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EFFECTS OF PROPIONATE ON FEEDING BEHAVIOR OF LACTATING DAIRY COWS

By

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ABSTRACT

EFFECTS OF PROPIONATE ON FEEDING BEHAVIOR OF LACTATING DAIRY COWS

By

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Increasing ruminal starch digestion often results in a sharp reduction in DMI of lactating dairy cows. Feeding ground high moisture corn increased true ruminally degraded organic matter by 1 kg/d and decreased dry matter intake (DMI) by 1.7 kg/d compared to dry ground corn when cows were fed a high starch diet. We hypothesized that excess propionate production in the rumen has hypophagic effects by stimulating oxidative metabolism in the liver. Intra-ruminal infusion of propionate decreased DMI, energy intake, meal size, and meal frequency in a dose-dependent manner. Hypophagic effects of propionate were greater when infused with ammonium compared to sodium although ammonium did not affect DMI when infused with acetate. Infusion of ammonium stimulated urea synthesis, and amino acid carbon generated from the urea cycle might have enhanced oxidative metabolism in the liver. We also hypothesized that hypophagic effects of propionate infusion are greater for cows fed more fermentable diets because of greater basal propionate production from ruminal fermentation. Contrary to our hypothesis, the magnitude of hypophagic effects of propionate was not affected by diets differing in ruminal starch digestibility of grains or forage to concentrate ratio, indicating that propionate flux from the rumen does not directly decrease feed intake. Propionate infusion did not cause hypophagia when it resulted in a large increase in plasma glucose

concentration. However, when propionate infusion resulted in slight increases in plasma glucose concentration, propionate decreased DMI. A possible explanation for these observations is that propionate stimulates oxidative metabolism in the liver to a greater extent when propionate is not extensively utilized for gluconeogenesis. This suggests that hypophagic effects of propionate are linked to glucose demand of animals and subsequent gluconeogenesis in the liver. Consistent with this speculation, propionate at higher rates of infusion decreased DMI to a greater extent in lower producing cows compared to higher producing cows. It is concluded that hypophagic effects of propionate in lactating dairy cows are consistent with its effects at stimulating oxidative metabolism in the liver and that a temporal pattern of oxidative metabolism in the liver might be related to senses of satiety and hunger.

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
BCS	Body Condition Score
BHBA	β-Hydroxy Butyric Acid
BW	Body Weight
CP	Crude Protein
DC	Dry Cracked Corn
DG	Dry Ground Corn
DM	Dry Matter
DMI	Dry Matter Intake
EL	Cows in Early Stage of Lactation
HF	High Forage
HM	High Moisture Corn
LF	Low Forage
ME	Metabolizable Energy
ML	Cows in Mid Stage of Lactation
MUN	Milk Urea Nitrogen
NDF	Neutral Detergent Fiber
NEFA	Non Esterified Fatty Acids
SCM	Solids-Corrected Milk
SF	Steam Flaked Corn
TRDOM	Truly Ruminally Degraded Organic Matter
VFA	Volatile Fatty Acids

INTRODUCTION

Maximizing energy intake is an important goal in nutritional management for high producing dairy herds. As the genetic potential for dairy cows to produce milk increases, maximum productivity and profitability of high yielding dairy herds have become more dependent on energy intake. Cows in early lactation often experience negative energy balance, and energy status at early lactation greatly affects peak milk yield and persistency of milk production. Because cows in early lactation mobilize body reserves and sustain high milk yield, they are more susceptible to metabolic diseases such as ketosis and fatty liver. One approach to increase energy intake is to increase energy density of diets by feeding more fermentable grains. However, greater starch digestibility can reduce feed intake (Allen, 2000), and may not necessarily increase energy intake. Maximizing dry matter intake (DMI) is another important approach to increase energy intake of lactating dairy cows. It is first necessary to elucidate the mechanisms regulating voluntary feed intake for lactating dairy cows to maximize energy intake.

Shifting site of starch digestion from the rumen to the intestines by feeding less fermentable grains often increases DMI of lactating dairy cows because excess fermentation in the rumen is considered to limit maximum voluntary feed intake in ruminants (Allen, 2000). Propionate is one of the major metabolic fuels for the ruminant liver, and Allen (2000) proposed that oxidative metabolism of propionate in the liver is a primary factor regulating feed intake of ruminants. Many studies on the effects of propionate infusion on feed intake have been reported in the literature. However, most of

previous studies failed to determine the specific effect of propionate on feed intake because effects of propionate were confounded with difference in pH or osmolarity of infusates. Most studies in the literature evaluated the effect of propionate on meal size only and did not evaluate its effect on intermeal interval although feed intake is regulated by satiety and hunger and animals can compensate decreased meal size by increasing meal frequency to maintain feed intake. In addition, some experiments did not discuss the effect of propionate on energy intake. It is important to determine whether propionate affects energy intake because our goal in nutritional management of dairy cows is to maximize energy intake. In addition, the role of propionate in feed intake regulation might differ depending on diets or energy requirement and physiological status of animals. Previous research did not evaluate the interaction of propionate with other factors extensively.

The objectives of this dissertation research were to evaluate effect of site of starch digestion on feeding behavior of lactating cows, to determine the specific effect of propionate on energy intake, and to investigate the mechanism by which propionate affects feed intake.

CHAPTER 1

LITERATURE REVIEW

Factors Affecting DMI

Mechanisms that regulate DMI in lactating dairy cows have been studied extensively. In general, cows are believed to consume feeds to meet their energy requirement. When cows are fed high energy diets which are palatable, digestible and low in fill, feed intake is thought to be regulated by the energy density of the ration (Mertens, 1994). According to the theory of Conrad et al. (1964), voluntary feed intake is regulated by either energy density of a diet, or physical fill depending on the digestibility of the diet consumed. However, this theory over-simplifies the complex mechanisms that limit DMI of dairy cows. For example, some studies in the literature (McCarthy et al., 1989; Casper et al., 1990; Overton et al., 1995) showed that less fermentable grains increase energy intake of lactating cows compared to more fermentable grains. Cows in those experiments increased DMI more than enough to compensate for the lower digestibility of less fermentable grains. Greater energy intake allowed them to increase milk production on less fermentable diets. If animals consume feeds to meet their energy requirement, we cannot explain these observations. Instead, it is more logical to think that greater fermentation in the rumen directly or indirectly sends a satiety signal to regulate DMI. Forbes (1995) suggested that feed intake is regulated by various factors depending upon metabolic status of animals, and integration of several

stimuli generally contributes to the regulation of voluntary feed intake. We need to understand what regulates voluntary feed intake of animals more specifically.

Voluntary DMI is regulated by humoral signals coordinating whole body metabolism. Ingvartsen and Andersen (2000) reviewed the humoral signals that affect DMI of lactating dairy cows, and suggested hormones such as estrogen, CCK, leptin, and cytokines might be involved in regulation of feed intake. The transient decrease in DMI observed in periparturient animals is of interest from the viewpoint of long-term feed intake regulation. A metabolic characteristic during the periparturient period is elevated plasma NEFA concentration. Fatty acid metabolism can be linked to the decrease in DMI during this period because low intake is particularly observed for obese animals compared to thin animals (Bines and Morant, 1983). Fatty acid oxidation in the liver has been shown to decrease feed intake in rats (Friedman et al., 1999). Leptin is primarily produced by adipocytes, and plasma leptin concentration increases as body reserve mass increases (Maffei et al., 1995). Significant evidence exists for regulation of feed intake by leptin in rats although little is known about the biology of leptin in ruminant animals.

Dietary factors that affect short-term regulation of feed intake in lactating dairy cows were recently reviewed (Allen, 2000). In that review, Allen (2000) showed that site of starch digestion often affects DMI. Starch can be digested either in the rumen or in the intestines. Starch fermented in the rumen produces volatile fatty acids, and starch digested in the small intestine is absorbed as glucose. When cows are fed grains that are rapidly fermented in the rumen, DMI sometimes decreased compared to when cows are

fed grains that are less fermented in the rumen. Greater ruminal fermentation is characterized by factors such as low ruminal pH, increased osmolarity of ruminal fluid, and greater fermentation acid production. Choi and Allen (1999) showed that propionate has greater hypophagic effects in lactating dairy cows compared to acetate, reduced ruminal pH, or increased osmolarity of ruminal fluid.

Effects of Site of Starch Digestion on DMI

Enhanced ruminal fermentation does not always decrease DMI (Theurer et al., 1999; Allen, 2000). A recent review by Theurer et al. (1999) reported that steam-flaked corn and sorghum increased starch digestibility compared to steam rolled corn (6 comparisons) and dry rolled sorghum (24 comparisons), respectively. However, neither steam-flaked corn compared to steam-rolled corn nor steam-flaked sorghum compared to dry-rolled sorghum affected DMI. Recent studies that measured starch digestibility in the rumen using cows with duodenal cannulas were summarized to determine the effect of ruminal starch digestibility on DMI (Allen, 2000). Although three comparisons (McCarthy et al., 1989; Overton et al., 1995) showed a significant decrease in DMI for treatments with greater ruminal starch digestibility, ruminal starch digestibility did not affect DMI for the others comparisons (Crocker et al., 1998; Knowlton et al., 1998a; Oliveira et al., 1995; Plascencia and Zinn, 1996; Poore et al., 1993). Other experiments (Herrera-Saldana and Huber, 1989; Wilkerson et al., 1997; Lycos et al., 1997; Grings et al., 1992; Callison et al., 2001; Oliveira et al., 1993) comparing different types of grain, processing methods, or conservation methods that are expected to result in different

ruminal degradability of starch failed to show the negative effects of ruminal starch (or non-structural carbohydrate) digestion on DMI.

These inconsistent observations might indicate that a threshold exists for the effect of fermentation acid production in the rumen on DMI. The concept for a threshold response agreed with observations of Moore et al. (1992). Moore et al. (1992) fed sorghum grains processed as either dry rolled (DR), steam flaked to the flake density of 0.40 kg/L (SF40), or steam flaked to the flake density of 0.27 kg/L (SF27) to dairy cows at 41.5% of dietary DM. Rate of enzymatic starch hydrolysis measured in vitro was greater for SF27 compared to SF40 and DR, and for SF40 compared to DR. However, DMI for DR, SF40, and SF27 was 25.7, 25.4, and 23.8 kg/d (SE = 0.5), respectively. Milk yield for DR, SF40, and SF27 was 31.0, 33.3, and 31.7 kg/d (SE = 0.6), respectively. Increasing starch digestibility from DR to SF40 did not affect DM intake and increased milk yield. However, further increase in starch digestibility from SF40 to SF27 decreased both DMI and milk yield. Although actual starch digestibility in the rumen was not measured in this experiment, it is speculated that the amount of fermentation acid produced in the rumen might not be high enough to affect DMI for cows fed SF40 but exceeded a threshold to affect DMI for the cows fed SF27

The threshold for ruminal fermentation to affect DMI, if it exists, might be altered by physiological status of animals, other dietary characteristics, or basal level of fermentation to which effects of treatment are compared. Some animal and dietary characteristics from recent experiments that reported significant difference in starch or

NSC digestibility in the rumen using duodenally cannulated cows were summarized for statistical evaluation (Table 1). Experiments were classified either Y or N; the experiments that report more fermentable grains decreased DMI were classified as Y and the experiments that report similar DMI regardless of fermentability of grain were classified as N. Treatment means were averaged within an experiment to characterize each study for comparison with others. Although some response variables (e.g. ruminal pH) were affected by treatments, the difference within an experiment was relatively less compared to the difference across the experiments. The objective of this statistical analysis was to determine factors affecting response in DMI to fermentability of grains. Data were analyzed by one-way ANOVA using JMP (SAS Institute, Cary, NC).

