Biohydrogenation, Duodenal Flow, and Intestinal Digestibility of *Trans* Fatty Acids and Conjugated Linoleic Acids in Response to Dietary Forage:Concentrate Ratio and Linseed Oil in Dairy Cows

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ABSTRACT

Duodenal flows of hydrogenation intermediates in response to changes in dietary forage:concentrate ratio (F:C) and linseed oil were evaluated using 4 lactating Holstein cows fed a low (65:35 forage to concentrate) or high (35:65) concentrate diet without (LC, HC) added oil or with linseed oil (LCO, HCO) at 3% of DM. A 4 × 4 Latin square design was implemented for 5 wk. Lower hydrogenation of 18:2 n -6 and 18:3 n -3 was observed with HC, but it increased with LCO or HCO. Duodenal flow of total conjugated linoleic acids (CLA) increased by 1.40 (LCO) to 3.01 (HCO) g/d with linseed oil. This response was associated with greater flows of *cis*9,*trans*11- (+0.21 to +0.55 g/d), *trans*11,*cis*13- (+0.33 to +0.36), *trans*11,*trans*13- (+1.01 to +1.15 g/d), and *trans*,*trans*-CLA (+0.12 to +0.72 g/d). *Trans*10,*cis*12-CLA flow averaged 0.08 g/d and was not affected by F:C or oil. *trans*11,*cis*15-18:2 flow increased by 8.5 (LCO) to 62 (HCO) g/d in response to linseed oil. Total *trans*-18:1 flow was 37 g/d in cows fed LC and increased to 81 g/d with HC. Feeding oil increased total *trans*-18:1 to the greatest extent with HCO. Flow of *trans*10-18:1 was lower with LC than with HC (1.46 vs. 20 g/d). Linseed oil increased *trans*11-18:1 flow by 40 (LCO) to 113 g/d (HCO). Feeding LCO and HCO also increased flows of *trans*6+7+8-, *trans*13+14-, *trans*15-, and *trans*16-18:1. Apparent intestinal digestibility of *trans*-18:1 isomers was largely unaffected by concentrate level and ranged between 67 and 95%. Linseed oil increased digestibility of nearly all isomers by 3 to 16 percentage units. Digestibility of *cis*9,*trans*11-CLA was greater in cows fed HC (55%) compared with cows fed LC (32%) and was not affected by linseed oil. Data suggest that high concen-

trate diets enhanced ruminal outflow of *trans*10-18:1. We provide initial in vivo evidence that supplemental 18:3 n -3 is hydrogenated to *trans*11,*cis*15-18:2, *trans*11-18:1, *trans*13+14-18:1, *trans*15-18:1, *trans*6+7+8-18:1, and *trans*16-18:1 primarily.

(**Key words:** hydrogenation, linseed oil, digestion, *trans* fatty acid)

Abbreviation key: CLA = conjugated linoleic acid, F:C = forage:concentrate ratio, HC = high concentrate diet (35:65 F:C ratio), HCO = HC with linseed oil at 3% of DM, LC = low concentrate diet (65:35 F:C ratio), LCO = LC with linseed oil at 3% of DM.

INTRODUCTION

Feeding high concentrate diets to dairy cows often reduces milk fat production (Doreau et al., 1999). Biohydrogenation of polyunsaturated fatty acids in the rumen is reduced when high concentrate diets are fed (Doreau and Ferlay, 1994; Kalscheur et al., 1997). This response is associated with shifts in bacterial populations (Latham et al., 1972), causing a reduction in the conversion of *trans*-18:1 isomers to 18:0 (Van Nevel and Demeyer, 1996).

Amounts of biohydrogenation intermediates produced in the rumen influence their concentrations in tissues or milk (Demeyer and Doreau, 1999; Chilliard et al., 2000). In cows fed high concentrate diets without (Pipero et al., 2002) or with (Griinari et al., 1998; Pipero et al., 2000) vegetable oils rich in linoleic acid, *trans*10-18:1 concentration in milk fat was greater than *trans*11-18:1. *Cis*9,*trans*11-, *trans*7,*cis*9-, *trans*8,*cis*10-, and *trans*10,*cis*12-18:2 concentrations in milk fat also were altered by changing forage:concentrate (F:C) ratio (Pipero et al., 2000, 2002). Vaccenic acid (*trans*11-18:1) and conjugated linoleic acids (CLA) (*cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2) in meat and milk are examples of biohydrogenation intermediates that may have beneficial implications in human health. Thus, additional knowledge concerning microbial biohydrogenation of unsaturated fatty acids in the rumen may benefit the nutrition and health of humans.

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Table 1. Ingredient and chemical composition of diets.

	Diet ¹			
	LC	LCO	HC	HCO
Ingredient, % of DM				
Grass hay, long cut	65.0	65.0	35.0	35.0
Soybean meal	7.0	17.0	0.0	5.0
Linseed oil	0.0	3.0	0.0	3.0
Concentrate mixture				
Ground wheat	14.0	7.5	32.4	28.4
Rapeseed meal	5.0	2.6	11.6	10.2
Sunflower meal	4.1	2.2	9.7	8.5
Wheat bran	2.8	1.5	6.5	5.8
Wet beet molasses	0.6	0.3	1.3	1.1
Distiller's bran	0.6	0.3	1.3	1.1
Limestone	0.7	0.4	1.6	1.3
Sodium chloride	0.1	0.1	0.3	0.3
Mineral-vitamin mix ²	0.1	0.1	0.3	0.3
Composition, % of DM				
OM	91.5	91.7	92.3	92.5
CP	16.9	19.1	17.4	18.2
NDF	44.5	43.3	33.7	32.8
ADF	24.7	24.1	18.3	17.9
Starch	11.1	6.6	23.9	21.1
Total fatty acids	1.6	4.7	1.9	5.1
Fatty acid profile, g/100 g total fatty acids				
12:0	0.23	0.07	0.11	0.04
14:0	0.64	0.24	0.38	0.17
16:0	21.7	10.8	17.8	10.1
<i>cis</i> 9-16:1	0.65	0.23	0.58	0.25
18:0	2.43	3.43	2.06	3.15
<i>cis</i> 9-18:1	17.9	15.5	29.1	20.6
18:2 <i>n</i> -6	30.7	20.2	36.0	23.0
18:3 <i>n</i> -3	25.8	49.6	14.0	42.6

¹LC = Diet with low concentrate (65:35 F:C), LCO = LC with linseed oil at 3% of DM, HC = diet with high concentrate (35:65 F:C), and HCO = HC with linseed oil at 3% of DM.

²Mineral-vitamin mix provided (g/kg premix): Ca, 240; P, 60; Mg, 50; Na, 20; Zn, 4; Mn, 3; Cu, 0.8; retinyl acetate, 1.5; cholecalciferol, 0.02; and DL- α -tocopherol acetate, 0.5.

Postruminal flows of *trans*-18:1 and CLA isomers were previously reported in lactating cows fed diets with 2 different F:C but without lipid supplementation (Piperoova et al., 2002). Interactions between F:C and unsaturated oils likely alter intestinal flows of hydrogenation intermediates. The primary objective of this study was to evaluate flows of *cis* and *trans* isomers of 18:1 and 18:2 during 5 wk of feeding high concentrate diets alone or in combination with linseed oil in lactating cows.

