Dose-Response Effects of Intraruminal Infusion of Propionate on Feeding Behavior of Lactating Cows in Early or Midlactation

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ABSTRACT

The objective of this experiment was to evaluate whether dose-response effects of intraruminal infusion of propionate on feeding behavior and dry matter intake (DMI) differ by stage of lactation. Six cows in early lactation (EL) and six cows in midlactation (ML) were assigned to blocks in a duplicated 6 × 6 Latin square design experiment. Treatments were mixtures of sodium propionate and sodium acetate containing sodium propionate at 0, 20, 40, 60, 80, and 100% of total volatile fatty acids (VFA), infused into the rumen continuously for 18 h starting 6 h before feeding at a rate of 21.7 mmol of sodium VFA/min. All cows were ruminally cannulated prior to the experiment. The diet was formulated to contain 30% NDF, and dry cracked corn was the major source of starch. We hypothesized that hypophagic effects of propionate infusion were greater for EL compared with ML because of greater plasma concentration of nonesterified fatty acids (275 vs. 76 μ Meq/L) and expected greater basal oxidative metabolism in the liver for EL compared to ML. Propionate infusion decreased DMI for EL and ML, but a quadratic effect of propionate infusion was observed for ML but not EL. This indicated a greater marginal reduction in DMI at higher doses of propionate for ML compared to EL, contrary to our hypothesis. Propionate infusion decreased meal size similarly for both stages of lactation, but linearly increased intermeal interval for ML but not EL. We speculate that lower milk yield for ML compared with EL (30.8 vs. 42.0 kg/d) decreased glucose demand by the mammary gland and increased the proportion of infused propionate oxidized in the liver for ML compared to EL.

(Key words: propionate infusion, feeding behavior, stage of lactation, glucose demand)

Abbreviation key: EL = cows in early lactation, ML = cows in midlactation.

INTRODUCTION

Maximizing energy intake is extremely important for nutritional management of cows in early lactation, not only for maximizing milk production, but also preventing metabolic disorders such as ketosis and hepatic lipidosis. However, mechanisms regulating voluntary feed intake are not well understood for cows in early lactation (EL). Physical fill can be the most dominant mechanism limiting DMI for high yielding cows around peak lactation (Allen, 2000), but it may contribute less in EL (Ingvarsen and Andersen, 2000). Low DMI in EL might be related to elevated concentration of plasma NEFA (Ingvarsen and Andersen, 2000). Extent of uptake of NEFA by the liver is determined by its concentration in plasma, which is elevated in early lactation (Emery et al., 1992). Langhans et al. (1985) proposed that metabolic fuels that are extensively metabolized in the liver have hypophagic effects, and fatty acid oxidation in the liver produces a satiety signal in rats (Langhans et al., 1987; Friedman et al., 1999). Furthermore, Koch et al. (1998) showed that temporal relationships exist between feed intake and hepatic ATP concentration in rats, supporting the hypothesis that oxidative metabolism in the liver is involved in feed intake regulation.

Propionate and NEFA are the primary metabolic fuels extensively utilized by the ruminant liver (Demigne et al., 1986; Emery et al., 1992). Glucose, acetate, and butyrate are the other major metabolic fuels for ruminants but have no, or inconsistent hypophagic effects (Allen, 2000), and they are not extensively utilized in the liver (Ballard, 1965; Ricks and Cook, 1981; Demigne et al., 1986). Allen (2000) proposed that hypophagic effects of propionate are from its oxidation in the liver. Although hypophagic effects of propionate are well documented, effects of propionate infusion on feed intake of ruminants have been inconsistent (Allen, 2000). Inconsistent hypophagic effects of propionate infusion might be because of unidentified differences in animal characteristics. Cows differing in physiological state might respond differently to intraruminal infusion of propionate. If propionate and NEFA decrease feed intake by stimulating oxidative metabolism in the

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Table 1. Ingredients and nutrient composition of experimental diet (% of dietary DM except for DM).

Diet ingredients	
Corn silage	24.3
Alfalfa silage	25.9
Dry cracked corn	24.8
Whole linted cottonseed	6.4
Protein mix ¹	13.9
Vitamin and mineral mix ²	4.7
Nutrient composition	
DM	48.4
OM	93.9
Starch	27.4
NDF	30.0
ADF	20.7
CP	18.1
Ether extract	5.2
Forage NDF	22.3

¹Protein mix contained 75% soybean meal, 20% distillers grains, and 5% blood meal.