The different response in DMI to fermentability of grains could not be explained by differences in days in milk, BW, or milk yield of cows across the experiments. However, ruminal pH and acetate concentration were lower ($P < 0.05$; 6.17 vs. 6.43 and $P < 0.01$; 58.1 vs. 63.0 mol/100 mole VFA, respectively) and propionate concentration was higher ($P < 0.01$; 28.0 vs. 21.8 moles/100 moles of VFA) in the experiments for which more fermentable grains decreased DMI compared to the experiments where more fermentable grains did not decrease DMI. Dietary crude protein concentration tended to be lower ($P < 0.11$; 16.3 vs. 18.2 %) for experiments for which DMI was decreased by more fermentable grains compared to the experiments that DMI was not affected by treatments. These results might indicate basal diet characteristics affected how animals responded to more fermentable grains. Lower ruminal pH and acetate concentration and higher propionate concentration in experiments for which DMI was decreased by greater

Table 1. Animal and dietary characteristics (mean across treatments) for experiments that reported significant difference in starch NSC digestibility in the rumen

	BW	DIM	Milk Yield	Diet		Ruminal	Ruminal VFA (mol/100 mol)		
	(kg)		(kg/d)	CP%	FC ratio	pH	Acetate	Propionate	Butyrate
McCarthy et al., 1989 [Y]	583	83	34.0	14.8	45:55	5.76	58.7	28.7	10.0
Overton et al., 1995 [Y]	538	154	25.8	16.2	45:55	5.84	57.7	30.0	9.8
Oba and Allen, 2000 [Y]	555	97	38.6	18.0	43:57	6.13	58.0	25.2	12.0
Poore et al., 1993 [N]	673	202	18.9	16.7	44:56	NR	NR	NR	NR
Oliveira et al., 1995 [N]	NR	149	27.8	18.9	35:65	NR	NR	NR	NR
Plascencia and Zinn, 1996 [N]	NR	140	24.9	NR	43:57	6.40	65.1	22.6	12.1
Knowlton et al., 1998a [N]	566	85	34.7	20.3	45:55	6.18	61.8	21.7	10.1
Crocker et al., 1998 [N]	506	83	31.1	17.6	45:55	6.36	64.1	20.3	12.8
Callison et al., 2001 [N]	520	100	25.0	17.2	50:50	5.95	61.8	23.1	11.2
Oba and Allen, 2000 [N]	555	97	33.9	18.3	66:34	6.29	62.0	21.4	11.8

DIM: Days in milk at the midpoint of experiment

FC ratio: Forage to concentrate ratio

NR: Not reported

[Y] The experiments that more fermentable grains decreased DMI

[N] The experiments that more fermentable grains did not decrease DMI

ruminal fermentation suggest that propionate production for the basal diet was close to the threshold for affecting DMI. If basal ruminal fermentation is close to a threshold to affect DMI, feeding more fermentable grains in the diet might readily decrease DMI. In addition, greater crude protein concentration might make animals more tolerant to the more fermentable diets (maintaining DMI with greater ruminal fermentation) possibly by increasing milk yield that is allowable by metabolizable protein.

Effects of Propionate on DMI

Hypophagic effects of propionate infusion in ruminants were extensively documented and summarized by Allen (2000). Some experiments in the literature reported that propionate infusion did not decrease feed intake (Deetz and Wangsness, 1981; Quigley and Heitmann, 1991; De Jong et al., 1981; Anil et al., 1993). Intrajugular infusions of propionate did not decrease DMI of sheep while infusion of insulin and glucagon did (Deetz and Wangsness, 1981). Portal infusion of propionate did not decrease DMI of sheep (Quigley and Heitmann, 1991). Infusion of VFA mixture either into the portal or the jugular vein did not affect feed intake of goats (De Jong et al., 1991). Anil et al. (1993) showed that infusion of Na propionate at the rate of 44.4 mmol/min decreased feed intake of dairy cows in one experiment. However, intraruminal infusions of Na propionate at rates of 22.2 mmol/min and 44.4 mmol/min had no effect on hay intake in two other experiments (Anil et al., 1993). Lactating cows used in these experiments differed in age and stage of lactation. Differences in glucose demand and energy balance of experimental animals probably affected intake response to propionate infusion in these experiments. It is not likely that a single factor limits

maximum feed intake. A variety of satiety signals can synergistically interact with each other to regulate maximum feed intake although one factor might become more dominant than the others.

Mbanya et al. (1993) suggested that DMI can be affected by combined effects of acid production and distention, not by one of the effects alone. They infused acetate, propionate, or both, with or without distention of the rumen by a balloon. Combination of VFA infusion and reticulo-rumen distention significantly depressed DMI while VFA infusion or distention alone did not. Integration of physical fill and metabolic satiety signals contributes to the regulation of voluntary feed intake. Similarly, Farningham et al. (1993) reported that portal infusion of either sodium propionate at 1.2 mmol/min or CCK had no effect on food intake of sheep, but together decreased feed intake by 44%. This reduction in feed intake was similar to when sodium propionate was infused at 2.4 mmol/min. These observations indicated that the response to propionate infusion in feed intake depends on infusion rate of propionate, and other dietary or physiological factors.

The hypophagic effect of propionate infusion might be altered by fermentability of the basal diets (Allen, 2000). Leuvenink et al. (1997) fed sheep with a pelleted grass, and reported that propionate infusion into the mesenteric vein of mature sheep at a rate of 2 mmol/min decreased intake but infusion at a rate of 1 mmol/min had no effect. In the experiment of Farningham and Whyte (1993), sheep were fed a pelleted diet containing 50% hay, 30% barley, and 10% molasses offered *ad lib.* Infusion of sodium propionate decreased feed intake linearly when infused over 3-h periods at rates between 0 and 83

mmol/min. The diet fed by Farningham and Whyte (1993) was more fermentable compared to the diet fed by Leuvenink et al. (1997). Inconsistent responses to propionate infusion in DMI may be partially because of differences in fermentability and propionate production in basal diets. Total amount of propionate that animals absorb and metabolize is the sum of propionate infused and propionate produced in the rumen from the diet. Allen (1997) reported that the amount of ruminally fermented organic matter and total VFA production in the literature ranged from 5.7 to 15.4 kg/d and 42 to 115 moles/d for lactating dairy cows, respectively. Propionate concentration in the rumen increases from 15 to 45% of total fermentation acids as amount of ruminally fermented OM increases (Davis, 1967), therefore propionate production is expected to range from 6.3 to 52 moles/d for lactating dairy cows.

Inconsistent hypophagic effects of propionate infusions might be explained by a concept of threshold response. A threshold for infused propionate to decrease DMI might be lower for animals fed more fermentable diets. A threshold for total propionate (sum of infusion and production in the rumen) to decrease DMI might be lower for animals fed more filling diets (Mbanya et al., 1993) or high fat diets (Farningham et al., 1983). In addition, energy requirement or physiological status of animals might also affect the threshold. If propionate regulates maximum DMI, as evidence in the literature suggests, it is important to know how propionate affects DMI and if a threshold for propionate exists.

Although hypophagic effects of propionate have been investigated extensively, more research is still needed. Evaluation of feeding behavior would help to understand the regulation mechanism of DMI by propionate because DMI is a function of both meal size and intermeal interval, which are determined by satiety and hunger, respectively. Most experiments in the literature that have evaluated the hypophagic effect of propionate monitored feed intake over very short periods ranging from 30 minutes to 3 hours, and essentially investigated the effect of propionate on meal size only. The interaction of hypophagic effect of propionate with other diet characteristics is another concern because physical fill or dietary fat supplementation sometimes dominate in regulation of feed intake (Allen, 2000). The majority of previous experiments focused on the effect of propionate on DMI, not on energy intake. Energy intake should be the response variable because maximization of energy intake is a major concern for practical nutritional management of dairy cows with high milk yield, and increasing DMI is an approach to maximize energy intake.

Mechanisms for Hypophagic Effect of Propionate

Hypophagic effects of propionate might be due to its negative effects on motility of the reticulo-rumen. McLeay and Pass (1966) showed that infusion of propionate and butyrate reduced the frequency and amplitude of reticulum and ruminal contractions compared to iso-osmotic infusion of acetate or NaCl. Mbanya et al. (1993) showed hypophagic effects of propionate infusion only for cows challenged by physical fill, but not for cows infused with propionate only. Thus, greater hypophagic effects of propionate might be because reduced motility increased physical fill in the rumen.

Hypophagic effects of propionate might be explained partially by greater insulin secretion. Increased serum glucose level and subsequent insulin secretion are thought to be the major satiety signals for non-ruminants. Infusion of insulin antibodies into the portal vein of rats increased meal size by 24 to 29%, indicating a role of insulin in feed intake regulation (Surina-Baumgartner et al., 1995). Similarly, insulin may regulate feed intake for ruminants because it is reported that insulin infusion decreased feed intake in sheep (Deetz et al., 1980; Deetz and Wangsness, 1981; Foster et al., 1991). There is much research that shows that propionate stimulates insulin secretion in ruminants. Infusion of propionate increased insulin secretion in sheep (Peters et al., 1983) and cows (Istasse et al., 1987). DeJong (1982) showed that infusion of propionate, n-butyrate, and n-valerate induced insulin secretion, while acetate is not an effective stimuli for insulin secretion. Intraruminal infusion of propionate increased insulin secretion while infusion of acetate did not (Gonda et al., 1997). Elevation of plasma insulin concentration can be observed under practical feeding situations. Lactating dairy cows responded to a high starch diet by elevating plasma insulin concentration (Lee et al., 1990), and insulin concentration increased immediately after feeding cows a high concentrate diet (Vasilatos and Wangsness, 1980). Infusion of propionate into the portal vein had greater hypophagic effects compared to the same rate of infusion into the jugular vein (Anil and Forbes, 1980). This might support the idea that hypophagic effects of propionate are mediated by insulin secretion because propionate concentrations at the pancreas might be higher and stimulate more insulin secretion when infused in the portal vein compared to when infused in the jugular vein (Allen, 2000).

It is the liver that senses propionate either directly or indirectly and generates satiety signals (Anil and Forbes, 1980; Anil and Forbes, 1988). Allen (2000) proposed that enhanced oxidative metabolism in the liver mediates hypophagic effects of propionate for ruminants. Although the primary metabolic pathway for propionate is gluconeogenesis in the liver (Figure 1), propionate has two pathways to stimulate oxidative metabolism in the liver. Propionate is metabolized to oxaloacetate and drives the TCA cycle in mitochondria stimulating complete oxidation of acetyl CoA (Figure 2). However, propionate itself is not oxidized in this metabolic pathway. Alternatively, propionate is metabolized to malate, shuttled out to the cytosol, and metabolized further to phosphoenol pyruvate. Phosphoenol pyruvate can be metabolized to acetyl CoA, which can be oxidized completely to carbon dioxide in the TCA cycle (Figure 3). Propionate oxidized in this pathway would not be utilized for gluconeogenesis.

Role of hepatic oxidative metabolism in feed intake regulation has been extensively studied for non-ruminants. Langhans et al. (1983, 1984, 1985a, 1985b, 1987a) showed that metabolic fuels that are not utilized by the liver do not have hypophagic effects while metabolic fuels that are extensively metabolized in the liver have hypophagic effects. Compared to non-ruminants, ruminant liver has unique metabolic characteristics. Glucokinase is absent in the ruminant liver (Ballard, 1965) and hepatic removal of glucose is negligible (Stangassinger and Giesecke, 1986). Similarly, ruminant liver does not utilize acetate (Reynolds, 1995) because of a lack of acetyl CoA

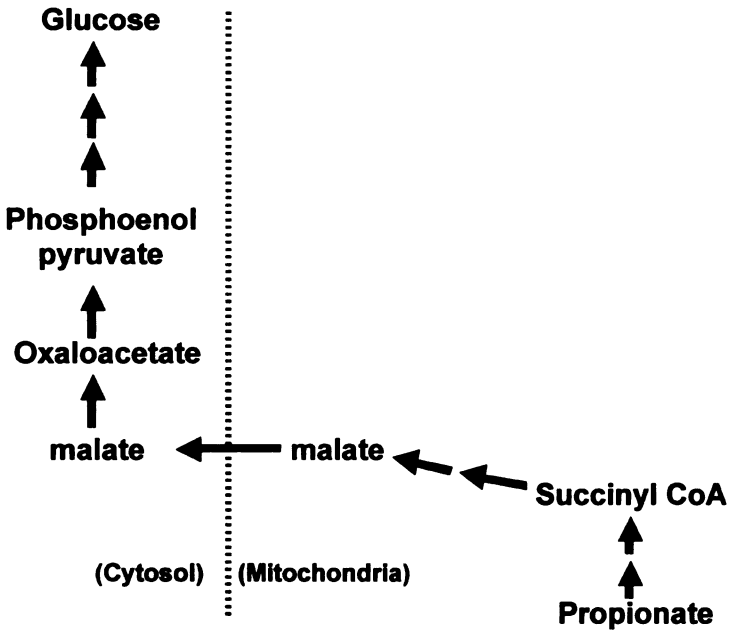

Figure 1. Propionate is utilized for gluconeogenesis.

