Effects of Substrate, Passage Rate, and pH in Continuous Culture on Flows of Conjugated Linoleic Acid and *Trans* C_{18:1}*

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ABSTRACT

A dual-flow continuous culture system consisting of 4 fermenters was used in a 4 × 4 Latin square design. The objective of the research was to evaluate the effects of solid dilution rate (SDR), pH, and concentration of linoleic acid (LA) in the feed mixture on the production of conjugated linoleic acid (CLA) and *trans*-C_{18:1}. The 4 treatments were 1) control = pH 6.5, 1% LA, 4%/h SDR; 2) high solid dilution rate (HSDR) = pH 6.5, 1% LA, 8%/h SDR; 3) high linoleic acid (HLA) = pH 6.5, 3% LA, 4%/h SDR; and 4) low pH (LPH) = pH 5.8, 1% LA, 4%/h SDR. Inoculum was collected 6 h after feeding from a cow fed 40% alfalfa hay and 60% grain. Liquid dilution rate was held at 0.12/h. All treatments except HLA contained 2% tallow. The LA was dissolved in buffer and continuously infused into the fermenters. The CLA flows were 16.5, 20.4, 23.2, and 25.2 mg/d for control, HSDR, HLA, and LPH, respectively. Compared with control, LPH increased flows of CLA, *cis*-C_{18:1}, and C_{18:2}, and decreased flow of C_{18:0}. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibilities were not affected by pH. The HSDR tended to increase CLA flow compared to control, possibly because a shorter solid retention time led to incomplete biohydrogenation (BH). The NDF and ADF digestibilities and bacterial numbers were reduced by HSDR. With more LA available as a substrate for CLA, HLA resulted in a higher flow of CLA than control. The HLA resulted in the highest acid detergent fiber and fatty acid digestibilities, bacterial numbers, and BH. Increasing solids passage rate, reducing pH, and increasing dietary LA appears to increase in vitro CLA production.

(**Key words:** conjugated linoleic acid, continuous culture, biohydrogenation)

Abbreviation key: BH = biohydrogenation, CLA = conjugated linoleic acid, FA = fatty acid, HLA = high linoleic acid, HSDR = high solid dilution rate, LA = linoleic acid, LPH = low pH, SDR = solid dilution rate, VA = vaccenic acid.

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. In the past 20 yr, data from animal studies and cell cultures have revealed that CLA possesses potentially beneficial effects to health by inhibiting cancer cell growth (Ip et al., 1994; Parodi, 1994, 1997; McGuire et al., 1997), preventing coronary heart disease (Lee et al., 1994), enhancing immune function (Miller et al., 1994), and diminishing fat deposition (Park et al., 1997). The CLA can be formed both ruminally by microbial activity during biohydrogenation (BH) of linoleic acid (LA) (Kepler et al., 1966) and endogenously from *trans*-vaccenic acid (VA), which also is an intermediate product of ruminal BH, by the activity of Δ -9 desaturase in tissue (Griinari et al., 2000; Corl et al., 2001). During BH, LA is first isomerized to CLA (*cis*-9, *trans*-11 C_{18:2}), the CLA is then quickly hydrogenated into VA (*trans*-11 C_{18:1}), and the VA can be further hydrogenated into stearic acid. The first 2 steps take a relatively short time to complete, but the last step is rate-limiting and usually takes a longer time to complete (Harfoot and Hazelwood, 1997). Under certain conditions, as with high concentration of LA, biohydrogenation of VA seems to be inhibited (Noble et al., 1974; Kim et al., 2000).

The hypotheses for this study were: 1) a higher solids dilution rate (SDR), resulting in a shorter retention time, will result in incomplete BH and thus will increase CLA and *trans* fatty acid (FA) flows from the fermenters; 2) a higher concentration of LA in the feed

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mixture will accordingly result in increased flows of CLA and *trans* FA; and 3) a decreased pH, which is believed to cause incomplete BH, will result in more production of CLA and *trans*-C_{18:1}. Factors like SDR and pH are difficult to control in vivo; therefore, a continuous culture system was used to evaluate the effects of SDR, pH, and concentration of LA in the feed mixture on the production of CLA and *trans*-C_{18:1}.