MATERIALS AND METHODS

Animals and Diets

Four peak lactation multiparous Holstein cows (55 to 87 DIM) were used in a 4 × 4 Latin square design with factorial arrangement of treatments during 4 5-wk periods to evaluate responses to feeding diets (Table 1) with a low (F:C = 65:35) or high (F:C 35:65) concentrate without (LC, HC) added oil or with linseed oil (Huilerie Van de Putte, Mouscron, Belgium) (LCO, HCO) supplemented at 3% of DM. Concentration (% of total fatty acids) of

18:3*n*-3, 18:2*n*-6, *cis*9-18:1, 18:0, and 16:0 in linseed oil was 59, 15, 16, 4, and 6%, respectively. During a 3-wk adaptation before the study, cows were fed a diet with equal amounts of forage and concentrate plus linseed oil at 1.5% of DM. Subsequently, the assigned diets were fed for 5 wk during each experimental period. The first 5 d of each period were used as a transition between treatments. Cows were housed in a tie stall barn during the experiment. The concentrate mixture with or without linseed oil was prepared daily and, along with the forage, was offered in equal amounts at 0900, 1330, and 1700 h for ad libitum consumption. Forage and concentrate refusals were weighed once daily, and quantities offered the following day were adjusted to maintain the desired F:C. Cows were milked at 0600 and 1700 h. The experimental protocol was approved by the Institut National de la Recherche Agronomique Animal Care and Use Committee. All experimental procedures were conducted in accordance with French Guidelines concerning the use of experimental animals including animal welfare (Anonymous, 1988).

Sampling, Measurements, and Analyses

Protocols for duodenal sample collection and analyses and data on OM, fiber, DM flows, and digestibility are presented in Ueda et al. (2003). Intake of DM, milk production, composition, and fatty acid profiles in milk fat are presented in Loor et al. (2002a). Briefly, DM digestibility was measured by total collection during the last 6 d of each period, and duodenal DM flow was assessed using YbCl₃ and polyethylene glycol. Both marker solutions (400 mg YbCl₃/L, 65 g polyethylene glycol/L) were continuously infused (100 mL/h) into the rumen during the last 5 d of each period beginning at 0900 h. Duodenal digesta (solid and liquid phases) samples (250 mL) from each cow were collected on d 26, 27, and 28 of each period to represent 3-h intervals within a 24-h period. A total of 16 samples of duodenal digesta were collected during the 3-d period. Samples for fatty acid analysis were obtained after reconstitution (based on duodenal DM flow) of the appropriate amount of residue and filtrate. Methods for separation of residue and filtrate and calculation of the reconstitution factor are presented in Ueda et al. (2003).

Fatty acids in linseed oil were directly methylated by in situ transesterification with 2 mL of 0.5N NaOCH₃ at 50°C for 10 min. Fatty acids in grass hay, soybean meal, and the concentrate mixture were methylated as described by Sukhija and Palmquist (1988). Fatty acids in duodenal digesta and feces were methylated essentially as described by Sukhija and Palmquist (1988) with modifications. Chloroform was chosen as the extracting solvent, and volume used was increased from 2 to 6 mL. The concentration of methanolic-HCl was decreased from 10%, as originally described, to 6.5%, and volume used was increased from 3 to 9 mL. Thus, the ratio of extracting solvent to methylation reagent was the same as in Sukhija and Palmquist (1988). Incubation time was increased from 2 to 2.5 h, and temperature was reduced from 80 to 65°C. Tubes were continuously checked for leaks during incubation and were repeated if gross leaks could not be controlled. These modified conditions minimized isomerization of *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 (Kramer and Zhou, 2001; Park et al., 2001), while ensuring complete recovery of total fatty acids in samples (Kramer and Zhou, 2001). In all cases, fatty acid methyl esters were recovered in 1 mL of hexane. Tricosanoate (Sigma, Saint-Quentin Fallavier, France) was used as the internal standard. Samples were injected by autosampler into a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (Varian, Les Ulis, France). Methyl esters from all samples were separated on a 100-m × 0.25-mm i.d. fused-silica capillary column (CP-Sil 88; Chrompack, Middelburg, The Netherlands). A custom preparation (kindly

donated by J. H. Herbein, Virginia Tech), thoroughly described in Loor and Herbein (2003), made with pure methyl esters was used for identification of *trans*-18:1, nonconjugated 18:2, and CLA isomers primarily. Odd and branched-chain fatty acids and 21:0 were identified with a commercial mixture containing 37 fatty acids (cat#47885-U; Supelco, Bellefonte, PA). Linolenic acid isomers were identified by comparison with a commercial mixture (cat# L6032; Sigma). *Trans*10-18:1, *trans*16-18:1, *trans*8,*cis*13-18:2, *cis*9,*trans*13-18:2, and *trans*11,*cis*15-18:2 were not available commercially. The *trans*-18:1 isomers were identified by order of elution as described in Griinari et al. (1998), and the 18:2 isomers according to Ulberth and Henninger (1994).

For hay, soybean meal, linseed oil, concentrate mixture, feces, and duodenal fatty acid analysis (0.5 to 1 µL methyl esters in hexane injected at a 50:1 to 120:1 split ratio), the injector temperature was maintained at 250°C, and the detector temperature was maintained at 255°C. The initial oven temperature was held at 70°C for 1 min, increased 5°C/min to 100°C (held for 2 min), then increased at 10°C/min to 175°C (held for 40 min), and 5°C/min to a final temperature of 225°C (held for 15 min) (as described originally by Loor and Herbein [2001]). Hydrogen was the carrier gas. Injector pressure was held constant at 158.6 kPa. We found no advantage in conducting various runs at different isothermal temperatures to separate 18:1 isomers, which is often needed because of the similar equivalent chain length value for certain isomers (Kramer et al., 2001). In our experience (also Loor and Herbein, 2001; Loor et al., 2002d; Loor et al., 2003), holding injector pressure constant rather than gas flow (Griinari et al., 1998; Duckett et al., 2002), allows for better peak resolution in the 18:1, 18:2, and CLA region with the column temperature conditions described previously. Figure 1 shows separations of 18:1 and nonconjugated 18:2 isomers during a single chromatographic run. Isomers in the CLA region eluted as shown in Kramer et al. (2001). Elution order between *trans*10,*cis*12-18:2 and 21:0, which may co-elute depending on temperature program (Kramer et al., 2001), was verified by comparing chromatograms of duodenal samples vs. those of milk fat obtained from the same cows before and after a 5-d infusion of 5 g/d pure *trans*10,*cis*12-18:2 mixture into the duodenum (Loor et al., 2002b).

Statistical Analysis

Data for fatty acid intake, duodenal fatty acid flow, apparent biohydrogenation, and apparent fatty acid digestibility are reported as least square means ± SEM. Data were analyzed as a Latin square with factorial arrangement of treatments using the MIXED procedure

Figure 1. Chromatograms illustrating the separation of *cis* (c) and *trans* (t) isomers of 18:1 (high concentrate diet) and nonconjugated 18:2 (high concentrate plus linseed oil) isomers in duodenal digesta samples during a single chromatographic run.

of SAS (SAS Inst., Inc., Cary, NC). The statistical model included cow, period, forage level, oil supplementation level, forage by oil interaction, and residual error. Fixed effects included period, forage level, oil supplementation level, and forage by oil interaction. Cow was the random effect. Overall differences between treatment means were considered to be significant when $P \leq 0.05$. Interactions for level of concentrate and oil were considered significant at $P \leq 0.10$ to guard against Type II error (Berndtson, 1991). This was deemed appropriate be-

cause of the low power of the experimental design to test for interactions rather than main effects combined with the a priori expectation for interactions.

