Apparent recovery of C18 polyunsaturated fatty acids from feed in cow milk: A meta-analysis of the importance of dietary fatty acids and feeding regimens in diets without fat supplementation

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ABSTRACT

A meta-analysis was conducted using the results of 82 experiments (78 publications, 266 treatments) to investigate the importance of dietary C18 fatty acids (FA) and feeding regimen for milk C18 FA profile and apparent recovery of selected FA relative to intake of these FA or their precursors. Feeding treatments based on lipid-supplemented diets were excluded. Feeding regimens were defined as grazing [including partial and full-time grazing, at dietary concentrate proportions from 0 to 44% dry matter (DM)], forage-based indoor feeding [$>65\%$ forage of total DM intake (DMI)], and concentrate-based indoor feeding (forage DMI $\leq 65\%$ of DMI). Linoleic acid (LLA), α -linolenic acid (ALA), and total C18 FA proportions in milk fat increased linearly with the respective dietary FA content in all feeding regimens tested. This effect was highest in the forage-based indoor feeding. Slopes were lowest for the grazing regimens, especially regarding ALA and the sum of all C18 FA, whereas the intercepts of the prediction equations of milk ALA and total C18 FA proportions were highest for grazing regimens. This indicates that, in grazing cows, factors other than dietary FA contents determine the C18 FA composition of the milk fat. At equal dietary LLA contents, the type of feeding regimen showed no significant effect on LLA proportion in milk fat. Milk fat proportions of rumenic acid and vaccenic acid were positively related to the sum of dietary ALA and LLA contents. Grazing regimens led to the strongest enrichment of rumenic acid and vaccenic acid in milk fat. The apparent recovery of ALA, LLA, and total C18 FA (secreted, % of intake), an estimate for transfer efficiency, decreased with increasing dietary content. This relationship followed a nonlinear decay function. When the dietary content of these FA ex-

ceeded a certain threshold (about 0.2, 0.8, and 2.8% of DM for ALA, LLA, and total C18 FA, respectively) the recovery in milk remained constant at about 5, 10, and 82% of the ingested ALA, LLA, and total C18 FA, respectively. At dietary proportions below 0.01% ALA and 1.5% total C18 FA of DM, their apparent recovery in milk fat exceeded 100%. In conclusion, a general inverse relationship between dietary C18 FA and the corresponding apparent recovery in milk fat seems to exist. Within this frame, the effect of different types of feeding regimens on the eventual milk C18 FA profile varies. Among them, grazing pasture appears to provide the most variable properties.

Key words: diet type, feed fatty acids, milk fat, conjugated linoleic acid, fatty acid transfer

INTRODUCTION

Linoleic acid (LLA; *cis*-9,*cis*-12 18:2) and α -linolenic acid (ALA; *cis*-9,*cis*-12,*cis*-15 18:3) are essential nutrients to all mammals because both FA cannot be endogenously synthesized in the body (Nakamura and Nara, 2003). An elevated content of PUFA in milk fat is commonly considered a determinant of a high nutritional quality of the milk (Woods and Fearon, 2009), and ALA plays an especially favorable role in this context (Barceló-Coblijn and Murphy, 2009). During ruminal fermentation, biohydrogenation eliminates large proportions of ALA and LLA and produces *inter alia* vaccenic acid (VA; *trans*-11 18:1) and rumenic acid (RA; *cis*-9,*trans*-11 18:2), the latter being an important natural isomer of the CLA (Jouany et al., 2007). Both VA and RA are also considered to be beneficial to human health (van Wijlen and Colombani, 2010).

Varying proportions of ALA and LLA from feed may be recovered intact in the milk of ruminants (Chilliard et al., 2007). Apart from fat supplementation, the principal diet type (e.g., forage proportion, grazing vs. conserved forage) considerably influences the levels of C18 PUFA in milk fat. For example, feeding pasture

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grass leads to higher contents of PUFA (Dewhurst et al., 2006) and dietary concentrates may enhance LLA proportion in milk fat (Ferlay et al., 2006; Leiber et al., 2015). The level of intake of the respective FA and, therefore, their content in the diet is an obvious cause being discussed. However, the focus was much less on their true efficiency (i.e., the proportion of these dietary PUFA actually reaching the milk fat). Besides, several studies did not find any (Leiber et al., 2004, 2005; Shingfield et al., 2005) or only a limited (Khiaosa-ard et al., 2010) positive relationship between PUFA intake and its content in milk fat. This can be explained by varying biological mechanisms affecting PUFA transfer from feed to milk fat. Alteration of the different steps of ruminal biohydrogenation determines the ruminal escape rates for LLA and ALA and, at the same time, the accumulation rates for VA and RA (Chilliard et al., 2007). Therefore, a higher supply of PUFA as substrates for ruminal biohydrogenation could increase the occurrence of these intermediates in milk fat, too. In addition, some experiments suggest that, when the intake of these FA is low, a kind of a homeostatic effect seems to exist, leading to an apparently larger transfer of LLA and ALA from feed to milk (Leiber et al., 2004; Shingfield et al. 2005; Khiaosa-ard et al., 2010). Biological changes often follow a nonlinear fashion, but individual studies were not able to cover broad ranges and multiple levels of FA intake in their response in milk FA secretion efficiency. Furthermore, the complexity of the underlying factors, which vary among individual studies, sometimes leads to apparently contradictory results. These findings underline that the efficiency of increasing FA intake to achieve elevated levels of ALA, LLA, VA, and RA in milk is highly variable and cannot yet be reliably predicted. However, in the past 20 yr, numerous feeding experiments investigating dietary effects on milk FA composition have been carried out. A statistically based evaluation of existing data sets, with the aim of grouping the effects of individual factors for their significance, has been carried out for milk FA composition (Coppa et al., 2013), but, as far as we know, not for the transfer efficiency of dietary FA based on known individual intake data. Therefore, the key questions, namely whether general patterns are underlying the efficiency of C18 PUFA transfer from feed to milk and whether these responses are indeed nonlinear, remain unanswered. In this respect, the considerations specified above indicate that especially the general type of feeding regimen could be important.

In the present investigation, general patterns of feeding effects on milk FA were analyzed by a meta-analysis approach. For that purpose, experiments from the literature were categorized into 3 common feeding regimens, namely (1) grazing, (2) indoor feeding of forage-based

diets, and (3) indoor feeding of concentrate-based diets. The comparison among regimens was done on the basis of dietary C18 FA concentration. In addition, the interaction between feeding regimen and dietary C18 PUFA contents was followed as it might help to elaborate specific recommendations concerning strategic feeding management options for enriching the milk with C18 PUFA. Treatments where diets were supplemented with lipids or feeds particularly rich in fat with the explicit intention to increase dietary fat content were not considered in the current study because the efficiency of fat supplements in modifying milk FA profile has been the subject of a previous meta-analysis (Glasser et al., 2008a).

