NUTRITION, FEEDING, AND CALVES

Effects of the Ratio of Ruminal Propionate to Butyrate on Milk Yield and Blood Metabolites in Dairy Cows

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ABSTRACT

Four Ayrshire cows ($\bar{X}$ = 56 DIM) were used in a 4 × 4 Latin square design to study the effects of the ratio of propionate to butyrate in the rumen on milk yield, milk composition, and blood metabolites. The cows were fed a basal diet (16.2% CP, 43.4% NDF) consisting of 50% grass silage, 6% grass hay, and 44% concentrate (percentage of DM). The diet supplied 44 Mcal/d of metabolizable energy and was supplemented with isoenergetic infusions of VFA (4.5 Mcal/d). Propionate (900 g/d) was replaced gradually with 33, 67, and 100% of butyrate on an energy basis. Replacement of propionate with butyrate in the infusate decreased propionate and increased butyrate concentrations in ruminal fluid and in blood plasma. Yields of milk and lactose decreased, and yield of milk fat increased, as butyrate increased. Milk fat content increased, and lactose content decreased, as butyrate increased. Increased ruminal supply of butyrate decreased plasma glucose concentration and increased blood ketone body concentration. When only butyrate was infused (750 g/d), either liver metabolism was changed or tissue mobilization was increased, as indicated by the increased production of long-chain milk fatty acids and increased plasma concentrations of acetate, Gly, and branched-chain AA. An increase in ruminal butyrate supply at the expense of propionate adversely affected milk yield and the repartitioning of nutrients between milk components. At a high percentage, increased butyrate might also adversely affect the overall metabolism of the cow.

(**Key words**: propionate, butyrate, milk yield, blood metabolites)

Abbreviation key: **ACAC** = acetoacetate, **AIA** = acid-insoluble ash, **BCAA** = branched-chain AA, **ME** = metabolizable energy.

INTRODUCTION

A decreased ratio of glucogenic (propionate) to lipogenic (acetate and butyrate) acids in ruminal VFA can decrease production of liver glucose, glucose supply to the mammary gland, and lactose and milk synthesis in high yielding dairy cows (6, 15). However, in some recent studies (7, 23), physiological glucose concentrations have not limited milk synthesis. The composition of the ration and milk yield determine the first-limiting nutrient.

Accordingly, milk synthesis of high yielding cows can be limited by diets that produce a ruminal fermentation pattern that is low in propionate and high in butyrate, which is often the situation for cows fed grass silage that is high in water-soluble carbohydrates, low in lactic acid, and supplemented with barley (18). With diets based on grass silage, replacing barley in the concentrate with fibrous by-products, such as sugar beet pulp (11), or feeding grass silages high in lactic acid and low in water-soluble carbohydrates (12) has increased the ratio of propionate to butyrate in ruminal VFA. Replacement of starchy concentrates with fibrous by-products at moderate supplementation levels in the diets of dairy cows has increased milk yield (10, 13, 33). The greatest relative increases have occurred generally in lactose yield, and the smallest have occurred in fat yield.

Intraruminal infusion of VFA is a widely used technique to study the effects of nutrient supply to the cow on milk secretion. In a previous study by Huhtanen et al. (15), increasing ruminal butyrate decreased the concentrations of plasma glucose and milk lactose but had no effect on milk yield. Butyrate also increased concentrations of blood ketones and seemed to have a specific effect on milk fat content. However, the interpretation of the results in that study (15) is somewhat difficult because the increase in butyrate was accompanied by decreases in acetate and in propionate. Also, the cows in that study

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yielded less than 20 kg/d and had a positive energy balance; therefore, glucose probably did not limit milk yield. The present experiment studied the effects of the ratio of propionate to butyrate in ruminal VFA on milk yield and blood metabolites of high yielding cows fed a diet based on grass silage and concentrate. To avoid possibly adverse effects of VFA infusions on energy intake, isoenergetic infusions were used.

MATERIALS AND METHODS

Cows and Management

Four Finnish Ayrshire cows in second or fourth lactation were used. The cows were 56 (SE ±10) DIM, had a mean milk yield of 26.6 kg/d, and weighed, on average, 560 kg at the beginning of the experiment. Each cow was fitted with a permanent ruminal cannula. The cows were housed individually in metabolism stalls and had free access to water. The daily allowance of feed was given in two equal meals at 0645 and 1545 h. The cows were milked each day at 0700 and 1600 h.

Experimental Diets

The basal diet consisted of 9 kg of DM/d of grass silage, 1 kg/d of grass hay, and 8 kg/d of concentrate mixture, which contained equal proportions of rolled barley, rolled oats, sugar beet pulp, and rapeseed meal. Metabolizable energy (**ME**) concentrations were assumed to be 2.5 Mcal/kg of DM for the silage, 1.9 Mcal/kg of DM for the hay, and 2.9 Mcal/kg of DM for the concentrate (24); thus, the basal diet supplied about 44 Mcal of ME/d. A commercial mineral mixture (17% Ca, 8.1% P, 6.0% Mg, and 6.9% Na) was given to meet the mineral requirements of the cows. The silage was a direct-cut timothy (*Phleum pratense*) ensiled with a formic acid-based additive (AIV-II; Valio Ltd., Helsinki, Finland) at a rate of 4 to 5 L/tonne.

