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Use of Principal Component Analysis to Investigate the Origin of Heptadecenoic and Conjugated Linoleic Acids in Milk

V. Fievez,* B. Vlaeminck,* M. S. Dhanoa,† and R. J. Dewhurst†
*Department of Animal Production, Ghent University,
Proefhoevestraat 10, 9090 Melle, Belgium
†Institute of Grassland and Environmental Research,
Plas Gogerddan, Aberystwyth SY23 3EB, U.K.

ABSTRACT

The aim of this paper was the application of principal component analysis (PCA) 1) to elucidate mutual metabolic relationships between milk fatty acids (FA) and 2) to illustrate the origin of milk FA, in particular $C_{17:1}$ and *cis*-9,*trans*-11 conjugated linoleic acid. Data were combined from 3 experiments with lactating Holstein-Friesian cows offered diets based on grass or legume silage and concentrates. Loading plots of PCA based on milk FA concentrations showed 4 groups of milk FA, having similar precursors or metabolic pathways in the rumen and/or mammary gland: medium-chain saturated FA, de novo synthesized from acetate and β-hydroxybutyrate; monoenoic milk FA, products of Δ^9-desaturase activity in the mammary gland; odd chain FA of rumen microbial origin and $C_{18:0}$, n-6 $C_{18:2}$, and n-3 $C_{18:3}$ of dietary origin or the result of rumen biohydrogenation. Loading plots of PCA based on both milk and duodenal FA concentrations as well as on milk FA yields and duodenal FA flows further illustrated the importance of postabsorptive synthesis of the milk medium chain saturated and monoenoic FA and the direct absorption from the blood stream of odd chain FA, $C_{18:0}$, n-6 $C_{18:2}$, and n-3 $C_{18:3}$. In all loading plots, milk oleic acid ($C_{18:1}$) appeared intermediate between clusters of 18-carbon FA and monoenoic FA, illustrating its dual (dietary and endogenous production) origin. Milk $C_{17:1}$ was suggested to be a desaturation product of $C_{17:0}$, in common with other milk monoenoic FA. Finally, the PCA technique, based on milk FA patterns of one experiment, was applied to investigate factors determining *cis*-9,*trans*-11 conjugated linoleic acid concentrations in milk. Within the range of diets and cows studied here, we showed changes in *cis*-9,*trans*-11 conjugated linoleic acid to be mainly dependent on vaccenic acid supply and to a lesser extent on variation in desaturase activity.

(**Key words:** principal component analysis; odd-chain fatty acid; conjugated linoleic acid; Δ^9-desaturase)

Abbreviation key: FA = fatty acids, **OCFA** = odd-chain fatty acids, **PCA** = principal component analysis.

INTRODUCTION

Milk odd-chain fatty acids (**OCFA**) (pentadecanoic acid, $C_{15:0}$; *iso* methyltetradecanoic acid, *iso* $C_{15:0}$; *anteiso* methyltetradecanoic acid, *anteiso* $C_{15:0}$; heptadecanoic acid, $C_{17:0}$; *iso* methylhexadecanoic acid, *iso* C17:0; *anteiso* methylhexadecanoic acid, *anteiso* $C_{17:0}$; and heptadecenoic acid, $C_{17:1}$) were suggested to originate principally from rumen microbes (Dewhurst et al., 2000). However, only trace amounts of $C_{17:1}$ were detected in pure rumen bacteria (Miyagawa, 1982; Minato et al., 1988). This raises the possibility of other sources of $C_{17:1}$ in milk. Because Δ^9-desaturase activity in the ruminant mammary tissue is responsible for the conversion of $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$ into $C_{14:1}$, $C_{16:1}$, and $C_{18:1}$ (oleic acid), respectively (Bickerstaffe and Annison, 1970), we hypothesised that $C_{17:1}$ could be produced endogenously from $C_{17:0}$ in the mammary gland.