RESULTS

Diets were not markedly different in OM content, but oil-supplemented diets had numerically greater CP content (Table 1). By design, NDF and ADF concentrations were lower, and starch greater, as level of dietary forage

Table 2. Fatty acid intake by Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Fatty acid, g/d	Diet				SEM	Effect ($P =$) ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc $\times$ Oil
12:0	0.73	0.66	0.45	0.42	0.07	0.01	0.46	0.78
14:0	2.04	2.17	1.50	1.77	0.17	0.01	0.26	0.70
16:0	68.6	97.3	70.3	105.0	3.93	0.24	0.01	0.44
<i>cis</i> 9-16:1	2.07	2.09	2.30	2.55	0.12	0.01	0.24	0.32
18:0	7.68	30.8	8.12	32.7	0.59	0.07	0.01	0.23
<i>cis</i> 9-18:1	56.4	139.1	114.9	214.0	4.75	0.01	0.01	0.11
18:2 <i>n</i> -6	96.7	180.9	142.2	238.7	5.71	0.01	0.01	0.30
18:3 <i>n</i> -3	82.1	444.9	54.7	442.7	7.22	0.05	0.01	0.20
Total	316.4	897.8	394.6	1037.8	20.8	0.01	0.01	0.17

¹ P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

decreased. Total fatty acid content of low and high concentrate diets alone averaged 1.7% compared with 4.9% when linseed oil was added.

Fatty Acid Intake

Feeding high concentrate diets increased ($P < 0.05$) total fatty acid intake by 109 g/d (Table 2). Oleic and linoleic acid intakes also were greater ($P < 0.05$) in response to high concentrate diets. Linolenic acid intake was lower (-29.6 g/d; $P < 0.05$) with HC vs. LC, but increased by 375 g/d in response to linseed oil.

Duodenal Fatty Acid Flow and Biohydrogenation

Total fatty acids. Feeding high concentrate diets resulted in an additional flow of 153 g fatty acids/d (Table 3). Linseed oil increased total duodenal fatty acid flow by 473 g/d. Net flow (intake – duodenal flow) of fatty acids was 44 g/d greater with high concentrate diets and 140 g/d greater with linseed oil.

18:3 isomers. *Cis*9,*cis*12,*cis*15-18:3 was the only 18:3 isomer provided in the diet (Table 2), but it accounted for 93% of total 18:3 isomers in duodenal lipids (Table 4). Its flow was not altered by concentrate level, but feeding HCO resulted in an increase (significant interaction, $P = 0.06$) of 20.1 g/d. Feeding low concentrate diets and addition of oil led to greater ($P \leq 0.05$) flows of *cis*9,*trans*12,*cis*15-18:3 and *cis*9,*trans*12,*trans*15-18:3. Linseed oil supplementation enhanced flow of *trans*9-*trans*12,*trans*15-18:3. We could not identify the presence of *cis*9,*trans*11,*cis*15-18:3 in duodenal lipids because of lack of a standard.

Conjugated 18:2 isomers. *Cis*9,*trans*11-CLA flow averaged 0.31 g/d with LC or HC (16% of total CLA flow). *Trans*10,*cis*12-CLA was not altered by concentrate or linseed oil level and averaged 0.08 g/d (3% of total CLA flow). Flow of *trans*9,*cis*11-, *trans*8,*cis*10-, *cis*10,*trans*12-, and *cis*11,*trans*13-CLA decreased with LCO but in-

creased with HCO ($P < 0.05$). *Trans*11,*cis*13- + *cis*9,*cis*11-, *trans*,*trans*-, and total CLA isomer flow was greater with linseed oil addition. Regardless of concentrate or oil level *trans*,*trans*-CLA were the most abundant isomers, followed by *cis*9,*trans*11-CLA, and *trans*11,*trans*13-CLA. Feeding linseed oil, regardless of concentrate level, increased ($P < 0.05$) flows of *cis*9-, *trans*11-, *trans*11,*cis*13-, *trans*11,*trans*13-, and *trans*,*trans*-CLA. In terms of absolute amounts, our values are consistent with those obtained by Piperova et al. (2002) with Ag⁺-HPLC, which showed for the first time that total conjugated *trans*,*trans*-18:2 are the most predominant isomers flowing into the small intestine of dairy cows.

Nonconjugated 18:2 isomers. *Cis*9,*cis*12-18:2 flow was 19 g/d greater in response to high concentrate diets (Table 4). Linoleic acid accounted for 35 to 84% of total nonconjugated 18:2 isomer flow depending on diet. *Trans*9,*cis*12- and *cis*9,*trans*13-18:2 flow was greater ($P < 0.05$) with high concentrate diets and increased ($P < 0.05$) further with linseed oil. These 2 isomers accounted for <1% of total nonconjugated 18:2 across diets. *Trans*11,*cis*15-18:2 was the second most abundant nonconjugated 18:2 isomer in duodenal lipids, accounting for 4% of total isomers in response to feeding LC or HC. Its proportion increased to 28 or 53% of total nonconjugated 18:2 isomers in cows fed LCO or HCO when flow increased by 9 or 62 g/d compared with unsupplemented diets.

Trans-18:1 isomers. *Trans*11-18:1 flow was not affected by concentrate level but increased ($P < 0.05$) by an average of 76.6 g/d with linseed oil supplementation (Table 5). High concentrate diets resulted in a 31-g/d increase in flow of *trans*10-18:1. Flows of *trans*4-, *trans*6+7+8-, *trans*13+14-, and *trans*15-18:1 also were greater ($P < 0.05$) because of the feeding of high concentrate diets. Linseed oil addition further increased flows of *trans*4- through *trans*6+7+8- and *trans*11- through *trans*16-18:1. A major response to linseed oil was a 29-

Table 3. Duodenal fatty acid flows (not including individual 18:1, 18:2, or 18:3 isomers) in Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Fatty acid, g/d	Diet				SEM	Effect ($P =$) ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc $\times$ Oil
12:0	1.69	2.02	1.77	2.82	0.32	0.19	0.05	0.29
14:0	6.32	6.61	6.49	10.7	1.27	0.11	0.09	0.14
<i>iso</i> -14:0	1.55	1.49	1.04	1.16	0.14	0.01	0.85	0.53
<i>cis</i> 9-14:1	0.07	0.12	0.07	0.16	0.02	0.37	0.01	0.30
15:0	9.01	11.3	6.97	9.89	0.83	0.05	0.01	0.69
<i>cis</i> 10-15:1	0.18	0.12	0.13	0.02	0.03	0.03	0.01	0.46
<i>iso</i> -15:0	11.0	12.3	10.8	13.9	1.3	0.59	0.09	0.46
<i>anteiso</i> -15:0	10.9	14.1	11.4	12.5	1.19	0.61	0.07	0.36
16:0	72.2	96.9	73.5	113.6	5.8	0.14	0.01	0.19
<i>iso</i> -16:0	2.25	1.90	1.65	1.20	0.37	0.03	0.15	0.86
<i>trans</i> 9-16:1 + <i>iso</i> -17:0	2.03	2.58	1.86	2.26	0.26	0.24	0.04	0.72
<i>cis</i> 9-16:1	1.52	4.16	2.11	3.26	0.79	0.84	0.03	0.33
17:0	3.80	4.46	3.62	4.82	0.38	0.76	0.02	0.40
<i>cis</i> 10-17:1	0.88	0.69	1.44	1.05	0.15	0.01	0.07	0.52
18:0	196.5	454.7	201.7	313.7	41.2	0.12	0.01	0.07
Total odd + branched ²	34.2	39.0	29.3	37.6	3.0	0.27	0.04	0.54
Total <i>trans</i> -18:1	37.1	144.6	80.7	303.9	37.1	0.02	0.01	0.14
Total <i>cis</i> -18:1	30.9	35.2	66.1	103.2	12.2	0.01	0.09	0.17
Total nonconjugated 18:2	26.0	34.0	43.0	120.8	22.5	0.05	0.09	0.15
Total conjugated 18:2	2.21	3.57	1.70	4.71	0.2	0.45	0.01	0.05
Total 18:3	9.69	15.3	9.30	31.7	5.6	0.06	0.01	0.05
19:0	0.53	0.67	0.38	1.12	0.14	0.30	0.01	0.05
20:0	4.44	4.88	4.19	4.70	0.37	0.57	0.22	0.92
<i>cis</i> 11-20:1	0.53	0.48	1.46	2.26	0.41	0.01	0.36	0.30
21:0	0.28	0.32	0.26	0.32	0.22	0.65	0.05	0.57
Total ³	426.6	841.4	521.6	1052.6	57.1	0.02	0.01	0.34