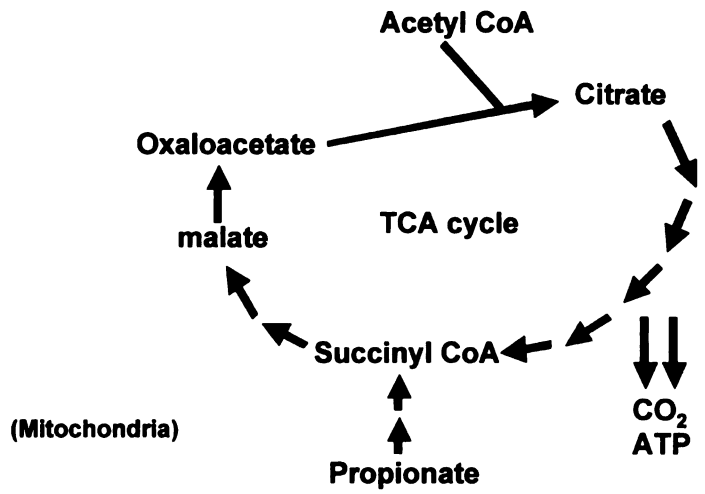

Figure 2. Propionate stimulates oxidative metabolism by driving the TCA cycle.

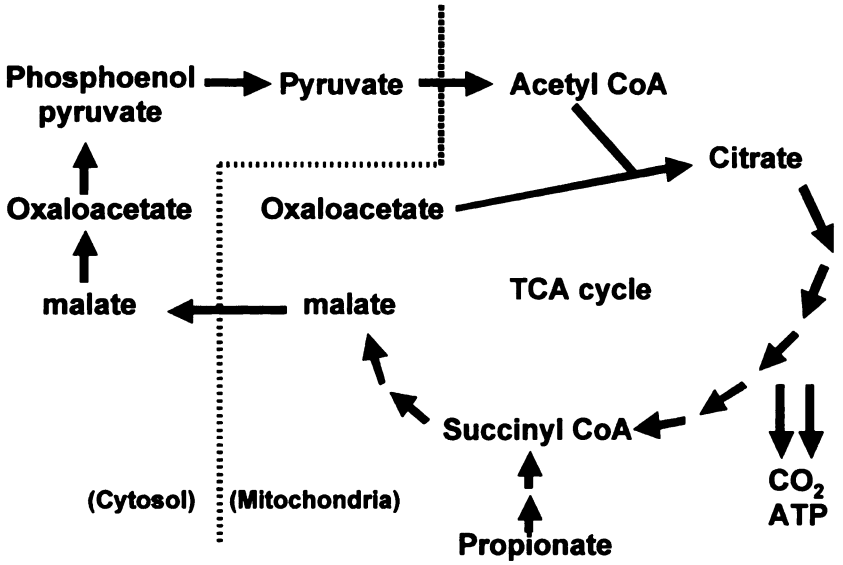

Figure 3. Propionate is oxidized completely to carbon dioxide after entering the TCA cycle as acetyl CoA.

synthetase that is required to activate acetate for further metabolism (Ricks and Cook, 1981). Butyrate is another fermentation acid produced in the rumen, but it is extensively metabolized to β-hydroxybutyrate in the ruminal epithelial tissues, and little is metabolized in the liver (Demigne et al., 1986). Contrarily, non-esterified fatty acids (NEFA) are the metabolic fuels extensively oxidized in the liver (Emery et al., 1992). Ruminant hepatocytes have high activity of propionyl CoA synthetase (Demigne et al., 1986) and are capable of utilizing propionate extensively. Hypophagic effects of propionate and fatty acids compared to other metabolic fuels such as glucose, acetate, and butyrate have been documented extensively for ruminants and have been reviewed recently (Allen, 2000). The relationship between metabolic fuels and their extent of utilization by the liver might indicate that propionate has hypophagic effects by stimulating oxidative metabolism in the liver (Allen, 2000).

Although most experiments in the literature suggest that glucose does not have a hypophagic effect (Allen, 2000), some reported that feed intake is decreased by postruminal infusion of glucose (Dhiman et al., 1993; Knowlton et al., 1998b). In the experiment of Dhiman et al. (1993), abomasal infusion of propionate (0.75 kg) and glucose (1.0 kg) decreased DMI for cows fed 98.2% alfalfa silage diet although glucose infusion did not decrease DMI when 1.2 kg of soy protein was infused with glucose. Knowlton et al. (1998b) reported that infusion of starch (1.5kg/d) into the rumen or abomasum decreased DMI to a similar extent. These inconsistent responses to glucose infusion or post-ruminal starch infusion have not been explained. However, glucose

absorbed in the small intestine is metabolized extensively to lactate by gut tissues. These inconsistent observations might be related to the extent of lactate metabolism in the liver.

Hypophagic effects of lactate have not been consistent for ruminants. Infusion of lactate into the jugular or mesenteric veins did not decrease feed intake (Baile and Forbes, 1974). Although Baile and Mayer (1969) reported that intra-ruminal infusion of lactate decreased feed intake, this might be because of metabolism of lactate to VFAs in the rumen. Lactate concentration in the portal vein can be high as glucose absorption in the small intestine increases because gut tissues metabolize glucose to lactate. Allen (2000) suggested that inconsistent hypophagic effects of lactate might depend on the extent of utilization in the liver because carbon balance and redox state of the liver affect extent of lactate extraction by the liver (Reynolds, 1995). Effects of lactate on feed intake regulation need to be investigated further.

Friedman and his colleagues conducted a series of experiment (Tordoff et al., 1988; Rawson and Frideman, 1994; Rawson et al., 1994a; Rawson et al., 1994b; Koch et al., 1998), and proposed that hepatic ATP concentration regulates feeding behavior. Dose of 2, 5-anhydro-D-mannitol increases food intake in rats (Tordoff et al., 1988) by decreasing ATP concentration in the liver (Rawson et al., 1994a). There are two possible mechanisms for 2, 5-anhydro-D-mannitol to decrease ATP concentration in the liver: trapping phosphate that would be available for ATP synthesis and decreasing glucose utilization. Similarly, ethionine decreases hepatic ATP concentration by trapping adenosine and increases feed intake in rats (Rawson et al., 1994b). Rawson and

Friedman (1994) reported that infusion of Na phosphate maintained ATP concentration in the liver and eliminated the hyperphagic effects of 2, 5-anhydro-D-mannitol. Recently, Koch et al. (1998) showed that temporal relationships exist between feed intake and hepatic ATP concentration.

Hepatic ATP concentration is determined by integrated hepatic metabolism. Friedman and Tordoff (1986) reported that intraperitoneal injection of 2-deoxyglucose (inhibitor of glucose utilization) or methyl palmoxirate (inhibitor of fatty acid oxidation) alone did not decrease feed intake significantly, but combined dose decreased feed intake synergistically. Friedman et al. (1999) evaluated the dose response effect of methyl palmoxirate, and reported that feed intake in rats increased only for the doses of 5 ppm and 10 ppm but not for the dose of 1 ppm. The ATP concentration in the liver was not affected by a 1ppm dose but decreased significantly at 10 ppm. The liver might be able to maintain hepatic energy status by oxidizing glycogen when challenged with 1 ppm of methyl palmoxirate because that level of methyl palmoxirate selectively decreased fatty acid oxidation only in the liver. However, the higher doses of methyl palmoxirate decreased fatty acid oxidation in the muscle as well as the liver, and increased glucose demand by peripheral tissues. The rats might not have continued to oxidize glycogen in the liver because liver glycogen reserves had been depleted. These observations suggest that inhibition of one type of metabolic fuel is sometimes not sufficient to decrease hepatic ATP concentration because the liver can utilize other metabolic fuels to some extent without affecting energy status of the liver.

Although extent of oxidative metabolism and energy status in the liver affect feed intake, it is still not known whether hepatic ATP concentration represents hepatic energy status. Rawson et al. (1996) reported that 2, 5-anhydro-D-mannitol increased feed intake for rats fed a high-carbohydrate diet, but not for rats fed a high-fat diet. Rawson et al. (1996) speculated that the type of diet affects the dominant metabolic fuel in the liver. Lack of hyperphagic effect of 2, 5-anhydro-D-mannitol for a high fat diet might indicate that hepatic oxidative metabolism was not altered by inhibition of glucose utilization in the liver for rats fed a high fat diet. However, the hyperphagic effect of 2, 5-anhydro-D-mannitol was previously attributed to decreased hepatic ATP concentration by trapping phosphate (Rawson et al., 1994a). If 2, 5-anhydro-D-mannitol increased feed intake by directly decreasing ATP concentration in the liver via trapping phosphate, it must have exerted similar hyperphagic effects for a high fat diet in the experiments of Rawson et al. (1996). Lack of hyperphagic effect of 2, 5-anhydro-D-mannitol for a high fat diet might indicate that hepatic ATP concentration is not the sole signal that the CNS senses hepatic energy status by. Reducing equivalent concentration or redox-state in the liver might send additional satiety signals to the CNS (Langhans et al., 1985a). In a recent experiment (Ji et al., 2000), 2, 5-anhydro-D-mannitol decreased ATP concentration in the liver, but did not increase feed intake. They reported that the combined dose of 2, 5-anhydro-D-mannitol and methyl palmoxirate increased feed intake by inhibition of both glucose utilization and fatty acid oxidation (Ji et al., 2000). Ji et al. (2000) suggested that ATP concentration in the liver might not necessarily reflect the availability or turnover rate of ATP. They proposed the phosphorylation potential calculated as ATP/(ADP x Pi) is more sensitive index of dynamic cellular energy status.

The satiety centers in the hypothalamus may sense signals originating in the liver via hepatic vagal afferents because hypophagic effect of glycerol, 3-hydroxybutyrate, malate, lactate and pyruvate were eliminated by selective hepatic vagotomy (Langhans et al., 1985c). Niijima (1983) reported that discharge rate of hepatic vagal afferents were reduced by glucose infusion in a dose-dependent manner. Langhans et al. (1985a) proposed that oxidative metabolism in the liver affects feed intake by hyperpolarizing membrane potentials of hepatocytes. Langhans and Scharrer (1987b) also found that ouabain, an inhibitor of the sodium pump, increased feed intake and suggested that oxidative metabolism within hepatocytes links to sodium pump activity and hepatocyte membrane potential.

Hyperpolarization of hepatocyte membrane caused by enhanced oxidative metabolism decreases discharge rate of hepatic vagus possibly because vagal afferents are in a close association with hepatocytes and some portion of liver parenchyma is innervated (Langhans, 1996). As described previously, hepatocyte membrane can be hyperpolarized by enhanced activity of sodium pump that is driven by ATP, but an alternative mechanism may exist. Langhans (1996) proposed that volumetrically controlled potassium channels might hyperpolarize hepatocyte membrane by efflux of potassium. Extraction of metabolic fuels by the liver may increase size of hepatocytes by drawing water by osmotic pressure, which is independent of oxidative metabolism in the liver. Hepatic vagus nerve has thermosensitive fibers and may sense the ATP concentration in the liver by the rise in liver temperature because oxidative

phosphorylation and oxidation of metabolic fuels are thermogenic (Langhans, 1996). In addition, vagal afferents may have mechanoreceptor to sense the change in size of hepatocytes.