MATERIALS AND METHODS

Creation of the Database

Sources of the Data Included. Data used in the present meta-analysis were obtained from original publications in the field of dairy cattle nutrition. A search on the database of the Web of Science using “milk,” “fatty acid,” and “diet” as keywords in the category “topic” was carried out. Additionally, the search included more specific keywords in the category “title” including “grazing,” “forage,” and “cow milk.” Only publications dealing with cattle were considered. Several other criteria were used to select data for the operational database. At first, to acknowledge the progress which took place in the analytical determination of FA and their isomers, only articles published from the year 2000 onwards were considered. Next, publications that did not provide data on all key variables needed for the evaluation, such as DMI, dietary FA content or fat content and FA composition, milk production traits, and milk FA composition, were not included in the database. Treatments where diets were supplemented with lipids or feeds particularly rich in fat were not taken into account for the analysis even when these treatments were part of the articles selected. This was also done to exclude possible confounding effects of (1) inhibitory effects of a high PUFA content on ruminal biohydrogenation, which is especially known for LLA (Beam et al., 2000), (2) the milk fat depression effect induced by diets containing highly unsaturated oils (Harvatine et al., 2009), and (3) to avoid the bias caused by a very high transfer rate when using rumen-protected fats. Some publications were from studies which attempted to strategically influence the biohydrogenation process by the use of marine products or monensin. These treatments were excluded as well. Experiments were treated individually even they originated from the same project or publication. In total, this left 82

experiments from 78 studies with 266 different dietary treatments in the final database.

Categorization, Coding, and Processing of Data Before Input into the Database. Dietary treatments were grouped according to 3 major feeding regimens, namely either grazing or feeding in barn with forage- or concentrate-based diets. In detail, any dietary treatments involving part-time or full-time grazing, independent of whether or not supplementary feeding was practiced, was categorized and termed as “Grazing” ($n = 50$ treatments). In this regimen, concentrate levels ranged from 0 to 44% of DM. The decision to include all grazing treatments despite sometimes high concentrate levels was made based on the evaluation of 2 subdata sets, with and without treatments with high concentrate levels ($>35\%$ of DM), where no major changes in the prediction equations and the effects of the factors investigated were found. All indoor feeding treatments where concentrate proportion was lower than 35% in DM were categorized as forage-based diet types and termed as “Forage indoors” ($n = 117$ treatments). This category included 22 dietary treatments (19% of forage indoors, 8% of total) where fresh forage was part of the diet (but only fed in barn). The decision to group diets containing fresh grass indoors with Forage indoors and not with Grazing was based on the assumption that grazing provides much more opportunity for plant selection and that this would be one main distinguishing characteristics of these feeding systems. The remaining treatments were considered as concentrate-based diets and named “Conc” ($n = 99$ treatments). This threshold has been used before in various cattle nutrition studies to define high- or low-forage diets (Loor et al., 2005; Glasser et al., 2007b; Sterk et al., 2011). Descriptive data of the experiments, which were included in the database, consisted of experimental duration, animal number per treatment, cow breed, DIM, DMI, concentrate proportion, and milk yield. These data are listed in Table 1.

Data on intake of total and individual FA were obtained directly from the publications where available. In all other cases, they were calculated from reported data on dietary FA contents and DMI. If dietary FA content of mixed diets was not specified, this variable was calculated from the ether extract (EE) content of the total diet as FA (% of DM) = $(1.03 \times \text{EE}) - 0.98$, following Allen (2000). The calculation was necessary for 31 total dietary treatments (9 for Forage indoors and 22 for Conc). In the case of Rego et al. (2004), for 1 of the forage-only treatments it was assumed that 43% of EE present in the forage leaves were FA (Palmquist and Jenkins, 2003). In 2 studies (Vanhatalo et al., 2007, $n = 4$ treatments; Lee et al., 2009, $n = 3$ treatments) the DM content of the concentrate supplemented, which was

not reported but necessary for FA intake calculation, was assumed to be 90%. Some publications reported dietary C18 unsaturated FA (i.e., 18:1, 18:2, and 18:3) without isomer specification. These FA were encoded as oleic acid (*cis*-9 18:1), LLA, and ALA, as these are typically the most prominent isomers. To standardize FA intake among studies, absolute FA intake was initially normalized for DMI and subsequently presented as dietary FA concentration (relative to DMI).

For milk FA, data input was restricted to ALA, LLA, VA, RA, and total C18 FA. In cases where not all of these FA or sums of FA were reported, these items were treated as missing values in the statistical evaluation. In 5 studies (Leiber et al., 2005; Bargo et al., 2006b; Jacobs et al., 2011; Khan et al., 2012; Kälber et al., 2013), VA was reported together with the *trans*-10 18:1 isomer as 1 summarized value. As the latter isomer is typically much less prevalent than VA in milk fat (Chilliard et al., 2007), these data were also encoded as VA in the present analysis. The FA, which was named generically as CLA by the authors without given isomer details, was encoded as RA. Finally, FA listed in the publications and indicated as not detected were given a zero value in data input.

Mostly, FA contents in milk fat were given, and composition was presented proportional to total FA (or FAME). In 1 study (Whiting et al., 2004; $n = 2$ treatments), milk FA composition was reported in relation to milk fat. When not reported directly, contents of FA proportions were estimated from their relation to milk fat content by, at the same time, assuming a FA content of 93.3% (Glasser et al., 2007a). Milk FA yield (g/d) was computed from FA content in milk and milk yield. The calculation of the apparent recovery of LLA, ALA, and total C18 FA in milk (% of intake) was performed as the proportion of individual milk FA yield (g/d) of intake of the respective FA (g/d) times 100. The values of the 2 biohydrogenation products analyzed, RA and VA, were related to the sum of LLA and ALA intake because both are the major products of ruminal biohydrogenation of LLA and ALA. The averages per feeding regimen of intake and milk variables were summarized in Tables 2 and 3.