MATERIALS AND METHODS

Continuous Culture System, Experimental Design, and Inoculation

A dual-flow continuous culture system (Stern and Hoover, 1990) consisting of 4 glass fermenters averaging 1781 mL in volume was used in a 4 × 4 Latin square design. Compared with control, each of the other 3 treatments varied in one of the 3 factors: SDR, concentration of LA (% of DM), and pH: 1) control = pH 6.5, 1% LA, and 4%/h SDR; 2) high SDR (**HS**DR) = pH 6.5, 1% LA, and 8%/h SDR; 3) high LA (**HL**A) = pH 6.5, 3% LA, and 4%/h SDR; and 4) low pH (**LP**H) = pH 5.8, 1% LA, and 4%/h SDR.

Each period consisted of 10 d, with the first 7 d for equilibration and the last 3 d for sample collection. Inoculum was collected from a ruminally cannulated Holstein cow 6 h after feeding 40% alfalfa hay and 60% grain. Ruminal contents were strained through 2 layers of cheese cloth. Once about 1600 mL of strained rumen fluid was obtained, it was evenly distributed into the 4 fermenters. After 3 such inoculations, the strained ruminal contents were washed with warm buffer solution containing urea (Weller and Pilgrim, 1974) and squeezed again. The fluid obtained was used to fill the fermenters until overflow occurred.

Feed and Fermenter Calibration

The feed mixtures consisted of 38% alfalfa hay and 62% concentrates (Table 1) and were ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 2-mm screen. All treatments except HLA contained 2.18% tallow. The feed mixtures were prepared at 0800 and 2000 h but continuously delivered into the fermenters over most of the day with an automatic feeding system. The addition of feed mixture DM to the fermenters was held at 120 g/d. Based on this DM addition, 3 and 1% of LA (95%, Nu-Check-Prep, Inc., Elysian, MN) in the free FA form was dissolved in buffer with an equivalent normality of KOH and continuously infused into the fermenters for HLA and the other 3 treatments, respectively.

The temperature of the fermenters was held at 39 ± 0.1°C. Each fermenter was continuously flushed with

Table 1. Ingredient and chemical composition of feed mixtures.

Item	Mixture 1 ¹	Mixture 2 ²
	(% of DM)	
Ingredient composition		
Alfalfa hay, ground	38.0	38.0
Dry shelled corn, ground	27.5	27.7
Soybean hulls	25.5	25.5
Soybean meal, 44% CP	1.50	1.50
Blood meal	1.47	1.47
Tallow	2.18	—
Linoleic acid ³	1.00	3.00
Feed grade urea	0.52	0.52
Dicalcium phosphate	0.75	0.75
Magnesium oxide	0.16	0.16
Trace mineral salt	0.42	0.42
Potassium sulfate	0.10	0.10
Sodium bicarbonate	0.79	0.79
Vitamin supplements ⁴	0.11	0.11
Chemical composition		
NDF	34.8	34.5
ADF	22.3	22.0
NFC ⁵	35.6	35.6
CP	17.8	17.7
NE _L , ⁶ Mcal/kg	1.74	1.74
Fatty acids, mg/g of DM		
Total	42.3	45.3
C _{16:0}	7.78	3.44
C _{18:0}	3.61	0.81
<i>Trans</i> -C _{18:1}	0.48	ND ⁷
<i>Cis</i> -C _{18:1}	7.32	2.72
C _{18:2}	15.0	34.6
C _{18:3}	2.30	2.42
CLA ⁸	0.36	ND

¹Mixture 1 was provided to fermenters for treatments designated as control, high solid dilution rate, and low pH.

²Mixture 2 was provided to the fermenter for the treatment designated as high linoleic acid.

³Continuously infused with buffer solution.

⁴Provided as 0.01% vitamin A (30,000 IU/g), 0.04% vitamin D (500,000 IU/g), and 0.06% vitamin E (44 IU/g).