Hepatocytes might release some neuromodulator to vagal afferent terminals. The ATP synthesized in hepatocytes may be secreted into interstitial fluid (Schlosser et al., 1996) and send satiety signals directly to the vagal afferents as a neurotransmitter. Alternatively, the N-methyl-D-aspartate receptor might be involved in transduction of satiety signals originating from the liver because blockage of this receptor by MK-801, non-competitive antagonists for N-methyl-D-aspartate receptor, increased meal size of rats (Burns and Ritter, 1998; Treece et al., 1998; Qian et al., 2000). This receptor exits in the caudal nucleus of the solitary tract, where vagal sensory fibers terminate (Treece et al., 1998), and peripherally on hepatic vagal afferents (Qian et al., 2000). However, it is not known whether the brain senses extent of oxidative metabolism in the liver by this neural pathway. Mechanisms how hepatocytes communicate with hepatic vagal afferents remain to be investigated (Langhans, 1996).

Summary

Feeding highly fermentable grain sometimes decreases voluntary feed intake in lactating dairy cows. Excess propionate production in the rumen is hypophagic and the mechanism might be related to the extent to which propionate stimulates oxidative metabolism in the liver. Research in the literature suggests that enhanced hepatic oxidative metabolism decreases feed intake by sending signals to the central nervous system via hepatic vagal afferents and that hepatic ATP concentration might be an indicator of hepatic oxidative metabolism. A temporal relationship between hepatic ATP variation and feeding behavior was observed in rats, but effects of propionate on hepatic ATP concentration and effects of hepatic oxidative metabolism on discharge rate of vagal afferents need to be investigated for ruminants. In lactating cows, propionate and NEFA have greater hypophagic effects compared to other metabolic fuels such as glucose and acetate. The distinction between the potent hypophagic metabolic fuels and less hypophagic metabolic fuels is whether or not they are metabolized in the liver. Propionate and NEFA are extensively metabolized in the liver while glucose and acetate are not metabolized in ruminant liver. Therefore, extent of oxidative metabolism in the liver likely plays an important role in regulation of feeding behavior in lactating dairy cows.

Hypophagic effects of propionate are documented extensively in the literature. However, it is not known how propionate exerts hypophagic effects and what factors may modulate the hypophagic effects of propionate. Feeding more fermentable grains in diets results in a variable response in feed intake. In addition, the extent of reduction in feed

intake by infusion of propionate is variable in the literature. It is speculated that a threshold exists for propionate to affect feed intake. The threshold response for the effect of propionate on satiety might be related to the balance among propionate flux to the liver and rate of propionate utilization for gluconeogenesis by the liver. If a greater amount of propionate is utilized for gluconeogenesis, less is oxidized in the liver and its hypophagic effects might be reduced. The flux of other metabolic fuels to the liver might also affect hypophagic effects of propionate because energy status of the liver might be maintained by utilizing other dominant metabolic fuels regardless of propionate flux. It is important to understand the mechanisms for regulation of feed intake in lactating cows fed highly fermentable diets. Maximizing energy intake in lactating dairy cows improves productivity of animals and profitability of dairy operations. The role of propionate metabolism in feeding behavior warrants further investigation.

CHAPTER 2

Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations

ABSTRACT

Effects of conservation method of corn grain and dietary starch concentration on DMI and productivity of lactating dairy cows were evaluated. Eight ruminally and duodenally cannulated Holstein cows (55 ± 15.9 days in milk; mean ± SD) were used in a duplicated 4 x 4 Latin square design with a 2 x 2 factorial arrangement of treatments. Experimental diets contained either ground high moisture corn (HM) or dry ground corn (DG) at two dietary starch concentrations (32 vs. 21%). Mean particle size and DM concentration of corn grain were 1863 μm and 63.2 %, and 885 μm and 89.7%, for HM and DG, respectively. Dry matter intake was lower for HM compared to DG treatment in high starch diets (20.8 vs. 22.5 kg/d), but similar for the HM and DG treatments in low starch diets (19.7 vs. 19.6 kg/d). This reduction in dry matter intake is attributed to smaller meal size for HM compared to DG in high starch diets (1.9 vs. 2.3 kg of DM for high starch diets; 2.1 vs. 2.0 kg of DM for low starch diets). Faster starch fermentation for HM in high starch diets might result in satiety sooner. Milk yield was greater when cows were fed high starch diets compared to low starch diets (38.6 vs. 33.9 kg/d) regardless of corn grain treatment. Solids-corrected milk yield was decreased by HM

compared to DG in high starch diets (33.2 vs. 35.2 kg/d), but not in low starch diets (32.5 vs. 31.9 kg/d). This is because of lower milk fat and protein concentration for cows fed HM in high starch diets. Reducing ruminal starch fermentation by substituting DG for HM can increase productivity of lactating cows fed high starch diets.

(**Key words:** conservation method of corn, intake, feeding behavior, nutrient utilization)

Abbreviation Key: HM = High moisture corn; **DG** = Dry ground corn; **TRDOM** = True ruminally degraded organic matter; **NEFA** = Non-Esterified Fatty Acids

INTRODUCTION

An important management goal in dairy nutrition is the maximization of energy intake. Cows in early lactation often experience negative energy balance, and energy status greatly affects peak milk yield and persistency of milk production. One approach to increase energy intake is to increase energy density of diets by feeding more fermentable grains in diets. Theurer et al. (1999) summarized studies comparing steam-flaked corn with steam-rolled corn and steam-flaked sorghum with dry-rolled sorghum, and concluded that steam-flake processing increases milk yield without decreasing DMI. They attributed the positive production responses to greater starch digestibility in the rumen and enhanced microbial protein flow to the duodenum. However, greater starch digestibility results in variable responses in animal productivity. A recent review (Allen, 2000) showed that greater starch digestibility in the rumen is sometimes associated with sharp reductions in DMI which can decrease energy intake. Inconsistent responses to

increased ruminally degraded starch might be because of differences in fermentability of basal diets and energy requirements of animals across the studies. However, the interaction between fermentability of grain and dietary starch concentration on productivity of lactating dairy cows has not been studied extensively.

Corn grain is the major source of dietary starch for lactating dairy cows in the United States. Ruminal starch digestibility of corn grain can be altered by fineness of grinding and by conservation method. Ying et al. (2002) reported that ruminal starch digestibility was reduced over 23% when dry ground corn (DG; mean particle size of 0.8 mm) was substituted for ground high moisture corn (HM; mean particle size of 2.0 mm) with no difference in total tract starch digestibility. We hypothesized that effects of starch digestibility of corn grain on productivity of dairy cows differ by concentration of starch in the diet. Greater ruminally degraded starch from HM is expected to increase productivity of lactating dairy cows compared to DG when cows are fed low starch diets, but decrease DMI and productivity when cows are fed high starch diets because of excess starch fermentation in the rumen. The objective of this experiment was to evaluate effects of high moisture and dry conservation methods of corn grain on DMI and productivity for lactating dairy cows fed two levels of dietary starch concentrations.

MATERIALS AND METHODS

Treatments and Cows

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (55 ± 15.9 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to duplicated 4 x 4 Latin squares balanced for carry over effects with a 2 x 2 factorial arrangement of treatments. Cows were cannulated ruminally and duodenally prior to calving, and assigned randomly to treatment sequence within a square. Treatments were dietary starch concentration (21% vs. 32%) and conservation method of corn grain (HM vs. DG). Treatment periods were 21 d with the final 10 d used to collect samples and data.

One corn hybrid (Pioneer 3730) was grown in 1998, and half of the field was harvested as HM at a DM concentration of 63.2%. High moisture corn was ground to a mean particle size of 1863 μm and ensiled in a 2.4 m x 9.0 m silage bag (Ag Bagger®, Ag Bag Corp., Blair, NE). The remaining half of the field was harvested as dry corn. Dry corn was finely ground to a mean particle size of 885 μm. Nutrient composition for corn grain treatments is shown in Table 1. Experimental diets contained either HM or DG, corn silage (50% of forage DM), alfalfa silage (50% of forage DM), a premix of protein supplements (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins (Table 2). All diets were formulated for 18% dietary CP concentration, and fed as total mixed rations.

TABLE 1. Nutrient composition of corn grains used to formulate experimental diets.

	HM[1]	DG[2]
DM%[3]	63.2	89.7
	-% of DM-	
Starch	67.0	70.5
NDF[3]	7.1	10.3
ADF	2.4	3.0
Crude Protein	9.1	9.1
Ether extract[4]	6.3	7.3
Ash[4]	1.6	1.3
Lactate	2.4	...
Ethanol	0.2	...
VFA	0.8	...
Organic acids	3.9	...
Mean particle size (μm)[3]	1863	885

[1] HM: High moisture corn

[2] DG: Dry ground corn

[3] Differ significantly between high moisture corn and dry ground corn ($P < 0.0001$).

[4] Differ significantly between high moisture corn and dry ground corn ($P < 0.05$).

TABLE 2. Ingredients and nutrient composition of experimental diets (% of dietary DM). [1]

	High starch		Low starch	
	HM[1]	DG[2]	HM	DG
Diet Ingredients				
Dry ground corn	...	31.6	...	10.8
High moisture corn	32.0	...	11.0	...
Corn silage	20.8	20.9	31.8	32.0
Alfalfa silage	22.2	22.3	34.0	34.1
Protein mix[4]	21.4	21.5	19.5	19.5
Vitamin & mineral mix[5]	3.6	3.7	3.7	3.6
Nutrient Composition				
DM	48.8[b]	53.0[a]	42.8[d]	43.8[c]
OM	93.4[a]	93.5[a]	92.2[b]	92.3[b]
Starch	31.1[a]	32.2[a]	21.0[b]	21.3[b]
NDF	23.1[b]	24.2[b]	30.1[a]	30.5[a]
ADF	15.2[b]	15.4[b]	20.8[a]	20.9[a]
Lignin	2.2[b]	2.2[b]	3.3[a]	3.3[a]
Indigestible NDF[6]	10.9[b]	11.2[b]	14.6[a]	14.7[a]
CP	18.0	18.0	18.3	18.3
Ether extract	5.2[b]	5.5[a]	4.8[c]	4.9[c]
Forage NDF	16.5[b]	16.5[b]	25.3[a]	25.4[a]
Corn grain starch (% of dietary starch)	68.8[a]	69.8[a]	35.0[b]	36.2[b]

[1] Means for nutrient composition in same row followed by different superscript letters differ significantly ($P < 0.05$).

[2] HM: High moisture corn

[3] DG: Dry ground corn

[4] Protein mix contained 70.2% soybean meal, 26.9% distillers grain, and 2.9% blood meal.

[5] Vitamin & mineral mix contains 50.0% dry ground corn, 15.6% sodium bicarbonate, 11.3% limestone, 10.4% dicalcium phosphate, 4.9% salt, 4.5% magnesium oxide, 2.5% trace mineral premix, 0.36% vitamin A, 0.31% vitamin D, and 0.18% vitamin E.

[6] Indigestible NDF: estimated after 120-h in vitro ruminal fermentation.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls, and fed once daily (1400 h) at 110% of expected intake. The amount of feed offered and orts were weighed for each cow daily during the collection period. Samples of all dietary ingredients (0.5 kg) and orts (12.5%) were collected daily and composited into one sample per cow per period. Cows were milked twice daily in their stalls during the feeding behavior monitoring period (d 16 to d 19), and in a milking parlor the rest of period. Milk yield was measured daily during the collection period and was averaged over the collection period. Milk was sampled at every milking on d 12, 16, and 19 of each period and analyzed for fat, crude protein, and lactose with infrared spectroscopy by Michigan DHIA (East Lansing). Body weight was measured on two consecutive days immediately prior to the start of the first period, and on d 19 and d 21 of each period. Empty body weight was measured after evacuation of ruminal digesta. The BCS was determined [(Wildman, 1982); five-point scale where 1 = thin to 5 = fat] by three trained investigators blinded to treatments immediately prior to the start of the first period and on d 21 of each period.

Feeding behavior and ruminal pH were monitored from d 16 through d 19 (96 h) of each period by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, water consumption, and ruminal pH were recorded for each cow every 5 sec. Daily means were calculated for number of meal bouts per day, interval between meals, meal size, eating time, ruminating time, and total chewing time. These response variables were calculated as daily means, then averaged

over the four days for each period. Blood samples and ruminal fluid samples were collected every 20 min for 24 h by automated sample collection system (Allen et al., 2000), starting at 1200 h on d 16. Blood was sampled from a jugular vein through a catheter inserted 1 d prior to sample collection.