Statistical Analyses

Evaluation of Dietary FA and Feeding Regimen Effects on FA Proportions in Milk Fat and Their Secretion with Milk. Data analysis on these variables was performed as outlined by Sauvant et al. (2008). The model used included both discrete (feeding regimen) and continuous predictor (FA intake, % of DM) variables and their interaction and random terms of experiment and their interactions with the fixed

Table 1. Sources of data, experimental details,¹ main production variables, and dietary treatments tested in the studies included in the present analysis

Reference	Exp. d	Exp. no.	Cow breed ²	DIM ³	DMI (kg/d)	Concentrate (%)	Milk (kg/d)	Feeding regimen ⁴
Adler et al. (2013)	21	4	O	118	17.7–17.9	30–34	15.9–18.0	F
Akbaridoust et al. (2014)	25	18	H	227	14.9–21.6	29–44	13.1–20.0	G
Akraim et al. (2007)	21	30	H	116	23	32	34.9	F
AlZahal et al. (2009)	28	3	H	140	22.0–24.2	26/44	32.6	F, C
Aprianita et al. (2014)	21	8	H	39	24.8	23	32.3	F
Arvidsson et al. (2012)	28	18	O	196	15.3–16.8	36–39	20.4–21.4	C
Avila et al. (2000)	14	4	H	103	23.9	5	32.7	C
Bargo et al. (2006a)	133	15	H	109	21.6–26.7	40–48	28.8–38.1	G, C
Bargo et al. (2006b)	21	20	H	101	18.3–24.8	0	19.1–29.9	G
Bartl et al. (2008)	17	6	O	67	4.6–15.4	0/13	1.9–8.2	F
Benchaar et al. (2012)	28	4	H	67	17.0/19.9	35/65	30.8/31.7	C
Boivin et al. (2013)	21	8	H	84	25.2–27.5	50–60	38.9–42.8	C
Bu et al. (2007)	63	10	H	170	16.2	41	21.7	C
Cabrita et al. (2007)	21	9	H	77	23.3–24.7	50	31.9–35.2	C
Cabrita et al. (2009)	21	16	H	87	22.0–22.9	50	36.2–37.4	C
Caroprese et al. (2010)	84	8	H	100	16.6	39	22.5	C
Chen et al. (2008)	84	30	H	43	18.3	55	29.3	C
Chichlowski et al. (2005)	70	12	H	29	21.5	48	34.9	C
Chilliard et al. (2009)	28	8	H	213	19.8	34	23	F
Dai et al. (2011)	66	10	H	120	21.3	54	25.5	C
Dewhurst et al. (2003)	28	18	H	64/82	17.9–22.6	17–37	24.9–33.2	F, C
Dhiman et al. (2000)	35	6	H	ND	20.6	45	27.4	C
Elgersma et al. (2004)	0–14	6	H	202	15.8–16.9	10	19.7–20.8	F
Falchero et al. (2010)	12	18	O	216	11.7–16.5	0	14.2–15.3	G
Ferlay et al. (2006)	77	8	O	103/83	13.7–15.8	0/65	13.6–16.5	G, F, C
Ferlay et al. (2010)	35	6	M	68	21.4	28	27.3	F
Gross et al. (2011)	0–35	20	H	100	10.0–22.5	13–39	27.2–33.4	F, C
Han et al. (2014)	63	12	H	136	18.8/20.3	40/60	19.2/21.1	C
He et al. (2005)	84	9	H	21	19.6	60	36.7	C
Höjer et al. (2012)	21	24	O	114/126	19.1–21.6	25–28	24.3–24.8	F
Huang et al. (2008)	28	6	H	ND	21.5	52	30.3	C
Jacobs et al. (2011)	28	28	H	153	18.3	25	28.8	G
Kälber et al. (2011)	25	6	M	162	13.4–17.8	11–14	18.8–21.4	F
Kälber et al. (2013)	15	6	M	233	13.5–15.6	12–14	16.0–18.5	F
Kälber et al. (2014)	17–23	5–6	M	228	11.9–16.1	11–15	14.6–19.3	F
Kay et al. (2004)	6	4	H	51	15.9	0	20.4	F
Kay et al. (2005)	21	10	H	267	16.0–20.0	0/55	12.5–19.8	G, C
Khan et al. (2012)	105	16	H	14	22.8–23.7	30–32	39.3–41.3	F
Khiaosa-ard et al. (2010)	22	6	M	215	13.4–15.1	0/55	14.3–15.3	F, C
Kliem et al. (2008)	28	8	H	159	19.1–23.9	46	29.7–33.1	C
Larsen et al. (2012)	14/42	12/48	H	140	20.6–21.8	30	31.0–32.4	G
Lee et al. (2009)	21	6	H	ND	18.2–19.6	10	32.1–32.9	F
Leiber et al. (2005)	42	6	O	94	13.7–18.3	0/50	12.8–22.2	G, F, C
Loor and Herbein, (2003)	7	4	H	52	25.6	40	47.5	C
Loor et al. (2005)	28	4	H	71	20.4	36/66	24.2–28.8	C
Lourenço et al. (2005)	21	4	H	249	16.1–18.4	25–27	20.2–21.9	F
Marchesini et al. (2009)	7	12	H	167	18.6	37	31.3	C
Mohammed et al. (2009)	21	6	H	76	15.6–18.7	14–17	16.1–24.6	G, F
Mohammed et al. (2010)	21	8	H	154	25.1–27.2	58	33.1–34.8	C
Moorby et al. (2009)	28	24	H	103	16.7–19.0	19–21	25.2–26.5	F
Morales-Almaráz et al. (2010)	21	5	H	94	22.7–23.3	27–37	33.6–35.4	G, C
Neveu et al. (2014)	16	21	H	93	22.1–22.3	37/41	30.9/31.5	C
Nielsen et al. (2006)	42	10	H	178	18.4–20.0	27–48	23.4–25.6	F, C
Patel et al. (2013)	ND	13–20	O	218	19.5–20.3	14–52	24.8–31.1	F, C
Petersen et al. (2011)	8	4	H	228	16.0–16.5	0	21.6–22.6	F, C
Petit et al. (2004)	35	4	H	38	23.8	48	24.8	C
Petit et al. (2009)	28	8	H	119	21.4	57	32	C
Ranathunga et al. (2010)	42	10	H	78	22.9–25.6	51	37.4–39.4	C
Rego et al. (2004)	22	12	H	130	17.1–21.0	0/40	24.1–25.0	G, C
Rego et al. (2008)	21	16	H	145	19.8–20.0	27	24.7–25.9	G
Roy et al. (2006)	19	6	H	95	17.7–21.6	36–73	29.5–34	C
Salawu et al. (2002)	28	9	H	67	140–16.4	31–47	20.8–23.7	F, C
Saliba et al. (2014)	28	6	H	168	21.5/25.6	30/70	25.5/32.2	F, C
Shingfield et al. (2005)	21	16	O	51	19.5–21.0	32–43	27.9–28.9	F, C

Continued

Table 1 (Continued). Sources of data, experimental details,¹ main production variables, and dietary treatments tested in the studies included in the present analysis