¹ P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.²Includes fatty acids with 14- to 19-carbons.³Total = sum of 12:0 to 21:0 including 18:1, 18:2, and 18:3 isomers.

g/d increase in flow of *trans*13+14-18:1 and an 11-g/d increase in flow of *trans*15-18:1. *Trans*11-18:1 flowing into the duodenum in response to linseed oil accounted for 37% of total *trans*-18:1 with LCO and 51% of total *trans*-18:1 with HCO. The proportion of *trans*13+14-18:1 increased from 11 to 17% of total *trans*-18:1 with linseed oil addition.

***Cis*-18:1 isomers.** Oleic acid accounted for 69% of total *cis*-18:1 flow in response to low concentrate diets, and its proportion decreased to 60% of total *cis*-18:1 with high concentrate diets (Table 5). This occurred despite a 28-g/d increase ($P < 0.05$) in oleic acid flow with high concentrate diets. Increases in flows of *cis*11-, *cis*12-, and *cis*13-18:1 also were associated with feeding high concentrate diets and accounted for the reduced proportion of oleic acid in duodenal lipids. *Cis*15-18:1 flow increased more markedly with HCO vs. LCO ($P < 0.05$). Linseed oil supplementation did not affect oleic acid flow. Isomers that responded to linseed oil included *cis*12- and *cis*13-18:1.

Odd-chain, branched-chain, and medium-chain monounsaturated fatty acids. Although total flow of odd- plus branched-chain fatty acids did not differ because of concentrate level (32 g/d), there were differences

in flows among some of these microbial-derived fatty acids (Table 3). Flows of *iso*-14:0, 15:0, *cis*10-15:1, and *iso*-16:0 were greater ($P < 0.05$) in cows fed low concentrate diets; whereas, flows of *cis*10-17:1 and *cis*11-20:1 were greater ($P < 0.05$) because of the feeding of high concentrate diets. Linseed oil supplementation resulted in an average increase ($P < 0.05$) of 7 g/d in the flow of odd- plus branched-chain fatty acids. This response was related to significantly greater ($P < 0.05$) flows of 15:0, 17:0, and 19:0 but also a tendency ($P = 0.07$) for greater flow of *anteiso*-15:0. Although not a major fatty acid in duodenal lipids, *cis*10-15:1 flow was reduced ($P < 0.05$) by feeding linseed oil. Supplemental linseed oil increased ($P < 0.05$) flows of *cis*9-14:1, *trans*9-16:1 + *iso*-17:0, and *cis*9-16:1.

Apparent ruminal biohydrogenation, stearic acid flow, and flow of total biohydrogenation intermediates. Ruminal biohydrogenation was estimated as the disappearance of dietary oleic, linoleic, or linolenic acid between mouth and duodenum (Table 6). As suggested by elevated flows of biohydrogenation intermediates and similar flows of 18:0 (Table 3), feeding high concentrate diets decreased biohydrogenation of linoleic and linolenic acid (Table 6).

Table 4. Duodenal flow of 18:2 and 18:3 isomers in Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer, g/d	Diet				SEM	Effect ($P =$) ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc $\times$ Oil
18:3								
<i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	8.93	12.9	8.82	29.6	4.10	0.06	0.01	0.06
<i>cis</i> 9, <i>trans</i> 12, <i>cis</i> 15	0.22	0.25	0.018	0.12	0.01	0.02	0.01	0.25
<i>cis</i> 9, <i>trans</i> 12, <i>trans</i> 15	0.19	0.29	0.081	0.27	0.02	0.01	0.01	0.52
<i>trans</i> 9, <i>trans</i> 12, <i>trans</i> 15	0.36	1.91	0.38	1.74	0.10	0.51	0.01	0.35
18:2 conjugated								
<i>cis</i> 9, <i>trans</i> 11	0.31	0.52	0.31	0.86	0.14	0.25	0.02	0.25
<i>trans</i> 9, <i>cis</i> 11	0.28	0.14	0.14	0.19	0.02	0.04	0.02	0.01
<i>trans</i> 8, <i>cis</i> 10	0.26	0.15	0.15	0.22	0.03	0.58	0.58	0.03
<i>trans</i> 10, <i>cis</i> 12	0.08	0.07	0.07	0.10	0.01	0.45	0.56	0.14
<i>cis</i> 10, <i>trans</i> 12	0.16	0.08	0.08	0.12	0.02	0.39	0.34	0.03
<i>cis</i> 11, <i>trans</i> 13	0.14	0.11	0.06	0.12	0.01	0.07	0.71	0.03
<i>trans</i> 11, <i>cis</i> 13 + <i>cis</i> 9, <i>cis</i> 11	0.26	0.59	0.12	0.48	0.06	0.08	0.01	0.81
<i>trans</i> 11, <i>trans</i> 13	0.33	1.34	0.22	1.37	0.16	0.75	0.01	0.57
<i>trans,trans</i>	0.47	0.59	0.55	1.27	0.18	0.05	0.03	0.11
18:2 nonconjugated								
<i>cis</i> 9, <i>cis</i> 12	21.8	20.2	36.5	42.8	3.95	0.01	0.52	0.29
<i>cis</i> 9, <i>trans</i> 12	0.39	1.51	0.69	1.99	0.45	0.38	0.02	0.83
<i>trans</i> 8, <i>cis</i> 13	2.12	1.52	2.80	1.57	0.50	0.44	0.07	0.50
<i>trans</i> 9, <i>cis</i> 12	0.04	0.56	0.38	3.93	0.91	0.05	0.04	0.11
<i>cis</i> 9, <i>trans</i> 13	0.02	0.63	0.22	2.39	0.45	0.05	0.01	0.12
<i>trans</i> 9, <i>trans</i> 12	0.37	0.16	0.37	4.06	1.45	0.18	0.23	0.18
<i>trans</i> 11, <i>cis</i> 15	0.99	9.47	2.11	64.1	13.8	0.10	0.05	0.13

¹ P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.²Isomer not detected in duodenal lipids.

Linseed oil supplementation, however, led to greater biohydrogenation of oleic, linoleic, and linolenic acid in the rumen.

Stearic acid flow, the major end product of biohydrogenation, increased by 112 g/d (HCO) to 258 g/d (LCO)

in response to linseed oil ($P = 0.07$) (Table 3). Flows of *trans*-18:1, *cis*-18:1, and nonconjugated 18:2 isomers were 101, 52, and 52 g/d greater ($P \leq 0.05$) in response to high concentrate diets. Total ruminal CLA and 18:3 isomer output increased primarily with HCO ($P = 0.05$).

