to stop the reaction. After phase separation had occurred, the upper phase was retrieved for FA analysis by GC and capillary column as described for FAs in feeds. The major FAs were identified based on a mixed FAME standard (Supelco 37 Component, Supelco Inc., Bellefonte PA, USA). Fatty acid concentration was calculated from the known amounts of the internal standard FAs (5 : 0 and 11 : 0). A second capillary column (200 m × 0·25 mm; CP7421, Varian Inc., CA, USA) was used on an identical GC to distinguish between *cis* and *trans* 18 : 1 isomers. Hydrogen was used as the carrier gas.

Statistical analysis

Analysis of variance of the milk data was performed with Procedure GLM of SAS (version 9.1, SAS Institute Inc., Cary NC, USA) with diet type (fixed factor) as the source of variance. The pre-experimental values, except for the ratios of FA intake and secretion with milk, were included as covariates to correct for initial differences within cows. Tukey's method was used for multiple comparisons among means. Milk data presented in the tables are Least Square Means, standard errors of the means and *P*-values. One cow of group M had to be excluded from evaluation due to feed intake problems.

Table 2. Effect of diet type on yield of milk and milk fat and on composition of short- and medium-chain fatty acids in milk†

Diet type	Hay (H)	Maize (M)	Barley (B)	SEM	*P*-value
Milk yield, kg /d	14·3	14·8	15·3	7·56	0·662
Milk fat yield, g/d	582	705	610	32·5	0·195
Fat concentration, g/kg	41·8	46·3	40·6	1·56	0·084
Fatty acids, g/kg total FAME					
4 : 0	26·5^{b}	30·0^{a}	26·9^{b}	0·68	0·010
6 : 0	29·5^{b}	33·0^{a}	32·2^{ab}	0·83	0·021
8 : 0	8·8	10·2	10·2	0·41	0·052
10 : 0	22·0^{b}	23·7^{b}	28·7^{a}	1·06	0·001
12 : 0	26·9^{b}	29·0^{b}	38·0^{a}	1·49	<0·001
13 : 0	1·3^{a}	0·9^{b}	1·4^{a}	0·04	<0·001
14 : 0	96^{b}	92^{b}	114^{a}	2·8	<0·001
14 : 1	8·5^{b}	10·0^{ab}	11·7^{a}	0·60	0·006
15 : 0	17·8^{a}	9·2^{c}	11·7^{b}	0·46	<0·001
16 : 0	274^{b}	250^{c}	318^{a}	6·2	<0·001
16 : 1	16·7	21·3	20·0	1·36	0·084
17 : 0	12·5^{a}	7·0^{b}	7·0^{b}	0·27	<0·001
17 : 1	4·7	4·5	4·1	0·46	0·601

†Means carrying no common superscript are different at $P<0·05$

Results

Feed DM intake was similar among the three diets, at 13·5, 13·4 and 15·1 kg/day. The total amounts of ingested FAs were similar between diets H and M. However, in diet B, FA ingestion was about 40% lower than in the other treatments. Intake of LA was highest with diet M and lowest (−70%) with diet H, whereas ALA intake was ten times higher with diet H, compared with diets M and B (Table 1). With diet M, a considerable amount of oleic acid (*cis*-9 18 : 1) was ingested.

Milk and milk fat yield did not significantly differ among the three diets (Table 2). There was a trend toward a higher milk fat concentration with diet M compared with the other two diets ($P<0·10$). The proportions of all short- and medium-chain saturated FAs in milk fat were significantly affected by diet type, but not in any clearly systematic manner. Proportions of the two major medium-chain FAs, 14 : 0 and 16 : 0, in milk fat were lower when cattle were fed diets H and M compared with diet B. The two monounsaturated medium-chain FAs, 16 : 1 and 17 : 1, in milk fat were not affected by diet type.