Reference	Exp. d	Exp. no.	Cow breed ²	DIM ³	DMI (kg/d)	Concentrate (%)	Milk (kg/d)	Feeding regimen ⁴
Solomon et al. (2000)	21	20	H	133	20.3–20.9	60	34.6–35.5	C
Staerfl et al. (2013)	26	6	H	112	14.4–15.7	0–4	14.7–19.1	F
Steinshamn and Thuen (2008)	191	12	O	74	13.9–19.9	0/38	22.0–28.1	F, C
Stergiadis et al. (2014)	42	60	H	ND	20.1/21.9	34/45	25.8/28.3	F, C
Van Dorland et al. (2008)	22	6	M	115/122	17.8–19.8	16–28	25.4–26.3	F
Vanhatalo et al. (2007)	21	5	O	77	19.4–21.3	40–44	26.5–27.7	C
Wales et al. (2009)	10	18	H	62	15.4–16.6	0–30	26.0–29.0	G
Ward et al. (2003)	21	12	H	ND	20	56	24.7	C
Whiting et al. (2004)	35	16	H	187	17.6–18.5	0	15.3–16.0	F
Whitlock et al. (2003)	28	12	H	63	26.9–28.1	40/50	35.5–36.8	C
Yan et al. (2010)	84	10	H	46	22.1	67	34.9–36.8	C
Ye et al. (2009)	63	12	H	150	20.8	50	25.4	C
Zachut et al. (2010)	>100	22	H	ND	26.1	66	49.5	C
Zunong et al. (2009)	28	4	H	175	22.1–22.6	34–36	20.5–20.8	G

¹Exp. d = experimental duration (d per run); Exp. no. = number of animals per experimental treatment.

²H = Holstein; O = other than Holstein; M = several cow breeds per treatment.

³DIM = DIM at the start of experiment (ND = no data or unclear).

⁴C = concentrate-based diet fed indoors; F = forage-based diet fed indoors; G = grazing.

factors (according to Sauvant et al., 2008). Initially, stage of lactation (3 levels: 0–100, 101–200, and >200 DIM) had been included in the model as well, but was omitted later as it was never significant. After stepwise removal of the random terms (St-Pierre, 2001; Sauvant et al., 2008), the statistical model applied was

$$Y_{ijk} = \mu + S_i + F_j + B_1X_{ij} + B_2X_{ij} + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall intercept, S_i is the random effect of the i th experiment, F_j is the fixed effect of the j th feeding regimen, X_{ij} is the value of the dietary level of the corresponding FA

Table 2. Averages of dietary FA contents, DMI, and C18 FA intakes across all experiments included in the analysis

Variable	Feeding regimen	n	Mean	SD	Minimum	Maximum	Median
Total C18 FA (% of DM)	All	264	1.78	0.853	0.51	5.62	1.63
	Grazing	50	1.98	0.759	0.64	3.62	1.86
	Forage indoors	116	1.52	0.744	0.51	5.56	1.45
	Concentrate	98	1.99	0.938	0.52	5.62	1.84
Linoleic acid (% of DM)	All	266	0.71	0.440	0.12	3.04	0.61
	Grazing	50	0.55	0.186	0.13	1.02	0.58
	Forage indoors	117	0.56	0.310	0.18	1.93	0.49
	Concentrate	99	0.97	0.534	0.12	3.04	0.92
α -Linolenic acid (% of DM)	All	266	0.61	0.502	0.02	2.87	0.52
	Grazing	50	1.09	0.680	0.12	2.87	0.88
	Forage indoors	117	0.62	0.404	0.02	1.95	0.58
	Concentrate	99	0.34	0.255	0.02	1.26	0.30
DMI (kg/d)	All	266	18.9	4.06	4.57	28.1	19.4
	Grazing	50	18.7	3.18	11.7	25.2	18.5
	Forage indoors	117	17.0	4.11	4.57	24.8	17.5
	Concentrate	99	21.1	3.19	13.4	28.1	20.8
Intake of total C18 FA (g/d)	All	264	347.3	200.0	27.8	1,377.8	307.3
	Grazing	50	375.9	153.01	77.7	691.8	372.0
	Forage indoors	166	267.3	150.31	27.8	1,023.0	260.0
	Concentrate	98	427.3	235.3	115.1	1,377.8	379.1
Intake of linoleic acid (g/d)	All	266	141.8	110.82	10.1	836.0	108.5
	Grazing	50	104.6	44.22	18.2	256.9	107.1
	Forage indoors	117	99.3	65.16	10.1	348.8	86.5
	Concentrate	99	210.7	139.83	25.2	836.0	190.9
Intake of α -linolenic acid (g/d)	All	266	111.9	89.72	0.73	517.4	93.9
	Grazing	50	204.3	124.42	17.8	517.4	160.9
	Forage indoors	117	107.7	67.4	0.73	309.7	100.44
	Concentrate	99	70.2	50.11	3.88	242.0	59.5

Table 3. Averages of yields of milk and selected milk C18 FA across all experiments included in the analysis

Variable	Feeding regimen	n	Mean	SD	Minimum	Maximum	Median
Milk (kg/d)	All	266	25.2	8.52	1.88	49.5	25.6
	Grazing	50	23.1	6.72	12.5	35.4	23.2
	Forage indoors	117	21.9	8.67	1.88	41.3	22.0
	Concentrate	99	30.1	6.70	13.8	49.5	30.7
Total milk FA (g/d)	All	266	933	297.3	75	1,745	967
	Grazing	50	835	228.6	366	1,297	849
	Forage indoors	117	861	337.3	75	1,586	872
	Concentrate	99	1,068	221.0	513	1,745	1,078
Linoleic acid (g/d)	All	266	16.8	9.94	0.78	57.5	14.7
	Grazing	50	13.1	6.92	3.83	32.5	10.5
	Forage indoors	117	13.3	7.36	0.78	29.4	12.7
	Concentrate	99	22.9	10.97	9.08	57.5	21.4
α -Linolenic acid (g/d)	All	266	5.57	3.179	0.20	18.2	4.91
	Grazing	50	6.59	3.546	3.11	17.6	5.31
	Forage indoors	117	5.96	3.661	0.20	18.2	5.23
	Concentrate	99	4.58	1.863	0.81	13.0	4.42
Vaccenic acid (g/d)	All	204	16.3	9.74	2.79	54.7	13.4
	Grazing	50	23.9	11.83	8.00	54.7	22.5
	Forage indoors	79	14.1	7.04	2.79	37.6	13.1
	Concentrate	75	13.5	7.95	3.18	43.8	11.9
Rumenic acid (g/d)	All	243	6.68	3.803	0.26	18.3	5.83
	Grazing	50	9.84	3.695	2.65	18.0	9.48
	Forage indoors	109	5.35	3.270	0.26	18.3	4.79
	Concentrate	84	6.53	3.437	2.00	18.3	5.34

(continuous predictor variable) of the j th feeding regimen of the i th experiment, B_1 is an overall regression coefficient of Y on X (a fixed effect), B_j is the effect of the j th feeding regimen on the regression coefficient (a fixed effect), and e_{ijk} is the residual error. According to St-Pierre (2001), nonsignificant interactions of fixed factors should be removed from the model. However, we did not follow this recommendation because the interaction term of some variables was close to the level of significance and the interaction effect was of particular interest for the present study.