Table 5. Duodenal 18:1 isomer flow in Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer, g/d	Diet				SEM	Effect ($P =$) ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc $\times$ Oil
18:1 <i>trans</i>								
4	0.37	1.06	0.88	1.87	0.21	0.01	0.01	0.47
5	1.29	3.12	1.81	3.36	0.19	0.07	0.01	0.47
6 + 7 + 8	1.83	6.75	5.98	16.2	2.48	0.02	0.01	0.27
9	1.38	3.89	2.96	13.1	3.30	0.12	0.08	0.26
10	1.46	6.61	20.2	50.6	12.1	0.04	0.20	0.38
11	21.4	61.7	26.0	138.8	17.2	0.10	0.01	0.17
12	1.93	8.32	3.78	9.57	1.24	0.21	0.01	0.81
13 + 14	4.17	29.6	10.3	42.9	2.75	0.01	0.01	0.19
15	1.95	12.1	4.84	16.8	1.52	0.02	0.01	0.56
16	2.34	11.5	3.98	10.7	1.39	0.75	0.01	0.39
18:1 <i>cis</i>								
9	24.0	21.6	47.1	54.9	9.0	0.01	0.73	0.51
11	5.20	4.80	11.1	18.7	2.43	0.01	0.13	0.10
12	1.17	3.35	4.70	10.5	1.79	0.01	0.03	0.28
13	0.35	0.81	0.57	1.54	0.18	0.02	0.01	0.18
15	0.60	4.65	2.61	17.6	2.23	0.01	0.01	0.02

¹ P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

Table 6. Biohydrogenation¹ of unsaturated fatty acids in the rumen of Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Fatty acid, %	Diet				SEM	Effect ($P =$) ²		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
<i>cis</i> 9-18:1	57.9	84.5	59.6	74.3	5.5	0.30	0.01	0.15
18:2 <i>n</i> -6	77.7	88.8	74.5	82.0	1.7	0.01	0.01	0.28
18:3 <i>n</i> -3	89.9	97.1	83.9	93.2	1.7	0.01	0.01	0.49
Total	77.3	92.9	70.7	85.7	2.1	0.01	0.01	0.89

¹Biohydrogenation (%) = [(intake of fatty acid – duodenal flow of fatty acid)/intake of fatty acid] × 100.² P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

Linseed oil increased ($P < 0.05$) flows of total *trans*-18:1 and total CLA and tended ($P = 0.09$) to increase total *cis*-18:1 and total nonconjugated 18:2 isomers.

Apparent Intestinal Digestibility of Fatty Acids

Nonconjugated 18:2, conjugated 18:2, and 18:3 isomers. Apparent intestinal digestibility of *cis*9-, *cis*12,*cis*15-18:3, *cis*9,*trans*12,*cis*15-18:3, *cis*9,*trans*12-, *trans*15-18:3, *trans*9,*trans*12,*trans*15-18:3, *cis*9-, *trans*11-18:2, *cis*9,*cis*12-, *trans*8,*cis*13-, *trans*9,*cis*12-, *cis*9,*trans*13-, and *trans*11,*cis*15-18:2 was greater ($P < 0.05$) with high concentrate diets (Table 7). Linseed oil

supplementation further increased ($P < 0.05$) apparent intestinal digestibility of *cis*9,*cis*12,*cis*15-18:3, *trans*9-, *trans*12,*trans*15-18:3, *trans*11,*trans*13-18:2, *trans*11,*cis*13-18:2, *cis*9,*trans*12-18:2, *trans*9,*cis*12-18:2, *cis*9,*trans*13-18:2, and *trans*11,*cis*15-18:2. *Cis*9-, *trans*11-18:2 apparent intestinal digestibility tended ($P = 0.10$) to increase with linseed oil supplementation.

Trans-18:1 and cis-18:1 isomers. Apparent intestinal digestibility of all *trans*-18:1 isomers, except *trans*4-18:1 (74%), without linseed oil supplementation was between 82 and 95% (Table 8). Dietary concentrate level affected apparent digestibility of only *trans*4-18:1 and *trans*12-18:1, which were greater ($P < 0.05$) in response

Table 7. Apparent intestinal digestibility¹ of 18:2 and 18:3 isomers in Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer, %	Diet				SEM	Effect ($P =$) ²		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
18:3								
<i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	76.2	81.1	82.1	91.9	1.5	0.01	0.01	0.14
<i>cis</i> 9, <i>trans</i> 12, <i>cis</i> 15	70.2	76.4	100	100	3.8	0.01	0.45	0.86
<i>cis</i> 9, <i>trans</i> 12, <i>trans</i> 15	73.4	77.8	100	81.3	4.6	0.01	0.57	0.21
<i>trans</i> 9, <i>trans</i> 12, <i>trans</i> 15	71.2	85.0	81.2	93.5	2.3	0.01	0.02	0.26
18:2 conjugated								
<i>cis</i> 9, <i>trans</i> 11	32.3	40.5	55.0	69.3	6.7	0.01	0.10	0.62
<i>trans</i> 9, <i>cis</i> 11	80.5	73.6	77.3	80.0	8.6	0.82	0.76	0.51
<i>trans</i> 8, <i>cis</i> 10	80.8	73.3	74.1	79.5	5.2	0.96	0.85	0.24
<i>trans</i> 10, <i>cis</i> 12	100 ³	100	100	100	...	...	...	...
<i>cis</i> 10, <i>trans</i> 12	100	100	100	100	...	...	...	...
<i>cis</i> 11, <i>trans</i> 13	100	100	100	100	...	...	...	...
<i>trans</i> 11, <i>cis</i> 13 + <i>cis</i> 9, <i>cis</i> 11	73.4	84.3	69.1	76.3	3.4	0.11	0.03	0.61
<i>trans</i> 11, <i>trans</i> 13	71.2	91.3	73.2	91.0	2.4	0.69	0.01	0.59
<i>trans</i> , <i>trans</i>	66.7	71.3	69.8	81.5	4.0	0.12	0.07	0.38
18:2 nonconjugated								
<i>cis</i> 9, <i>cis</i> 12	85.4	87.0	90.9	92.2	1.3	0.01	0.26	0.89
<i>cis</i> 9, <i>trans</i> 12	72.6	86.0	78.4	85.9	2.9	0.35	0.01	0.33
<i>trans</i> 8, <i>cis</i> 13	59.4	60.5	72.9	68.5	4.2	0.02	0.67	0.47
<i>trans</i> 9, <i>cis</i> 12	55.1	88.8	85.5	94.6	1.7	0.01	0.01	0.01
<i>cis</i> 9, <i>trans</i> 13	...	85.5	64.6	91.6	1.6	0.01	0.01	0.01
<i>trans</i> 9, <i>trans</i> 12	91.3	66.9	88.8	89.5	5.8	0.10	0.06	0.06
<i>trans</i> 11, <i>cis</i> 15	69.2	92.2	77.4	95.5	1.8	0.01	0.01	0.21

¹[(g/d of duodenal fatty acid – g/d of fecal fatty acid)/g/d of duodenal fatty acid] × 100.² P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.³Isomer not detected in fecal lipids.⁴Isomer not detected in duodenal or fecal lipids.