⁵NFC = Nonfiber carbohydrates; 100 - %NDF - %CP - (%FA/0.9) - %ash.

⁶Calculated according to NRC (1989).

⁷ND = Not detected.

⁸CLA = Conjugated linoleic acid.

nitrogen gas, and the contents were continuously mixed. The pH was controlled within a range of ±0.05 units by an automatic pH monitoring system, which was calibrated with a portable pH meter on a daily basis. Before each time the feed mixture was added, solids and liquid dilution rates were measured, and the filtrate pump and buffer input rates were adjusted accordingly.

Collection and Storage of Samples

On each morning of the last 3 d of each period, contents from filtrate and overflow containers were mixed for each fermenter, and 1000 mL of the effluent mixture was placed into a plastic jug. Samples from the 3 consec-

utive sampling days were composited and frozen. After freeze-drying, these samples were ground and analyzed for DM, NDF, ADF, protein, and FA. At 1500 h of d 9, 20 mL of contents from each fermenter was collected as inoculum for the most probable number procedure (Dehority et al., 1989) to estimate the numbers of total and cellulolytic bacteria. On d 10 and after the morning sampling of effluent, the contents that remained in each fermenter were collected and filtered through 8 layers of cheesecloth; 50 mL of the fluid was collected, and 3 mL of 6 N HCl was added to stop fermentation. The 53 mL of fluid was frozen for later analysis of VFA and $\text{NH}_3\text{-N}$. The rest of the fluid was then centrifuged to harvest bacteria, samples of which were kept frozen until later analysis of DM, OM, purines, N, and FA.

Laboratory Analyses

Representative feed, effluent, and harvested bacteria samples taken during the collection period were lyophilized and ground through a 2-mm screen in a Wiley mill. Feed, effluent, and bacteria samples were dried at 105°C for determination of DM and ashed in a muffle furnace at 550°C for determination of OM. Purine concentrations of effluent and bacteria samples were used to determine effluent microbial flow (Ushida et al., 1985; Zinn and Owens, 1986). Nitrogen content of the samples was determined according to Bremner and Mulvaney (1982) using a Tecator Digestion System 20, 1015 Digestor and a Tecator Kjeltac System, 1026 Distilling Unit (Tecator AB, Hoganas, Sweden). Analysis of NDF and ADF components was according to Goering and Van Soest (1970). To minimize the interference by fat with NDF analysis (Van Soest et al., 1991), all feed and effluent samples were filtered with 100 mL of boiling ethanol prior to treatment in 30 mL of 8 M urea and 0.2 mL of α -amylase (Sigma A-5426; Sigma Chemical Co., St. Louis, MO).

The stored fluid samples were thawed, mixed, and centrifuged at $15,000 \times g$, and the supernatant was filtered through Whatman #1 paper. The filtered supernatant was saved for later analyses of VFA and $\text{NH}_3\text{-N}$. A Hewlett Packard 5890, Series II GLC (Hewlett-Packard Company, Avondale, PA) with a HP 3396A Integrator (Hewlett-Packard Company) was used for all VFA analyses. The GLC was equipped with a 1.8-m glass column packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW (Supelco, Inc., Bellefonte, PA). The internal standard used was 2-ethylbutyric acid, and N was the carrier gas. Injector port temperature was 185°C, and the detector port was set at 195°C. The column was held at 115°C for 8 min.

The FA content of feed, effluent, and bacterial samples was analyzed according to the procedure described

by Sukhija and Palmquist (1988). The GLC was equipped with a 30-m, 0.25-mm i.d., 10% SP-2380 fused silica capillary column (Supelco, Inc.) for analysis of FA in all samples of feed, effluent, and bacteria. The injector port temperature was 230°C, and the detector port was set at 250°C. The column was held at 165°C for 13 min and then increased at 2.5°C/min to 200°C and held for an additional 2 min.