²Vitamin and mineral mix contains 66.4% dry ground corn, 20.8% dicalcium phosphate, 7.8% salt, 2.4% magnesium oxide, 1.9% trace mineral premix, and 0.71% vitamin ADE premix.

liver, an interaction between hypophagic effects of propionate and mobilized NEFA is of interest to elucidate regulation mechanisms for feed intake in EL.

The objective of this experiment was to determine whether dose-response effects of intraruminal infusion of propionate on feeding behavior differ by stage of lactation.

MATERIALS AND METHODS

Experimental Design and Treatments

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Six multiparous Holstein cows in early lactation and six multiparous Holstein cows in midlactation (ML) were used in this experiment. However, one cow in early lactation and another cow in midlactation had adverse reactions to infusions (temporary metabolic alkalosis, hypocalcemia, and hypokalemia) and were removed from the experiment. Days in milk were 9 ± 6 and 192 ± 17 (Mean $\pm$ SD) for five cows in early lactation and for five cows in midlactation, respectively. Cows in early lactation were ruminally cannulated at least 30 d prior to calving, and cows in midlactation ruminally cannulated for previous experiments were selected from Michigan State University Dairy Cattle Teaching and Research Center. Experimental diets contained dry cracked corn, corn silage, alfalfa silage, cottonseeds, a premix of protein supplements (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins (Table 1). Dietary NDF, CP, and starch concentrations were 30.0, 18.1, and 27.4%, respectively. Dry cracked corn (mean

particle size = 3.6 mm) was used as the major source of starch to limit propionate production from the basal diet. Throughout the experiment, cows were housed in tie stalls and fed a TMR once daily at 110% of expected intake.

The total length of the experiment was 28 d, consisting of 14 d for diet adaptation, 3 d for data and sample collection to characterize cows at each stage of lactation, and 11 d for data and sample collection to determine the effect of infusion treatments. Treatments were continuous intraruminal infusion of mixtures of sodium propionate and sodium acetate containing sodium propionate at six different concentrations. During the final 11 d of experiment, cows were assigned to duplicate incomplete 6×6 Latin squares balanced for carryover effects with 2 d per subperiod: one square for cows in early lactation and the other square for cows in midlactation. Each 2-d subperiod had 18 h of infusion followed by 30 h of recovery except for the period 6, in which recovery for next infusion was not required. Treatment solutions were prepared by diluting 28.1 mol sodium VFA to 18 L with deionized water. Concentrations of propionate were 0, 20, 40, 60, 80, and 100% of total VFA infused. Sodium acetate was added to keep the osmolality and pH of infusates constant across the treatments to isolate specific effects of propionate relative to acetate on feeding behavior of dairy cows. Concentrations of total VFA were 1.56 M across the treatments, and 15 L of each solution was infused over 18 h starting 6 h prior to feeding. Infusion rate was 13.9 ml/min, providing 21.7 mmol of sodium VFA/min. Solutions were infused using four-channel peristaltic pumps (#78016-30, Cole-Parmer Instrument, IL) and Tygon tubing (7.5 m $\times$ 1.6 mm i.d.). Infusion started 6 h prior to feeding so that VFA concentrations in the rumen reached steady state (assuming absorption and passage rates of 20 and 15%/h, respectively) by feeding time when feeding behavior monitoring started.

Data and Sample Collection

The amount of feed offered and orts were weighed for each cow daily. Samples of all dietary ingredients (0.5 kg) were collected daily during the 3-d collection period and on the feeding behavior monitoring days during the infusion period (d 18, 20, 22, 24, 26, and 28) and composited to one sample. Samples of orts (12.5%) were collected daily during the 3-d collection period (d 15 to 17) and composited into one sample per cow. Body weight and BCS [(Wildman, 1982); five-point scale where 1 = thin to 5 = fat] were measured on d 17 of each period. Cows were milked twice daily in the milking parlor except for the evening milking on feeding behavior monitoring days (d 18, 20, 22, 24, 26, and 28).

when cows were milked in their stalls. Milk yield was measured daily during the 3 d collection period and averaged to characterize cows in each stage of lactation. Milk was sampled at every milking during the 3-d collection period and analyzed for fat, true protein, lactose, SNF with midinfrared spectroscopy by Michigan DHIA (AOAC, 1997).

