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Apparent Recovery of Duodenal Odd- and Branched-Chain Fatty Acids in Milk of Dairy Cows

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ABSTRACT

This study compared flows of odd- and branched-chain fatty acids (OBCFA) at the duodenum with corresponding yields in milk. Four mid-lactation Holstein-Friesian dairy cows were offered 4 dietary treatments, based on different ratios of ryegrass silage and concentrates (80:20, 65:35, 50:50, and 35:65 on a dry matter basis), in a 4 × 4 Latin square design experiment with 4-wk periods. Samples of milk and duodenal digesta were collected during the final week of each period and analyzed for fatty acids. Biohydrogenation of linoleic and α-linolenic acids (C18:2 and C18:3) was extensive for all treatments, with a tendency to be lower for C18:3 with increased concentrate feeding. The proportion of duodenal flows of these fatty acids that appeared in milk declined with increasing concentrate feeding. There was little change in the yield of OBCFA in milk in response to increasing level of concentrate inclusion and no significant relationship with the yield of microbial protein at the duodenum. The efficiency of transfer of *iso* C15:0 and *anteiso* C15:0 from the duodenum to milk was similar to that for C18:3, with a reduced proportion transferred into milk at higher flows. Yields of C15:0, C17:0, and *iso* C17:0 in milk were higher than duodenal flows, confirming synthesis in animal tissues.
Key words: dairy cow, fatty acids, biohydrogenation, milk composition

INTRODUCTION

Rumen microbial protein is often the main component of MP supply to dairy cows (NRC, 2001). A large number of studies over the last 40 yr have estimated microbial protein flow from the rumen using marker techniques and cannulated animals. Odd- and branched-chain fatty acids (**OBCFA**) are distinctive components of rumen microorganisms (Harfoot, 1978) and have recently been used as markers of the microbial protein content within rumen and duodenal digesta (Vlaeminck et al., 2005, 2006).

One of the major difficulties when estimating the composition of duodenal flows is obtaining representative samples of microorganisms leaving the rumen. For example, there is relatively little information about the relative proportions of solid- and liquid-associated bacteria in duodenal digesta (Vlaeminck et al., 2006). The relatively constant ratio of OBCFA:N within solid- and liquid-associated bacteria (Vlaeminck et al., 2006) means that OBCFA may be more robust than conventional markers, such as purines (Vlaeminck et al., 2005).

Odd- and branched-chain fatty acids are significant components of milk fat, which has led to speculation that milk OBCFA might provide a noninvasive technique for estimating microbial protein flows from the rumen (Dewhurst et al., 2000). The main OBCFA in milk are isomers of pentadecanoic acid (C15:0, *iso* C15:0, and *anteiso* C15:0), and heptadecanoic acid (C17:0, *iso* C17:0, and *anteiso* C17:0). Although there is some evidence of a useful relationship between milk OBCFA and microbial protein flow from the rumen (Vlaeminck et al., 2005), there has been no robust evaluation of the relationship between OBCFA flowing at the duodenum and their yield in milk.

The objective of this work was to evaluate the relationship between duodenal flows of OBCFA and their yield in milk, for a series of diets differing widely in forage:concentrate ratio.

MATERIALS AND METHODS

Experimental Design

The experimental design and feed composition have been described previously (Moorby et al., 2006). All procedures were regulated by the UK Home Office under the Animals (Scientific Procedures) Act of 1986. Briefly, 4 multiparous Holstein-Friesian dairy cows [starting at a mean 90 DIM (SD = 33.6)], with simple cannulas

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in the rumen and the proximal duodenum, were used in a 4 × 4 Latin square design experiment with 4-wk periods. Four dietary treatments composed of ryegrass silage and dairy concentrate (NDF, CP, and starch were 24.8, 22.8, and 22.1% of DM, respectively) were offered at different forage-to-concentrate (F:C) ratios (80:20, 65:35, 50:50, and 35:65 on a DM basis). The concentrate comprised (% as fed): wheat (30), rapeseed meal (15), corn gluten feed (15), linseed meal (12), sunflower meal (11), sugar beet pulp (6), molasses (6), and palm kernel meal (2). Sodium bicarbonate was added to all diets at the rate of approximately 1.7% of total DM, mixed in with the concentrate ration, which was offered in 2 equal portions per day, 1 at each milking.