Table 8. Apparent intestinal digestibility¹ of 18:1 isomers in Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer, %	Diet				SEM	Effect ($P =$) ²		
	LC	LCO	HC	HCO		Conc	Oil	Conc $\times$ Oil
18:1 <i>trans</i>								
4	66.6	83.4	82.2	89.4	2.2	0.01	0.01	0.06
5	95.2	98.0	94.7	97.5	0.5	0.32	0.01	0.95
6 + 7 + 8	91.2	94.0	90.8	95.5	1.1	0.68	0.01	0.46
9	90.0	93.7	90.1	95.3	1.1	0.44	0.01	0.52
10	90.5	94.2	91.5	95.2	1.4	0.50	0.03	0.96
11	87.9	92.9	85.0	93.3	1.3	0.36	0.01	0.22
12	82.0	92.4	86.7	93.8	1.2	0.03	0.01	0.20
13 + 14	91.5	94.0	87.6	94.1	1.4	0.21	0.01	0.16
15	92.7	93.3	89.1	93.0	1.8	0.29	0.23	0.37
16	91.1	90.7	86.2	90.6	2.3	0.31	0.41	0.34
total	87.9	92.7	88.4	93.8	2.0	0.42	0.04	0.31
18:1 <i>cis</i>								
9	80.3	78.8	85.5	87.4	2.0	0.01	0.93	0.31
11	78.4	77.1	80.6	87.9	1.9	0.01	0.09	0.02
12	83.1	90.0	91.9	95.8	1.2	0.01	0.01	0.23
13	86.3	90.2	88.4	93.1	1.1	0.04	0.01	0.68
15	93.0	95.3	92.8	95.5	1.2	0.99	0.06	0.85
total	84.2	86.3	87.8	91.9	1.8	0.02	0.09	0.21

¹[(g/d of duodenal fatty acid – g/d of fecal fatty acid)/g/d of duodenal fatty acid] × 100.

²*P* value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

to feeding high concentrate diets. Linseed oil supplementation resulted in greater ($P < 0.01$) apparent intestinal digestibility of all *trans*-18:1, except *trans*-16:18:1, and ranged from 89 to 98%. Apparent intestinal digestibility of *cis*-18:1 isomers ranged from 77 to 96%. Except for *cis*-15:18:1, apparent intestinal digestibility of other *cis*-18:1 isomers responded to dietary concentrate level. Apparent intestinal digestibility of *cis*-9-, *cis*-11-, *cis*-12-, and *cis*-13:18:1 was greater ($P < 0.05$) with high concentrate diets. Feeding linseed oil enhanced ($P < 0.05$) apparent intestinal digestibility of *cis*-12- and *cis*-13:18:1 and tended ($P = 0.06$) to enhance *cis*-15:18:1.

Odd- and branched-chain fatty acids, medium-chain fatty acids, stearic acid, and 20:0. Apparent intestinal digestibilities of *iso*-14:0, 15:0, 16:0, 17:0, 18:0, 19:0, and 20:0 were greater with HCO vs. LCO ($P \leq 0.10$; Table 9). Supplemental linseed oil increased ($P < 0.05$) apparent intestinal digestibilities of 12:0, *iso*-15:0, and *anteiso*-15:0. Apparent intestinal digestibility of 14:0 tended ($P = 0.08$) to be greater with linseed oil supplementation.

DISCUSSION

Duodenal Flow of Odd-Chain and Branched-Chain Fatty Acids

Decreased flow of some odd (15:0, *cis*-10-15:1) and branched-chain (*iso*-14:0, *iso*-16:0) fatty acids with the high concentrate diets might have been associated with the prevalence of microbial species that thrive in the

rumen when diets of high forage content are fed (Tajima et al., 2001). From a review of the literature, it was shown that dietary fiber content is positively related to percentage of odd-chain and branched-chain fatty acids in ruminal bacteria (Sauvant and Bas, 2001). In the present study, molar proportions of acetate, *iso*-butyrate, and *iso*-valerate were lower in ruminal fluid from cows fed high concentrate diets (Ueda et al., 2003). Lower ruminal VFA availability might have been associated with decreased microbial synthesis of fatty acids. Results from the present study confirmed previous evidence, indicating that feeding unsaturated lipids or oils to ruminants very often reduces the percentage of odd-chain and branched fatty acids in duodenal lipids (Pantoja et al., 1996; Sauvant and Bas, 2001). This effect could be related to a direct inhibition of unsaturated fatty acids on microbial fatty acid synthesis, which may result in their reduced flows, or a dilution effect, by greater amounts of other fatty acids.

Biohydrogenation of *Cis*-9-18:1, 18:2*n*-6, and 18:3*n*-3

Lower apparent biohydrogenation of 18:2*n*-6 in response to feeding high concentrate diets was consistent with its greater flow to the duodenum, as observed by Kalscheur et al. (1997). Despite lower 18:3*n*-3 biohydrogenation when high concentrate diets were fed, its flow to the duodenum was not enhanced, and this response was similar to that observed by Kalscheur et al. (1997).

Table 9. Apparent intestinal digestibility¹ of selected fatty acids (not including individual 18:1, 18:2, or 18:3 isomers) in Holstein cows fed a high or low concentrate diet without supplemental oil (HC, LC) or supplemented at 3% of DM with linseed oil (HCO, LCO).

Fatty acid, ³ %	Diet				SEM	Effect (<i>P</i> =) ²		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
12:0	59.4	71.7	62.4	80.7	3.3	0.11	0.01	0.40
14:0	67.7	70.1	63.1	73.0	3.2	0.79	0.08	0.26
<i>iso</i> -14:0	63.2	65.6	49.0	63.4	3.1	0.02	0.02	0.07
15:0	67.6	70.9	58.9	71.6	2.5	0.15	0.01	0.10
<i>iso</i> -15:0	78.4	81.4	74.6	80.3	1.4	0.08	0.01	0.31
<i>anteiso</i> -15:0	78.7	80.6	77.7	82.9	1.6	0.64	0.04	0.29
16:0	77.7	81.5	73.8	84.2	1.3	0.68	0.01	0.04
17:0	54.6	50.7	13.4	40.5	4.3	0.01	0.01	0.01
18:0	90.9	86.5	75.6	85.3	3.2	0.03	0.41	0.04
19:0	87.0	82.3	74.7	86.9	2.6	0.18	0.19	0.01
20:0	63.1	65.6	59.5	71.4	2.6	0.68	0.02	0.10
<i>cis</i> 11-20:1	73.3	73.8	91.7	94.2	1.9	0.01	0.33	0.52
21:0	49.7	55.9	50.6	56.7	4.7	0.85	0.21	0.99
Total ⁴	77.4	81.6	78.9	86.1	3.6	0.35	0.04	0.15

¹[(g/d of duodenal fatty acid – g/d of fecal fatty acid)/g/d of duodenal fatty acid] × 100.

²*P* value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

³Fatty acids with negative digestibility are not included.

⁴Total = fatty acids with 12 to 21 carbons including 18:1, 18:2, and 18:3 isomers.

Flows of oleic and linoleic acid were only moderately increased and not statistically significant despite numerically lower biohydrogenation when linseed oil was fed with high concentrate diets. Although 18:3*n*-3 biohydrogenation increased with linseed oil, the extent of the increase in biohydrogenation was lower (93% vs. 97%) with the high concentrate diet, which might have partly accounted for the greater flow of 18:3*n*-3 with HCO (+236%) compared with LCO (+44%). Feeding a 75% concentrate diet to lactating cows reduced biohydrogenation of 18:2*n*-6 and 18:3*n*-3 but did not affect *cis*9-18:1 hydrogenation (Kalscheur et al., 1997). In contrast, in ewes fed a constant amount of fatty acids from soybean oil in combination with graded increments (18.4 to 72.9%) of dietary forage, biohydrogenation of oleic, linoleic, and linolenic acid increased in proportion with dietary forage (Kucuk et al., 2001).