Samples of feces, ruminal fluid, and blood were collected every 9 h on d 15 to 17. Ruminal fluid samples were collected from five different sites in the rumen, squeezed through a nylon screen, and pH was determined immediately after collection. Samples were frozen at -20°C until further analysis. Blood samples were collected from coccygeal vessels into two Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ): one with sodium heparin and the other with potassium oxalate and sodium fluoride as a glycolytic inhibitor. Both were centrifuged at $2000 \times g$ for 15 min immediately after sample collection, and plasma was harvested and frozen at -20°C until analysis.

On feeding behavior monitoring days (d 18, 20, 22, 24, 26, and 28), infusion started at 0800 h, 6 h prior to feeding, and continued for 18 h. Cows were not allowed access to feed between 1000 to 1400 h to minimize confounding effects of ruminal fermentation from the previous feeding. This was consistent throughout the experiment for adaptation of feeding behavior. Feeding behavior was monitored for 12 h (1400 to 0200 h) by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, and voluntary water consumption were recorded for each cow every 5 s, and meal bouts, interval between meals, meal size, eating time, ruminating time, and total chewing time were calculated. At the end of feeding behavior monitoring period (0200 h), ruminal fluid and blood were sampled and processed as described previously. Additionally, blood samples mixed with sodium heparin were analyzed for pH and concentrations of ionized Ca, Na, K, and Cl using a blood gas analyzer (Stat Profile 4, Nova Biomedical, Waltham, MA) according to manufacturer's recommendation.

Sample and Statistical Analysis

Diet ingredients, orts, and fecal samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1 mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, indigestible NDF, ADF, CP, and starch according to the methods described previously (Oba and Allen, 2003a). Concentrations of all nutrients except for DM were expressed as percentages of DM determined from drying at 105°C in a forced-air oven. Indigestible NDF was used as an

internal marker to calculate apparent total tract digestibility (Cochran et al., 1986). Metabolizable energy intake from diet was calculated according to National Research Council (2001) based on actual digestibility of diets.

Ruminal fluid samples were analyzed for VFA concentrations according to the method described previously (Oba and Allen, 2003a). Plasma samples were processed to determine concentrations of acetate and propionate in a similar manner as described for ruminal fluid (Oba and Allen, 2003a). Commercial kits were used to determine plasma concentration of glucose (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA), NEFA (NEFA C-kit; Wako Chemicals USA, Richmond, VA), and β -hydroxy butyrate (BHBA; kit #310-A; Sigma Chemical Co.).

All data from the 3-d collection period ($n = 10$) were analyzed to characterize cows at each stage of lactation using the ANOVA procedure of JMP (version 4.0, SAS Inc., Cary, NC) according to the following model:

$$Y_{ij} = \mu + S_i + e_{ij}$$

where

μ = overall mean,

S_i = fixed effect of lactation stage ($i = 1$ to 2), and

e_{ij} = residual, assumed to be normally distributed.

All data for infusion effects from 11 d of infusion period ($n = 60$) were analyzed using the fit model procedure of JMP (version 4.0, SAS Inc.) according to the following model:

$$Y_{ijklm} = \mu + S_i + C(S)_{j(i)} + P_k + L_l + Q_l + SL_{il} + SQ_{il} + e_{ijklm}$$

where

μ = overall mean,

S_i = fixed effect of lactation stage ($i = 1$ to 2),

$C(S)_{j(i)}$ = random effect of cow nested in a lactation stage ($j = 1$ to 5),

P_k = fixed effect of period ($k = 1$ to 6),

L_l = linear effect of infusion,

Q_l = quadratic effect of infusion,

SL_{il} = interaction between lactation stage and linear effect of infusion,

SQ_{il} = interaction between lactation stage and quadratic effect of infusion, and

e_{ijklm} = residual, assumed to be normally distributed.

Treatment effects were declared significant at $P < 0.05$, and tendency for treatment effects was declared at $P <$

0.10. Treatment effects were analyzed for each stage of lactation separately if *P*-value for interaction was less than 0.10.

RESULTS

During the 3-d collection period before infusion, DMI tended to be lower for EL compared with ML (22.4 vs. 24.5 kg/d; $P < 0.06$; Table 2), although milk yield was greater for EL than for ML (42.0 vs. 30.8 kg/d; $P < 0.001$). Dry matter intake during the first 12 h after feeding was more than 70% of daily intake for both groups, and DMI during this period also tended to be lower for EL than for ML (16.2 vs. 18.3 kg/12h; $P < 0.09$). Plasma NEFA concentration was greater for EL compared with ML (275 vs. 76 $\mu\text{Meq/L}$; $P < 0.001$). Plasma concentrations of glucose (52.8 vs. 59.0 mg/dl; $P < 0.01$) and insulin (5.7 vs. 11.9 $\mu\text{IU/ml}$; $P < 0.001$) were lower for EL compared to ML.