Milk and Digesta Sampling

Cows were milked twice a day, at approximately 0800 and 1600 h, throughout the experiment. Milk samples were taken for fatty acid analysis during the final week of each period. Afternoon and morning milk samples were analyzed separately and composited by day before statistical analysis.

Duodenal digesta was sampled over 2 d in the final week of each period, as described by Dewhurst et al. (2003). Ytterbium acetate and chromium EDTA were used as particulate and liquid markers, respectively, to allow reconstitution of true digesta composition (Faichney, 1975).

Sample Handling and Fatty Acid Analysis

Milk, feed, and digesta samples were stored frozen (–18°C) without preservative. Fatty acid methyl esters (**FAME**) were prepared from freeze-dried feed and digesta samples using a one-step extraction and methylation procedure (Sukhija and Palmquist, 1988). Analysis of feed and digesta FAME used a 'Select for FAME' column (Varian Ltd., Oxford, UK; 100 m × 0.25 mm i.d.) on a CP-3800 gas chromatograph (Varian Ltd.). The carrier gas was helium, and detector and injector temperatures were 255 and 250°C, respectively. Peaks were identified from external standards and quantified using an internal standard (C19:0).

The digesta FAME were run with the following temperature program: hold at 70°C for 1 min, increase to 100°C at 5°C/min, hold for 2 min, increase to 175°C at 10°C/min, hold for 34 min, increase to 225°C at 4°C/min, and hold for 20 min. Carrier gas was run at constant pressure with one increase to avoid falling off the Van Deemter plateau: 275 kPa for 52.5 min, increase at 2.75 kPa/min to 310 kPa, and hold for 20.5 min. This complex temperature and pressure program was used to maximize separation of C18:1 and C18:2 isomers.

Milk fat from thawed milk was extracted in 3 steps according to Vlaeminck et al. (2005). In the first step, samples were extracted with ammonium hydroxide solution, ethanol, diethyl ether, and petroleum ether. In the second step, samples were extracted with ethanol, diethyl ether, and petroleum ether; in the final step, only diethyl ether and petroleum ether were used. Extracts were combined and brought up to a final volume of 20 mL with a mixture of diethyl ether and petroleum ether (1:1, vol/vol). Fatty acids were methylated and analyzed by gas chromatography on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard Co., Brussels, Belgium) with a CP-Sil88 column for FAME (100 m × 0.25 mm × 0.2 μm; Chrompak Inc., Middelburg, the Netherlands) as described by Vlaeminck et al. (2005). We were unable to resolve *anteiso* C17:0 with confidence from C16:1 and so results are not presented for these fatty acids.

Statistical Analysis

Results were analyzed using the Genstat 7 software package (Release 7.1, 2003; Lawes Agricultural Trust, Rothamsted, UK). The ANOVA model used a treatment structure of F:C ratio (which was partitioned into linear, quadratic, and other effects; 3 df), and a blocking structure of period + cow (3 df each). There were 15 df in total (4 cows × 4 periods), and so 6 residual df. Statistical significance was declared at the 95% confidence level, although trends (90% confidence level) were also noted.

RESULTS

Results for feed intake, milk production and composition, rumen function, rumen and total tract digestion, and nitrogen partitioning have been presented previously (Moorby et al., 2006). Rumen ammonia concentration increased significantly with increasing concentrate feeding, but there was no significant effect on rumen pH, and significant changes in acetate molar percentage (decrease) and butyrate molar percentage (increase) were numerically small.