Results from the present study confirmed those of Kalscheur et al. (1997), indicating that a low F:C alone can reduce rates of biohydrogenation and alter profiles of 18:1, 18:2, and 18:3 intermediates flowing to the small intestine. Data from *in vivo* (Kalscheur et al., 1997) and *in vitro* (Van Nevel and Demeyer, 1996) studies suggested that low ruminal pH reduces rates of ruminal biohydrogenation. In the present study, however, we did not observe an effect of concentrate or linseed oil on ruminal pH, which averaged 6.38 ± 0.12 (Ueda et al., 2003). We propose that changes in the extent of biohydrogenation when high concentrate diets are fed may be independent of pH. For example, pH averaged 5.83 in the study of Kalscheur et al. (1997), but dietary starch was 37% of DM compared with 23% of DM in the present

study (Table 1). Ruminal biohydrogenation may be sensitive to subtle changes in microbial populations induced by dietary starch content (Latham et al., 1972; Tajima et al., 2001) even if ruminal pH is not reduced.

Duodenal Flow of CLA Isomers

Total CLA flow increased by 73% in dairy cows when dietary concentrate increased from 40 to 75% of DM (Piperova et al., 2002). Concomitantly, *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 flow increased by 60 and 200%. The proportion of *trans*10,*cis*12-18:2 increased from 8 to 14% of total CLA in response to the 75% concentrate diet (Piperova et al., 2002). The proportion of *cis*9,*trans*11-18:2 did not change, however, and averaged 30% of total CLA. A number of *trans,trans*-18:2 isomers also increased their proportions in duodenal lipids from cows fed the 75% concentrate diet (Piperova et al., 2002). With the exception of *trans*10,*cis*12-18:2 flow, results from the present study with the high concentrate diet are similar to those observed by Piperova et al. (2002) despite differences in dietary concentrate level (75% vs. 65%). Thus, a likely factor contributing to the discrepancy in *trans*10,*cis*12-18:2 flow between both studies could be the absolute amount of dietary starch which was higher (40% vs. 23%) in the study of Piperova et al. (2002). Starch source in the concentrate (corn grain vs. wheat) and/or forage(s) (corn plus alfalfa silage vs. grass hay) also may be important in terms of manipulating ruminal environment for *trans*10,*cis*12-18:2 production. Forages used by Piperova et al. (2002) clearly provided an addi-

tional amount of starch compared with grass hay in the present study.

In ewes, duodenal flow of *cis*9,*trans*11-18:2 increased, but *trans*10,*cis*12-18:2 decreased in response to graded increments of dietary forage at a constant level (7.4% of diet DM) of supplemental fatty acids from soybean oil (Kucuk et al., 2001). *Cis*9,*trans*11-18:2 accounted for 35 to 66% of total CLA and *trans*10,*cis*12-18:2 for 26 to 3% as dietary forage level increased from 18.4 to 72.9% of DM (Kucuk et al., 2001). In steers fed a typical finishing diet (78% corn grain), *cis*9,*trans*11-18:2 averaged 29%, whereas *trans*10,*cis*12-18:2 and *trans,trans*-18:2 averaged 6 or 41% of total CLA in duodenal lipids (Duckett et al., 2002). Although addition of 2.4% corn oil to the diet resulted in greater flows of the 3 CLA isomers, the proportion of *trans*10,*cis*12-18:2 was 26% compared with 21 or 37% for *cis*9,*trans*11-18:2 or *trans,trans*-18:2 (Duckett et al., 2002). Overall, data suggest that CLA profile in ruminal outflow is affected by F:C, type of unsaturated oil, and their interaction.

To our knowledge, no in vivo experiment has evaluated flows of *trans*10,*cis*12-18:2 in response to graded increases of dietary grain or concentrate. Recently, it was demonstrated that replacing incremental portions of orchardgrass or red clover with corn grain in dual-flow continuous culture fermenters resulted in linear increases in the output of *trans*10,*cis*12-18:2 (but also *cis*9,*trans*11-18:2 and *trans,trans*-18:2) into effluent (Loor et al., 2003). This result indicates that incremental grain or concentrate have the potential to enhance ruminal production of *trans*10,*cis*12-18:2. However, data with ruminants fed high concentrate diets alone show that production of *trans*10,*cis*12-18:2 may be highly variable. What seems evident from recent experiments (Piperova et al., 2000; Duckett et al., 2002) is that a supply of high linoleic acid oil along with low dietary F:C has the greatest potential to enhance flow of *trans*10,*cis*12-18:2. In contrast, our results indicate that a supply of high linolenic acid oil should lead to enhanced ruminal production of *trans*11,*cis*13-, *trans*11,*trans*13-, and *trans,trans*-18:2.

An important effect associated with feeding the high concentrate diets that has direct repercussions on ruminal biohydrogenation is the potential changes in populations of microorganisms. The ratio of cellulolytic (primarily *Butyrivibrio fibrisolvens*) to propionogenic, lactogenic, and amylolytic bacteria in the rumen of lactating cows was severely reduced by feeding a high concentrate:low forage diet (80:20) (Latham et al., 1972). Counts of *Anaerovibrio lipolytica* also decreased markedly because of the feeding of a high concentrate diet (Tajima et al., 2001). In vitro, strains of *Megasphaera elsdenii* isolated from a dry cow fed a 90% corn grain diet produced more *trans*10,*cis*12-18:2 from pure linoleic acid than controls

(Kim et al., 2002). In the absence of "normal" rates of lipolysis, which may reduce biohydrogenation and increase the level of free linoleic acid in the rumen, there would be a greater opportunity for microorganisms such as *Megasphaera elsdenii* to isomerize linoleic acid to *trans*10,*cis*12-18:2. Such an effect could explain the observed increases in the concentration of this isomer in milk fat from cows fed high concentrate diets (Piperova et al., 2002). Ruminal environment in our study, however, may not have changed sufficiently to enhance growth of bacteria capable of isomerizing linoleic acid to *trans*10,*cis*12-18:2. Availability of 18:2*n*-6 (180 to 240 g/d, with 50% derived from grass hay) also may not have been high enough to increase *trans*10,*cis*12-18:2 synthesis in the rumen. Alternatively, rapid hydrogenation of *trans*10,*cis*12-18:2 to *trans*10-18:1 (Loor and Herbein, 2001) could have reduced the amount of the CLA and increased *trans*10-18:1 flowing into the duodenum.

Duodenal Flows of Nonconjugated 18:2 Isomers

We present the first estimates of *trans*11,*cis*15-18:2 flow to the small intestine in ruminants. *Trans*11,*cis*15 to 18:2 was the primary 18:2 produced during hydrogenation of 18:3*n*-3 in vitro (Kemp et al., 1975), and this was confirmed in the present study. Other *cis/trans*-18:2 and *trans/cis*-18:2 isomers identified in duodenal lipids were most likely produced as a result of isomerization of dietary 18:2*n*-6 (Kemp et al., 1975). Data (Table 6) also suggest that the numerical reduction in 18:2*n*-6 (−7 percentage units) and 18:3*n*-3 (−4 percentage units) biohydrogenation when linseed oil was fed in combination with the high concentrate diet contributed to the accumulation of *trans*9,*cis*12-18:2, *cis*9,*trans*13-18:2, *trans*9,*trans*12-18:2, and *trans*11,*cis*15-18:2 intermediates in the rumen.