The BH was calculated according to the equation of Tice et al. (1994), in which the number of double bonds was considered: $\text{BH} = 100 - (100 \times ((\text{D18:1} + (\text{D18:2} \times 2) + (\text{D18:3} \times 3))/(\text{D18:0} + \text{D18:1} + \text{D18:2} + \text{D18:3}))/((\text{I18:1} + (\text{I18:2} \times 2) + (\text{I18:3} \times 3))/(\text{I18:0} + \text{I18:1} + \text{I18:2} + \text{I18:3})))$, where D = fermenter flow (g/d), and I = input (g/d).

Statistical Analysis

Statistical analyses were performed using the general linear models procedure of SAS (1999). Effects of fermenter, period, and treatment were tested. Mean separation was performed using the least significant difference procedure when the treatment effect was significant. Significance was declared when $P < 0.05$ and tendency was identified when $0.05 < P \leq 0.10$.

RESULTS

Feed mixtures contained 34.7% NDF, 22.2% ADF, 35.6% nonfiber carbohydrates, and 17.8% CP (Table 1). Concentration of total FA in the feed mixtures was similar (43.8 mg/g DM), and LA was 2.3 times higher in the feed mixture for the HLA treatment. The concentration of LA in this feed mixture was less than 3.0 times higher than reflected by supplemental LA because of the LA contributed by tallow.

Although attempted, no other *trans*- $\text{C}_{18:1}$ and CLA isomers were detected except *trans*-11 $\text{C}_{18:1}$ (VA) and *cis*-9, *trans*-11 $\text{C}_{18:2}$; thus only total CLA was reported. Compared with control, HSDR tended to increase CLA flow but decreased *trans*- $\text{C}_{18:1}$ flow (Table 2). Providing high LA increased the flows of CLA and $\text{C}_{18:0}$ but decreased the flow of *trans*- $\text{C}_{18:1}$ compared with control. The low pH treatment increased the flows of CLA, *cis*- $\text{C}_{18:1}$, and $\text{C}_{18:2}$ but decreased the flow of total FA and $\text{C}_{18:0}$. However, *trans*- $\text{C}_{18:1}$ flow with LPH was similar to the control, although it was higher than the other 2 treatments.

The HSDR resulted in lower $\text{NH}_3\text{-N}$, total VFA, and propionate concentrations but higher acetate and isovalerate concentrations and acetate-to-propionate ratio than control (Table 3). The HLA increased total VFA concentration. However, the proportions of acetate and propionate and the acetate-to-propionate ratio were

Table 2. Effects of solid dilution rate, level of linoleic acid, and pH on fatty acid (FA) flows in continuous culture.¹

	Treatment ²				SE
	Control	HSDR	HLA	LPH	
	mg/d				
Total FA	5652 ^a	5124 ^{ab}	5208 ^a	4824 ^b	330
C _{16:0}	920	778	520	836	113
C _{18:0}	1947 ^b	2316 ^{ab}	2552 ^a	804 ^c	204
<i>Trans</i> -11 C _{18:1}	629 ^a	263 ^b	239 ^b	712 ^a	57
<i>Cis</i> C _{18:1}	556 ^b	443 ^b	404 ^b	822 ^a	75
C _{18:2} n6	237 ^b	203 ^b	245 ^b	570 ^a	61
C _{18:3} n3	24.8	24.0	19.2	19.2	2.3
Total CLA ³	16.5 ^c	20.4 ^{bc}	23.2 ^{ab}	25.2 ^a	1.3

^{a,b,c}Means in the same row with different letters differ ($P < 0.05$).

¹DM flows were 118, 120, 116, and 120 g/d for control, HSDR, HLA, and LPH, respectively, before correction of minerals from buffer solution.

²Control = pH 6.5, 1% linoleic acid (LA), and 4%/h solid dilution rate (SDR); HSDR = high SDR (8%/h); HLA = high LA (3%); and LPH = low pH (5.8).

³CLA = Conjugated linoleic acid.

similar between HLA and control. The HLA decreased isovalerate concentration compared with control. Compared with the other 3 treatments, LPH resulted in the highest proportions of propionate and valerate and the lowest NH₃-N, total VFA, acetate, and isobutyrate concentrations and acetate-to-propionate ratio.