Principal component analysis (**PCA**) is often used to reduce the dimensionality of data profiles containing intercorrelated variables. Moreover, PCA aims to display the maximum amount of variation in a data profile within a few principal components. Hence, pairwise score plots derived from PCA are useful to find similarities and contrasts between samples, whereas correlations among variables can be identified in loading plots. The latter were used earlier to highlight similarity in metabolic pathways (Massart-Leën and Massart, 1981). Consequently, the aim of this study was to test our hypothesis of endogenous production of $C_{17:1}$ using PCA. A further objective was to apply this tool to investigate the importance of mammary Δ^9-desaturase activity in the production of milk conjugated linoleic acid (*cis*-9,*trans*-11 $C_{18:2}$). Indeed, postabsorptive synthesis from vaccenic acid (*trans*-11 $C_{18:1}$) has been suggested as the predominant source of milk *cis*-9,*trans*-11 $C_{18:2}$ (Griinari et al., 2000). This suggestion has led to increased

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Corresponding author: V. Fievez; e-mail: veerle.fievez@UGent.be.

interest in stimulating mammary Δ^9-desaturase activity, particularly as a potential means to enhance *cis*-9,*trans*-11 $C_{18:2}$ concentrations of dairy products (e.g., Lock and Garnsworthy, 2000).

MATERIALS AND METHODS

Experimental Design and Diets

The current study combined data from 3 experiments. All experiments were conducted according to a 4-period incomplete or complete changeover design. Each experimental period lasted for 28 d. All diets were based on grass or legume silage and concentrate and offered to 4 or 6 lactating Holstein-Friesian cows with duodenal and rumen cannulas.

Experiment 1. Experimental design and diets are as described by Dewhurst et al. (2003a, 2003b). Briefly, the experiment was according to a 4-period incomplete changeover design, in which 6 cows in the beginning of the lactation were used to test 6 dietary treatments. Each cow was offered 4 different diets. Cows received 8 kg/d of a standard dairy concentrate, in 3 portions: 3 kg at milking (0730 and 1600 h) and 2 kg at 1200 h. Concentrates contained 52 g of fatty acids (**FA**) per kilogram of DM, with $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, n-6 $C_{18:2}$, and n-3 $C_{18:3}$ being the most abundant FA, representing 4.2, 15.7, 3.1, 25.0, 29.9, and 2.0% of total FA, respectively. Cows had ad libitum access to one of the 6 silages: grass, red clover, white clover, alfalfa, and 50/50 (DM basis) mixtures of grass and red clover and grass and white clover. Each forage treatment comprised a proportional mixture of all cuts taken in the year. Fresh forage was distributed daily at 0900 h. The FA levels in the different silages were similar (16 g/kg DM), although slightly higher for white clover silage (22 g/kg DM). $C_{16:0}$ (19.3 to 25.2%), $C_{18:2}$ (16.0 to 23.1%), and $C_{18:3}$ (41.0 to 55.0%) represented over 90% of the FA (Dewhurst et al., 2003b). Average daily silage DMI was 10.2, 13.9, 12.1, 14.9, 13.6, and 12.4 kg for the grass, grass-red clover, red clover, grass-white clover, white clover, and alfalfa silage based diet, respectively (Dewhurst et al., 2003b).

Experiment 2. This experiment was a 4 × 4 Latin square. Four dairy cows in midlactation were offered diets varying in forage-to-concentrate ratio. Dietary treatments were based on ad libitum access to ryegrass silage and a standard dairy concentrate with forage/concentrate ratios of 80/20, 65/35, 50/50, 35/65 on a DM basis (Dewhurst et al., 2002; Moorby et al., 2002). Concentrates and grass silage contained 43 and 12 g of FA per kilogram DM, respectively. Predominant FA (% of total FA) in concentrates were $C_{12:0}$ (4.8%), $C_{16:0}$ (16.9%), $C_{18:1}$ (26.6%), n-6 $C_{18:2}$ (35.4%), and n-3 $C_{18:3}$ (5.3%), whereas grass silage FA mainly consisted of $C_{16:0}$ (22.5%), n-6 $C_{18:2}$ (17.8%), and n-3 $C_{18:3}$ (44.8%). Average daily DMI were 13.2, 15.5, 18.4, and 20.7 kg for diets with forage to concentrate ratios of 80/20, 65/35, 50/50, 35/65, respectively (Moorby et al., 2002).