Experimental Procedures

All cows received the basal diet. The experiment was designed as a balanced 4 × 4 Latin square with 14-d periods. The treatments were intraruminal infusions of 900 g/d of propionic acid of which 33, 67, or 100% was replaced on an energy basis with butyric acid. All four treatments supplied 4.5 Mcal of energy. Sodium hydroxide (10*N*) was used to adjust the pH to approximately 4. The VFA were mixed with tap water to a total volume of 20 L/d and infused continuously into the rumen with a peristaltic pump (Watson Marlow Ltd., Falmouth, England).

Feed samples were taken when the feed was weighed, and the samples were composited to provide one sample per period for the silage and one sample over two periods for the hay and the ingredients of the concentrate mixture. The DM content of the experimental feeds was analyzed weekly.

Milk yield and feed intake were recorded daily. Milk was sampled from the last four consecutive milkings of each period and analyzed for fat, protein, and lactose contents by an infrared milk analyzer (Milkoscan 605; A/S N Foss Electric, Hillerød, Denmark). The results are based on intakes and milk yields for the last 7 d of each period.

The digestibility of the diets was calculated using acid-insoluble ash (**AIA**) as an internal marker and Cr as an external marker. Six grams of Cr_2O_3 paper (26% Cr) were given via ruminal cannula twice daily at 0700 and 1600 h. Fecal grab samples were taken on d 8 to 12 of each period at 0700 and 1900 h. To determine liquid dilution rate, 15 g of Co-EDTA were infused into the rumen within 30 min on d 13 of each period. Liquid outflow rate from the rumen was calculated as the slope of the regression of the natural logarithm of the Co concentration against time after a single dose of Co-EDTA into the rumen. The Co-EDTA was prepared as described by Udén et al. (35). Ruminal samples were taken before the morning feeding on d 13 of each period; thereafter, 8 samples for determination of ruminal fermentation and 10 samples for determination of liquid dilution rate were taken at 1.5-h intervals. The pH was measured immediately, and strained samples were stored frozen for VFA analyses. For Co analyses, the samples were centrifuged, diluted with a mixture of 2.25 *M* nitric and hydrochloric acids, and stored frozen for analyses.

Blood samples were taken on d 14. Ten hourly samples (approximately 20 ml each), starting 0.5 h before the morning feeding, were taken through indwelling polyethylene catheters that had been placed in the jugular vein on the previous day. The samples for plasma AA analyses were composited on an equal volume basis to provide one sample for each cow during each period. Otherwise, the samples were treated as described by Miettinen and Huhtanen (22).

Analytical Procedures

The chemical composition of the feed and fecal samples was analyzed as described by Huhtanen et al. (15). The Co concentration of ruminal fluid samples and the Cr concentration of feed samples were determined by atomic absorption spectrometry (5100PC; Perkin Elmer Corp., Norwalk, CT) (38). The AIA was measured in 2*N* hydrochloric acid solution using the method described by Van Keulen and

TABLE 1. Chemical composition and feeding values of the experimental feeds.

	Silage[1]	Hay	Barley	Oats	SBP[2]	RSM[3]
DM, %	24.0	83.5	89.0	88.1	90.8	88.8
	(% of DM)					
Ash	7.4	6.8	2.4	3.0	9.2	8.0
CP	14.8	10.3	12.9	14.4	12.5	35.8
NDF	54.8	66.8	22.6	24.4	32.4	28.4
ADF	27.9	31.7	5.1	9.1	17.8	18.9
ADL[4]	1.6	1.7	0.9	1.4	2.0	8.4

[1]pH 3.87; DM: 3.5% water-soluble carbohydrates, 6.6% lactic acid, 1.6% acetic acid, and 0.1% butyric acid; total N: 4.9% ammonia N and 63.0% soluble N.

[2]Sugar beet pulp.

[3]Rapeseed meal.

[4]Acid detergent lignin.

Young (36). The VFA of ruminal fluid samples were determined according to Huida (16).

The concentrations of urea and acetone in milk, acetoacetate (**ACAC**), and BHBA in whole blood, urea, glucose, insulin, and VFA in plasma were analyzed by the methods described by Huhtanen et al. (15). Plasma AA were analyzed by an AA analyzer (model LC 5001; Biotronic, Pucheim, Germany). Composition of milk fatty acids was determined by the method of Laakso et al. (20) using GLC.