During the 11-d infusion period, a tendency for interaction between effect of lactation stage and quadratic effect of infusion was observed for DMI ($P < 0.10$; Table 3). Propionate infusion decreased DMI linearly for EL ($P < 0.001$; Table 4 and Figure 1) but quadratically for ML ($P = 0.03$). Propionate infusion quadratically decreased meal size for EL and ML to a similar extent (Figure 2). However, an interaction between effect of lactation stage and linear effect of infusion tended to be significant for intermeal interval ($P < 0.10$); propionate infusion linearly increased intermeal interval for ML ($P < 0.01$) but not for EL (Figure 3). Propionate infusion linearly increased plasma insulin concentration for both EL and ML ($P < 0.05$; Table 5). Plasma concentration of ionized Ca and K decreased linearly and plasma pH increased linearly as propionate infusion increased. Interactions between effect of lactation stage and infusion treatment were observed for plasma concentrations of NEFA, glucose, BHBA, and acetate. Propionate infusion decreased plasma NEFA concentration linearly for EL ($P < 0.001$) but not for ML. The marginal increase in plasma glucose concentration at lower rates of propionate infusion was greater for EL compared to ML (Table 5; Figure 4). Plasma BHBA concentration was greatly reduced from 15.0 mg/dl with infusion of 0% propionate solution to 3.5 mg/dl with infusion of 40% propionate solution for EL (Figure 5). Similarly, plasma acetate concentration was decreased by lower rates of propionate infusion to a greater extent for EL compared with ML.

DISCUSSION

Feeding Behavior

Propionate infusion decreased DMI linearly for EL but quadratically for ML, indicating less marginal re-

duction in DMI at lower doses of propionate but greater marginal reduction in DMI at higher doses of propionate for ML compared to EL. The different responses in DMI between EL and ML (linear vs. quadratic) to propionate infusion are supported by observed feeding behavior. Propionate infusion decreased DMI by both decreasing meal size and increasing intermeal interval in ML while decreasing meal size only for EL. Therefore, the greater marginal reduction in DMI at higher rates of propionate infusion for ML compared with EL is because propionate reduced hunger for ML but not EL. Effects of propionate infusion on plasma concentration of ionized Ca and K were probably because of the combined effects of DMI depression caused by propionate and metabolic alkalosis caused by infusion of sodium VFA.

The different response in intermeal interval to propionate infusion might be related to the kinetics of propionate metabolism in the liver. Postmeal propionate flux to the liver is expected to be similar for EL and ML at respective infusion treatments because the same diet was fed to both groups of cows, and meal size was similar between groups. In addition, ruminal propionate concentration and apparent total tract OM digestibility were not affected by stage of lactation during the 3-d collection period before infusion. We speculate that glucose demand of the body affects propionate metabolism in the liver; infused propionate is used for gluconeogenesis to a greater extent and less propionate is oxidized in the liver as glucose demand of the body increases. Milk yield observed in this experiment is equivalent to 3.0 vs. 2.2 kg/d of estimated glucose demand by the mammary gland for EL and ML, respectively (Amaral-Phillips et al., 1993). Propionate infusion did not affect intermeal interval for EL possibly because greater glucose demand from greater milk yield stimulated gluconeogenesis and decreased the relative proportion of infused propionate used for oxidative metabolism in the liver. However, infused propionate might be oxidized in the liver if glucose demand of the body is relatively low, reducing hunger. Intermeal interval increased linearly by propionate infusion for ML possibly because of lower glucose demand related to lower milk yield compared to EL.

The idea that glucose demand of the body affects extent of hypophagia caused by propionate does not explain the linear reduction in DMI observed for EL at lower rates of propionate infusion. Plasma glucose concentration was greatly increased from 51.9 mg/dl with infusion of 0% propionate solution to 60.2 mg/dl with infusion of 40% propionate solution for EL. In another experiment using cows in midlactation, propionate did not cause hypophagia when plasma glucose concentration was greatly increased by infusion (Oba

Table 2. Effects of stage of lactation on feeding behavior, ruminal fermentation, blood metabolites, nutrient digestibility, and milk production.