The model described above was applied to all FA contents and yields studied, except for the LLA proportion in milk fat where the random interaction terms were significant and, hence, were considered in the model. In this situation, the equation derived from the analysis can very well explain the observations, but any call for predictions of future outcomes could be poor because of the unknown effect of the individual experiments (Sauvant et al., 2008). The number of animals was used to weight the means reported (Jayanegara et al., 2012). Analysis of the data was conducted using the MIXED procedure of SAS (version 9.1, SAS Institute, Cary, NC). For each model, root mean square error (RMSE) was also indicated; this parameter was provided through the INFLUENCE option. Data summarization and descriptive statistics for intake and milk production were calculated by the MEANS procedure of SAS.

Evaluation of Dietary FA Effects on Apparent Recovery of FA in Milk. Based on preliminary

graphs, it appeared that, overall, the response of apparent FA recovery to intake level did not follow a linear pattern. Therefore, the NLMIXED procedure of SAS would have been best for examining the prediction equations because it offers a flexible and powerful environment for fitting nonlinear models, where both fixed and random effects are permitted to have a nonlinear relationship to the response variable. However, from the preliminary graphs it appeared that the area with very low FA intake was critical for developing the nonlinear effect of intake and the differences among feeding regimens. Unfortunately, the Conc regimen data set contained insufficient numbers of observations for very low LLA intake. This made it inappropriate to statistically compare such nonlinear effects among the feeding regimens. However, when visually considering the available data at higher intake levels and very low intake of the other 2 groups, the recovery data of all feeding strategies appeared to be at a similar scale. Thus, it was assumed that all feeding regimens followed a nonlinear response and their differences, even when statistically significant, might not have been biologically relevant. Based on this assumption, it was decided to determine the best-fit nonlinear equation of data pooled across all feeding strategies. An exponential decay relationship with 3 parameters was selected for the analysis and the respective statistical model was

$$Y_{ij} = c + ae^{-bX_{ij}} + e_{ij}$$

Table 4. Prediction of proportions of selected milk FA (% of total FA) from the dietary content of the respective FA (% of DM)¹ in the different feeding regimens

Variable	Total n	Parameter estimate						Effects (<i>P</i> -value)			
		Intercept	SE _{intercept}	<i>P</i> _{intercept}	Slope	SE _{slope}	<i>P</i> _{slope}	RMSE ²	Feeding	FA intake	Interaction
Total C18 FA	256							9.124	0.002	0.017	0.110
Grazing		41.21	2.830	<0.001	0.274	1.1762	0.816				
Forage indoors		32.59	1.446	<0.001	1.733	0.7387	0.020				
Concentrate		30.83	1.517	<0.001	2.599	0.7032	<0.001				
Linoleic acid	266							0.7100	0.364	<0.001	0.945
Grazing		1.205	0.2082	<0.001	0.705	0.3319	0.036				
Forage indoors		1.156	0.1310	<0.001	0.797	0.1820	<0.001				
Concentrate		1.414	0.1534	<0.001	0.825	0.1681	<0.001				
α-Linolenic acid	266							0.6460	0.007	<0.001	<0.001
Grazing		0.665	0.0860	<0.001	0.115	0.0579	0.048				
Forage indoors		0.340	0.0566	<0.001	0.575	0.0703	<0.001				
Concentrate		0.416	0.0462	<0.001	0.131	0.0921	0.157				
Vaccenic acid	204							1.2475	0.002	<0.001	0.311
Grazing		2.107	0.2695	<0.001	0.530	0.1234	<0.001				
Forage indoors		1.058	0.2313	<0.001	0.434	0.1507	0.005				
Concentrate		0.991	0.2262	<0.001	0.263	0.1536	0.089				
Rumenic acid	243							0.5536	<0.001	<0.001	0.921
Grazing		0.909	0.1143	<0.001	0.200	0.0535	<0.001				
Forage indoors		0.423	0.0890	<0.001	0.208	0.0598	<0.001				
Concentrate		0.354	0.0917	<0.001	0.181	0.0633	0.005				

¹Rumenic acid and vaccenic acid are relative to the dietary content of their precursors (sum of linoleic acid and α-linolenic acid).

²RMSE = maximum root mean square error.

where Y_{ij} represents apparent recovery (%) of a FA at the i th dietary level of FA of the j th study, X_{ij} is the value of the i th intake of the corresponding FA of the j th study, a and b are parameters varying among studies, c is the overall intercept, and e_{ij} is the residual error. Distributions of both the data and the random effect (study effect) were assumed to be normal. It was also assumed that the random effect among studies (s) entered the model through only 1 parameter, either as $a + s$, $b + s$, or $c + s$, where $s \sim N(0, \sigma_s^2)$, where σ_s^2 represents the variance among studies. Using more than 1 random effect in the parameters did not lead to convergence. The NLMIXED procedure of SAS was applied to analyze the data. Data were weighted by the number of observations per treatment using the REPLICATE statement (Binder et al., 2005). The NLMIXED procedure does not provide RMSE; residual values were used to compute sums of squares for error using the MEAN procedure of SAS and, subsequently, RMSE were calculated (Robbins et al., 2006).

RESULTS

Milk FA Proportions

All parameter estimates and the effects of test factors are shown in Table 4. Data of individual and total C18 FA proportions of total milk FA were plotted against the corresponding FA content in the diets (Figures 1

and 2). The figures illustrate the data fits across studies within each feeding regimen after taking into account variance between studies.

Total C18 FA. Clear effects of the dietary C18 FA content ($P = 0.017$) and the feeding regimen ($P = 0.002$) on milk C18 FA content were found. The parameter estimate (Table 4) suggested that for Grazing the milk C18 FA accounted for 41.8% of total milk FA independent of dietary C18 FA content. By contrast, for Forage indoors and Conc, milk mean values of C18 FA contents were lower (35.2 and 36.0% of total milk FA, respectively), and increased significantly with dietary C18 FA content (Figure 1A). Still, the interaction of dietary FA and feeding regimen was not significant ($P = 0.11$).