Statistical Methods

The data were subjected to ANOVA; cow, period, and treatment were included in the statistical model. The data from analyses of ruminal fluid and the blood were calculated by split-plot ANOVA using the following model: $Y_{ijkl} = \mu + C_i + P_j + T_k + e_{ijk} + H_l + (CH)_{il} + (PH)_{jl} + (TH)_{kl} + e_{ijkl}$, where C, P, T, and H are cow, period, treatment, and time effects, respectively; e_{ijk} is the main plot error (6 df); and e_{ijkl} is the subplot error. Orthogonal polynomials were used to test linear, quadratic, and cubic responses to the gradual replacement of propionate with butyrate in the VFA infusion. Significance was declared at $P < 0.10$ unless otherwise stated.

RESULTS

Diet Composition, Intake, and Digestibility

The chemical composition of the dietary ingredients is shown in Table 1. The mean OM, CP, and NDF contents of the basal diet were 93.4, 16.2, and 43.4% of DM, respectively. The mean content of GE was 4.35 Mcal/kg of DM. The silage was high quality (low pH and low concentrations of fermentation acids and ammonia N). All cows received the same amount of the basal diet, and the small differences in DMI reflected differences in the amount of orts (Table 2).

TABLE 2. Effect of ruminal VFA infusions on feed intake.

	Infusion[1]				
	P	PB	BP	B	SEM
	(kg of DM/d)				
DMI[2]					
Silage	8.05	8.43	8.82	8.66	0.342
Hay	0.95	0.93	0.96	0.91	0.039
Barley	1.73	1.78	1.72	1.77	0.022
Oats	1.73	1.78	1.72	1.77	0.022
SBP[3]	1.73	1.78	1.72	1.77	0.022
RSM[4]	1.73	1.78	1.72	1.77	0.022
Total	16.11	16.72	16.83	16.88	0.420

[1]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[2]Linear, quadratic, and cubic responses to VFA infusions were nonsignificant ($P > 0.10$).

[3]Sugar beet pulp.

[4]Rapeseed meal.

TABLE 3. Effect of ruminal VFA infusions on digestibility of dietary constituents.

	Infusion[1]				
	P	PB	BP	B	SEM
	(%)				
DM[2]	68.8	67.7	68.0	68.6	0.67
OM	70.7	69.7	70.0	70.8	0.60
CP	70.7	69.8	69.3	70.0	0.74
NDF	58.6	57.3	58.4	60.4	1.07

[1]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[2]Linear, quadratic, and cubic responses to VFA infusions were nonsignificant ($P > 0.10$).

The VFA infusions had no effect on the digestibility of dietary constituents when determined using either AIA or Cr (results not shown) as digestibility markers (Table 3). The mean DM digestibilities were similar when determined using either AIA or Cr (68.3% vs. 68.6%), but the standard error of the means of the digestibility values was smaller when AIA was used as the digestibility marker. Calculated energy balance of cows was positive during the experiment (0.7 Mcal of ME/d), and there were no significant differences between the treatments.

Ruminal Fermentation

Ruminal pH was maintained at >6.0 during the entire feeding cycle and was not affected by infusions (Table 4). The responses to gradual replacement of propionate with butyrate in VFA infusions in ruminal fermentation pattern were as expected; the molar percentage of propionate decreased ($P < 0.001$), and that of butyrate increased ($P < 0.001$), as the amount of butyrate infusion increased. The ratio of propionate to butyrate decreased from 1.85 to 0.78 ($P < 0.001$) as butyrate increased. The ratio of lipogenic to glucogenic VFA [(acetate + butyrate):propionate] increased from 2.9 to 5.1 (linear effect, $P < 0.001$; quadratic effect, $P < 0.01$) as butyrate increased. The molar percentage of valerate decreased ($P < 0.10$) and that of caproate increased ($P < 0.10$) as butyrate increased.

Liquid dilution rate ($\overline{X}$ = 0.164/h) and ruminal fluid volume ($\overline{X}$ = 81.3 L) were not affected significantly by treatments (Table 4).

TABLE 4. Effect of ruminal VFA infusions on ruminal pH, total VFA concentration, molar percentages of individual fatty acids,[1] liquid dilution rate, and ruminal volume.

	Infusion[2]					Contrast[3]		
	P	PB	BP	B	SEM	L	Q	C
						P		
pH	6.40	6.32	6.32	6.35	0.044	NS[4]	NS	NS
Total VFA, m*M*	113	116	113	110	2.2	NS	NS	NS
	(mol/100 mol)							
Acetate	58.1	58.2	57.8	58.5	0.60	NS	NS	NS
Propionate	24.8	22.9	19.9	16.1	0.41	***	†	NS
Isobutyrate	0.75	0.73	0.74	0.74	0.014	NS	NS	NS
Butyrate	13.6	15.3	18.7	21.8	0.57	***	NS	NS
Isovalerate	0.78	0.79	0.82	0.75	0.060	NS	NS	NS
Valerate	1.41	1.35	1.31	1.29	0.029	†	NS	NS
Caproate	0.48	0.54	0.56	0.77	0.060	†	NS	NS
Liquid dilution rate, /h	0.168	0.167	0.164	0.157	0.0064	NS	NS	NS
Ruminal volume, L	81.3	78.5	81.2	84.2	3.46	NS	NS	NS

[1]Means of seven sampling times.