Experiment 3. The experiment was designed as a 4 × 4 Latin square experiment (Hindle et al., 2003). Cows in early lactation were offered grass silage/beet pulp/concentrate in ratios of 43/27/30 on a DM basis. Diets were distributed once daily as a TMR. All diets were isoenergetic and isonitrogenous and based on the animals' requirements for energy and protein. The four concentrates differed in amount of (protected) starch. Diets contained between 71 and 121 g of FA/kg DM with $C_{12:0}$ (2.2 to 4.9%), $C_{14:0}$ (1.2 to 2.3%), $C_{16:0}$ (14.4 to 15.8%), $C_{18:0}$ (1.9 to 3.0%), $C_{18:1}$ (4.3 to 10.0%), n-6 $C_{18:2}$ (15.8 to 28.2%) and n-3 $C_{18:3}$ (29.6 to 51.9%) representing over 90% of total FA. Average daily DMI was relatively constant and ranged from 18.1 to 19.6 kg.

Sampling

In all three experiments, feed, duodenal, and milk samples, taken during the final week of each experimental period were used in the statistical analysis. In experiments 1 and 2, silage (composite of three) and concentrate samples were stored frozen and freeze-dried prior to chemical analysis. In experiment 3, silage and concentrates were sampled together at feeding (TMR) and stored frozen. Daily samples were thawed and mixed prior to chemical analysis. Duodenal sampling in experiments 1 and 2 was performed over 2 consecutive days using the automated equipment described by Evans et al. (1981), 24-h duodenum samples were stored frozen and freeze dried prior to analysis. In experiment 3, duodenal samples were collected every 4 h during 2 consecutive days and the 12 individual samples were mixed prior to freezing. Analysis was performed on freeze-dried samples. Milk samples were taken from 4 (experiments 1 and 2) or 8 (experiment 3) consecutive milkings, stored frozen without preservative and freeze-dried (experiments 1 and 2) or thawed (experiment 3) prior to FA analysis.

In experiment 1, ytterbium acetate (mean 650 mg of Yb/d) was infused into the rumen continuously as a marker to allow estimation of flows at the duodenum (Dewhurst et al., 2003a). Duodenal flows in experiments 2 and 3 were determined based on the double marker technique as described by Faichney (1992). In all 3 experiments, milk yields were recorded throughout the experiment, and mean values from the final week of each period were used for calculations of milk FA yields.

Fatty Acid Analysis

Feed, duodenal, and milk samples were used for extraction and methylation of FA and GLC analysis of

FA methyl esters. In experiments 1 and 2, extraction and methylation of FA were based on the methods described by Sukhija and Palmquist (1988). Fatty acid methyl esters in feed and milk samples were analyzed by GLC using an Innowax column (30 m × 0.32 mm i.d.) (Phenomonex, Macclesfield, UK). For duodenal digesta of experiment 1, a CP-Sil88 column (50 m × 0.25 mm i.d.) (Chrompack, The Netherlands) was used, whereas FA methyl esters in duodenal samples of experiment 2 were separated by a chemically bonded CP-Select for FAME (100 m × 0.25 mm i.d.) (Varian, Walton-on-Thames, UK). Extraction of milk FA in experiment 3 was according to the method of the International Organization for Standardization (ISO-3889). Extraction of feed and duodenal FA, methylation and GLC analysis of FA methyl esters were as described by Raes et al. (2001). Compared to the latter, the GLC temperature program was modified for milk FA analysis (70°C for 4 min, 13°C/min until 175°C, 175°C for 27 min, 4°C/min until 215°C, 215°C for 31 min). Thirteen FA were identified in common in all three experiments and both in duodenal and milk samples, i.e., $C_{14:0}$, $C_{14:1}$, *iso* $C_{15:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, *iso* $C_{17:0}$, $C_{17:0}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1}$, n-6 $C_{18:2}$, and n-3 $C_{18:3}$. As *anteiso* $C_{15:0}$ seemed to coelute with an unidentified FA on a 30 m column (experiments 1 and 2), this OCFA was excluded from the analysis. *Anteiso* $C_{17:0}$ was not considered either, as its concentrations in duodenal samples of experiment 1 seemed inexplicably high. For each of the 13 individual FA, means were calculated per cow and diet from 2 (duodenal samples of experiments 1 and 2), 4 (milk samples of experiments 1 and 2) or 8 (milk samples of experiment 3) FA analyses. Overall, we had duodenal and milk FA patterns from 56 experimental units (x_{ij}, with i = 1, 13; j = 1, 56). Unless otherwise stated, individual milk and duodenal FA were expressed as a proportion of total FA (% of total FA), the latter being the sum of the 13 commonly determined FA.