LLA. The meta-analysis showed that the proportion of LLA in total milk FA was affected by the dietary LLA content ($P < 0.001$), but not by feeding regimen, and that no interaction occurred between these 2 factors (Table 4). The slope found with increasing dietary LLA content was significant with all feeding regimens (Figure 1B). Based on the regressions it can be estimated that, at a mean dietary LLA proportion of 0.71% of DM (Table 2), the proportions of milk LLA were quite similar for Grazing (1.70%) and Forage indoors (1.72%) and only slightly higher (2.00%) for Conc.

ALA. Linear regressions (Table 4) showed that the response of the ALA proportion was affected by both dietary ALA level ($P < 0.001$) and feeding regimen ($P = 0.007$). Across all feeding regimens, an increase of

Figure 1. Total C18 FA (A), linoleic acid (B), and α -linolenic acid (C) proportions of milk fat in relation to its contents in diets of cows subjected to feeding regimens based on grazing ($\bullet$), forage-based diets fed indoors (Δ), and concentrate-based diets fed indoors ($\circ$). Figure 1 displays adjusted means and regression lines across all treatments per feeding regimen [grazing (—), forage-based diets fed indoors ($\cdots$), and concentrate-based diets fed indoors (---)]. The corresponding parameter estimates are given in Table 4.

Figure 2. Vaccenic acid (A) and rumenic acid (B) proportions of milk fat in relation to the contents of linoleic acid (A) and α -linolenic acid (B) in diets of cows subjected to feeding regimens based on grazing ($\bullet$), forage-based diets fed indoors (Δ), and concentrate-based diets fed indoors ($\circ$). Figure 2 represents adjusted means and regression lines across all treatments per feeding regimen [grazing (—), forage-based diets fed indoors ($\cdots$), and concentrate-based diets fed indoors (---)]. The parameter estimates are given in Table 4.

dietary ALA proportion resulted in a higher proportion of ALA in total milk FA. Within feeding regimens, only Forage indoors showed a steep slope, which resulted in an interaction of the 2 factors ($P < 0.001$; Figure 1C, Table 4).

VA. The dietary contents of both LLA and ALA together ($P < 0.001$) and the feeding regimen ($P < 0.01$) were positively associated with the proportion of VA in total milk FA (Table 4). No interaction of the 2 factors was observed. Among the feeding regimens, Grazing led to the highest proportions of VA in total milk FA (highest intercept), and its slope was similar to that of Forage indoors (Figure 2A, Table 4). To a lesser extent, Conc tended to lead to a slope significantly different from zero ($P = 0.089$).

Figure 3. Transfer efficiency of total C18 FA (A), linoleic acid (B), and α -linolenic acid (C) from feed to milk in relation to their dietary proportions (% of DM) in cows subjected to feeding regimens based on grazing (●), forage-based diets fed indoors (Δ), or concentrate-based diets fed indoors (○). The line corresponds to the equation obtained from the adjusted apparent transfer efficiency calculated across all feeding strategies. Parameter estimates: a = scale parameter; b = rate parameter. RMSE = root mean square error.

RA. The proportion of RA in total milk FA was affected by feeding regimen ($P < 0.001$) and the sum of dietary LLA plus ALA contents ($P < 0.001$); no interaction was found between these factors (Table 4) and the slopes were similar in magnitude (Figure 2B). It was obvious that Grazing was more effective in this respect than the other 2 feeding regimens, as it had the highest intercept.

Recovery of Total C18 FA, ALA, LLA, or Derivatives in Milk Fat in Relation to Dietary FA

The apparent FA recovery (serving as an estimate for transfer efficiency) was plotted against the corresponding dietary FA proportions (% of DM). The parameter estimates as well as the regression curves are shown in Figure 3. All recoveries of total C18 FA, LLA, and ALA were different ($P < 0.01$) from zero. The response can be divided into 3 phases consisting of the highest declining rate of the curve at a very low intake (and, thus, dietary content) of these FA (phase I), a lower declining rate at low to moderate intake (phase II), and a constant rate at moderate to high intake (phase III).

For total C18 FA, at low dietary content (<1.5% of DM) the apparent recovery in milk fat exceeded 100% (Figure 3). Regarding the pattern of change described above, in phase I, when dietary C18 FA increased from 0.5 to 0.9% of DM, the total C18 FA apparent recovery in milk fat drastically decreased from 3.84 to 1.82 times of intake. In phase II, where the dietary proportion of C18 FA increased from 1.0 to 1.8% of DM, the apparent recovery decreased from 1.58- to 0.90-fold of intake. Beyond 1.8% of DM (phase III), the recovery rate stayed quite constant, with approximately 81 to 90% of total C18 FA ingested by the animal being secreted with the milk. The apparent recovery of LLA decreased markedly from 54 to 34% when the dietary LLA proportion was increased from 0.1 to 0.2% of DM. Then, the recovery slowly decreased from 23 to 11% as the LLA proportion in diet increased from 0.3 to 0.6% of DM before reaching a constant at approximately 10% with further increasing dietary LLA proportions. For ALA, the apparent recovery would exceed 100% at very low intake (i.e., at less than 0.01% of DM). From our database, where the lowest dietary proportion was 0.016% of DM, the maximum predicted ALA transfer was 92%. Then, it dropped to 52 and 18% when the dietary ALA proportion increased to 0.04 and 0.09% of DM, respectively. Next, the apparent ALA recovery efficiency declined, albeit with a slower rate, from 15 to 6% as the dietary ALA proportion increased from 0.10 to 0.20% of DM, respectively. After that, it remained constant at about 5%.

DISCUSSION

Although PUFA contents of milk fat would increase with increasing PUFA intake (Leiber et al., 2011; Sterk et al., 2011), this statement cannot be totally generalized, particularly for high-forage diets (Leiber et al., 2005; Shingfield et al., 2005; Dewhurst et al., 2006). Forage type and proportion play an important role in this respect (Sterk et al., 2011; Coppa et al., 2013). Especially when grazing is practiced, further factors seem to have an effect on this relationship (Kraft et al., 2003; Leiber et al., 2005). Besides alterations of ruminal biohydrogenation, which might *inter alia* be caused by plant secondary compounds from the forages (Cabiddu et al., 2010; Jayanegara et al., 2011a) or by modifications of the ruminal pH (Fuentes et al., 2011), a general adjusting metabolic mechanism, which maintains certain PUFA levels in the functional tissues (including milk), appears probable (Kälber et al., 2011; Willems et al., 2014). It seems important to determine whether general mechanisms occur across many different feeding experiments. This would elucidate the significance of PUFA content of the diet and, with that, PUFA intake for milk PUFA profile in relation to forage feeding regimen and to forage-to-concentrate ratio. This was the main question dealt with in the current meta-analysis.