The proportion of 18 : 0 was about 38% higher in milk fat of cows fed diet H compared with the other two treatments (Table 3). With diet M, milk fat proportions of *trans* 18 : 1 FAs were significantly higher than with diet B, but were similar for with diet H, except for *trans*-9 and

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Table 3. Effect of diet type on composition of long-chain fatty acids in milk (g/kg total FAME) and on selected milk fatty acid ratios†

Diet type	Hay (H)	Maize (M)	Barley (B)	SEM	*P*-value
18 : 0	104·6[a]	77·8[b]	72·3[b]	4·85	<0·001
18 : 1 *trans*					
t-4	0·16[a]	0·17[a]	0·11[b]	0·010	0·002
t-5	0·14[a]	0·14[a]	0·08[b]	0·012	0·007
t-(6-8)	1·51[a]	1·70[a]	0·86[b]	0·071	<0·001
t-9	2·60[b]	2·86[a]	1·90[c]	0·063	<0·001
t-10	1·69[b]	2·38[a]	1·77[b]	0·107	0·001
t-11	23·53[a]	22·67[a]	9·10[b]	1·201	<0·001
t-12	1·70[a]	1·56[a]	1·11[b]	0·081	<0·001
18 : 1 *cis*					
c-9	223·5[b]	273·1[a]	199·0[b]	10·2	0·001
c-10	0·26	0·31	0·30	0·023	0·359
c-11	5·01[b]	7·46[a]	6·98[a]	0·330	0·001
c-12	1·06[b]	1·57[a]	1·37[a]	0·077	0·001
c-13	1·03[ab]	1·22[a]	0·93[b]	0·065	0·039
c-14	2·66[a]	1·34[b]	0·94[b]	0·108	<0·001
c-15	0·83[a]	0·32[b]	0·25[b]	0·028	<0·001
c-16	0·96[a]	0·78[b]	0·54[c]	0·042	<0·001
18 : 2 *n*-6	12·94[b]	30·15[a]	25·83[ab]	1·548	0·027
18 : 2 *c*-9, *t*-11	11·44[b]	15·82[a]	6·01[c]	0·539	<0·001
18 : 2 *t*-11, *c*-15	5·40[a]	1·44[b]	0·96[b]	0·217	<0·001
18 : 3 *n*-3	12·57[a]	3·72[b]	3·86[b]	0·537	<0·001
18 : 3 *n*-6	0·53	0·48	0·45	0·059	0·635
20 : 0	2·14[a]	1·77[ab]	1·58[b]	0·131	0·030
20 : 1	0·40[b]	0·68[a]	0·63[a]	0·032	<0·001
20 : 2 *n*-6	0·46	0·44	0·40	0·028	0·358
20 : 3 *n*-6	0·62[b]	1·07[ab]	1·36[a]	0·129	0·003
21 : 0	0·70[a]	0·32[b]	0·40[b]	0·051	<0·001
20 : 4 *n*-6	1·17[b]	1·50[ab]	1·63[a]	0·110	0·022
20 : 3 *n*-3	0·24[a]	0·11[b]	0·12[b]	0·017	<0·001
20 : 5 *n*-3	1·22[a]	0·79[b]	0·82[b]	0·043	<0·001
22 : 0	1·12[a]	0·58[b]	0·81[b]	0·065	<0·001
22 : 1	0·13	0·09	0·14	0·023	0·494
22 : 2	1·28[a]	0·36[b]	0·16[b]	0·069	<0·001
23 : 0	0·46[a]	0·26[b]	0·28[b]	0·035	0·003
24 : 0	0·48	0·34	0·38	0·045	0·120
24 : 1	0·29[a]	0·20[b]	0·30[a]	0·019	0·005
Fatty acid ratios					
n-6/*n*-3	1·30[b]	7·04[a]	5·54[a]	0·347	<0·001
18 : 2 *c*-9, *t*-11/18 : 1 *t*-11	0·473[b]	0·702[a]	0·678[a]	0·033	<0·001

† Means carrying no common superscript are different at $P<0·05$

trans-10 18 : 1 (both of these were significantly lower with diet H). For *cis*-9 to *cis*-13 18 : 1, concentrations in milk fat were highest with diet M; while proportions of *cis*-14 to *cis*-16 18 : 1 were highest with diet H. The concentration of LA in milk fat was 133% higher in diet M compared with H ($P<0·05$), whereas no statistically significant difference was found for LA between H and B. The milk fat proportion of RA (*cis*-9, *trans*-11 18 : 2) was significantly different among diets in the order of M>H>B. With diet H, ALA proportion in milk fat was threefold higher than with diets M and B. The longer chain *n*-6 FA, 20 : 3 and AA in milk fat occurred in significantly higher proportions with diets M and B compared with H, while for the *n*-3 category, 20 : 3 and 20 : 5 (eicosapentaenoic acid, EPA), higher levels were found with diet H than with diets M and B. Consequently, the ratio of *n*-6 : *n*-3 was substantially higher with diets M and B than with diet H. The ratio of RA to VA (*trans*-11 18 : 1) in milk fat, partly reflecting the degree of mammary desaturation of VA, was significantly lower in diet H compared with the diets M and B.