The digestibility of NDF and numbers of total and cellulolytic bacteria were reduced by HSDR (Table 4). The HLA increased ADF digestibility, total and cellulolytic bacteria, and BH compared with control. The LPH treatment increased FA digestibility but decreased true N digestibility, total and cellulolytic bacteria, and BH compared to control. Although they were numerically lower, the NDF and ADF digestibilities were not significantly decreased by the low pH.

DISCUSSION

Effects of SDR on CLA Flow

One of the hypotheses was that a shorter solid retention time would lead to a more incomplete BH; thus, HSDR should have resulted in higher flows of CLA and *trans*-C_{18:1} than control. A trend ($P = 0.06$) occurred for HSDR to increase CLA flow in the effluent. However, *trans*-C_{18:1} flow was decreased. This may have occurred for 2 reasons. First, the filtrate, which had a constant dilution rate, also contains some small feed particles, which might partially alleviate the difference between fermenters with high and low SDR. Second, all fermenters were provided with the same amount of feed mixture DM. After reaching steady-state conditions, DM con-

Table 3. Effects of solid dilution rate, level of linoleic acid, and pH on NH₃-N and VFA concentrations in continuous culture.

	Treatment ¹				SE
	Control	HSDR	HLA	LPH	
NH ₃ -N, mg/dL	16.6 ^a	12.8 ^b	17.2 ^a	10.1 ^c	0.5
Total VFA, mM	126 ^b	116 ^c	139 ^a	105 ^d	1
Acetate, mol/100 mol	70.9 ^c	73.7 ^{ab}	72.0 ^{bc}	62.3 ^d	0.6
Propionate, mol/100 mol	11.8 ^b	9.91 ^c	12.6 ^b	20.0 ^a	0.50
Butyrate, mol/100 mol	11.0	10.0	10.0	12.3	0.9
Isobutyrate, mol/100 mol	0.68 ^{ab}	0.59 ^b	0.74 ^a	0.16 ^c	0.03
Valerate, mol/100 mol	1.27 ^b	1.21 ^b	1.18 ^b	1.64 ^a	0.08
Isovalerate, mol/100 mol	4.32 ^b	4.67 ^a	3.58 ^c	3.68 ^c	0.08
Acetate:propionate	6.03 ^b	7.45 ^a	5.75 ^b	3.13 ^c	0.15

^{a,b,c,d}Means in the same row with different letters differ ($P < 0.05$).

¹Control = pH 6.5, 1% linoleic acid (LA), and 4%/h solid dilution rate (SDR); HSDR = high SDR (8%/h); HLA = high LA (3%); and LPH = low pH (5.8).

Table 4. Effects of solid dilution rate, level of linoleic acid, and pH on feed digestibilities, bacteria number, and biohydrogenation in continuous culture.

	Treatment ¹				SE
	Control	HSDR	HLA	LPH	
Apparent digestibility, %					
OM	32.6	26.2	32.4	34.8	1.7
NDF	53.8 ^{ab}	39.1 ^c	57.8 ^a	46.1 ^{bc}	3.3
ADF	50.1 ^b	30.8 ^b	58.4 ^a	42.7 ^{ab}	5.2
N	18.4	8.1	19.0	14.4	4.3
FA ²	-9.5 ^b	-0.2 ^{ab}	13.3 ^a	6.9 ^a	4.2
True digestibility, %					
OM	48.9 ^{ab}	41.4 ^b	54.8 ^a	47.4 ^{ab}	2.5
N	64.4 ^a	58.9 ^{ab}	69.6 ^a	51.8 ^b	3.5
Efficiency of microbial protein synthesis, g of N/kg of OM truly digested	31.4	43.6	31.3	26.4	4.7
Log ₁₀ bacteria number					
Total	9.85 ^b	9.22 ^c	10.39 ^a	9.27 ^c	0.09
Cellulolytic	8.53 ^b	8.09 ^c	9.63 ^a	8.14 ^c	0.10
BH ³ , %	61.1 ^b	72.8 ^{ab}	81.1 ^a	30.4 ^c	3.8

^{a,b,c}Means in the same row with different letters differ ($P < 0.05$).