[2]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[3]Linear (L), quadratic (Q), and cubic (C) responses to VFA infusions.

[4]$P > 0.10$.

†$P < 0.10$.

***$P < 0.001$.

Milk Yield and Composition

Milk yield decreased ($P < 0.10$) linearly as butyrate increased, but, because milk fat content increased ($P < 0.01$) at the same time, 4% FCM yield was not affected (Table 5). The increase in milk fat content was greatest at the highest butyrate infusion. Milk lactose content ($P < 0.01$) and yield ($P < 0.05$) decreased, and fat yield increased ($P < 0.05$) linearly, as butyrate increased. Milk protein yield varied quadratically ($P < 0.10$), reaching a minimum with the 67% treatment. Milk acetone concentration increased ($P < 0.10$) as butyrate increased (from 0.025 to 0.140 m*M*), especially at the highest infusion. Milk urea content was, on average, 3.33 m*M* and was not affected by the treatments.

Composition of milk fatty acids and the yields of individual fatty acids are shown in Tables 6 and 7. The proportions of butyric (C_4), caproic (C_6), and caprylic (C_8) fatty acids increased, and the percentage of stearic acid ($C_{18:0}$) varied (linear and quadratic effects, $P < 0.05$), as butyrate increased.

As expected from the increased supply of milk fat precursors from butyrate, the yield of short-chain fatty acids (C_4 to C_{14}) increased as butyrate increased. The relative differences were more pronounced for butyric and caproic acids than for the other short-chain fatty acids that were synthetized de novo in the mammary gland. The production of palmitic ($C_{16:0}$), linoleic ($C_{18:2}$), stearic, and linolenic ($C_{18:3}$) acids increased (at least $P < 0.10$) with increasing butyrate infusion. The increases in butyric, palmitic, and total C_{18} fatty acids were greatest at the highest butyrate infusion.

Blood Metabolites

The effects of the VFA infusions on blood metabolites are shown in Table 8. The blood concentrations of ACAC and BHBA increased linearly ($P < 0.01$), and plasma glucose concentration decreased linearly ($P < 0.01$), as ruminal butyrate infusion increased. The ratio between blood ACAC and BHBA remained nearly constant across treatments. Blood ketones increased postprandially as butyrate increased (Figure 1), and the interaction of treatment by time was highly significant ($P < 0.001$). The effect of butyrate on blood ketones and plasma glucose tended to be curvilinear; the difference was greatest between the two highest butyrate infusions. The differences between the treatments in plasma propionate and butyrate concentrations reflected infusion and ruminal concentrations. Although the infusion had no effect on ruminal acetate concentrations, the acetate concentration in plasma increased linearly ($P < 0.05$) as the rate of butyrate infusion increased. The ratio of propionate to butyrate in VFA infusion had no effect on concentrations of insulin or urea in plasma.

The effects of the infusions on plasma AA concentrations are given in Table 9. The concentrations of the branched-chain AA (**BCAA**; Ile, Leu, and Val) increased linearly ($P < 0.05$) as rate of butyrate infusion increased. Although the linear effect of

TABLE 5. Effect of ruminal VFA infusions on milk yield, milk composition, and yields of milk constituents.

	Infusion[1]					Contrast[2]		
	P	PB	BP	B	SEM	L	Q	C
						P		
Milk, kg/d	27.2	26.5	25.3	26.0	0.53	†	NS	NS
4% FCM, kg/d	26.1	26.2	25.5	28.0	0.72	NS[3]	NS	NS
Milk composition, %								
Fat	3.82	4.02	4.16	4.61	0.134	**	NS	NS
Protein	3.20	3.17	3.18	3.25	0.060	NS	NS	NS
Lactose	4.55	4.53	4.40	4.37	0.032	**	NS	NS
Yield, g/d								
Fat	1015	1037	1028	1172	39.4	*	NS	NS
Protein	860	831	792	832	16.0	NS	*	NS
Lactose	1232	1200	1114	1136	25.7	*	NS	NS
Energy, Mcal/d	19.1	19.0	18.3	19.9	0.46	NS	NS	NS

[1]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[2]Linear (L), quadratic (Q), and cubic (C) responses to VFA infusions.

[3]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

butyrate on plasma BCAA was significant, only the highest butyrate infusion markedly increased the concentration of individual or total BCAA. For nonessential AA, increased butyrate infusion significantly decreased Ala and Gln and increased ($P < 0.001$) the concentration of Gly.

DISCUSSION

Feed Intake and Digestibility

Propionate infusion has been shown to reduce feed intake (17). Although no differences in feed intake among the treatments were statistically significant, the intake of grass silage was numerically less when propionate alone was infused (Table 2), and, consequently, calculated energy balance was also slightly negative for this treatment. The mean energy balance (0.7 Mcal of ME/d) was not significantly different from zero. In contrast to results of the previous study by Huhtanen et al. (15), the digestibility of the diet did not improve with the increased rate of butyrate infusion.