Statistics

Fatty acid proportions (% of total FA) in duodenal digesta and milk were compared using the general linear model (GLM) procedures (univariate) according to: $Y_{ijk} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + \varepsilon_{ijk}$, with Y_{ijk} = FA proportions; A_i = diet effect; B_j = animal effect; C_k = sample origin (i.e., duodenal digesta or milk); AB_{ij}, AC_{ik}, BC_{jk} = interactions between different factors; ε_{ijk} = residual error.

Relationships between milk FA were evaluated from the loading plots of PCA, based on the correlation matrix (consisting of 13 variables), using SPSS (SPSS software for Windows, release 11.0, SPSS Inc.). Levels (% of total FA) of the 13 milk FA from the 3 experiments were used in the latter PCA. Similarly, loading plots, based on FA concentrations (% of total FA) as well as duodenal flows or milk yields (g/d) of FA, were used to illustrate the origin of milk FA. Duodenal concentrations of $C_{14:1}$ and $C_{17:1}$ were below the detection limit in duodenal samples of experiments 1 and 2. Hence, including these duodenal FA in PCA may result in a (physiologically meaningless) contrast between samples of experiments 1 and 2 on the one hand and experiment 3 on the other. Hence, these duodenal FA were excluded from the latter PCA. Accordingly, the correlation matrix consisted of 24 variables (13 milk FA and 11 duodenal FA). Because *trans*-11 $C_{18:1}$ and *cis*-9,*trans*-11 $C_{18:2}$ were not determined in milk from experiments 1 and 2, only data from experiment 3 could be used to investigate the importance of mammary Δ^9-desaturase activity in the production of milk *cis*-9,*trans*-11 $C_{18:2}$. For this PCA, FA concentrations were expressed relative to 17 identified milk FA ($C_{14:0}$, $C_{14:1}$, *iso* $C_{15:0}$, *anteiso* $C_{15:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, *iso* $C_{17:0}$, *anteiso* $C_{17:0}$, $C_{17:0}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1}$, n-6 $C_{18:2}$, n-3 $C_{18:3}$, *trans*-11 $C_{18:1}$, and *cis*-9,*trans*-11 $C_{18:2}$). Grouping of FA in pairwise loading plots was evaluated based on squared Euclidean distances. Fatty acids with squared distances below 0.100 were considered to belong to the same group.

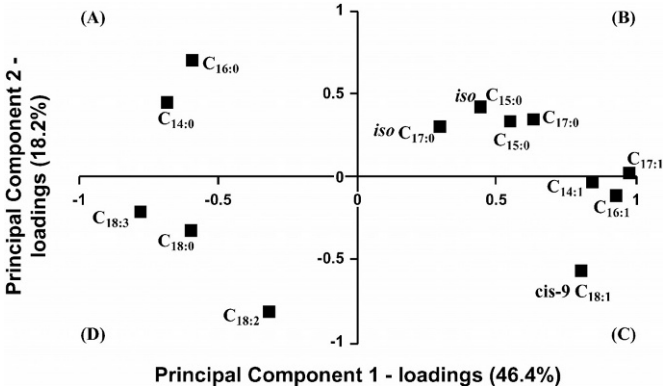

Figure 1. Loading plot, describing the relationships among milk fatty acids derived from a principal component analysis based on proportions (percentage of total fatty acids) of C14 to C18 fatty acids in milk from 3 experiments (n = 56).

RESULTS AND DISCUSSION

The first (PC 1) and second (PC 2) principal components described 64.6% of the total variation in milk FA patterns of samples from the 3 experiments (Figure 1). In this loading plot, 4 groups of milk FA could be distinguished: $C_{14:0}$ and $C_{16:0}$ in quadrant (A); *iso* $C_{15:0}$, *iso* $C_{17:0}$, $C_{15:0}$ and $C_{17:0}$ in quadrant (B); $C_{18:0}$, n-3 $C_{18:3}$, and n-6 $C_{18:2}$ in quadrant (D); and $C_{14:1}$, $C_{16:1}$, and $C_{17:1}$ between quadrant (B) and (C). It is likely that FA form-