Ruminal fluid was centrifuged at 2,000 x g for 15 min immediately after collection, and supernatants were frozen at -20° C until analysis. Blood samples were collected into two tubes: one with lithium heparin and the other with potassium oxalate and sodium fluoride as a glycolytic inhibitor. Both were centrifuged at 2,000 x g for 15 min immediately after sample collection, and plasma was harvested and frozen at -20° C until analysis.

Sample and Statistical Analysis

Diet ingredients and orts 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 (1mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, ADF, lignin, indigestible NDF, CP, and starch. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. Concentrations of NDF and ADF were determined [(VanSoest et al., 1991); method A for NDF]. Crude protein was analyzed according to Hach et al. (1985). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, and glucose concentration was measured using a glucose oxidase (Glucose kit #510; Sigma Chemical Co., St. Louis, MO) and absorbance was determined with micro-plate reader

(SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Indigestible NDF was estimated as NDF residue after 120-h in vitro fermentation (Goering and VanSoest, 1990). Concentrations of all nutrients except for DM were expressed as percentages of DM determined by drying at 105° C in a forced-air oven. Corn grain was dry sieved (Sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan), using a sieve shaker (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant, and mean particle size of corn grain was calculated (ASAE, 1968). The TRDOM was calculated as described by Oba and Allen (2002a).

Ruminal fluid was analyzed for VFA and lactate concentrations. Samples were centrifuged at 26,000 × *g* for 15 min, and supernatant (600 μL) was mixed with 600 μL $Ca(OH)_2$ and 300 μL of $CuSO_4$ containing crotonic acid as an internal marker in 1.7 ml micro centrifuge tubes. Samples were centrifuged at 12,000 × *g* for 10 min, and supernatant (1000 μl) was taken and mixed with 28 μl of H_2SO_4 in 1.5 ml micro centrifuge tubes. Samples were frozen and thawed twice, and centrifuged at 12,000 × *g* for 10 min. Supernatant was transferred to HPLC vials. Concentrations of VFA and lactate of the supernatant were determined by HPLC as described by Dado and Allen (1995). Rate of VFA production (moles/d) was estimated from the measured true ruminally degraded organic matter (Oba and Allen, 2002a) and microbial efficiency (Oba and Allen, 2002b) according to Allen (1997).

Plasma samples were analyzed for concentrations of acetate, glucose, non-esterified fatty acid (NEFA), insulin, and growth hormone. Plasma was processed to

quantify acetate concentration as described for ruminal fluid. Due to greater protein concentration for plasma samples, the first stage of sample processing was duplicated to obtain enough supernatant (1000 µl) to be mixed with 28 µl of H_2SO_4 in 1.5 ml micro centrifuge tubes. Plasma growth hormone concentration was determined by radioimmunoassay (Gaynor et al., 1995). Commercial kits were used to determine plasma concentration of glucose (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), NEFA (NEFA C-kit; Wako Chemicals USA, Richmond, VA), and insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Frequency and amplitude of insulin peaks were quantified according to Merriam and Wachter (1982).

All data were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + e_{ijkl}$$

where

μ = overall mean,

C_i = random effect of cow (j = 1 to 8),

P_j = fixed effect of period (k = 1 to 4),

T_k = fixed effect of treatment (l = 1 to 4),

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

Period x treatment interaction was originally evaluated, but it was removed from the statistical model because interaction was not significant for response variables of primary interest. Orthogonal contrasts were made for the effect of dietary starch

concentration, conservation method of corn grain, and interaction of dietary starch concentration and conservation method. Treatment effects and their interaction were declared significant at $P < 0.05$ and $P < 0.10$, respectively, and tendency for treatment effects and their interaction were declared at $P < 0.10$ and $P < 0.15$, respectively.

RESULTS AND DISCUSSION

DMI and Ruminal Fermentation

Cows fed high starch diets had greater DMI compared to cows fed low starch diets ($P < 0.001$; Table 3), and this might be attributed to greater physical fill in the rumen for low starch diets. Low starch diets contained more forage NDF compared to high starch diets (25.3 vs. 16.5 % DM), and forage NDF is a primary factor reducing DMI by physical fill in the rumen (Allen, 2000). Interaction of dietary starch concentration and conservation method of corn grain was significant for DMI ($P < 0.07$). The DG treatment increased DMI by 1.7 kg (22.5 vs. 20.8 kg/d) compared to HM treatment when fed in high starch diets but had no effect (19.6 vs. 19.7 kg/d) when fed in low starch diets. Consistent with treatment effects on DMI, meal size was greater for DG treatment compared to HM treatment (2.3 vs. 1.9 kg) when cows were fed high starch diets, but not when cows were fed low starch diets (2.0 vs. 2.1 kg).

The number of meal bouts per day was greater for high starch diets compared to low starch diets ($P < 0.04$). The HM treatment tended to increase the number of meal

TABLE 3. Effects of corn grain conservation method at two dietary starch contents on feeding behavior.

	High starch		Low starch			*P* value		
	HM[1]	DG[2]	HM	DG	SE	Starch[3]	Corn[4]	INT[5]
DMI (kg/d)	20.8	22.5	19.7	19.6	0.5	< 0.001	0.12	0.07
Meal bouts (/d)	11.4	9.9	9.7	9.5	0.5	0.04	0.09	0.16
Meal length (min)	28.0	31.1	36.2	35.0	1.9	< 0.01	0.56	0.28
Intermeal interval (min)	93.9	105.0	103.5	101.2	4.5	0.57	0.33	0.17
Meal size (kg)								
DM	1.9	2.3	2.1	2.0	0.1	0.53	0.21	0.06
Starch	0.59	0.74	0.44	0.43	0.03	< 0.0001	0.03	0.02
NDF	0.44	0.54	0.61	0.59	0.03	< 0.01	0.15	0.06
Eating time (min/d)	253.2	258.9	300.4	287.0	9.2	< 0.001	0.77	0.38
Ruminating time (min/d)	427.1	438.2	493.4	478.3	12.5	< 0.001	0.87	0.31
Total chewing time (min/d)	680.2	697.0	793.8	765.3	16.2	< 0.001	0.77	0.22
Eating chews (/d)	17411	18226	20891	19452	794	< 0.01	0.83	0.23
Ruminating chews (/d)	25530	26403	30342	28699	1036	< 0.01	0.72	0.25
Total chews (/d)	42941	44632	51233	48150	1601	< 0.01	0.71	0.17

[1]HM: High moisture corn
[2]DG: Dry ground corn
[3]Starch: Effect of dietary starch content
[4]Corn: Effect of conservation method of corn grain
[5]INT: Interaction of dietary starch content and conservation method of corn grain.

bouts compared to DG treatment ($P < 0.09$), and the difference between HM and DG treatments was numerically greater (interaction $P < 0.16$) when cows were fed high starch diets (11.4 vs. 9.9 /d) compared to low starch diets (9.7 vs. 9.5 /d). Although DMI is a function of meal size and meal frequency, greater DMI for DG treatment in high starch diets is explained solely by greater meal size because DG treatments did not increase meal frequency. On the contrary, when high starch diets were fed, cows consuming HM corn tended to eat more frequently, but were not able to compensate for the decreased meal size, resulting in an overall depression in DMI.

The reduction in DMI and meal size for HM treatment in high starch diets can be attributed to greater ruminal fermentation. Because dietary forage NDF concentration was similar between HM and DG treatment within the same dietary starch concentration, differences in physical fill were not likely to be responsible for differences in DMI. For cows fed high starch diets, true ruminally degraded OM (TRDOM) was greater for HM compared to DG treatment (11.3 vs. 10.3 kg/d; Table 4), and calculated rate of VFA production was greater for cows fed HM corn compared to DG corn (68.8 vs. 52.0 moles/d). A reduction in DMI with increased ruminal fermentation has been reported previously. McCarthy et al. (1989) increased starch digestibility in the rumen by replacing ground shelled corn with steam rolled barley in diets containing more than 40% starch at DM basis, and reported approximately 3 kg depression in DMI. Overton et al. (1995) also showed that increased substitution of barley for dry corn linearly decreased DMI for lactating dairy cows.

Lower DMI for HM treatment when cows were fed high starch diets might be explained by higher propionate production in the rumen. Propionate has greater hypophagic effect compared to acetate (Farningham and Whyte, 1993; Sheperd and Combs, 1998). Intra-ruminal infusion of propionate decreased DMI compared to acetate on both an iso-molar (Farningham and Whyte, 1993) and an iso-energetic basis (Sheperd and Combs, 1998). Although greater fermentation in the rumen is often associated with low ruminal pH and greater osmolarity of ruminal fluid, their hypophagic effects have not been shown. Choi and Allen (1999) showed that ruminal pH *per se* does not have a direct effect on DMI and feeding behavior of lactating dairy cows. They also found that infusion of hyper-osmotic solutions of NaCl decreased meal size, but did not affect DMI because cows compensated for smaller meal size by increasing meal frequency.

When cows were fed low starch diets, HM treatment did not decrease DMI compared to DG treatment. Greater starch digestibility in the rumen does not necessarily decrease DMI (Theurer et al., 1999; Knowlton et al., 1998; Grings et al., 1992; Callison et al., 2001). Inconsistent effect of ruminal starch digestibility on feed intake might imply that a threshold exists for propionate to affect DMI. Feeding behavior might be controlled by a dominant mechanism related to stimulation of tension receptors by ruminal fill until a mechanism related to propionate begins to dominate on highly fermentable diets.

High starch diets decreased molar ratio of acetate ($P < 0.0001$; Table 4), iso-butyrate ($P < 0.001$), and iso-valerate ($P < 0.03$) compared to low starch diets. High

TABLE 4. Effects of corn grain conservation method at two dietary starch contents on rumen fermentation.

	High starch		Low starch			*P* value		
	HM[1]	DG[2]	HM	DG	SE	Starch[3]	Corn[4]	INT[5]
Ruminal pH								
Daily mean	6.12	6.13	6.25	6.32	0.05	< 0.01	0.41	0.48
Daily variance	0.120	0.121	0.109	0.070	0.016	0.08	0.23	0.20
TRDOM[6] (kg/d)	11.3	10.3	9.3	7.7	0.6	< 0.001	0.03	0.60
Total VFA (mM)	101.8	105.5	102.3	97.0	2.9	0.18	0.78	0.14
VFA (mol/100 mol)								
Acetate	58.1	57.8	61.5	62.4	0.8	< 0.0001	0.68	0.44
Propionate	24.7	25.6	21.6	21.2	1.0	< 0.001	0.80	0.52
Iso-butyrate	1.09	1.08	1.20	1.30	0.04	< 0.001	0.20	0.32
Butyrate	12.1	11.8	12.1	11.5	0.4	0.68	0.25	0.66
Iso-Valerate	1.76	1.68	1.89	2.00	0.10	0.03	0.90	0.31
Valerate	2.29	2.04	1.78	1.63	0.15	< 0.01	0.19	0.74
Acetate: Propionate ratio	2.51	2.38	2.91	3.02	0.11	< 0.0001	0.92	0.28
VFA production[7] (moles/d)								
Total	68.8	52.0	58.0	43.7	5.7	0.13	0.01	0.89
Acetate	40.1	29.9	36.1	27.4	3.3	0.37	0.01	0.88
Propionate	16.8	13.4	12.2	9.3	1.6	0.02	0.05	0.99
Butyrate	8.4	6.2	6.9	5.0	0.7	0.07	< 0.01	0.91

[1]HM: High moisture corn

[2]DG: Dry ground corn

[3]Starch: Effect of dietary starch content

[4]Corn: Effect of conservation method of corn grain

[5]INT: Interaction of dietary starch content and conservation method of corn grain.