	EL ¹	ML ²	SE	P-value
Feeding behavior				
DMI (kg/d)	22.4	24.5	0.7	0.06
DMI (kg/12 h)	16.2	18.3	0.8	0.09
Meal bouts (/12 h)	7.8	7.2	0.5	0.38
Intermeal interval (min)	63.8	60.9	3.7	0.59
Meal size (kg DM)	2.1	2.6	0.2	0.12
ME intake (Mcal/12 h) ³	34.0	41.8	3.2	0.11
Chewing time				
Eating (min/12 h)	198	195	15	0.92
Ruminating (min/12 h)	224	235	9	0.45
Total (min/12 h)	422	430	20	0.78
Water intake (L/12 h)	52.2	59.0	4.5	0.32
Ruminal fermentation				
pH	6.04	6.18	0.05	0.11
Total VFA (mM)	129	132	3	0.50
Acetate (%)	61.4	61.8	0.6	0.65
Propionate (%)	22.1	21.5	0.3	0.26
iso-Butyrate (%)	0.98	1.07	0.04	0.14
Butyrate (%)	12.0	11.9	0.4	0.84
iso-Valerate (%)	1.82	1.80	0.05	0.83
Valerate (%)	1.70	1.85	0.04	*
A:P ratio ⁴	2.78	2.87	0.07	0.38
Plasma				
Acetate (mM)	1.26	1.32	0.10	0.64
Propionate (mM)	0.23	0.22	0.01	0.47
Glucose (mg/dl)	52.8	59.0	1.3	**
Insulin (μ IU/ml)	5.7	11.9	0.9	***
NEFA (μ Meq/L)	275	76	16	***
BHBA (mg/dl)	6.0	5.5	0.6	0.57
Apparent total tract digestibility (%)				
DM	54.3	53.0	1.2	0.45
OM	55.6	54.6	1.1	0.52
NDF	26.1	26.5	1.6	0.84
Starch	80.8	79.1	1.3	0.39
CP	61.6	59.2	1.2	0.20
Ether extracts	61.5	62.7	3.1	0.79
Milk production				
Milk yield (kg/d)	42.0	30.8	1.3	***
Milk fat (%)	4.06	3.80	0.19	0.36
Milk protein (%)	2.69	3.08	0.09	**
Milk lactose (%)	4.78	4.76	0.08	0.88
Milk SNF (%)	8.36	8.75	0.12	*
Milk energy (Mcal/d)	29.9	21.9	0.9	***
BW (kg)	650	631	27	0.63
BCS	3.00	2.50	0.20	0.11

¹Cows in early lactation.²Cows in midlactation.³Metabolizable energy intake was calculated according to NRC (2001) based on actual digestibility of diets.⁴Acetate-to-propionate ratio.* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.

and Allen, 2003b). Therefore, the linear reduction in DMI for EL was not expected, especially at lower rates of propionate infusion, and an alternative or additional explanation is needed for DMI response in EL. We speculate that propionate at lower rates of infusion stimulated oxidative metabolism in the liver for EL by increasing concentrations of tricarboxylic acid cycle inter-

mediates and oxidation of acetyl CoA, whereas infused propionate was utilized for gluconeogenesis.

In support of our speculation, a sharp reduction in plasma BHBA concentration was observed for EL at lower rates of propionate infusion. One possible interpretation of this reduction in plasma BHBA is that the pool of acetyl CoA in the liver from oxidation of NEFA

Table 3. Dose-response effects of intraruminal infusion of sodium propionate relative to sodium acetate on feeding behavior and energy intake for cows in early and midlactation.