Ruminal Fermentation

Based on similar ruminal pH, molar percentages of acetate, liquid dilution rates, and ruminal volumes among the four infusions, fermentation of the basal diet and ruminal turnover rate were probably not affected by the ratio of propionate to butyrate in the infusate. Molar percentages of propionate and butyrate were within the range observed with practical diets with the possible exception of the highest butyrate infusion (11, 18).

The ruminal VFA profile was modified as expected. The percentage of propionate decreased, the percentage of butyrate increased, and there was no change in acetate, as the amount of butyrate in the infusate increased. The changes in the concentration and in the molar percentage were linear for butyrate ($r = 0.99$) and also for propionate, except for the highest infusion rate.

Using an approach that was similar to that in a previous study (15), daily production of propionate and butyrate from the basal diet was calculated to be 16.9 and 14.6 mol, respectively. The rate of ruminal butyrate production at 310 mmol/Mcal of ME was higher than the 255 mmol/Mcal of ME in the previous study of Huhtanen et al. (15) or the 201 mmol/Mcal of ME calculated from the data of Krehbiel et al. (19). The production rate of butyrate in this study (1.3 mol/kg of digestible DM) was also higher than the mean 0.5 to 0.9 mol/kg of digestible DM given by Sutton (32). The probable reason for this difference

TABLE 6. Effect of ruminal VFA infusions on milk fatty acid composition.

Fatty acid	Infusion[1]				SEM	Contrast[2]		
	P	PB	BP	B		L	Q	C
	(g/100 g of fat)					*P*		
C_4	3.25	3.53	3.85	4.23	0.05	***	NS[3]	NS
C_6	1.95	2.40	2.60	2.58	0.02	*	NS	NS
C_8	1.35	1.50	1.63	1.53	0.05	*	†	NS
C_{10}	3.53	3.75	3.98	3.45	0.01	NS	*	NS
C_{12}	4.43	4.53	4.75	4.00	0.02	NS	*	NS
C_{14}	12.65	12.83	12.83	12.20	0.02	NS	NS	NS
Total C_4 to C_{14}	27.15	28.53	29.63	28.00	0.50	NS	*	NS
C_{16}	34.18	32.33	32.28	34.25	0.50	NS	**	NS
$C_{18:0}$	9.33	10.50	10.80	10.50	0.26	*	*	NS
cis-$C_{18:1}$	14.73	14.70	14.15	14.45	0.70	NS	NS	NS
trans-$C_{18:1}$	2.65	2.78	2.63	2.78	0.07	NS	NS	NS
$C_{18:2}$	2.00	2.00	2.05	2.08	0.07	NS	NS	NS
$C_{18:3}$	0.43	0.48	0.53	0.48	0.01	NS	NS	*
Total C_{18}	29.3	30.6	30.1	30.2	8.3	NS	NS	NS

[1]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[2]Linear (L), quadratic (Q), and cubic (C) responses to VFA infusions.

[3]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

was a ruminal fermentation pattern that was rich in butyrate in the diets used in our study. In the present trial, ruminal propionate production, as a proportion of DMI, was 7.8%, which was lower than the 9% based on the absorption of propionic acid in portal blood (27). The lower value in our study was likely to have resulted from a ruminal fermentation pattern that was low in propionate. However, the estimated propionate production rate in this study (1.5 mol/kg of digestible DM) was well within the range (1.3 to 1.8 mol/kg of digestible DM) presented by Sutton (32).

Milk Yield and Composition

Moderate levels of concentrates, which increased the ratio of ruminal propionate to butyrate in cattle (11), often increased yields of milk, protein, and lactose and decreased milk fat content of dairy cows (10, 13, 14, 33). Similarly, grass silages that contained high amounts of lactate have been shown to decrease milk fat and protein contents compared with effects of restrictively fermented silage (12).

Accordingly, in this study, replacing propionate with butyrate decreased milk and lactose yields, decreased lactose, and increased milk fat content. These results are also in agreement with results of Blauwiekel et al. (6). However, at a lower milk yield, increasing butyrate in VFA mixture had no effect on milk yield (15). Different effects of butyrate on milk yield might have been related to energy balance of the cows, which was more positive in the study of Huhtanen et al. (15). In the present study, the cows were close to energy balance, assuming an efficiency of 0.62 for utilization of ME above maintenance for milk yield. In contrast to our results, Hurtaud et al. (17) reported a trend toward a lower milk yield with the infusion of propionic acid than with the infusion of a VFA. In their study (17), the percentage in propionate of ruminal VFA was much higher than in our study, even at the highest propionate infusion (33.5% vs. 24.8%), which might be related to increased insulin secretion that directs milk precursors to body tissues.

The decrease in milk fat content, in response to increasing amounts of propionate in the VFA mixture or a corresponding increase in response to increased butyrate, was in agreement with previous data (17, 34). Propionic acid decreased, and butyric acid increased, milk fat content with (6, 15, 17) and without (34) isoenergetic VFA supplementation. In this present experiment, the increase in milk fat content and yield with the decreasing ratio of propionate to butyrate was a consequence of increased precursor supply rather than of fat depression by propionate. Production of short-chain fatty acids that were syn-

TABLE 7. Effect of ruminal VFA infusions on production of individual milk fatty acids.