Duodenal Flow of *Trans*-18:1, *Cis*-18:1, and 18:0

Responses in *trans*11-18:1 and *trans*10-18:1 flow with the high concentrate diets are similar to those observed in cows fed a 75% concentrate diet (Piperova et al., 2002). As indicated for CLA isomers, starch availability and its effects on buffering capacity and(or) alterations in the microbial ecosystem in the rumen must be linked with a shift in the production of isomers with a *trans*11- to a *trans*10- double bond. The *trans*10-18:1 isomer could arise via hydrogenation of *trans*10,*cis*12-18:2, as shown initially by Loor and Herbein (2001), or via isomerization of *cis*9-18:1 (Mosley et al., 2002). An interesting response to linseed oil regardless of concentrate level was the marked increase in flow of *trans*13+14-, *trans*15-, and *cis*15-18:1 to the duodenum. We hypothesize that they might have arisen during biohydrogenation of 18:3*n*-3.

*Trans*13-18:1 might have been an end product of *trans*11,*trans*13-18:2 (which increased markedly with linseed oil supplementation) hydrogenation. *Trans*15-18:1 might have accumulated during sequential reductions of *trans*9,*trans*12,*trans*15-18:3, whereas *cis*15-18:1 might have accumulated via hydrogenation of *trans*11,*cis*15-18:2.

Similar to previous results from Kalscheur et al. (1997), feeding high concentrate diets resulted in greater duodenal flow of *cis*9-18:1. In both studies, the response might have been partly associated with greater intake of oleic acid and a lack of change in its apparent biohydrogenation. Under those circumstances, greater amounts of oleic acid in the rumen might have resulted in a portion being isomerized to *trans*4-, *trans*5-, and *trans*6+7+8-18:1 as shown in vitro (Mosley et al., 2002). Such a response might account for the greater flow of these *trans*-18:1 isomers observed in the present study with the high concentrate diets. Several *cis*-18:1 isomers may be formed during isomerization and hydrogenation of 18:2*n*-6 or 18:3*n*-3 (Kemp et al., 1975). Results from the present study confirm the accumulation of various *cis*-18:1 isomers, particularly *cis*15-18:1, during hydrogenation of polyunsaturated fatty acids in vivo.

Despite similar 18:0 flow regardless of concentrate level, the large increase in ruminal outputs of total *trans*-18:1, *cis*-18:1, and nonconjugated 18:2 isomers (Table 3) with high concentrate diets provides evidence of altered ruminal biohydrogenation. When linseed oil was fed, the extent of the response in 18:0 flow was clearly associated with dietary concentrate level ($P = 0.07$ for concentrate by oil interaction). Thus, flow of 18:0 was +131% greater with LCO compared with +56% with HCO. This response seemed to be related with lower hydrogenation of 18:2*n*-6 and 18:3*n*-3 in the oil fraction of the high concentrate diet.

Apparent Intestinal Digestibility of Saturated Fatty Acids

Similar to our results, apparent intestinal digestibility of 18:0 in dairy cows increased linearly with degree of unsaturation of the lipid supplement, regardless of dietary concentrate level (Pantoja et al., 1996). Palmitic acid digestibility was shown to increase with its greater intake (Weisbjerg et al., 1992) as in the current study. Hindgut synthesis of odd- plus branched-chain fatty acids by microbes or hydrogenation of unsaturated fatty acids would lead to lower apparent digestibility of microbial-derived fatty acids and 18:0.

From an extensive review of the literature, it was concluded that, on average, the digestibility of saturated fatty acids increases moderately with chain length and that unsaturated fatty acids are more digestible than

saturated fatty acids (Doreau and Ferlay, 1994; Doreau and Chilliard, 1997). Weisbjerg et al. (1992), however, showed that saturated fatty acid digestibilities in cows fed 500 g/d of a palmitic acid-rich fat source was greater compared with feeding the same amount of tallow. Digestibilities decreased as the amount of fat fed increased to 1000 g/d. Rates of micelle formation and(or) bile production may vary in response to the degree of unsaturation and(or) chain length. Alterations in digestive and(or) absorptive capacities of the small intestine also may affect fatty acid digestion.

Apparent Intestinal Digestibility of 18:1 Isomers

Biohydrogenation in the hindgut might have resulted in a small underestimation of saturated fatty acid digestibility and an overestimation of unsaturated fatty acid digestibility, because digestibility in the small and large intestine are relatively close (Doreau and Ferlay, 1994). Average values for digestibilities of total *trans*-18:1 and total *cis*-18:1 regardless of concentrate level in the present study were 88 and 86%, respectively. Digestibilities of total *trans*-18:1 and total *cis*-18:1 isomers in response to feeding linseed oil averaged 93 and 89%, respectively. Overall, our average digestibility values for total 18:1 are greater than the average digestibility (85%) reported from a compilation of literature data by Doreau and Ferlay (1994), but similar to those found more recently with high concentrate diets and(or) different lipid supplements with varying degrees of unsaturation (Kalscheur et al., 1997; Loor et al., 2002c; Scollan et al., 2001). Responses to oilseed supplementation may not be comparable with those for free oils because the seed coat may present an additional obstacle for normal digestion and absorption of fatty acids.

Apparent Intestinal Digestibility of 18:2 and 18:3 Isomers

Few data exist regarding digestibility of individual 18:2 or 18:3 isomers. In ewes, 18:2*n*-6 digestibility decreased linearly in response to gradual increases in dietary forage level (Kucuk et al., 2001). In contrast, 18:3*n*-3 digestibility increased. In the present study, greater digestibilities of 18:2*n*-6 and 18:3*n*-3 with the high concentrate diet is in agreement with previous results. This response also applies to *trans*8,*cis*13-18:2, *trans*9,*cis*12-18:2, *trans*11,*cis*15-18:2, *cis*9,*trans*11-18:2, and all 18:3 isomers. It is important to note that, similar to results found with sheep (Doreau et al., 2003), overall apparent intestinal digestibility of *cis*9,*trans*11-18:2 was very low. With forages, in particular, these values may not be accurate because this CLA isomer (and most other ones) was detected in very low amounts. Net synthesis of this

CLA in the hindgut from 18:2*n*-6 also would lead to lower apparent digestibility of this isomer, but this has not been demonstrated experimentally. At least for *trans*9,*cis*12-18:2 and *trans*11,*cis*15-18:2, higher apparent intestinal digestibility seemed to be associated with greater duodenal flow when the high concentrate diet was fed.

In summary, a low dietary F:C should be sufficient to reduce unsaturated fatty acid biohydrogenation and increase *trans*-18:1 flow into the small intestine. Linseed oil supplementation to high concentrate diets may result in further increases in the duodenal flow of *trans*-18:1 and certain CLA. Independently of changes in pH, high levels of grain or concentrate in the rumen could induce a shift in the production of *trans*11-18:1, the major *trans*-18:1 intermediate, to *trans*10-18:1. Limitations in 18:2*n*-6 substrate and dietary starch may preclude enhanced ruminal outflow of *trans*10,*cis*12-CLA. Input of linolenic acid when hydrogenation is incomplete may result in enhanced ruminal outflow of *trans*11,*cis*15-18:2, *trans*11-18:1, *trans*13+14-18:1, and *trans*15-18:1. *Cis*9-, *trans*11-CLA flow into the small intestine will be enhanced marginally with linseed oil because of its low linoleic acid content. However, linseed oil may increase endogenous synthesis of *cis*9,*trans*11-CLA in tissues by enhancing postabsorptive availability of *trans*11-18:1 (Lor et al., 2002a).

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