	EL ¹						ML ²						SE	P-value		
	0 ³	20	40	60	80	100	0	20	40	60	80	100		S ⁴	L ⁵	Q ⁶
Actual volume infused (L)	14.8	14.7	14.7	14.5	14.9	14.8	14.9	14.9	14.8	15.0	14.3	14.8	0.2	0.73	0.49	0.46
Feeding behavior																
DMI (kg/12 h) ^{SQ}	13.5	11.9	11.0	10.8	9.5	8.4	13.6	12.0	11.5	12.7	9.4	7.1	0.7	0.58	***	0.15
Meal bouts (/12 h)	7.7	6.1	7.5	7.0	6.3	7.0	7.9	7.4	6.7	7.1	5.5	7.0	0.5	0.87	0.06	0.23
Intermeal interval (min) ^{SL}	73.4	105	77.7	91.5	87.0	76.1	60.2	74.0	70.6	79.9	109	86.4	12.4	0.51	0.15	0.29
Meal size (kg DM)	1.9	2.1	1.6	1.8	1.6	1.3	1.8	1.6	1.8	1.8	1.6	1.0	0.2	0.99	***	*
ME intake (Mcal/12 h)																
Diet ^{7,SQ}	28.5	25.2	23.4	22.6	20.3	17.8	32.4	29.1	27.6	30.4	22.7	17.4	1.6	0.33	***	0.13
Infusion ⁸	3.2	3.7	4.2	4.6	5.2	5.7	3.2	3.7	4.2	4.8	5.0	5.6	0.1	0.76	***	0.95
Total ^{SQ}	31.7	28.9	27.6	27.2	25.5	23.5	35.6	32.8	31.9	35.1	27.7	23.0	1.6	0.33	***	0.13
Chewing time (min/12 h)																
Eating	182	142	148	151	133	114	176	153	151	154	138	92	10	0.72	***	0.25
Ruminating	138	125	121	125	125	126	118	109	116	137	130	105	15	0.79	0.88	0.86
Total	319	268	269	276	259	216	294	262	265	291	268	197	20	0.75	***	0.22
Water intake (L/12h) ^{SQ}	85.5	75.7	78.7	75.7	69.3	74.2	76.5	76.5	73.8	82.1	71.2	61.3	4.2	0.61	**	0.43

¹Cows in early lactation.²Cows in midlactation.³Propionate content in infusates (%).⁴Effect of stage of lactation.⁵Linear effect of intraruminal infusion of propionate.⁶Quadratic effect of intraruminal infusion of propionate.⁷Metabolizable energy intake from diet was calculated according to NRC (2001) based on actual digestibility of diets.⁸Metabolizable energy intake from infusates assumed energy density of 0.2094 and 0.3672 Mcal/mol for acetate and propionate, respectively.* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.^{SL} $P < 0.10$ for interaction of lactation stage and linear effect of propionate.^{SQ} $P < 0.10$ for interaction of lactation stage and quadratic effect of propionate.

was decreased by oxidation in the tricarboxylic acid cycle as the proportion of propionate in infusates increased to 40%. Although gluconeogenesis is the major

metabolic pathway for propionate in the liver, increased concentration of tricarboxylic acid intermediates from greater propionate flux to the liver can result in in-

Table 4. Dose-response effects of intraruminal infusion of sodium propionate relative to sodium acetate on DMI and intermeal interval for cows in early and midlactation.

	0 ¹	20	40	60	80	100	SE	P-value	
								L ²	Q ³
DMI (kg/12 h)									
Cows in early lactation	13.5	11.9	11.0	10.8	9.5	8.4	0.9	***	0.99
Cows in midlactation	13.6	12.0	11.5	12.7	9.4	7.1	1.7	***	*
Intermeal interval (min)									
Cows in early lactation	73.4	105.0	77.7	91.5	87.0	76.1	15.3	0.97	0.64
Cows in midlactation	60.2	74.0	70.6	79.9	109.0	86.4	9.3	**	0.52

¹Propionate content in infusates (%).²Linear effect of intraruminal infusion of propionate.³Quadratic effect of intraruminal infusion of propionate.* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.

Figure 1. Dose-response effect of intraruminal propionate infusion on DMI (kg/12h) for cows in early lactation (EL; —) and midlactation (ML; - - -). Values are least square means $\pm$ SE.

creased oxidation of acetyl CoA. Greater propionate flux to the liver might have stimulated hepatic oxidative metabolism without oxidation of propionate. Net oxidation of propionate requires metabolism to pyruvate and reentry into the tricarboxylic acid cycle as acetyl CoA. However, net oxidation of propionate in the liver would be inhibited with excess acetyl CoA because acetyl CoA stimulates pyruvate carboxylase and increases oxaloacetate formation from pyruvate (Ballard et al., 1969). Therefore, infused propionate might stimulate gluconeogenesis and oxidative metabolism in the liver simultaneously for EL, increasing plasma glucose concentration and decreasing DMI linearly.

Propionate can also decrease ketogenesis by increasing insulin secretion and decreasing lipolysis in adipose tissue. In this experiment, propionate infusion linearly decreased plasma NEFA concentration for EL. Therefore, reduction in BHBA at lower rates of propionate infusion does not exclusively indicate enhanced oxidative metabolism in the liver. However, the extent of reduction in plasma concentration of BHBA at 20%

Figure 2. Dose-response effect of intraruminal propionate infusion on meal size (kg) for cows in early lactation (EL; —) and midlactation (ML; - - -). Values are least square means $\pm$ SE.