Effects of Dietary C18 FA on Milk C18 FA Proportions

Milk FA can originate from 4 main sources: (1) from *de novo* synthesis in the mammary gland, (2) from feed, (3) from ruminal microbial metabolism (biohydrogenation and microbial lipids), and (4) from mobilization of body reserves (Chilliard et al., 2007). The first mechanism generates short- to medium-chain FA (4:0 to 14:0) and approximately half of 16:0 from acetate and BHBA mainly derived from ruminal fiber digestion (Chilliard et al., 2007). Origin in the diet contributes the remaining portion of 16:0 and all longer-chain FA ($\geq$ C18) occurring in the milk lipids. Under typical conditions, dietary FA are the determining source for C18 FA in milk. As expected, in the present study, overall proportions of C18 FA in milk FA linearly increased with their intake, although the extent of this relationship (for ALA and total C18 FA) was less close in the grazing regimen. The response to intake of proportions of individual C18 FA was of different magnitude. For instance, LLA was more closely related to its intake than ALA, and this occurred independently from feeding regimen. One main explanation is the generally higher extent of ALA biohydrogenation in the rumen (Fievez et al., 2007). Jouany et al. (2007) assumed that

the difference in susceptibility to biohydrogenation is caused by a preferential uptake of LLA by bacteria, thus protecting LLA from biohydrogenation, or by differences in microbial isomerase or saturase affinity between ALA and LLA. In addition, it was shown that the intestinal absorption of ALA was lower than that of LLA (Doreau and Ferlay, 1994). Moreover, it was stated that LLA is preferentially used by the mammary tissue for triglyceride synthesis (Grummer, 1991). Conversely, ALA supply to milk synthesis is hormonally regulated in humans (Burdge, 2004), and probably in all mammals, which may imply that this FA is endogenously higher regulated than LLA and contents in milk are maintained partly independently from intake, as is apparent for other functional tissues (Willems et al., 2014).

The intercept of the regression equation in Table 4 can be considered as an estimate of LLA and ALA secretion at zero intake, reflecting the endogenous contribution from tissue mobilization. The contribution of FA from body reserves can approach some 20% of milk FA when cows are in a negative energy balance (Lock and Bauman, 2004). However, it is important to note that the majority of studies in our database were conducted with cows that were not in a negative energy balance. Selective mobilization of body reserves of functional FA (such as ALA and LLA) and their transport to the mammary gland is known to be driven by hormonal regulation in lactating women (Burdge, 2004), which might well be the case in cattle, too. This could be the reason for endogenous ALA and LLA contribution to milk fat, even in cows with positive energy balance and even though ALA is lower concentrated in adipose tissue than in milk fat (Leiber et al., 2011). In general, adipose tissues of cattle contain a large proportion of total C18 FA (about 60% of total FA; Douglas et al., 2007; Leiber et al., 2011), whereas total C18 FA proportions in milk are lower (mostly 30–45% of total FA in our database); thus, a considerable depot for supplying milk synthesis with these FA exists.

The present meta-analysis revealed that contribution might be even higher than stated by Lock and Bauman (2004) from the body reserves when considering only the C18 FA fraction in milk fat. The estimation of milk LLA and ALA secretion with zero intake would be as high as 7.4 to 14.1 g/d of LLA and 3.1 to 5.4 g/d of ALA. These amounts, to be released in case of body fat mobilization, would account for more than half of the estimated mean secretion of the FA (13.1–22.9 g/d for LLA and 4.6–6.6 g/d for ALA) to be expected with average intakes of these FA. However, as no data with zero intakes was in our database, the prediction of milk fat composition at zero intake of the respective FA could be overestimated.

Effects of Feeding Regimen on Milk C18 FA Proportions

The present evaluation showed that, at the same dietary LLA level, the feeding regimen had no significant effect on milk LLA proportions. Therefore, the current results suggest that further comparisons among studies for any dietary effects on milk LLA should be adjusted to LLA intake. Unlike LLA, the ALA proportion in milk FA was largely affected by feeding regimen. At lower dietary ALA contents, the effect of grazing regimen on ALA enrichment in milk fat was greatest (highest intercept); whereas, at the same time, its slope was more flat than that of the Forage indoors regimen. When the dietary ALA level was beyond 1% of DM, the Forage indoors regimen effect got superior in enhancing milk ALA compared with the others. Considering that both the indoor forage group and the grazing group were high in forage DMI, it is obvious that not the content but rather the type of forages seem to have made the difference.

Increasing ALA intake by using (mostly) forages containing higher levels of this FA with the indoor forage regimen was the most efficient approach to enrich milk ALA. The steep slope of the equation for Forage indoors can be less explained by plant secondary compounds, because in the present database the experimental plants used for indoor feeding, which resulted in high ALA intake >1.2% of DM, were mostly grass-species dominated (3 observations from grass-based fresh, 4 grass-based conserved, 1 clover-based fresh, 1 clover-based conserved, and 3 natural grassland hay). As the contents of plant secondary compounds in grasses are low compared with herbs (Jayanegara et al., 2011b; Kälber et al., 2011), the main effect in grass-dominated diets appears to be directly related to the dietary ALA content.

It is still unclear if ALA from plants either fed in the barn to or grazed by cows escapes ruminal biohydrogenation differently, or whether any differences exist in postruminal metabolism. For instance, Ferlay et al. (2006) noted a higher extent of ALA biohydrogenation in grazing cows compared with cows offered hay diets. One important factor could be the different pattern of eating, ruminating, and resting of dairy cows in barn or on pasture (Kaufmann et al., 2011), which might well influence ruminal fermentation and, thus, lipolysis and biohydrogenation. Other factors which might be involved include differences in DMI (Leiber et al., 2005), rumen fill, rumen pH, rumen microbial populations, and digesta kinetics (Mohammed et al., 2009). Moreover, factors affecting ruminal digestibility of forages (e.g., maturity or conservation method) could affect biohydrogenation and the subsequent ALA

transfer to milk as well (Shingfield et al., 2005; Vanhatalo et al., 2007). Halmemies-Beauchet-Filleau et al. (2013a) showed that, compared with fresh grass, hay contained less C18 PUFA, but concomitantly lowered the extent of lipolysis and biohydrogenation of these FA in the rumen, which resulted in similar flows to the omasum independent of forage conservation method. However, fresh grass elevated LLA and ALA in milk fat more than hay (Halmemies-Beauchet-Filleau et al., 2013b). This difference in effect was associated with a lower (but still positive) energy balance, an increased uptake of preformed FA, and a decreased *de novo* FA synthesis in the mammary gland of cows fed fresh grass (Halmemies-Beauchet-Filleau et al., 2013b).