Concentrations of the major fatty acids in the feeds used in this study are presented in Table 1. Linoleic, oleic, and palmitic acids were the predominant fatty acids in the concentrate, with lower levels of α-linolenic acid supplied by the linseed meal. The total level of fatty acid, as well as the proportion of C18:3 fatty acid (45%), was at the lower end of the usual range for ryegrass silage. Odd- and branched-chain fatty acid comprised only 1.13 and 0.27% of the total fatty acids in the grass silage and concentrates, respectively.

Duodenal flows of individual fatty acids are presented in Table 2. There was a substantial increase (approxi-

Table 1. Concentrations of odd- and branched-chain fatty acids and other significant fatty acids in the feeds used in the experiment (g/kg of DM)

Fatty acid	Grass silage	Concentrates
C4:0	0.378	0.018
C6:0	0.088	0.054
C8:0	0.012	0.176
C10:0	0.004	0.160
C12:0	0.046	2.07
C14:0	0.116	0.788
Iso C15:0	0.013	0.006
Anteiso C15:0	0.039	0.014
C15:0	0.031	0.036
C16:0	2.78	7.28
C16:1	0.075	0.166
Iso C17:0	0.010	0.006
Anteiso C17:0	0.011	0.007
C17:0	0.035	0.047
C18:0	0.313	1.410
C18:1	0.503	13.02
C18:2	2.19	15.29
C18:3	5.52	2.29
Total fatty acids	12.3	43.2

mately 75%) in the duodenal flow of *anteiso* C15:0 with increasing concentrate proportion, and smaller increases (10 to 35%) for other OBCFA apart from C15:0 (for which the level was similar across F:C ratios). Biohydrogenation values were calculated as the loss of the fatty acid of interest between the diet and duodenal flow as a percentage of the intake of the fatty acid. There was no significant effect of F:C ratio on biohydrogenation of C18:2 (mean = 90.5%), but a tendency ($P < 0.1$) for biohydrogenation of C18:3 to reduce with increasing concentrate feeding (95.0, 94.1, 92.8, and 93.3%; SED = 0.77).

Daily yields of individual fatty acids in milk are presented in Table 3. The proportions of major fatty acids (C14:0 = 10.5%; C16:0 = 30.3%; C18:0 = 9.8%; C18:1 = 20.4%) were well within normal ranges (Jensen, 2002). Total milk fatty acids are those reported in Table 3 as well as low levels of other C18:1 isomers, C20 and C22 fatty acids (19 different peaks, which together made up 2.3% of total fatty acids). Levels of C18:3 in milk (0.33%) were in the normal range for cows fed silage and concentrates (Dewhurst and Lee, 2005), whereas levels of conjugated linoleic acid (*cis*-9, *trans*-11; 0.70%) were at the upper end of the range for cows fed silage-based diets. Odd- and branched-chain fatty acids made up only a small percentage of milk fatty acids: C15:0 = 1.23%; *iso* C15:0 = 0.27%; *anteiso* C15:0 = 0.46%; C17:0 = 0.57%; *iso* C17:0 = 0.21%; and C17:1 = 0.28%.

Figure 1 shows the relationship between duodenal flows and yields in milk for each OBCFA (and C18:3 for comparison), by treatment mean. It is immediately apparent that there were not strong relationships between duodenal flows and yields in milk for individual fatty acids. Furthermore, yields of some of the OBCFA in milk were greater than corresponding flows at the duodenum. Table 4 presents these results in terms of the apparent recovery of duodenal OBCFA in milk.