Figure 3. Dose-response effect of intraruminal propionate infusion on intermeal interval (min) for cows in early lactation (EL; —) and midlactation (ML; - - -). Values are least square means $\pm$ SE.

propionate treatment compared to 0% propionate treatment was not supported by similar changes in plasma NEFA concentration, indicating that reduction in lipolysis does not solely account for the sharp reduction in plasma BHBA concentration.

Data obtained from this experiment need to be interpreted with caution because propionate was infused for a relatively short period (18 h), and propionate infusion for a longer period may give different results. In this experiment, the infusion period needed to be short enough to conduct the experiment while the cows were in a specific stage of lactation, especially for EL. Although plasma NEFA concentration decreased linearly as propionate infusion increased, plasma NEFA concentration for 100% propionate treatment in EL was still much greater than that for 0% propionate treatment in ML. Propionate infusion over a longer period might have decreased NEFA concentration to a greater extent. In addition, propionate can inhibit β -oxidation of NEFA by decreasing activity of fatty acyl-CoA dehydrogenase (Shaw and Engel, 1985; Emery et al., 1992) or by decreasing fatty acid transport to mitochondria (Jesse et al., 1986). If animals were given sufficient time to adapt enzyme activity over a longer infusion period, propionate might decrease oxidative metabolism of mobilized NEFA, causing less hypophagia for EL compared to ML.

Acetate Utilization

Plasma glucose concentration for ML was increased by infusion of 100% sodium acetate solution (0% propionate treatment) compared with that of the 3-d collection period before infusions (61.2 vs. 59.0 mg/dL) in spite of a 15% decrease in energy intake. This was similar to observations for cows in midlactation in other experiments using a similar infusion protocol (Oba and Allen, 2002b); sodium acetate infusion increased plasma glu-

Table 5. Dose-response effects of intraruminal infusion of sodium propionate relative to sodium acetate on metabolites in the rumen and plasma for cows in early and midlactation.

	EL ¹						ML ²						SE	P-value		
	0 ³	20	40	60	80	100	0	20	40	60	80	100		S ⁴	L ⁵	Q ⁶
Ruminal fermentation																
Total VFA (mM)	135	129	125	125	130	124	124	132	120	123	118	124	5	0.52	0.14	0.38
Acetate (%) ^{SL}	75.2	69.6	64.0	59.3	50.4	44.7	72.6	68.6	64.2	58.3	53.6	49.4	1.8	0.99	***	0.43
Propionate (%) ^{SL}	13.8	19.8	25.0	29.7	37.0	44.5	16.4	21.3	25.0	30.0	34.8	39.6	1.8	0.90	***	0.38
iso-Butyrate (%)	0.37	0.44	0.50	0.57	1.17	0.87	0.46	0.55	0.49	0.64	0.90	0.89	0.14	0.92	***	0.56
Butyrate (%)	8.6	7.6	8.0	7.7	6.1	7.0	8.2	6.8	7.6	7.7	7.5	7.0	0.7	0.98	*	0.77
iso-Valerate (%)	1.16	1.26	1.38	1.40	1.52	1.53	1.32	1.53	1.40	1.54	1.68	1.60	0.08	0.37	***	0.43
Valerate (%)	0.92	1.07	1.25	1.39	1.30	1.45	1.12	1.31	1.36	1.57	1.53	1.43	0.09	0.09	***	*
A:P ratio ^{7, SL}	5.50	3.60	2.55	2.01	1.36	1.02	4.51	3.22	2.58	1.97	1.58	1.37	0.18	0.88	***	***
Plasma																
Acetate (mM) ^{SL, SQ}	6.3	2.9	1.3	1.2	1.0	0.9	2.1	1.6	1.6	1.5	0.8	0.8	0.5	0.62	***	**
Propionate (mM)	0.19	0.20	0.20	0.27	0.30	0.34	0.17	0.17	0.22	0.25	0.26	0.32	0.02	0.76	***	0.15
Glucose (mg/dl) ^{SL}	51.9	58.6	60.2	62.4	62.6	63.8	61.2	64.2	65.1	64.9	65.6	65.6	1.1	**	***	***
Insulin (μIU/ml)	4.3	6.0	6.2	6.1	8.3	8.0	9.0	9.7	10.4	10.6	9.7	10.1	1.3	0.06	*	0.53
NEFA (μMeq/L) ^{SL}	307	349	200	196	167	174	80	79	118	66	78	80	27	***	***	0.57
BHBA (mg/dl) ^{SL, SQ}	15.3	7.8	3.5	3.5	3.6	2.3	5.0	3.1	4.0	3.7	3.0	2.9	1.0	0.98	***	***
pH	7.51	7.55	7.53	7.53	7.53	7.57	7.52	7.53	7.54	7.54	7.53	7.55	0.01	0.94	**	0.56
Na ⁺ (mM)	149	150	149	149	149	149	149	149	149	148	148	149	0.7	0.81	0.90	0.95
K ⁺ (mM)	3.91	3.64	3.78	3.64	3.48	3.32	3.75	3.51	3.38	3.46	3.43	3.12	0.11	0.21	***	0.67
Cl ⁻ (mM)	93.8	94.3	92.9	93.9	93.5	93.6	95.1	95.1	95.5	94.9	94.1	94.6	0.8	0.38	0.32	0.98
Ca ⁺⁺ (mM)	1.11	1.04	1.05	1.04	1.06	0.99	1.07	1.02	1.03	1.02	1.00	1.00	0.02	0.51	***	0.66