ing a cluster follow a common metabolic pathway (Massart-Leën and Massart, 1981). $C_{14:0}$ and $C_{16:0}$, showing negative loadings for PC 1 and positive loadings for PC 2 are (partially) de novo-synthesized from acetate and β-hydroxybutyrate. These FA were separated by PC 2 from the 18-carbon FA, absorbed directly from the blood stream, and of dietary origin or the result of rumen biohydrogenation. The 4 milk OCFA showed positive loadings for both PC 1 and PC 2. Monoenoic milk FA, which are predominantly produced by Δ^9-desaturase activity, had high positive loadings for PC 1 but were not correlated with PC 2. $C_{18:1}$ is close to this cluster, although showing a negative loading for PC 2, which might be due to the dual origin of milk $C_{18:1}$, i.e., directly absorbed from the circulatory system as well as endogenous production. Clustering of $C_{17:1}$ with $C_{14:1}$ and $C_{16:1}$ supports our hypothesis of endogenous production from $C_{17:0}$. In summary, 2 clusters were mainly determined by dietary factors or processes in the rumen, i.e., biohydrogenation of dietary FA and de novo synthesis of OCFA by rumen microbes. The 2 other groups were related to metabolic processes in the mammary gland. The importance of postabsorptive synthesis of $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{17:1}$, and $C_{18:1}$ is further confirmed by their significantly higher proportions in milk than in duodenal samples (Table 1) ($P < 0.001$) and illustrated in the loading plot based on PCA of both milk and duodenal FA (Figure 2). Indeed, clustering of duodenal and milk n-6 $C_{18:2}$ and n-3 $C_{18:3}$ on the one hand and milk and duodenal OCFA on the other hand illustrates the positive correlation between duodenal and milk levels of these FA. This could be expected as these FA are absorbed directly from the blood stream and do not undergo further transformation in the udder. Neither $C_{14:0}$ and $C_{16:0}$ nor monoenoic acids in milk clustered with duodenal $C_{14:0}$ and $C_{16:0}$ or monoenoic acids respectively. This illustrates milk levels of these FA are mainly determined by de novo synthesis in the udder and not by their duodenal supply. The negative loading for PC 1 of 18-carbon FA on the one hand and the positive loading of OCFA and monoenoic FA in milk on the other hand demonstrates that these FA are negatively correlated. Indeed, increased dietary fat supply (mainly 18-carbon FA and $C_{16:0}$) reduces the proportion of OCFA in duodenal contents and milk as de novo synthesis of odd chain FA by rumen bacteria remains constant or is partially inhibited at higher dietary fat levels (Demeyer et al., 1978). The negative correlation between 18-carbon FA and monoenoic milk FA could be the result of a partial inhibition of the Δ^9-desaturase activity through increased concentrations of n-3 $C_{18:3}$ and n-6 $C_{18:2}$ (Bickerstaffe and Annison, 1970). A similar picture emerged when running the statistical analysis with duodenal FA flows and milk FA yields, with

Table 1. Mean concentrations of fatty acids in milk and duodenal digesta (% of total fatty acids[1]) from 3 experiments [mean ± SE].

Experiment	$C_{14:0}$	$C_{14:1}$	*iso* $C_{15:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	*iso* $C_{17:0}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	cis-9 $C_{18:1}$	n-6 $C_{18:2}$	n-3 $C_{18:3}$
Duodenal digesta													
1 (n = 23)	3.14 ± 0.062	ND[2]	0.60 ± 0.021	1.94 ± 0.023	23.4 ± 0.21	0.33 ± 0.016	0.35 ± 0.013	0.87 ± 0.021	ND[2]	57.1 ± 0.59	6.05 ± 0.203	4.07 ± 0.152	2.08 ± 0.127
2 (n = 16)	1.52 ± 0.040	ND[2]	0.94 ± 0.046	1.99 ± 0.151	19.7 ± 0.11	0.16 ± 0.014	0.40 ± 0.023	0.81 ± 0.051	ND[2]	64.7 ± 0.54	5.12 ± 0.336	3.48 ± 0.339	1.17 ± 0.074
3 (n = 16)	1.96 ± 0.137	0.08 ± 0.007	0.52 ± 0.030	1.50 ± 0.052	19.2 ± 0.38	0.20 ± 0.023	0.15 ± 0.014	1.49 ± 0.113	0.10 ± 0.020	59.6 ± 1.02	7.02 ± 0.225	5.55 ± 0.205	2.63 ± 0.257
Milk													
1 (n = 24)	14.4 ± 0.16	1.60 ± 0.046	0.27 ± 0.010	1.77 ± 0.021	35.6 ± 0.44	2.52 ± 0.057	0.15 ± 0.008	0.73 ± 0.009	0.39 ± 0.009	11.8 ± 0.18	27.9 ± 0.40	1.98 ± 0.066	0.76 ± 0.052
2 (n = 16)	13.9 ± 0.32	1.81 ± 0.093	0.34 ± 0.014	1.86 ± 0.065	37.0 ± 0.97	2.74 ± 0.123	0.26 ± 0.010	0.82 ± 0.022	0.50 ± 0.024	10.9 ± 0.45	27.9 ± 0.86	1.54 ± 0.120	0.43 ± 0.021
3 (n = 16)	15.7 ± 0.32	0.75 ± 0.057	0.27 ± 0.005	1.48 ± 0.060	45.2 ± 1.50	1.25 ± 0.12	0.19 ± 0.010	0.69 ± 0.021	0.17 ± 0.014	14.8 ± 1.10	16.6 ± 0.93	1.80 ± 0.095	1.01 ± 0.035