[6]TRDOM: True ruminally degraded organic matter

[7]VFA production rate (moles/d) was estimated from the measured true ruminally degraded organic matter (Oba and Allen, 2002a) and microbial efficiency (Oba and Allen, 2002b) according to the calculation method described by Allen (1997)

starch diets increased molar ratio of propionate ($P < 0.001$) and valerate ($P < 0.01$) concentration compared to low starch diets. Volatile fatty acid profile was not affected by conservation method of corn grain. Total volatile fatty acid concentration was not affected by treatments. It is noteworthy that the concentration of total VFA in ruminal fluid did not reflect the amount of OM truly fermented in the rumen. Although treatment means for TRDOM varied from 7.7 to 11.3 kg/d in this experiment, no relationship was observed between TRDOM and total VFA concentration (Figure 1). Total VFA concentration did not indicate fermentation acid production in the rumen because treatment effect on rates of absorption and passage compensated for effects of production rates on VFA concentration.

Daily mean ruminal pH was lower for high starch diets compared to low starch diets ($P < 0.01$), but was not affected by corn grain treatment. In this experiment, daily mean ruminal pH was above 6.1 even for high starch diets regardless of corn grain treatments, and it was close to the pK_a for bicarbonate, the major buffer secreted in saliva. This indicates that diet fermentability does not negatively affect ruminal pH if buffering capacity of ruminal digesta exceeds fermentation acid production in the rumen (Allen, 1997). In this experiment, eating time, ruminating time, and total chewing time were greater for low starch diets compared to high starch diets ($P < 0.001$). These observations were consistent with higher ruminal pH for low starch diets because of greater salivary buffer secretion and less fermentation acid production in the rumen for low starch diets compared to high starch diets. However, chewing activities were not affected by corn grain treatment. Despite greater fermentation in the rumen, HM

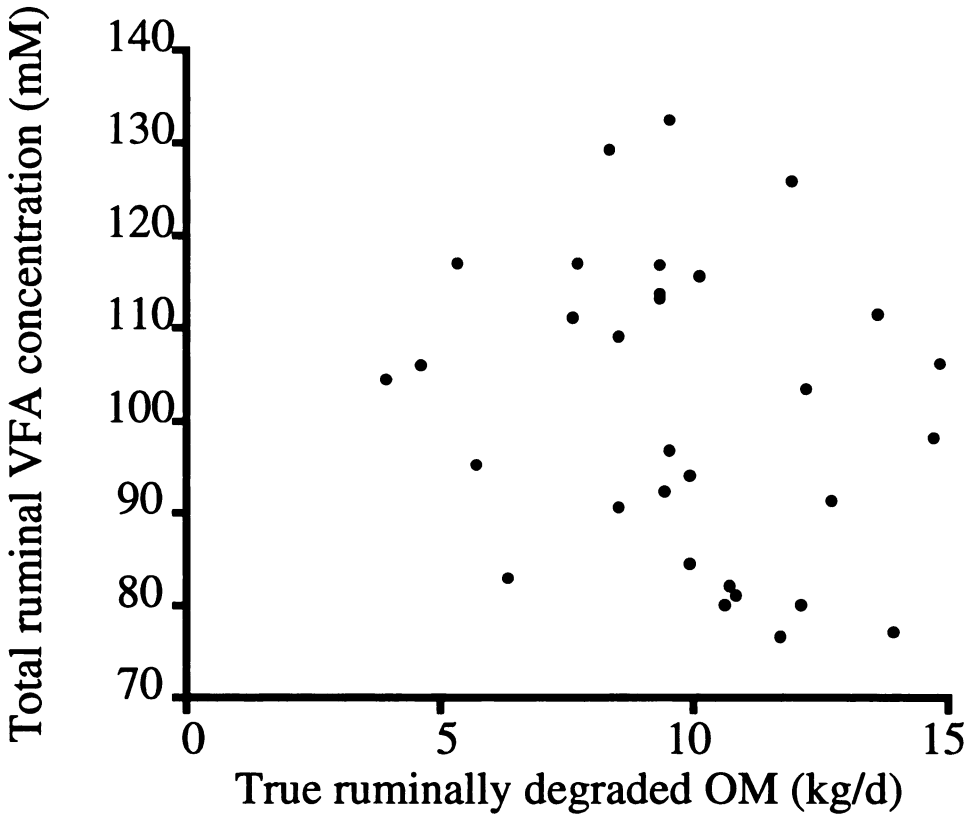

Figure 1. Relationship between true ruminally degraded OM (TRDOM) and total ruminal VFA concentration ($P > 0.21$, $r^2 = 0.05$).

treatment did not decrease ruminal pH possibly because buffering capacity of ruminal digesta was maintained by sufficient chewing activity and saliva flow. Because ruminal pH and total VFA concentration were not directly related to fermentation acid production in the rumen, neither explained hypophagic effects of the HM treatment.

Milk Production and Plasma Metabolites

Milk yield and SCM yield were greater for high starch diets compared to low starch diets ($P < 0.0001$ and $P < 0.02$; Table 5). An interaction of dietary starch concentration and conservation method of corn grain was detected for SCM yield ($P < 0.10$). The DG treatment increased SCM yield by 2.0 kg (35.2 vs. 33.2 kg/d) when fed in high starch diets but decreased SCM by 0.6 kg (31.9 vs. 32.5 kg/d) when fed in low starch diets. Significant interactions for main effects were also observed for milk fat concentration ($P < 0.06$) and milk protein concentration ($P < 0.07$). The DG treatment increased milk fat concentration (3.59 vs. 3.05 %) and milk protein concentration (3.02 vs. 2.98 %) compared to HM treatment when cows were fed high starch diets. However, HM treatment increased milk fat concentration (3.95 vs. 3.73 %) and milk protein concentration (2.94 vs. 2.87 %) compared with DG treatment when cows were fed low starch diets.

Cows fed low starch diets benefited more from consuming HM compared to DG possibly because of greater ruminal fermentation. However, when DG replaced HM in high starch diets, productivity of lactating cows was enhanced from higher DMI. In

TABLE 5. Effects of corn grain conservation method at two dietary starch contents on productivity.

	High starch		Low starch			*P* value		
	HM[1]	DG[2]	HM	DG	SE	Starch[3]	Corn[4]	INT[5]
Yield (kg/d)								
Milk	38.8	38.4	33.4	34.3	0.9	< 0.0001	0.78	0.45
FCM (3.5%)	35.7	38.7	35.7	35.4	1.0	0.12	0.21	0.14
SCM	33.2	35.2	32.5	31.9	0.8	0.02	0.40	0.10
Milk fat	1.17	1.35	1.33	1.27	0.06	0.51	0.31	0.04
Milk protein	1.14	1.13	0.98	0.97	0.02	< 0.0001	0.70	0.82
Milk lactose	1.90	1.87	1.63	1.67	0.05	< 0.0001	0.94	0.52
Milk Composition (%)								
Milk fat	3.05	3.59	3.95	3.73	0.17	< 0.01	0.37	0.06
Milk protein	2.98	3.02	2.94	2.87	0.03	< 0.01	0.67	0.07
Milk lactose	4.93	4.93	4.83	4.87	0.02	< 0.001	0.21	0.42
BW change (kg/d)	0.36	0.21	-0.68	-0.80	0.29	< 0.01	0.76	0.83
BCS change (/21d)	0.10	0.04	-0.09	-0.12	0.06	< 0.01	0.47	0.75

[1]HM: High moisture corn

[2]DG: Dry ground corn

[3]Starch: Effect of dietary starch content

[4]Corn: Effect of conservation method of corn grain

[5]INT: Interaction of dietary starch content and conservation method of corn grain.

agreement with our observation, Moore et al. (1992) reported greater milk yield when starch digestibility was increased by replacing dry-rolled sorghum with steam-flaked sorghum (flake density of 0.40 kg/L). However, flaked sorghum with a density of 0.27 kg/L (associated with more rapid starch hydrolysis) decreased DMI and milk yield. Theurer et al. (1999) showed that greater starch digestibility by steam-flake processing of corn and sorghum grain increased milk yield with similar DMI. Greater starch digestibility in the rumen might improve milk yield if DMI is maintained and if extra energy is not directed to body reserves by greater insulin secretion. Experiments in which a reduction in milk yield was associated with enhanced starch digestibility in the rumen also reported reduced DMI with greater ruminal starch digestion (MaCarthy et al., 1989; Overton, 1995). Optimal ruminal starch digestibility is dependent upon starch concentration and fermentability of diets.

High starch diets decreased milk fat concentration, but increased BW gain ($P < 0.01$) and body condition score (BCS) gain ($P < 0.01$) compared to low starch diets. High starch diets decreased daily mean ruminal pH, but increased daily variance for ruminal pH. We previously proposed that partitioning of absorbed fuels to milk or body reserves is influenced by variation in ruminal pH because it determines pattern of supply of metabolic fuels from the rumen to the blood circulation (Oba and Allen, 2000). Rate of fermentation acid absorption from the rumen is a function of ruminal pH. Ruminal pH with less daily fluctuation might result in more consistent supply of metabolic fuels from the rumen to the blood circulation while a greater fluctuation in ruminal pH might indicate more pulsatile energy supply. A more pulsatile energy supply may stimulate

insulin secretion, increasing energy metabolite utilization in adipose tissues more than milk fat synthesis (Oba and Allen, 2000). In agreement with this theory, high starch diets increased insulin concentration ($P < 0.01$; Table 6). Diurnal pattern for plasma insulin concentration (Figure 2) shows that high starch diets consistently increased plasma insulin concentration compared to low starch diets. Greater daily means for plasma insulin concentration for high starch diets are attributed to a greater baseline of insulin secretion ($P < 0.001$) and enhanced amplitude of insulin peaks ($P < 0.001$) compared to low starch diets.

A significant interaction of dietary starch concentration and conservation method of corn grain was observed for milk fat concentration and milk fat yield, indicating milk fat was depressed for HM treatment only for cows fed high starch diets. However, the reasons for this milk fat depression are not known. Corn grain treatment did not affect mean plasma insulin concentration although the change in plasma insulin concentration during a meal was greater for cows fed HM compared to DG ($P < 0.03$). Corn grain treatment affected insulin secretion only transiently. *Trans*-$C_{18:1}$ fatty acids produced in the rumen can decrease milk fat yield, and increased *trans*-$C_{18:1}$ fatty acid production in the rumen was related to low ruminal pH when cows were fed high concentrate diets (Kalscheur et al., 1997; Kennelly et al., 1999). However, ruminal pH was not different between HM and DG treatments for cows fed high starch diets. Therefore, the milk fat depression in this experiment might not be caused by enhanced production of *trans*-$C_{18:1}$ fatty acids in the rumen although they were not measured.

TABLE 6. Effects of corn grain conservation method at two dietary starch contents on plasma metabolite and hormone concentration.

	High starch		Low starch			*P* value		
	HM[1]	DG[2]	HM	DG	SE	Starch[3]	Corn[4]	INT[5]
Glucose (mg/dl)								
Daily mean	61.0	60.7	59.6	57.9	0.8	< 0.01	0.53	0.76
Daily variance	15.5	15.5	18.4	13.1	4.0	0.96	0.44	0.44
Acetate (mM)								
Daily mean	0.91	0.88	0.98	0.98	0.04	0.04	0.64	0.71
Daily variance	0.14	0.10	0.18	0.15	0.04	0.20	0.38	0.89
NEFA[6] (meq/L)								
Daily mean	154	158	214	177	21	0.07	0.44	0.35
Daily variance	1081	1499	2693	2462	531	0.19	0.35	0.29
Growth hormone (ng/ml)								
Daily mean	2.09	2.00	2.68	2.70	0.21	< 0.01	0.86	0.81
Daily variance	3.30	2.51	4.08	4.22	1.10	0.14	0.67	0.61
Insulin (μIU/ml)								
Daily mean	14.8	13.6	11.1	10.3	0.9	< 0.001	0.54	0.51
Daily variance	37.6	21.4	14.4	12.7	7.7	0.04	0.25	0.31
At initiation of meal	12.8	12.7	9.3	9.5	0.8	< 0.001	0.99	0.81
At end of meal	18.4	15.7	12.7	11.8	1.4	< 0.01	0.18	0.49
Change during meal	5.5	3.0	3.4	2.3	0.8	0.07	0.03	0.35
Baseline	9.5	9.0	7.0	7.1	0.6	< 0.001	0.77	0.60
Numbers of peak (/d)	20.6	20.4	20.4	20.5	0.9	0.93	0.93	0.81
Peak amplitude	8.7	7.5	5.6	5.4	0.7	< 0.001	0.26	0.43
Peak length (min)	46.3	47.6	48.4	47.2	2.9	0.73	0.99	0.62
Inter-peak interval (min)	70.0	70.2	72.5	70.0	3.2	0.71	0.69	0.65

[1]HM: High moisture corn; [2]DG: Dry ground corn; [3]Starch: Effect of dietary starch content; [4]Corn: Effect of conservation method of corn grain; [5]INT: Interaction of dietary starch content and conservation method of corn grain.