¹Cows in early lactation.²Cows in midlactation.³Propionate content in infusates (%).⁴Effect of stage of lactation.⁵Linear effect of intraruminal infusion of propionate.⁶Quadratic effect of intraruminal infusion of propionate.⁷Acetate to propionate ratio.* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.^{SL} $P < 0.10$ for interaction of lactation stage and linear effect of propionate.^{SQ} $P < 0.10$ for interaction of lactation stage and quadratic effect of propionate.

Figure 4. Dose-response effect of intraruminal propionate infusion on plasma glucose concentration (mg/dl) for cows in early lactation (EL; —) and midlactation (ML; - - -). Values are least square means $\pm$ SE.

cose concentration by 8%. These observations suggest that acetate spared glucose utilization in some tissues for ML, decreasing rate of glucose clearance from the blood because acetate is not a precursor for gluconeogenesis. However, plasma glucose concentration was not increased by infusion of the 100% acetate solution for EL, and plasma acetate concentration increased from 1.3 mM for the 3 d collection period before infusion to 6.3 mM with infusion of the 100% acetate solution. The different response to acetate infusion for EL and ML suggests that acetate spares glucose utilization to a lesser extent for EL compared to ML. Although the mechanism for lower utilization of acetate in EL is not known, activity of enzymes needed to utilize acetate as a metabolic fuel or for fatty acid synthesis might be lower in EL. Activity of acetyl CoA carboxylase that catalyzes the limiting step for de novo fatty acid synthesis might not be sufficient due to low concentration of plasma insulin in EL (Vernon et al., 1991). In addition, Guesnet et al. (1991) reported that insulin stimulated

Figure 5. Dose-response effect of intraruminal propionate infusion on plasma β -hydroxy butyrate (BHBA; mg/dl) for cows in early lactation (EL; —) and midlactation (ML; - - -). Values are least square means $\pm$ SE.

incorporation of acetate into fatty acids to a lesser extent for ewes in early lactation compared to nonlactating ewes in midstage of pregnancy.

CONCLUSION

Propionate infusion decreased DMI for both EL and ML, but a quadratic effect of propionate infusion was observed only for ML, indicating a greater marginal reduction in DMI at higher rates of propionate infusion for ML compared with EL. The different response in DMI between EL and ML can be attributed to effects of propionate on intermeal interval. Propionate infusion linearly increased intermeal interval for ML but not EL while propionate infusion decreased meal size similarly for EL and ML. Lower milk production relative to ME intake for ML might have decreased demand for gluconeogenesis and increased the proportion of infused propionate oxidized in the liver at higher rates of propionate infusion compared to EL. Although plasma NEFA concentration was greater for EL compared to ML, the hypophagic effects of propionate were not greater for EL at higher rates of infusion, and meal size decreased similarly for EL and ML. These results might be attributed to greater glucose demand and greater gluconeogenesis in EL. However, a sharp reduction in plasma BHBA concentration and linear reduction in DMI were observed for EL at lower rates of propionate infusion, suggesting that short-term propionate infusion might have caused hypophagia by increasing oxidation of acetyl CoA in the liver. Future research needs to examine independent effects of different glucose demands and different rates of basal oxidative metabolism in the liver on hypophagic effects of propionate.

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