[1]Total fatty acids = $C_{14:0}$ + $C_{14:1}$ + $C_{15:0}$ + *iso* $C_{15:0}$ + $C_{16:0}$ + $C_{16:1}$ + $C_{17:0}$ + *iso* $C_{17:0}$ + $C_{17:1}$ + $C_{18:0}$ + cis-9 $C_{18:1}$ + n-6 $C_{18:2}$ + n-3 $C_{18:3}$.
[2]ND = not detectable.

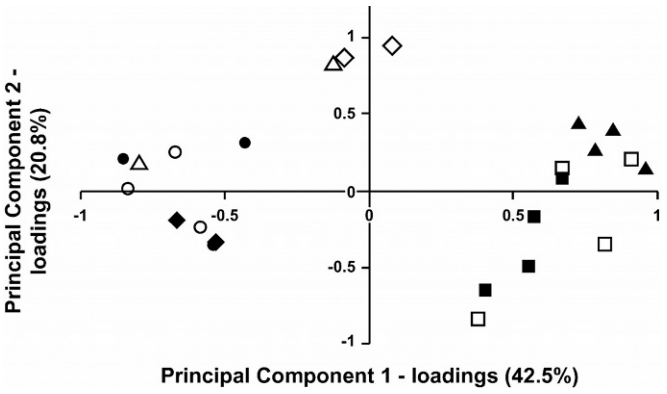

Figure 2. Loading plot, describing relationships between milk (full symbols) and duodenal (open symbols) fatty acids (% of total fatty acids). $C_{18:0}$, n-6 $C_{18:2}$, and n-3 $C_{18:3}$ are represented by circles; $C_{14:0}$ and $C_{16:0}$ by diamonds; $C_{15:0}$, $C_{17:0}$, *iso* $C_{15:0}$, and *iso* $C_{17:0}$ by squares and mono-unsaturated fatty acids, i.e., $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, and $C_{18:1}$ by triangles. Duodenal concentrations of $C_{14:1}$ and $C_{17:1}$ were not included in the principal component analysis, as they were below the detection limit in experiments 1 and 2.

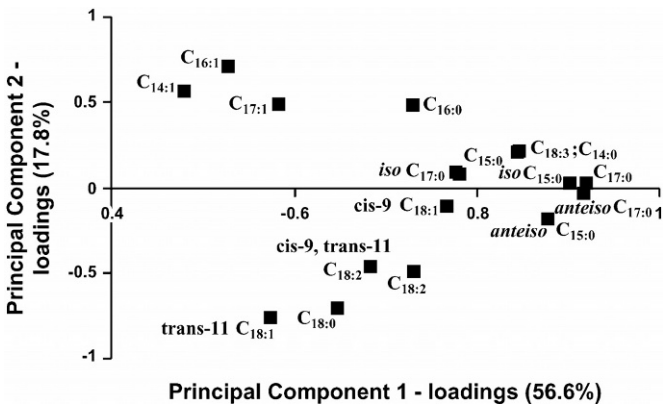

Figure 3. Loading plot, describing the relationships among milk fatty acids derived from a principal component analysis based on concentrations (% of total fatty acids) of C14 to C18 fatty acids (including vaccenic acid, *trans*-11 $C_{18:1}$ and conjugated linoleic acid, cis-9,*trans*-11 $C_{18:2}$) in milk from experiment 3 (n = 122).