When compared with the amounts of ALA ingested daily, the secretion of ALA, RA, VA, EPA and total *n*-3 FA in milk fat was the lowest with diet H (Table 4), even though milk fat ALA and EPA concentrations were highest with this diet. When daily LA ingestion was compared, the relative secretion of LA, RA, VA, AA, and total *n*-6 FA was higher with diet H compared with diets M and B. The amounts of 18 : 0 secreted in milk were approximately six to seven times higher than the amounts ingested with diets B or H. For diet M, this ratio was significantly lower.

Discussion

Influences of dietary factors on the transfer of dietary ALA and LA from feed to milk fat

Comparison of the roughage only diet (hay, H) with the two mixed roughage and concentrate diets (maize, M; barley, B) enabled investigation of the effects of concentrates, independent of the energy and protein density, because these diets were isoenergetic and isonitrogenous. The lower LA and the higher ALA concentrations in milk fat of group H, compared with groups M and B, clearly corresponded to the different intake levels of the respective FAs with these diets. Thus, the dietary amount of individual FAs was a major factor in determining the ultimate ALA and LA concentrations in milk fat. However, the occurrence of ALA in milk, relative to its intake, was lowest in the group fed the hay diet. This finding was in agreement with experiments of Shingfield et al. (2005) and of Van Dorland et al. (2008) who also showed decreased ALA recovery rates in milk following increased ALA intakes. The dietary fibre concentration, which possibly could have played a role in the present study and in the study of Shingfield et al. (2005), was not varied in the study of Van Dorland et al. (2008) and was ruled out as an influencing factor by AlZahal et al. (2009).

The differing levels of easily degradable starch could also have been a confounding factor in the present study. However, Leiber et al. (2004, 2005) and Van Dorland et al. (2008) showed that lower ALA and LA intakes were always associated with higher relative recovery rates of these FA in milk, and vice versa. Differing dietary starch levels had not been applied in those studies indicating, that this effect occurs independently of starch.

In alpine studies (Leiber et al. 2004, 2005) that compared roughages from lowland and alpine pastures, differences in FA transfer were confounded by differences in

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Table 4. Ratios of fatty acid amounts secreted with milk (g/d) to that of either 18 : 3 *n*-3 or 18 : 2 *n*-6 or 18 : 0 ingested with the diet (g/d)†

Diet type	Hay (H)	Maize (M)	Barley (B)	SEM	*P*-value
Relative to 18 : 3 *n*-3 in diet					
18 : 3 *n*-3	0·036[c]	0·101[b]	0·150[a]	0·0090	<0·001
18 : 2 *c*-9, *t*-11	0·032[c]	0·412[a]	0·252[b]	0·0216	<0·001
18 : 1 *t*-11	0·066[c]	0·575[a]	0·386[b]	0·0272	<0·001
20 : 5 *n*-3	0·003[c]	0·021[b]	0·032[a]	0·0014	<0·001
Total *n*-3	0·040[c]	0·124[b]	0·187[a]	0·0095	<0·001
Relative to 18 : 2 *n*-6 in diet					
18 : 2 *n*-6	0·133[a]	0·076[b]	0·117[a]	0·0111	0·009
18 : 2 *c*-9, *t*-11	0·103[a]	0·041[b]	0·031[b]	0·0067	<0·001
18 : 1 *t*-11	0·214[a]	0·057[b]	0·047[b]	0·0081	<0·001
20 : 4 *n*-6	0·011[a]	0·004[c]	0·008[b]	0·0007	<0·001
Total *n*-6	0·154[a]	0·084[b]	0·133[a]	0·0122	0·004
Relative to 18 : 3 and 18 : 2 in diet					
18 : 2 *c*-9, *t*-11	0·024[b]	0·037[a]	0·027[b]	0·0025	0·010
18 : 1 *t*-11	0·051	0·052	0·042	0·0031	0·082
18 : 0	0·235[ab]	0·184[b]	0·300[a]	0·0235	0·016
Relative to 18 : 0 in diet					
18 : 0	6·782[a]	3·742[b]	6·153[a]	0·5426	0·004

† Means carrying no common superscript are different at $P<0·05$

feed energy density. A lack of energy at the rumen level was hypothesised to have limited the capacity for microbial biohydrogenation (Leiber et al. 2005). In the present study, care was taken not to alter energy densities between the diets. Thus, the compensatory increase of LA or ALA recovery in milk could be clearly attributed to the lower intakes of these FAs.