Fatty acid	Infusion[1]				SEM	Contrast[2]		
	P	PB	BP	B		L	Q	C
	(g/d)					P		
C_4	33.0	36.7	39.1	49.4	1.4	***	†	NS[3]
C_6	19.6	25.0	26.6	30.1	2.0	**	NS	NS
C_8	13.8	15.6	16.6	17.8	0.5	**	NS	NS
C_{10}	35.9	38.7	41.2	40.4	1.6	†	NS	NS
C_{12}	45.2	46.7	49.6	46.9	2.2	NS	NS	NS
C_{14}	128.6	132.4	132.8	143.2	5.7	NS	NS	NS
Total C_4 to C_{14}	276.1	295.1	305.9	327.9	11.2	*	NS	NS
C_{16}	346.5	337.1	332.0	401.4	17.2	†	†	NS
$C_{18:0}$	94.5	108.9	110.3	123.4	4.0	**	NS	NS
cis-$C_{18:1}$	149.3	152.3	144.0	169.4	9.2	NS	NS	NS
trans-$C_{18:1}$	26.8	28.7	27.0	32.5	1.7	NS	NS	NS
$C_{18:2}$	21.1	21.1	20.6	23.3	1.4	†	NS	NS
$C_{18:3}$	4.8	5.4	4.8	5.8	0.2	*	NS	NS
Total C_{18}	296.6	316.4	306.8	354.6	13.7	*	NS	NS

[1]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[2]Linear (L), quadratic (Q), and cubic (C) responses to VFA infusions.

[3]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

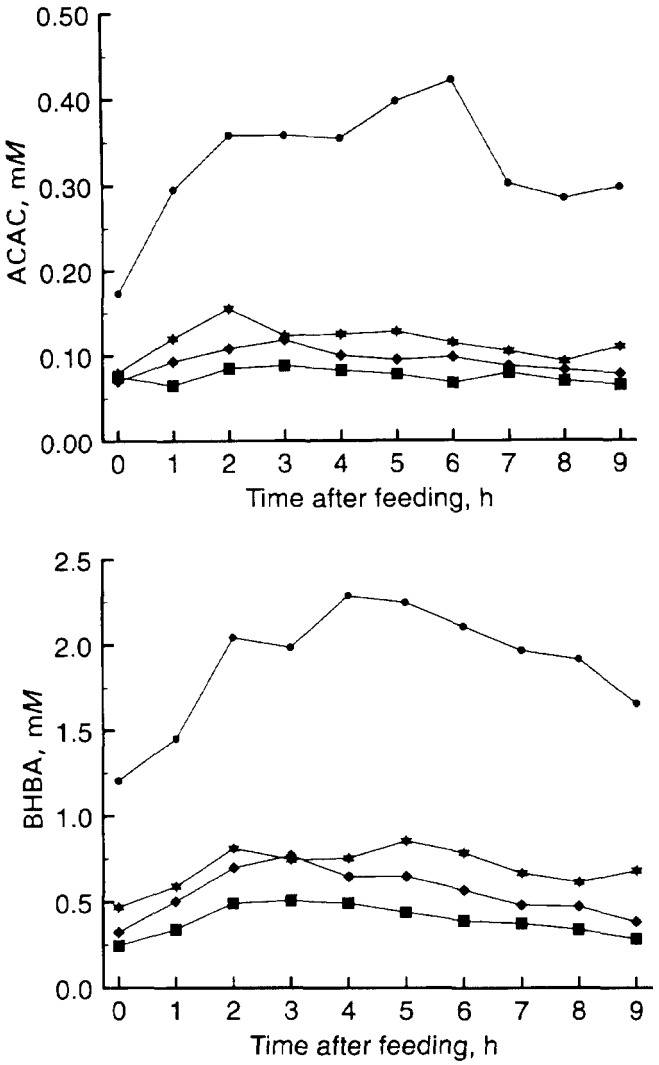

Figure 1. The effect of the ratio of propionate to butyrate [100:0 (■), 67:33 (♦), 33:67 (★), 0:100 (●)] in the ruminal infusate on the pattern of diurnal changes in concentrations of blood acetoacetate (ACAC) and BHBA. Pooled SEM for ACAC was 0.040 and for BHBA was 0.217.

thesized de novo in the mammary gland increased linearly as the rate of butyrate infusion increased. Relative increases in fatty acid yield decreased as chain length increased. This result was consistent with decreasing relative specific radioactivities with fatty acids of shorter chain length after injection of radioactive butyrate (25) and with the increase in inhibition of the synthesis of short-chain fatty acids when the amount of either mobilized or dietary long-chain fatty acids was increased (26). The production of C_{16} and C_{18} fatty acids remained fairly constant at the three lowest butyrate infusions but increased markedly at the highest butyrate infusion. This result might have been related to the increased mobilization of adipose tissue or changes in the liver metabolism of long-chain fatty acids in cows infused with only butyrate (22, 26). Consistent with the results of Hurtaud et al. (17), the ratio of $C_{18:1}$ to $C_{18:0}$ was highest with propionate infusion, suggesting increased desaturase activity in the mammary gland.