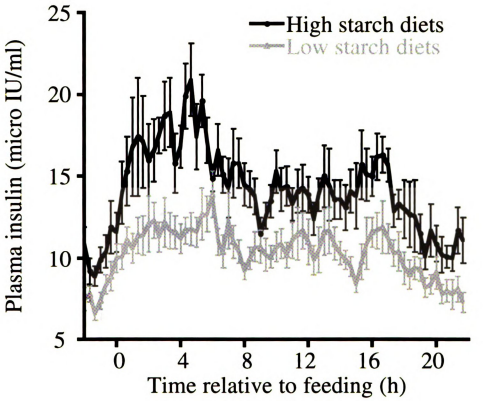

Figure 2. Effect of dietary starch concentration on plasma insulin concentration relative to feeding time.

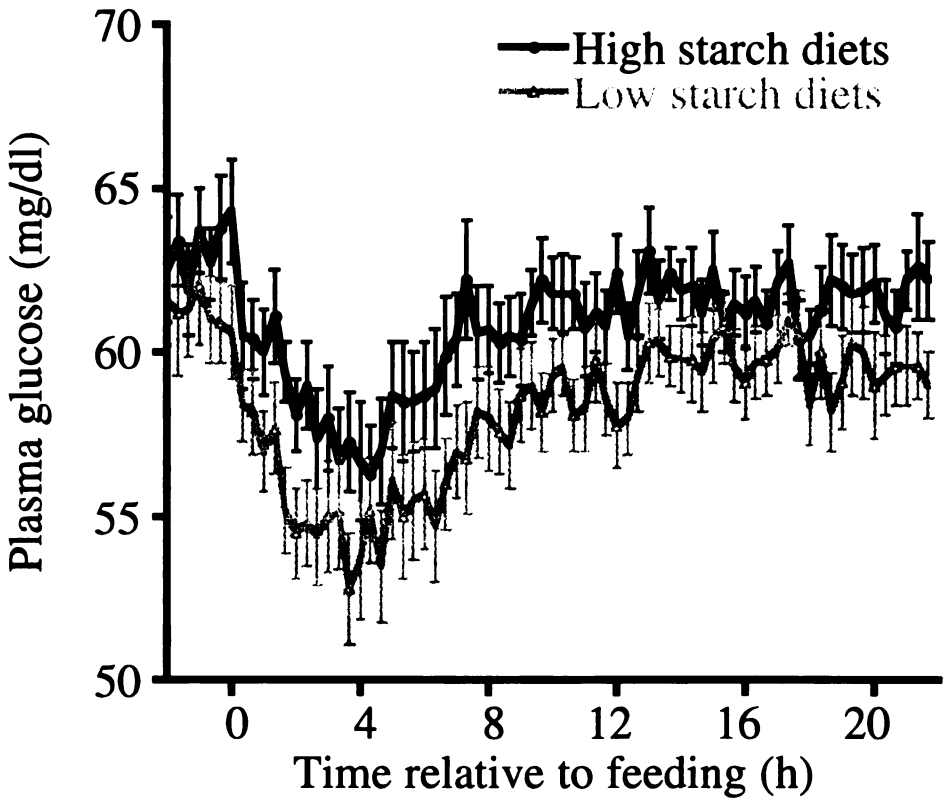

Figure 3. Effect of dietary starch concentration on plasma glucose concentration relative to feeding time.

High starch diets decreased plasma concentrations of growth hormone ($P < 0.01$) and NEFA ($P < 0.07$) compared to low starch diets. Lower plasma acetate concentration ($P < 0.04$) for high starch diets might be because of less acetate flux from the rumen and greater acetate utilization in peripheral tissues from stimulation by insulin compared to low starch diets. Plasma concentration of glucose was greater for high starch diets compared to low starch diets ($P < 0.01$; Figure 3), but it is noteworthy that plasma glucose concentration decreased after feeding regardless of diet. This reduction in plasma glucose concentration is partially attributed to an increase in plasma insulin concentration after feeding. Because insulin decreases gluconeogenesis and increases glycogen synthesis in the liver, we speculate that absorbed propionate is not directly metabolized to glucose but transiently utilized for glycogen synthesis after feeding, and that glucose is released from glycogen storage over time.

CONCLUSION

Substitution of DG for HM reduced ruminal fermentation and increased DMI and SCM yield compared to HM in high starch diets. This is consistent with the theory that propionate production in the rumen can affect satiety and DMI. Although DG treatment decreased TRDOM, DG treatment increased productivity of lactating cows fed high starch diets because of greater DMI. However, cows fed low starch diets increased productivity when consuming HM compared to DG. Optimal ruminal starch digestibility is dependent upon the starch concentration and fermentability of diets.

CHAPTER 3

Dose-response effects of intra-ruminal infusion of propionate on energy intake and feeding behavior of lactating dairy cows

ABSTRACT

Dose-response effects of intra-ruminal infusion of propionate on feeding behavior of lactating dairy cows were evaluated with eight ruminally cannulated Holstein cows past peak lactation. In experiment 1, treatments were mixtures of propionic acid and acetic acid infused into the rumen continuously for 14 h at a rate of 16.7 mmol/min. Treatment solutions contained propionic acid at 8 different concentrations. In experiment 2, treatments were mixtures of sodium propionate and sodium acetate infused into the rumen continuously for 14 h at a rate of 25 mmol/min. Treatment solutions contained sodium propionate at 4 different concentrations. Treatment solutions contained acetic acid and sodium acetate, respectively for experiment 1 and experiment 2, to keep the osmolarity and pH (but not energy concentration) of infusates constant across the treatments within an experiment. Experimental diets were formulated to contain 29% NDF, and dry cracked corn (mean particle size = 3.6 mm) was the major source of starch. Infusion started 2 h before feeding and ended 12 h after feeding, and feeding behavior was monitored for 12 h after feeding using a computerized data acquisition system. Total metabolizable energy (ME) intake was calculated by adding the energy of infusates to

dietary energy intake. Experimental designs were an 8 x 8 Latin square for experiment 1 and a duplicated 4 x 4 Latin square for experiment 2. In experiment 1, as infusion rate of propionate increased, dry matter intake ($P < 0.01$) and total ME intake ($P < 0.05$) decreased linearly. In Experiment 2, as infusion of propionate increased, dry matter intake ($P < 0.0001$) and meal size ($P = 0.03$) decreased linearly, and number of meal bouts tended to decrease linearly ($P = 0.08$). Total ME intake also decreased linearly ($P < 0.0001$) as proportion of propionate of the VFA infused increased. This indicates that the reduction in dietary energy intake due to propionate infusion was greater than the energy supplied from propionate infusions. Our results demonstrate that propionate plays an important role in feed intake regulation by affecting both satiety and hunger.

(**Key words:** propionate infusion, feed intake, feeding behavior)

Abbreviation Key: ME = metabolizable energy

INTRODUCTION

Maximizing energy intake is an important goal for nutritional management for high producing dairy cows. Although feeding more fermentable grains in diets increases energy density of diets, excess fermentation in the rumen sometimes decreases DMI and does not necessarily increase energy intake in lactating cows (Allen, 2000). However, greater ruminal fermentation is more desirable to increase microbial protein production as well as energy intake unless DMI is decreased. Therefore, it is important to understand mechanisms that regulate voluntary feed intake when cows are fed high grain diets.

Greater ruminal fermentation is characterized by a variety of factors such as low ruminal pH, increased osmolarity of ruminal fluid, and more fermentation acid production. Choi and Allen (1999) showed that propionate has greater hypophagic effects in lactating dairy cows compared to acetate although pH of ruminal fluid per se did not affect DMI and feeding behavior. In addition they reported that intra-ruminal infusion of hyper-osmotic solution decreased meal size but did not decrease DMI because cows increased meal frequency. Hypophagic effects of propionate have been documented extensively for ruminants (Anil and Forbes, 1980; Elliot et al., 1985; Farningham and Whyte, 1993; Mbanya et al., 1993; Hurtaud et al., 1993; Wu et al., 1994; Sheperd and Combs, 1998).

However, some experiments in the literature reported that propionate infusion did not decrease feed intake (Deetz and Wangsness, 1981; Quigley and Heitmann, 1991; De Jong et al., 1981; Anil et al., 1993). Inconsistent hypophagic effects of propionate might be explained by a threshold response of propionate for feed intake regulation. Infusion of propionate might not affect DMI and feeding behavior unless amount of propionate exceeds a threshold. This concept agrees with observations that feeding more fermentable grains does not always decrease DMI in lactating dairy cows (Allen, 2000). Dose response effects of propionate on feed intake were previously investigated for lactating dairy cows (Anil et al., 1993) and sheep (Farningham and Whyte, 1993). Feed intake decreased linearly as infusion rate of propionate increased for experiments of Anil et al. (1993) and Farningham and Whyte (1993), and a threshold for infused propionate to

decrease feed intake was not detected. However, it is difficult to interpret their results because propionate was infused for only 3 h in those experiments (Anil et al., 1993; Farningham and Whyte, 1993), so hypophagic effects of propionate were evaluated essentially on meal size only. Hypophagic effects of propionate should be evaluated by monitoring feeding behavior for a longer period because cows are able to compensate for smaller meal size by increasing meal frequency (Choi and Allen, 1999). Evaluation of feeding behavior would also help to understand the regulatory mechanism of propionate on DMI because DMI is a function of both meal size and intermeal interval, which are determined by satiety and hunger, respectively.

Although maximization of energy intake is a primary concern for practical nutritional management, the majority of previous experiments that studied hypophagic effects of propionate by infusion focused on effect of propionate on DMI not on energy intake. Energy intake should be evaluated because animals are supplied extra energy from infused propionate in those studies. The objectives of this experiment were to evaluate dose response effects of intra-ruminal infusion of propionate on feeding behavior and energy intake in lactating dairy cows and to determine if a threshold exists for effects of propionate infusion on feed intake.

MATERIALS AND METHODS

Experimental procedures were approved by All University Committee on Animal Use and Care at Michigan State University.

Experiment 1

Eight multiparous Holstein cows (113 ± 26 DIM; mean ± SD) cannulated ruminally for previous experiments were selected from the Michigan State University Dairy Cattle Teaching and Research Center. Treatments were continuous intra-ruminal infusion of mixtures of propionic acid and acetic acid at 8 different ratios. Treatment solutions were prepared by diluting 16.8 moles of VFA (propionic acid and acetic acid at ratios of 0:7, 1:6, 2:5, 3:4, 4:3, 5:2, 6:1, and 7:0) to 18 L with de-ionized water. Acetic acid was added to keep the osmolarity 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 0.93 M across the treatments, and 15 L of each solution was infused over 14 h. Infusion rate was 17.9 ml/min, which is equivalent to infusion of 16.7 mmol of VFA/min. The solutions were infused using 4-channel peristaltic pumps (#78016-30, Cole-Parmer Instrument, IL) and Tygon® tubing (7.5 m x 1.6mm I.D.). Infusion started 2 h before feeding so that treatments could influence feeding behavior from the first meal immediately after feeding. Treatment periods were 2 d with 14 h of infusion followed by 34 h of recovery.

Experimental diets contained dry cracked corn (mean particle size of 3.6 mm), corn silage, alfalfa silage, 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 29.0, 15.9, and 30.8%, respectively. Dry cracked corn (mean particle size = 3.6 mm) was the major source of starch to minimize propionate production from the basal diet. Diet adaptation period was 14 d, and the final 3 d were used for data collection for DMI and milk yield to characterize the cows used in this experiment. The BW and BCS [(Wildman, 1982); a five-point scale where 1 = thin to 5 = fat] were determined on the last day of the diet adaptation period. Means for BW, BCS, DMI and milk yield were 623 kg, 2.6, 25.4 kg/d, and 36.4 kg/d, respectively. After 14 d of diet adaptation, cows were assigned to an 8 x 8 Latin square balanced for carry over effects for infusion treatments.