Comparing the milk fat composition of the cows fed diets M or B revealed the same effect of ALA and LA intake on the respective recovery rates. With these diets, the roughage to concentrate ratio (an effect found to be influential by Tsiplakou & Zervas, 2008), the dietary fibre and the starch proportions were all constant. Again, the main factor altering LA and ALA transfer to milk was the amounts of these dietary FAs that were ingested.

Influences of dietary factors on biohydrogenation products in milk

Vaccenic acid is an intermediate product of ruminal biohydrogenation of both LA and ALA (Jenkins et al. 2008). Vaccenic acid was significantly elevated in milk fat with diets H and M when compared with diet B, indicating that the amount of plant-derived PUFAs ingested, rather than the kind of dietary FA (LA or ALA) or the proportion of concentrate or fibre as such, determined the VA concentration in milk fat. This was underlined by the rather constant ratio of milk VA to the sum of LA and ALA ingested across all three diets. However, ruminal VA is also the major precursor for milk RA, which is partly synthesised by Δ^9 desaturation in the mammary gland of lactating cows (Griinari et al. 2000). Therefore, milk VA levels do not reflect the entire amount of VA derived from ruminal biohydrogenation, and this limits use of VA as an indicator of ruminal processes.

Unlike the VA levels, RA proportion was higher in milk fat with diet M compared with diet H. In the rumen, RA is the first product of the ruminal biohydrogenation pathway of LA (Chilliard et al. 2007; Jenkins et al. 2008) and total RA may be hydrogenated to VA (Moate et al. 2008). In contrast, ruminal biohydrogenation of ALA does not result in RA as a major intermediate (Jenkins et al. 2008). Thus, the lower RA concentration found in milk fat following consumption of diet H, which had a high ALA content and less LA, suggests that not only Δ^9 desaturation in the mammary gland but also ruminal RA levels are decisive in determining the final RA concentration in milk fat. A high dietary proportion of a roughage rich in ALA therefore does not necessarily lead to an increased RA concentration in milk even if it increases VA concentrations in milk. Rather, a high dietary intake of LA would appear to be required for RA increases to be observed.

The low proportion of *trans*-10 18 : 1 in milk fat with diets B and H (which both supplied lower amounts of LA) compared with diet M (which supplied a higher LA amount) suggests that this isomer was mainly a product of incomplete ruminal LA biohydrogenation. This suggestion is consistent with the literature (Jouany et al. 2007; Leiber et al. 2010). Comparison between diets B and M also showed that neither the roughage to concentrate ratio nor the fibre supply, as such, were relevant to the increased *trans*-10 18 : 1 concentrations noted in the present data. The high proportion of several other *trans* 18 : 1 isomers found in milk from cows fed diet M may have at least partially resulted from the high dietary 18 : 1 *n*-9 supply (Mosley et al. 2002). The high proportion of *cis*-15 18 : 1 in milk fat of cows fed diet H reflects that this FA is a typical product of ALA biohydrogenation (Jenkins et al. 2008).

The generally high secretion of 18 : 0 (stearic acid) with milk in relation to its intake demonstrated that large amounts of this FA are produced along the transfer pathway from diet to milk, mainly in the rumen (Chilliard et al. 2007). As indicated by the secretion to intake ratio, it seemed that diet M, when compared with diets H and B, in some way inhibited the terminal step of ruminal biohydrogenation, possibly due in some way to the high dietary LA concentrations (Moate et al. 2008). Thus, in addition to plant secondary compounds (Khiaosa-Ard et al. 2009), increased dietary LA supplementation could be a tool for inhibiting the terminal biohydrogenation step and enhancing the VA and RA levels in milk.

The present study demonstrated that if constant dietary energy and protein levels are maintained, the rate of transfer of LA and ALA directly from the diet into the milk is reciprocal to the intake of the respective FA. The transfer is apparently independent of the concentrate and fibre proportions. Furthermore, the concentrations of VA and, to a lesser degree, of RA in milk fat will depend on the sum of

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ingested LA and ALA, regardless of their proportions in the diet. Finally, the conclusion can be drawn that the complete ruminal biohydrogenation of FAs to stearic acid may be partly inhibited by feeding elevated amounts of LA.