DISCUSSION

The relatively low level of total fatty acids and the low proportion of α-linolenic acid in the grass silage

Table 2. Effects of dietary forage:concentrate ratio on flows (g/d) of major fatty acids at the duodenum of lactating dairy cows

	Forage:concentrate ratio					Significance[1]	
Fatty acid	80:20	65:35	50:50	35:65	SED	Linear	Quadratic
C8:0	0.436	0.547	0.634	0.572	0.0674	†	NS
C10:0	0.370	0.418	0.533	0.469	0.0462	*	NS
C12:0	1.24	1.80	3.14	3.36	0.246	***	NS
C14:0	3.57	4.67	6.24	6.35	0.213	***	*
Iso C15:0	2.57	3.05	3.49	3.41	0.144	***	*
Anteiso C15:0	4.50	5.32	6.80	6.47	0.221	***	*
C15:0	6.24	6.56	6.68	6.12	0.132	NS	**
C16:0	43.0	58.3	83.4	87.5	2.76	***	*
Iso C17:0	1.14	1.31	1.42	1.44	0.0546	**	NS
C17:0	2.45	2.54	2.86	2.73	0.035	***	**
C18:0	140.6	191.8	272.1	275.4	9.84	***	*
C18:1 *trans*-11	12.3	16.5	21.9	25.0	1.19	***	NS
C18:1 *cis*-9	8.2	14.3	25.4	32.0	3.73	***	NS
C18:1 *cis*-11	1.34	2.18	3.81	4.30	0.305	***	NS
C18:2	5.19	9.43	18.39	21.24	2.655	***	NS
C18:3	3.08	3.93	4.99	4.71	0.436	**	NS
CLA[2] *cis*-9, *trans*-11	0.078	0.144	0.189	0.234	0.0405	**	NS
CLA *trans*-10, *cis*-12	0.092	0.100	0.142	0.162	0.0409	†	NS

[1]Significance of linear and quadratic treatment effects: †$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[2]CLA = conjugated linoleic acid.

Table 3. Effects of dietary forage:concentrate ratio on yields (g/d) of major fatty acids in milk

	Forage:concentrate ratio					Significance[1]	
Fatty acid	80:20	65:35	50:50	35:65	SED	Linear	Quadratic
C4:0	27.4	31.1	32.3	37.9	2.02	**	NS
C6:0	18.3	21.1	22.6	25.8	1.44	**	NS
C8:0	9.35	10.40	11.54	13.10	0.880	**	NS
C10:0	17.3	20.8	23.0	25.6	1.99	**	NS
C12:0	16.9	19.4	21.8	24.2	2.33	*	NS
Iso C13:0	0.187	0.200	0.227	0.274	0.0288	*	NS
Anteiso C13:0	0.502	0.542	0.618	0.693	0.0851	†	NS
Iso C14:0	0.704	0.657	0.715	0.694	0.0738	NS	NS
C14:0	69.7	77.8	82.0	88.4	4.84	**	NS
C14:1	8.24	8.35	9.05	9.69	0.841	NS	NS
Iso C15:0	2.09	2.09	2.03	1.95	0.190	NS	NS
Anteiso C15:0	3.27	3.43	3.58	3.61	0.339	NS	NS
C15:0	9.88	9.38	8.78	8.72	0.942	NS	NS
Iso C16:0	1.28	1.35	1.56	1.65	0.0952	**	NS
C16:0	222	237	225	231	11.03	NS	NS
Iso C17:0	1.44	1.51	1.41	1.65	0.174	NS	NS
C17:0	4.45	4.35	4.01	4.18	0.232	NS	NS
C17:1	2.43	2.19	1.76	2.03	0.231	†	NS
C18:0	57.9	72.0	79.7	87.0	2.21	***	†
C18:1 *trans*-6–8[2]	0.823	1.197	1.557	2.161	0.163	***	NS
C18:1 *trans*-9	1.04	1.30	1.65	2.23	0.189	***	NS
C18:1 *trans*-10	4.25	4.98	5.82	6.57	0.433	***	NS
C18:1 *trans*-11	5.19	6.54	7.90	10.98	1.36	**	NS
C18:1 *cis*-9	129	148	155	182	11.10	**	NS
C18:1 *cis*-11	1.85	2.13	2.74	3.47	0.311	***	NS
C18:2	5.68	6.78	9.64	13.48	1.60	**	NS
CLA[3] *cis*-9, *trans*-11	4.38	4.81	5.46	6.80	0.451	***	NS
CLA *trans*-10, *cis*-12	0.413	0.269	0.184	0.127	0.0639	**	NS
C18:3	2.27	2.41	2.61	2.97	0.231	*	NS
C20:0	1.04	1.26	1.32	1.34	0.052	**	*
C20:1	1.04	1.11	1.24	1.32	0.127	†	NS

[1]Significance of linear and quadratic treatment effects: †$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[2]Sum of *trans*-6, *trans*-7, and *trans*-8 C18:1, which coeluted.