PC 1 and PC 2 describing 77.8% of the total variation in FA flows and yields. All variables had positive loadings for PC 1 (figure not shown). Direct absorption from the blood stream of milk OCFA, $C_{18:0}$, n-6 $C_{18:2}$, and n-3 $C_{18:3}$ was confirmed by the clustering of duodenal and milk OCFA as well as duodenal and milk 18-carbon FA, showing high loadings for PC 1 (0.86, 0.81, 0.90, and 0.87, respectively). These milk and duodenal FA were clearly separated from mutually clustering monoenoic milk FA ($C_{14:1}$, $C_{16:1}$, and $C_{17:1}$) with average loading for PC 1 of 0.04. The PC 1 loading of milk $C_{18:1}$ was intermediate (0.37). Duodenal flows and milk yields of $C_{14:0}$ and $C_{16:0}$ were separated based on PC 1 (0.67 vs. 0.96) and PC 2 (–0.45 vs. 0.10). Contrary to PCA based on FA concentrations (% of total FA), FA of microbial and dietary origin could not be distinguished in the PCA based on duodenal FA flows and milk FA yields (g/d). The contrast between OCFA and 18-carbon FA in the former PCA illustrated the decreasing relative importance of OCFA in total microbial FA when readily available long chain FA are supplied. Nevertheless, absolute duodenal flows of OCFA are mainly determined by outflow of rumen microbes. In the three experiments currently considered, milk yield, dietary fat intake as well as intake of rumen fermentable OM were highest in experiment 3, resulting in increased flow and yields of both 18-carbon FA as well as microbial OCFA.

Interest in mammary Δ^9-desaturase is growing as *cis*-9,*trans*-11 $C_{18:2}$ in milk was shown to be predominantly of endogenous origin (e.g., Griinari et al., 2000; Lock and Garnsworthy, 2002; Piperova et al., 2002). Low *cis*-9,*trans*-11 $C_{18:2}$ concentrations in duodenal digesta compared to milk (Table 2) and the close correlation between duodenal *trans*-11 $C_{18:1}$ and milk *cis*-9,*trans*-11 $C_{18:2}$ ($r_{pearson} = 0.876$, $P < 0.001$, n = 16) confirm the importance of endogenous *cis*-9,*trans*-11 $C_{18:2}$ production in the mammary gland. Clusters in the loading plot based on milk FA concentrations observed in experiment 3 (Figure 3) were not as obvious as when combining the 3 experiments (Figure 1), probably due to lower dietary variation. Nevertheless, clustering of $C_{17:1}$ with $C_{14:1}$ and $C_{16:1}$ reconfirms the importance of

Table 2. Mean (± SE), minimum and maximum concentrations of vaccenic acid (*trans*-11 $C_{18:1}$) and *cis*-9,*trans*-11 conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$) in duodenal digesta (n = 15) and milk (n = 16) (% of total fatty acids[1]) and ratio $C_{14:1}/C_{14:0}$ in milk (g/g) of cows in experiment 3.

	Duodenal digesta		Milk		
	trans-11 $C_{18:1}$	*cis*-9, *trans*-11 $C_{18:2}$	*trans*-11 $C_{18:1}$	*cis*-9, *trans*-11 $C_{18:2}$	$C_{14:1}/C_{14:0}$
Mean	3.18 ± 0.201	0.036 ± 0.008	1.25 ± 0.116	0.32 ± 0.013	0.048 ± 0.001
Min	1.94	ND[2]	0.56	0.14	0.020
Max	4.46	0.11	2.32	0.51	0.080

[1]Total fatty acids = $C_{14:0}$ + $C_{14:1}$ + *iso* $C_{15:0}$ + *anteiso* $C_{15:0}$ + $C_{15:0}$ + $C_{16:0}$ + $C_{16:1}$ + *iso* $C_{17:0}$ + *anteiso* $C_{17:0}$ + $C_{17:0}$ + $C_{17:1}$ + $C_{18:0}$ + $C_{18:1}$ + n-6 $C_{18:2}$ + n-3 $C_{18:3}$ + *trans*-11 $C_{18:1}$ + *cis*-9,*trans*-11 $C_{18:2}$.

[2]ND = not detectable.