¹Control = pH 6.5, 1% linoleic acid (LA), and 4%/h solid dilution rate (SDR); HSDR = high SDR (8%/h); HLA = high LA (3%); and LPH = low pH (5.8).

²FA = Fatty acid.

³BH = Biohydrogenation, calculated according one of the equations of Tice et al. (1994), which includes the number of double bonds.

tent in each fermenter remained constant; the HSDR treatment could have decreased the retention time of the solids but could not ensure a significantly higher total DM outflow than other treatments, thus reducing the extent of any increase in CLA flow.

Effects of Level of LA on CLA Flow

With more LA available as a substrate for CLA production, HLA resulted in the second highest CLA flow among treatments (Table 2). However, *trans*-C_{18:1} flow was less than half of that of control, and the flow of C_{18:0} was much higher for HLA than that of control. Interestingly, HLA also resulted in the highest concentration of total VFA, ADF digestibility, bacteria numbers, and BH. These results are consistent with the finding in previous studies that feeding oil or oilseeds providing LA to cows can be an effective way to increase CLA concentration in milk fat. Chouinard et al. (2001) fed cows with 4% Ca salt of FA (Ca salt of FA prepared for this study may have had low rumen protection) either from canola oil, soybean oil, or linseed oil. Although all 3 treatments increased CLA concentration in milk (13.0, 22.0, and 19.0 mg/g of fat, respectively) compared with control (3.5 mg/g of fat), cows fed the Ca salt of soybean oil had the highest CLA concentration. Kelly et al. (1998) reported that feeding high LA oil (sunflower) increased CLA concentrations to 24.4 mg/

g of milk fat compared with values of 13.3 and 16.7 mg/g of fat for high oleic acid and high linolenic acid oils, respectively. Dhiman et al. (1999) found that 3.6% soybean oil in a diet resulted in the highest CLA concentration in milk fat (20.4 mg/g), whereas feeding roasted soybean and linseed oil also increased CLA concentration in milk fat but to a lesser extent. In another study, Dhiman et al. (2000) observed a linear effect of feeding soybean oil (0 to 4.0%) on CLA concentration in milk fat (5.1 to 21.3 mg/g). The present study confirms that feeding oil or oilseeds providing LA to cows can contribute to the increase in milk CLA by increasing duodenal flow of CLA. However, most of the LA added in HLA was hydrogenated to C_{18:0}, with only a small amount of CLA escaping BH. This indicates that CLA originating in the rumen may not be the most significant source of CLA in milk. This is consistent with the findings by Griinari et al. (2000) and Corl et al. (2001) that endogenous synthesis of CLA accounts for most of the CLA produced in milk.

Kepler et al. (1966) and Noble et al. (1974) found that accumulation of VA in the rumen seemed to occur most consistently when the concentration of free C_{18:2} is high in ruminal contents. Beam et al. (2000) found the overall rate of BH of C_{18:2} in vitro was 14.3%/h but declined 1.2%/h for each percentage unit increase in C_{18:2} added to the substrate. They concluded that high LA concentrations in the diet would possibly reduce BH and in-

crease the postruminal flow of this unsaturated FA. Kalscheur et al. (1997b) studied the effects of fat source (high oleic sunflower oil, high linoleic sunflower oil, and partially hydrogenated vegetable shortenings) on duodenal flow of *trans*-C_{18:1} and milk fat production in dairy cows. The flow of *trans*-C_{18:1} to the duodenum was higher for cows fed diets supplemented with fat than for cows fed the control diet. More incomplete BH may have accounted for the increased flow to the duodenum and concentration in milk of *trans*-C_{18:1} for cows fed diets containing high oleic and high linoleic sunflower oils. In the present study, however, LA flow was similar and *trans*-C_{18:1} flow lower in the effluent of HLA compared with control rather than increased as suggested by other research. Moreover, stearic acid flow was increased by HLA, consistent with the extent of BH being the highest for this treatment. This may have occurred because continuous infusion of LA into the fermenters lessened any potential adverse effects on bacteria and allowed more accessibility of the bacteria to FA for BH. Kim et al. (2000) observed that *B. fibrisolvens*, an important microorganism in BH and CLA production, could not initiate growth at a relatively low concentration of LA and CLA if unadapted. However, when the LA concentration was increased in a stepwise fashion, the organism tolerated more CLA.