[3]CLA = conjugated linoleic acid.

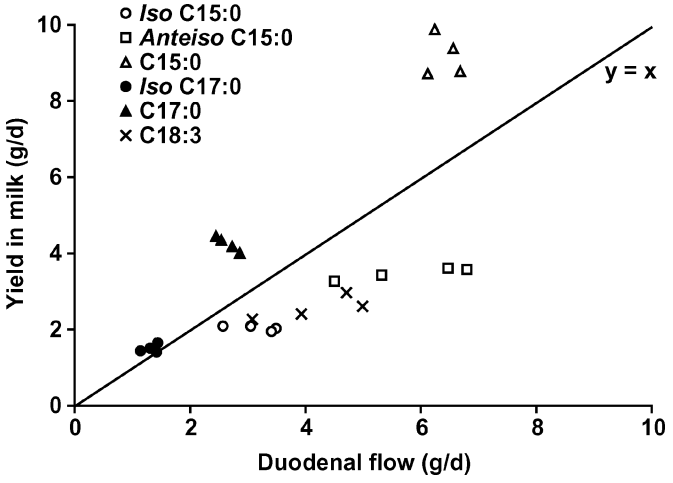

Figure 1. Relationship between flow at the duodenum and yield in milk for individual odd- and branched-chain fatty acids, as well as α-linolenic acid. Treatment means are based on 4 animals per treatment and represent forage:concentrate ratios of 20:80, 35:65, 50:50, and 65:35 (DM basis).

(Table 1) reflect the relatively advanced stage of maturity (Dewhurst et al., 2001), which is also reflected in its high NDF content.

Fatty Acid Biohydrogenation

Biohydrogenation of C18:2 and C18:3 was much more extensive in the current study than in the studies of Kalscheur et al. (1997) and Loor et al. (2004). It is not possible to assign causes for this difference, although it may be a feature of the different forages used in these studies (grass silage vs. grass hay or corn silage and alfalfa haylage; Chilliard et al., 2000).

The tendency for a decrease in biohydrogenation, particularly for C18:3, with increasing concentrate proportion is consistent with, but not as dramatic as, earlier results (Kalscheur et al., 1997; Loor et al., 2004). However, the fact that differences in biohydrogenation were so small, despite a 5-fold range of dietary starch supply (Moorby et al., 2006), does not support the suggestion of Loor et al. (2004) that starch supply has an important direct effect on biohydrogenation. Indeed, neither ru-

Table 4. Effects of dietary forage:concentrate ratio on apparent recoveries (%) of duodenal odd- and branched-chain fatty acids in milk

	Forage:concentrate ratio					Significance[1]	
Fatty acid	80:20	65:35	50:50	35:65	SED	Linear	Quadratic
Iso C15:0	81.2	69.5	59.0	59.3	5.72	**	NS
Anteiso C15:0	72.1	64.5	53.1	56.1	6.16	*	NS
C15:0	159.9	143.3	133.7	144.7	14.02	NS	NS
Iso C17:0	127.5	121.6	102.6	122.9	14.82	NS	NS
C17:0 and C17:1	284.8	265.8	206.0	236.2	14.95	**	†
C18:1 *trans*-11	41.5	42.9	37.1	44.6	3.92	NS	NS
C18:2	107.2	74.8	54.4	62.4	8.91	**	*
C18:3	74.0	63.5	53.0	66.0	5.20	†	*

[1]Significance of linear and quadratic treatment effects: †$P < 0.1$; *$P < 0.05$; **$P < 0.01$.

men pH nor dietary starch supply provided a consistent explanation for effects of F:C ratio and buffers on milk fat yield across these studies. The proportion of duodenal flows of C18:2 and C18:3 that appeared in milk declined with increasing flows of these fatty acids, in agreement with previous work (Chilliard et al., 2000).