Butyric acid appeared to have a specific effect on milk fat content. Increased butyrate supply increased fat content, despite a constant ratio of acetate to propionate of approximately 3.5 and the ratio of (acetate + butyrate) to propionate >4.0 (15), which is considered to be a threshold for milk fat content (31). On the basis of our VFA infusion studies, the ratio of ruminal propionate to butyrate affects milk content more profoundly than ratios of acetate to propionate or (acetate + butyrate) to propionate. The increase in milk fat content was 0.09% per percentage unit increase in molar percentage of butyrate in the present study; the corresponding increase was 0.055% in the previous study of Huhtanen et al. (15). The greater effect in the present study might have been related to the depressive effect of propionate on milk fat content.

The present results confirm the depressive effect of butyrate on milk lactose content (6, 15). Hurtaud et al. (17) attributed higher lactose content from propionate infusion to the glucogenic properties of propionic acid. In our studies, the reduced lactose content from increased butyrate infusion was associated with reduced plasma glucose concentration and lactose yield. This result suggests that the limited supply of glucose caused by decreased propionate availability decreased lactose content rather than suggesting that the glucogenic effect of propionic acid increased it. Glucose balance can be estimated by subtracting the amount of glucose required from the theoretical amount of glucose derived from ruminal propionate. Glucose balance, calculated according to MacRae et al. (21), was positive, except at the highest rate of butyrate infusion, when it was negative (241 g/d). Amaral-Phillips et al. (2) found that cows can tolerate a negative glucose balance of this magnitude for a short time, but, in this study, the experimental period was 14 d, which was obviously beyond the capacity of cows to tolerate a large glucose shortage without metabolic changes.

Infusions had a quadratic effect on milk protein yield; however, the protein yield was highest with propionate infusion, in agreement with results of earlier infusion studies in which propionate was shown to increase, and butyrate was shown to decrease, milk protein yield (29, 30). One possible explanation is the AA sparing effect of propionate in liver gluconeogenesis.

Blood Metabolites

Similar to results of earlier studies (15, 19), increased infusion of butyrate into the rumen or a decreased dietary ratio of propionate to butyrate (22) increased concentrations of plasma butyrate and blood ketones (ACAC and BHBA). However, the increase in the blood concentration of BHBA and ACAC was greater than the increase in plasma butyrate, leading to the decreased ratios of plasma butyrate to blood BHBA and ACAC when ruminal butyrate increased (from 0.149 to 0.044 and from 0.768 to 0.258, respectively). This result indicated that the ketogenic capacity of ruminal epithelium or liver was not exceeded but, on the contrary, was increased as ruminal butyrate increased. Other studies (19, 37) have suggested that the enzyme systems for ketogenesis in the ruminal epithelium and in the liver might be exceeded with high ruminal butyrate, leading to an increase of butyrate in the portal and peripheral blood. However, at the highest butyrate infusion, the shortage of glucose and fatty acids that were mobilized from the adipose tissue might have increased liver ketogenesis. The ratio of ACAC to BHBA remained stable, indicating that the capacity of the liver to convert ACAC to BHBA was not exceeded, which was consistent with the results of Reynolds et al. (28).

The great increase in the concentration of acetate at the highest butyrate infusion may be an indication of increased mobilization of NEFA from the adipose tissue (3) or of release of acetate from the liver, because the postprandial increase in plasma acetate was also greatest with the highest butyrate infusion.

The decrease in plasma glucose concentration from increasing ruminal butyrate supply has also been reported in other studies (8, 15, 19). This effect might be due to decreased gluconeogenesis from propionate in the liver because of decreased absorption from the gut and possibly also from the negative effects of butyrate on the utilization of propionate in liver (1).

The increase in ruminal propionate supply from 1248 g/d with the basal diet to 2148 g/d, the highest rate of propionate infusion, increased plasma propionate from 26.6 to 37.7 m*M*; however, this increase had no effect on plasma insulin concentrations. The increase of ruminal butyrate from 1287 to 2037 g/d did not have an effect on the plasma insulin concentration either, although plasma butyrate concentration increased from 58.4 to 83.5 m*M*. These results confirm the earlier findings (15, 19) that increased ruminal production of propionate or butyrate in physiological concentrations has no effect on plasma insulin concentration. However, the effects of propionate and butyrate on insulin secretion remain obscure because the peripheral insulin concentrations merely indicate the net difference in secretion rate and liver clearance (9).