Interestingly, providing high LA also resulted in the highest apparent FA digestibility, but the digestibilities were low as expected. The negative balance of FA in the rumen has been attributed to factors such as variations in estimating digesta flow associated with application of digestion markers and synthesis of FA by bacteria (Harfoot and Hazlewood, 1997). In the present *in vitro* study, flow was measured gravimetrically, but a small extent of FA digestion was still noted for HLA and LPH.

Effects of pH on CLA Flow

Although factors altering ruminal fermentation and the microbial population are undoubtedly keys to controlling the regulation of BH and CLA synthesis (McGuire et al., 1997), very few studies have directly associated CLA production with ruminal pH. Some researchers have investigated the effect of forage-to-concentrate ratio on the concentration of CLA in milk, but no detailed information on ruminal pH was given and the results were not consistent (Jiang et al., 1996; Chouinard et al., 1998). However, effects of dietary factors on production of *trans*-C_{18:1} have been more extensively studied. When the proportion of grain (fermentable starch) is >50% of DMI (Gaynor et al., 1995), ruminal pH is most likely reduced, and a depression in milk fat percentage generally occurs. The milk fat depression is

usually accompanied by an elevated concentration of *trans*-C_{18:1} and LA in duodenal contents and milk fat, which indicate an inhibited ruminal BH (Palmquist et al., 1993). Kalscheur et al. (1997a) found that a diet containing low forage and no buffer decreased ruminal pH and increased duodenal flow of *trans*-C_{18:1} and resulted in more milk with lower fat percentage. Although Griinari et al. (1998) later revealed that milk fat depression caused by feeding high concentrate diets or the addition of unsaturated FA may involve *trans*-10 C_{18:1} rather than *trans*-11 C_{18:1}, *trans*-11 C_{18:2} concentration and flow to the duodenum may increase when ruminal fermentation is altered, especially when pH is reduced.

Reduced ruminal pH adversely affects microbial populations, especially cellulolytic bacteria, and reduces ruminal BH (Harfoot and Hazlewood, 1997). Data derived from the present study revealed reduced total and cellulolytic bacteria numbers, accompanied by reduced acetate-to-propionate ratio and BH when pH was reduced. Thus, reducing ruminal pH increases CLA flow, possibly by altering the ruminal bacterial population and limiting the extent of ruminal BH.

Effects of SDR, LA, and pH on VA Flow

Compared with control, LPH resulted in similar but HSDR and HLA lower VA flows. Stearic acid flows were higher for HLA, tended to be higher for HSDR, and were lower for LPH compared with control. The HSDR and HLA may not have limited ruminal BH as much as LPH; thus, most of the VA was biohydrogenated into stearic acid with LPH. Based on current information, increasing the production of VA in the rumen may be more important than increasing CLA because most of the CLA in milk is derived by endogenous synthesis from VA by Δ -9 desaturase (Griinari et al., 2000; Corl et al., 2001; Qiu et al., 2002).

CONCLUSIONS

Providing additional LA as a precursor of CLA, reducing pH to hinder BH, and increasing solid passage rate to reduce the extent of BH increased the flow of CLA from the fermenters, although the effect of passage rate was minimal. However, reducing ruminal pH, increasing SDR, and adding free LA did not increase the flow of VA, possibly because LA was continuously supplied to the fermenter. Most of the LA added was BH, with a small amount of CLA escaping BH, indicating that CLA originating in the rumen may not be a significant source of CLA in milk. Further *in vivo* studies are needed to evaluate the relationship and interaction of solid passage rate, level of LA, and pH on CLA production in the rumen, flow to the small intestine, and incorporation into milk fat.

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