OBCFA

There was little change in the yield of OBCFA in milk in response to increasing levels of concentrate inclusion. This was in contrast to highly significant effects on yields of even-chain fatty acids, including short- and medium-chain de novo synthesized fatty acids and many of those derived from dietary 18-carbon fatty acids. This lack of relationship was surprising, because there was a substantial increase in microbial yield at the duodenum in response to increasing concentrates (Moorby et al., 2006). Nonetheless, there was a strong correlation between the flows of purine bases and of OBCFA in duodenal digesta in this study (Vlaeminck et al., 2006). This suggests that, in contrast to the results of Vlaeminck et al. (2005), milk OBCFA are not useful microbial markers.

There are a number of possible explanations for the differences in the apparent transfer of OBCFA from duodenum into milk. It is possible that treatment differences in mobilization of body fat reserves could contribute to milk OBCFA. However, these cows were studied after peak lactation and the cows were gaining body reserves (Moorby et al., 2006). It is more likely that these effects relate to differences in synthesis and oxidation of fatty acids in cow tissues. As noted above, Chilliard et al. (2000) showed a decreasing transfer of duodenal fatty acids into milk with increasing duodenal flows of fatty acids. This may relate to changes in patterns of fatty acid oxidation, differential incorporation of OBCFA into body fat reserves, or other factors. Interestingly, *iso* C15:0 and *anteiso* C15:0 behaved in a very similar way to C18:3, with similar transfer efficiencies and corresponding declines in apparent recoveries from duodenum to milk with increasing concentrate feeding level (Table 4). This is consistent with the decrease in fatty acid oxidation with increasing chain length and increase with increasing unsaturation noted by DeLany et al. (2000).

Yields of C15:0, C17:0, and *iso* C17:0 fatty acids in milk exceeded duodenal flows substantially (Figure 1 and Table 4). Comparison of the ratios of C15:0 to *anteiso* C15:0 fatty acids in jugular plasma (0.94) and milk fat (1.99) in the study of Loor et al. (2005) also suggests a relative increase in C15:0 fatty acid in milk, although there was no corresponding increase for C17:0. Earlier studies have shown that the straight-chain fatty acids (C15:0 and C17:0) can be synthesized de novo from propionate (Scaife et al., 1978; Massart-Leën et al., 1983; Rigout et al., 2003). Rigout et al. (2003) showed a relatively smaller response in C17:0 than C15:0 when infusing propionic acid into the rumen, perhaps reflecting a more limited ability to elongate C15:0 to C17:0, although desaturation of C17:0 to C17:1 might also have reduced the apparent effect (Fievez et al., 2003).

There was no evidence for increased synthesis of OBCFA in the mammary gland with increased concentrate feeding, which may reflect the fact (Moorby et al., 2006) that the high-starch diet in this study did not elicit the very high levels of propionate production of the studies by Scaife et al. (1978), or differences in species and metabolic state. It is possible that there were counterbalancing effects of F:C ratio, with both synthesis and oxidation of these OBCFA changing with increased concentrate feeding level.

CONCLUSIONS

There was no significant relationship between the duodenal flow of OBCFA and their yield in milk. The efficiency of transfer of *iso* C15:0 and *anteiso* C15:0 from duodenum to milk was similar to that for C18:3, with

a reduced proportion transferred into milk at higher flows. Yields of C15:0, C17:0, and *iso* C17:0 in milk were greater than duodenal flows, suggesting de novo synthesis in animal tissues. It is not possible to determine whether the lack of apparent diet effect on duodenum to milk recovery for C15:0 and *iso* C17:0 is masked by increased tissue synthesis of these fatty acids with increased concentrate feeding.

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