A further important factor of grazing could be the opportunity for selection on pasture, which might be particularly important concerning plants rich in secondary metabolites (Villalba et al., 2004), as these seem to have a high influence on ruminal biohydrogenation (Khiaosa-ard et al., 2009; Jayanegara et al., 2011a; Petersen and Jensen, 2014). Likewise, previous studies have associated the effect of grazing on enrichment of ALA in ruminant tissues with botanical and thus biochemical diversity, in particular with a diversity of plants with elevated contents of secondary compounds (Leiber et al., 2005; Ferlay et al., 2006; Cabiddu et al., 2010; Willems et al., 2014), which can influence ruminal biohydrogenation. Generally, in the present as well as in other meta-analyses (Coppa et al., 2013, 2015), grazing appears as a feeding strategy that has a particular strong influence on the milk FA profiles.

Vaccenic acid is the major intermediate of ruminal biohydrogenation of both ALA and LLA, and milk VA is mostly of ruminal origin (Chilliard et al., 2007). From the present study, it can be expected that in the grazing regimen ALA had a greater influence on VA production than LLA did because the ALA intake was greater. Besides, milk LLA concentrations suggest a similar biohydrogenation rate of LLA among feeding regimens. Based on this assumption, we found that the effect of the grazing regimen was exceptional because, with increasing ALA intake, milk ALA proportions remained almost unchanged whereas a large accumulation of VA in milk occurred. This suggests an increase in the intensity of the first step of biohydrogenation (isomerization of *cis*-12 double bond of ALA), but a low extent of the final step (conversion of VA to stearic acid) at increasing dietary ALA content. The increase in VA supply likely explains the similar response to dietary PUFA found in milk RA proportion with the grazing regimen, as a large part of milk RA originates from endogenous desaturation of VA (Richter et al., 2012). Elevated VA and RA proportions were also found in the rumen fluid lipids of grazing animals (Lourenço et al., 2007; Khiao-

sa-ard et al., 2011), confirming that a certain modification of biohydrogenation took place. This means that a high grass DMI, and thus some components in fresh pasture, either favor the production of VA or limit the final step of biohydrogenation (Bargo et al., 2006a). However, considering the different results found with high-forage DMI in grazing and indoor forage regimens in our evaluation, grass DMI was not likely to be the major factor of influence. Plant secondary compounds, such as tannins, have been indicated as a ruminal VA enhancer (Khiaosa-ard et al., 2009; Vasta et al., 2009), but these components are not substantially present in grass-dominated pastures anyway. Again, we hypothesized that, besides selection, differences in intake (i.e., rumen fill) and rumination patterns (Kaufmann et al., 2011) could play a key role.

Effects of Feeding Regimen and Dietary C18 FA on the Extent of C18 FA Transfer from Diet to Milk

Several studies showed that an increase of dietary C18 PUFA proportions could elevate their contents in the milk fat (Mohammed et al., 2009; Wales et al., 2009; Khiaosa-ard et al., 2010). However, there are also indications that the C18 PUFA transfer efficiency is not always positively related to the intake (Leiber et al., 2005; Bargo et al., 2006a; Khiaosa-ard et al., 2010) or to the available amount at the duodenum (Bickerstaffe et al., 1972). The present meta-analysis, covering a broad range in C18 FA intake from diets not supplemented with fat, confirmed both of these apparently contradictory observations, because it turned out that the response was not linear and its characteristics depended on dietary contents. A homeostatic effect (i.e., constant mammary uptake of the C18 PUFA at intake rates above a certain level, mobilization of body reserves below certain intake levels for specific FA, and positive correlation with incorporation in milk between these intake levels) could be an explanation for the relationships found.

From the present evaluation it appears that at low dietary PUFA levels, variation in transfer efficiency is high, whereas it becomes more predictable as PUFA intake increases. This indicates that there might be a metabolic programming to maintain certain levels of C18 FA, particularly the unsaturated representatives, in milk fat. In a feeding condition where energy intake covers requirements, body fat mobilization is considered negligible and dietary C18 FA are the major source of these FA in milk (Richter et al., 2012). However, even in cases of excessive lipid intake from oil supplementation, transfer of C18 FA from duodenum to milk decreased to sustain a maximum threshold value of about 52% C18 FA of total milk FA (Glasser et al., 2008b). In turn, the

present meta-analysis revealed that, when C18 PUFA intake was very limited, their transfer apparently exceeded 100%, assuming that intakes reported in these studies were correct. In the present data set, many C18 FA transfer levels were calculated to be in the range of apparently 1- to 2-fold of intake. As a consequence, this means that, when C18 FA are not sufficiently available from the digestive tract, they must be mobilized endogenously. Preferential release of C18 PUFA from body tissues has been reported for wild ruminants (Soppela and Nieminen, 2002) and the turnover of adipose tissue in cows is generally high (McNamara, 1989), which makes the supply of milk fat with PUFA from body tissues plausible even when the animal is not in a catabolic stage. In agreement with our assumption, the recent study by Halmemies-Beauchet-Filleau et al. (2013b) revealed changes in blood lipid composition with forage type, which indicate variations in body fat mobilization in lactating cows even during positive energy balance. It is, however, difficult to relate changes in milk FA to distinct events of mobilization of adipose tissue because nutritional history plays a role in determining FA profiles of adipose tissue (Leiber et al., 2011). Moreover, regardless of nutrition, the FA profiles differ largely among adipose tissue sites, and mobilization may be specific for different adipose tissue sites and different FA within adipose tissue site (Chilliard et al., 2000). More research should be done to determine plasticity of the adipose tissue mobilization in response to the mammary demand for distinct FA.

CONCLUSIONS

An increasing dietary content of total and individual C18 FA was confirmed to be a means to elevate these FA and biohydrogenation intermediates in milk fat. Feeding regimens that are characterized by differences in botanical source of FA and feeding pattern were particularly influential on the secretion of ALA and its intermediates with milk. Increasing ALA intake with fresh and conserved forages offered in barn to cows was the most direct approach for enhancing ALA proportions in milk fat. Grazing on pastures was obviously associated with other factors, which overruled the significance of the dietary ALA content for its level in milk fat. These factors still have to be identified to understand why grazing and which pasture types affect milk ALA and its intermediates in such a particular way. Furthermore, grazing led to enrichment of RA and VA in milk fat regardless of C18 PUFA supply. The present meta-analysis, covering a broad range of diets, confirmed that C18 FA transferred were from feed to milk nonlinearly and inversely responded to the dietary contents of these FA. The extent to which mobilization

from endogenous sources contributes to total milk C18 PUFA under different conditions remains to be seen. Within this frame, different feeding regimens had a different potential to modify milk PUFA composition.

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