TABLE 8. Effect of ruminal VFA infusions on the concentrations of blood and plasma metabolites.[1]

	Infusion[2]					Contrast[3]		
	P	PB	BP	B	SEM	L	Q	C
						P		
Blood								
ACAC,[4] m*M*	0.076	0.093	0.115	0.324	0.040	**	†	NS[5]
BHBA, m*M*	0.39	0.55	0.70	1.89	0.217	**	†	NS
VFA, μM								
Acetate	635	734	721	1029	96.8	*	NS	NS
Propionate	37.5	33.0	27.8	26.6	3.35	*	NS	NS
Isobutyrate	9.0	8.8	8.9	10.7	1.16	NS	NS	NS
Butyrate	58.4	70.3	78.3	83.5	6.80	*	NS	NS
Glucose, m*M*	3.72	3.57	3.46	3.03	0.103	**	NS	NS
Urea, m*M*	3.71	3.99	4.01	3.68	0.217	NS	NS	NS
Insulin, mU/L	15.8	14.9	15.0	15.4	2.57	NS	NS	NS

[1]Means of 10 sampling times.

[2]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[3]Linear (L), quadratic (Q), and cubic (C) responses to VFA infusions.

[4]ACAC = Acetoacetate.

[5]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

Plasma concentrations of Ala and Gln decreased as ruminal butyrate increased, especially at the highest butyrate infusion. Arterial concentration of Ala was also decreased in a previous experiment (28) in which n-butyrate was infused into the mesenteric vein of beef steers, which might indicate increased utilization of these AA in liver gluconeogenesis (5). The increase in the plasma concentrations of Gly and BCAA as butyrate supply increased probably indicated increased tissue mobilization (4).

CONCLUSIONS

Decreases in the ratio of propionate to butyrate from exogenous intraruminal isoenergetic infusions repartitioned milk energy from lactose and protein to fat without affecting feed intake, diet digestibility, or milk energy yield. Ruminal butyric acid apparently had specific effect on milk fat content. The ratio of ruminal propionate to butyrate seemed to have had a greater effect on milk fat than did the ratio of acetate to propionate or (acetate + butyrate) to propionate. High ruminal production of butyrate can have adverse effects on glucose production and lactose synthesis in dairy cows fed diets based on grass silage. The ketogenic capacity of the gut wall and liver was probably not exceeded, despite high ruminal production of butyrate.

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TABLE 9. Effect of ruminal VFA infusions on jugular vein plasma AA concentration.

AA[3]	Infusion[1]				SEM	Contrast[2]		
	P	PB	BP	B		L	Q	C
	(μM)					*P*		
Arg	63.5	69.6	76.8	72.1	4.9	NS[4]	NS	NS
His	19.8	26.2	23.1	26.8	2.5	NS	NS	NS
Ile	81.8	77.9	78.5	117.7	9.2	*	†	NS
Leu	46.2	56.3	53.3	77.3	5.7	*	NS	NS
Lys	47.4	55.3	53.7	51.2	3.2	NS	NS	NS
Met	11.3	12.6	10.8	9.2	1.3	NS	NS	NS
Phe	40.3	39.6	38.1	39.0	1.4	NS	NS	NS
Thr	93.0	105.4	96.6	87.2	4.4	NS	†	NS
Trp	46.1	44.8	47.5	43.3	3.2	NS	NS	NS
Val	148.9	148.0	148.6	195.1	9.3	*	*	NS
Ala	216.6	197.9	189.3	144.9	20.1	†	NS	NS
Asn	20.8	22.0	21.4	19.8	1.1	NS	NS	NS
Asp	13.9	15.6	14.6	13.2	1.1	NS	NS	NS
Glu	75.2	81.8	85.2	82.6	3.8	NS	NS	NS
Gln	215.8	227.0	213.7	179.9	11.6	†	†	NS
Gly	202.0	214.8	229.5	264.7	5.7	***	†	NS
Pro	64.6	70.9	71.8	68.8	4.5	NS	NS	NS
Ser	85.9	94.9	97.1	91.5	3.2	NS	†	NS
Tyr	54.4	49.6	52.3	51.7	2.6	NS	NS	NS
Cit	53.6	52.8	54.3	49.8	3.6	NS	NS	NS
Orn	31.2	32.1	33.4	32.3	2.6	NS	NS	NS
Tau	33.3	30.2	33.0	35.1	1.3	NS	†	NS
BCAA	277	282	280	390	23.3	*	†	NS
EAA	598	636	627	718	32.4	†	NS	NS
NEAA	945	974	975	917	36.7	NS	NS	NS
Total (EAA + NEAA)	1543	1610	1602	1635	58.8	NS	NS	NS

[1]The VFA infusion rate: P = 900 g/d of propionate, PB = 600 g/d of propionate plus 250 g/d of butyrate, BP = 300 g/d of propionate plus 500 g/d of butyrate, and B = 750 g/d of butyrate.

[2]Linear (L), quadratic (Q), and cubic (C) responses to VFA infusions.

[3]BCAA = Sum of Leu, Ile, Val; EAA = sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val; and NEAA = nonessential AA.

[4]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

***$P < 0.001$.

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