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References

ALP (Agroscope Liebefeld-Posieux) 2008 [Feeding Recommendations and Nutrient Tables] (in German). Online version. (Ed ALP, Posieux, Switzerland). http://www.alp.admin.ch/dokumentation/00611/00631/index.html?lang=de. Accessed on April 2, 2009

AlZahal O, Or-Rashid MM, Greenwood SL, Douglas MS & McBride BW 2009 The effect of dietary fiber level on milk fat concentration and fatty acid profile of cows fed diets containing low levels of polyunsaturated fatty acids. *Journal of Dairy Science* **92** 1108–1116

Barceló-Coblijn G & Murphy EJ 2009 Alpha-linolenic acid and its conversion to longer chain *n*-3 fatty acids: Benefits for human health and a role in maintaining tissue *n*-3 fatty acid levels. *Progress in Lipid Research* **48** 355–374

Benjamin S & Spener F 2009 Conjugated linoleic acids as functional food: an insight into their health benefits. *Nutrition & Metabolism* **6** 36

Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J & Doreau M 2007 Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology* **109** 828–855

Dewhurst RJ, Shingfield KJ, Lee MRF & Scollan ND 2006 Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* **131** 168–206

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV & Bauman DE 2000 Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9–desaturase. *Journal of Nutrition* **130** 2285–2291

Jenkins TC, Wallace RJ, Moate PJ & Mosley EE 2008 Board invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *Journal of Animal Science* **86** 397–412

Jouany J-P, Lassalas B, Doreau M & Glasser F 2007 Dynamic features of the rumen metabolism of linoleic acid, linolenic acid and linseed oil measured in vitro. *Lipids* **42** 351–360

Khiaosa-Ard R, Bryner SF, Scheeder MRL, Wettstein H-R, Leiber F, Kreuzer M & Soliva CR 2009 Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. *Journal of Dairy Science* **92** 177–188

Klevenhusen F, Bernasconi SM, Kreuzer M & Soliva CR 2010 Experimental validation of the IPCC default values for ruminant-derived methane and its carbon isotope signature. *Animal Production Science* in press

Leiber F, Scheeder MRL, Wettstein HR & Kreuzer M 2004 Milk fatty acid profile of cows under the influence of alpine hypoxia and high mountainous forage quality. *Journal of Animal and Feed Sciences* **13** (Suppl 1) 693–696

Leiber F, Kreuzer M, Nigg D, Wettstein HR & Scheeder MRL 2005 A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. *Lipids* **40** 191–202

Leiber F, Tsvetkova V, Petrova I, Kreuzer M & Scheeder MRL 2010 Two cases of grazing sheep with abundance of *trans* octadeca fatty acids in milk fat. *Milchwissenschaft – Milk Science International* **65** 73–76

Moate PJ, Boston RC, Jenkins TC & Lean IJ 2008 Kinetics of ruminal lipolysis of triacyglycerol and biohydrogenation of long-chain fatty acids: new insights from old data. *Journal of Dairy Science* **91** 731–742

Mosley EE, Powell GL, Riley MB & Jenkins TC 2002 Microbial biohydrogenation of oleic acid to *trans* isomers in vitro. *Journal of Lipid Research* **43** 290–296

Shingfield KJ, Salo-Väänänen P, Pahkala E, Toivonen V, Jaakkola S, Piironen V & Huhtanen P 2005 Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. *Journal of Dairy Research* **72** 349–361

Suter B, Grob K & Pacciarelli B 1997 Determination of fat content and fatty acid composition through 1-min transesterification in the food sample; principles. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* **A 204** 252–258

Tsiplakou E & Zervas G 2008 Comparative study between sheep and goats on rumenic acid and vaccenic acid in milk fat under the same dietary treatments. *Livestock Science* **119** 87–94

Van Dorland HA, Kreuzer M, Leuenberger H & Wettstein HR 2008 Comparative potential of white and red clover to modify the milk fatty acid profile of cows fed ryegrass-based diets from zero-grazing and silage systems. *Journal of the Science of Food and Agriculture* **88** 77–85

Van Soest PJ, Robertson JB & Lewis BA 1991 Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74** 3583–3597