Δ^9-desaturase activity in the mammary gland for the production of $C_{17:1}$ (Figure 3). Despite the fact that up to 75% of *cis*-9,*trans*-11 $C_{18:2}$ was reported to be produced endogenously (Griinari et al., 2000; Lock and Garnsworthy, 2002; Piperova et al., 2002), it did not cluster with $C_{14:1}$, $C_{16:1}$ and $C_{17:1}$. On the contrary, *cis*-9,*trans*-11 $C_{18:2}$ was located close to its precursor, *trans*-11 $C_{18:1}$ and a very strong correlation was observed between these 2 milk FA ($r_{pearson} = 0.808$, $P < 0.001$, n = 122). Close linear relationships between milk fat *trans*-11 $C_{18:1}$ and *cis*-9,*trans*-11 $C_{18:2}$ has been observed across a wide range of diets (e.g., review by Bauman et al., 1999), which was also visualized in a PCA loading plot previously (Jiang et al., 1996). Correlations between milk monoenoic FA and their precursors were far less strong ($C_{14:0}$ vs. $C_{14:1}$, $r_{pearson} = 0.197$, $P = 0.030$; $C_{16:0}$ vs. $C_{16:1}$, $r_{pearson} = 0.433$, $P < 0.001$; $C_{17:0}$ vs. $C_{17:1}$, $r_{pearson} = 0.355$, $P < 0.001$; $C_{18:0}$ vs. cis-9 $C_{18:1}$, $r_{pearson} = 0.383$, $P < 0.001$, n = 122). Apparently, within the range of cows and diets studied in experiment 3, differences in milk *cis*-9,*trans*-11 $C_{18:2}$ were determined mainly by variation in the duodenal supply of *trans*-11 $C_{18:1}$ rather than by variation in Δ^9-desaturase activity. The latter is further confirmed by the absence of any correlation between milk *cis*-9,*trans*-11 $C_{18:2}$ and $C_{14:1}/C_{14:0}$ ($r_{pearson} = -0.127$, $P = 0.165$, n = 122), which has been identified as a good indicator of Δ^9-desaturase activity (Lock and Garnsworthy, 2002). Correlations between $C_{14:1}/C_{14:0}$ and monoenoeic acid concentrations ($C_{14:1}$, $C_{16:1}$, $C_{17:1}$, and $C_{18:1}$) were dramatically higher ($r_{pearson} = 0.958$, 0.522, 0.769, and 0.541, respectively; $P < 0.001$; n = 122). Solomon et al. (2000) proposed substantial differences among individual cows in milk cis-9,trans-11 $C_{18:2}$ could be due to: 1) differences in the production of *trans*-11 $C_{18:1}$ in the rumen, 2) differences in the rumen accumulation of *cis*-9,*trans*-11 $C_{18:2}$, or 3) differences between individual cows in tissue activity of Δ^9-desaturase. Individual cows in the current experiment showed considerable differences in Δ^9-desaturase activity, as suggested by $C_{14:1}/C_{14:0}$ ratios across treatments varying between 0.032 and 0.062. However, both the separation of *cis*-9,*trans*-11 $C_{18:2}$ from $C_{14:1}$, $C_{16:1}$, and $C_{17:1}$ in the loading plot and its strong correlation with duodenal and milk *trans*-11 $C_{18:1}$ suggest that the conversion of *cis*-9,*trans*-11 $C_{18:2}$ was mainly precursor driven, rather than dependent on variation in desaturase activity. Nevertheless, differences in tissue activity of Δ^9-desaturase might play a secondary role, as suggested from the significant partial correlation between milk *cis*-9,*trans*-11 $C_{18:2}$ and $C_{14:1}/C_{14:0}$ when controlling for milk *trans*-11 $C_{18:1}$ concentration ($r_{partial} = 0.533$, $P < 0.001$, n = 122).

CONCLUSIONS

Loading plots of PCA appear to offer an interesting approach to indicate mutual metabolic relationships between milk FA and to illustrate origin of milk FA. Using this tool, $C_{17:1}$ was identified to be a desaturation product of its saturated precursor, $C_{17:0}$, in common with other milk monoenoic FA. Moreover, this study revealed milk *cis*-9,*trans*-11 $C_{18:2}$ concentrations to be mainly dependent on the *trans*-11 $C_{18:1}$ supply, rather than determined by the Δ^9-desaturase activity, at least within the range of diets and cows studied here.

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