Throughout the experiment, cows were housed in tie-stalls, and fed once daily (1030 h) at 110% of expected intake. Cows were not allowed access to feed from 0830 h to 1030 h. The amount of feed offered and orts were weighed for each cow daily. On every infusion day, samples of all dietary ingredients (0.5 kg) were collected, and treatment solutions were infused from 0830 h to 2230 h. Cows were milked twice daily in the milking parlor except for the evening milking on infusion day, for which cows were milked in their stalls. Feeding behavior was monitored from 1030 h to 2230 h on every infusion day by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, and water consumption were recorded for each cow every 5 sec, and meal bouts, interval between meals, meal size, eating time,

TABLE 1. Ingredients and nutrient composition of experimental diets (% of dietary DM except for DM).

Diet Ingredients	
Corn silage	27.0
Alfalfa silage	25.4
Dry cracked corn	25.9
Whole linted cottonseed	6.8
Protein mix[1]	9.9
Vitamin & mineral mix[2]	5.0
Nutrient Composition	
DM	49.5
OM	93.0
Starch	30.8
NDF	29.0
ADF	20.8
CP	15.9
Ether extract	3.8
Forage NDF	21.2
Metabolizable energy (Mcal/kg)[3]	2.72

[1] Protein mix contained 75% soybean meal, 20% distillers grain, and 5% blood meal.

[2] Vitamin & mineral mix contains 66.4% dry ground corn, 20.4% dicalcium phosphate, 7.8% salt, 2.4% magnesium oxide, 1.9% trace mineral premix, 0.34% vitamin A, 0.29% vitamin D, and 0.08% vitamin E.

[3] Metabolizable energy was calculated from book values according to NRC (1989)

ruminating time, and total chewing time were calculated. Total metabolizable energy (ME) intake was calculated by adding the ME from infusates to the ME from the diet. The experimental diet was assumed to contain 2.72 Mcal/kg of ME based on book values from NRC (1989). Intake of ME was evaluated instead of intake of NE_L because the efficiency of energy conversion from ME to NE_L for infused acetate and propionate is not known and it could be different depending on milk fat concentration. In addition, the effect of propionate on energy intake was our primary concern, and evaluation of ME intake is appropriate to accomplish our objective of this experiment. Acetate and propionate were assumed to contain 0.2094 and 0.3672 Mcal/mol of ME, respectively (Sheperd and Combs, 1998). Infusates were weighed before and after infusion, and actual amount of solutions infused into the rumen was calculated. The ME from infusates was calculated by multiplying ME concentration of infusates by the amount infused into the rumen for 14 h or 12 h. Total ME intake was calculated using ME intake from infusion over 14 h and 12 h, but results were similar with same statistical significance. Therefore, ME intake from 12 h of infusion is discussed.

Diet ingredients 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 (1mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, ADF, CP, and starch. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. The NDF and ADF concentrations were determined [(VanSoest et al., 1991); method A for NDF]. Crude protein was analyzed according to Hach et al. (1985). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with

sodium hydroxide, and glucose concentration was measured using a commercial kit (Glucose kit #510; Sigma Chemical Co., St. Louis, MO). Concentrations of all nutrients except for DM were expressed as percentages of DM determined from drying at 105° C in a forced-air oven. Corn grain was dry sieved through 8 sieves (Sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan), using a sieve shaker (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant and, mean particle size of corn grain was calculated (ASAE, 1968).

All data for experiment 1 were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + L_k + Q_l + e_{ijklm}$$

where

μ = overall mean,

C_i = random effect of cow (j = 1 to 8),

P_j = fixed effect of period (k = 1 to 8),

L_k = linear effect of treatment,

Q_l = quadratic effect of treatment, and

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

Actual amount of solution infused into the rumen was not affected by treatments, and it was not included in the statistical model. Linear and quadratic effects of treatments were

evaluated. Treatment effects were declared significant at $P < 0.05$, and tendency for treatment effects was declared at $P < 0.10$.

Experiment 2

The cows used for experiment 1 were used for experiment 2 at a later stage of lactation (159 ± 26 DIM; mean ± SD). Cows were fed the same diet described for experiment 1, and were assigned to duplicated 4 x 4 Latin squares balanced for carry-over effects. Treatments were continuous intra-ruminal infusion of mixtures of sodium propionate and sodium acetate at 4 different ratios. The infusion rate of VFA for experiment 2 was higher than that for experiment 1 (25.0 vs. 16.7 mmol/min). Thus, acetate and propionate were infused as sodium salts for experiment 2 to avoid risk of ruminal acidosis, which is different than experiment 1 in which they were infused as acids. Treatment solutions were prepared by diluting 25.2 moles of VFA salt (sodium propionic acid and acetic acid at ratios of 0:3, 1:2, 2:1, and 3:0) to 18 L with de-ionized water. Sodium acetic acid was added to keep the osmolarity of infusates constant across the treatments to isolate specific effects of propionate relative to acetate on feeding behavior of dairy cows. Concentration of total VFA was 1.4 M across the treatments, and 15 L of each solution was infused over 14 h. Infusion rate was 17.9 ml/min, which is equivalent to infusion of 25.0 mmol of VFA/min. Infusion protocol, methods for data and sample collection, methods for data and sample analysis were as described for experiment 1. Additionally, milk yield was recorded and milk samples were taken at both milking on every infusion day for experiment 2. Milk samples were analyzed for

fat, true protein, lactose, solids-non-fat, milk urea nitrogen concentration with infrared spectroscopy by Michigan DHIA (East Lansing).

All data for experiment 2 were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijkl} = \mu + S_i + C(S)_{j(i)} + P_k + L_l + Q_m + Cov_{INF} + e_{ijkl}$$

where

μ = overall mean,

S_i = fixed effect of square (i = 1 to 2)

$C(S)_{j(i)}$ = random effect of cow nested in a square (j = 1 to 8),

P_k = fixed effect of period (k = 1 to 4),

L_l = linear effect of treatment,

Q_m = quadratic effect of treatment,

Cov_{INF} = effect of actual amount of solution infused into the rumen as covariate, and

e_{ijklmn} = residual, assumed to be normally distributed

One pump was used for each square of 4 cows, and random effect of cow was nested in a square that shared the same infusion pump. Interactions of square x treatment and period x treatment were originally evaluated, but they were removed from the statistical model because interactions were not significant for response variables of interest. Actual amount of solution infused into the rumen was included in the statistical model as a

covariate because actual amount of infusates tended to differ by treatments (quadratic effect of treatments: < 0.09). Linear and quadratic effects of treatments were evaluated. Treatment effects was declared significant at $P < 0.05$, and tendency for treatment effects was declared at $P < 0.10$.

RESULTS

Experiment 1

As infusion rate of propionate increased, DMI decreased linearly from 15.1 kg/12h for 0% propionate to 13.2 kg/12h for 100% propionate treatment ($P < 0.01$; Table 2). Intermeal intervals tended to be longer ($P < 0.07$) and meal size tended to be smaller ($P < 0.09$) as dose of propionate increased. Total ME intake decreased linearly by infused propionate from 43.4 Mcal/12h for 0% propionate to 40.2 Mcal/12h for 100% propionate ($P < 0.05$). Quadratic effects of treatment were not observed for any response variable.

Experiment 2

As proportion of propionate in infusate increased, DMI decreased linearly from 15.0 kg/12h for 0% propionate to 8.3 kg/12h for 100% propionate treatment ($P < 0.0001$; Table 3). Similarly, propionate decreased meal size linearly from 2.5 kg for 0% propionate to 1.5 kg/12h for 100% propionate treatment ($P < 0.03$). Number of meal bouts over 12h tended to decrease linearly ($P < 0.08$) with increasing propionate although

TABLE 2. Dose response effects of intra-ruminal infusion of propionate relative to acetate on feeding behavior for experiment 1.

	Propionate : Acetate Ratio									*P* value	
	0 : 7	1 : 6	2 : 5	3 : 4	4 : 3	5 : 2	6 : 1	7 : 0	SE	L[1]	Q[2]
Actual volume infused (L)	14.7	14.6	14.8	14.8	14.5	14.7	14.8	14.8	0.2	0.45	0.79
Feeding behavior											
DMI (kg/12h)	15.1	14.9	14.3	15.2	13.5	13.0	13.6	13.2	0.6	< 0.01	0.87
Meal bouts (/12h)	8.3	6.9	6.9	8.1	7.6	7.2	7.5	7.1	0.5	0.58	0.81
Intermeal interval (min)	65.1	76.6	83.6	71.6	73.1	74.7	79.1	85.5	5.3	0.07	0.91
Meal size (kg DM)	1.9	2.3	2.2	2.0	1.8	2.0	1.9	1.9	0.1	0.09	0.76
Metabolizable energy intake											
Diet (Mcal/12h)	40.9	40.5	38.8	41.2	36.7	35.3	36.8	35.9	1.7	< 0.01	0.87
Infusion (Mcal/12h)	2.5	2.7	3.0	3.3	3.5	3.8	4.1	4.3	0.03	< 0.0001	0.64
Total (Mcal/12h)	43.4	43.2	41.8	44.5	40.2	39.1	40.9	40.2	1.7	0.05	0.87
Chewing time											
Eating (min/12h)	158	163	148	173	160	159	149	149	9.5	0.36	0.32
(min/kg DMI)	10.6	10.9	10.5	11.4	11.8	11.6	10.9	11.4	0.6	0.11	0.31
Ruminating (min/12h)	220	195	209	223	219	211	232	228	12	0.11	0.57
(min/kg DMI)	15.0	13.4	14.8	15.0	16.8	16.3	17.4	18.5	1.5	< 0.01	0.44
Total (min/12h)	379	377	357	411	379	373	381	377	14	0.83	0.72
(min/kg DMI)	25.8	25.6	25.4	27.3	28.7	26.9	28.2	30.0	1.8	< 0.01	0.70
Drinking behavior											
Water intake (L/12h)	49.9	48.9	48.4	46.9	51.3	47.6	48.3	48.2	2.1	0.61	0.87
Drinking bouts (/12h)	11.4	11.1	10.5	9.6	10.0	10.3	11.3	9.3	0.6	0.06	0.47
Drinking interval (min)	67.9	65.6	70.3	69.6	89.3	66.9	62.9	74.5	8.3	0.66	0.43
Drink size (L/bout)	5.0	4.6	4.8	5.3	6.3	5.2	4.8	5.8	0.4	0.13	0.63

[1] Linear effect of treatments

[2] Quadratic effect of treatments

TABLE 3. Dose response effects of intra-ruminal infusion of propionate relative to acetate on feeding behavior and milk production for experiment 2.

	Propionate : Acetate Ratio					*P* value	
	0 : 3	1 : 2	2 : 1	3 : 0	SE	L[1]	Q[2]
Actual volume infused (L)	15.2	15.0	14.9	15.1	0.2	0.58	0.09
Feeding behavior							
DMI (kg/12h)	15.0	13.3	11.5	8.3	0.7	< 0.0001	0.26
Meal bouts (/12h)	7.4	7.4	6.0	6.1	0.6	0.08	0.92
Intermeal interval (min)	75.4	76.3	87.7	90.1	9.6	0.21	0.91
Meal size (kg DM)	2.5	2.0	2.1	1.5	0.3	0.03	0.71
Metabolizable energy intake							
Diet (Mcal/12h)	40.7	36.1	31.3	22.4	1.9	< 0.0001	0.26
Infusion (Mcal/12h)	3.8	4.8	5.5	6.7	0.08	< 0.0001	0.33
Total (Mcal/12h)	44.5	41.0	36.8	29.1	1.9	< 0.0001	0.27
Chewing time							
Eating (min/12h)	178	161	153	110	10	< 0.001	0.23
(min/kg DMI)	12.0	11.9	13.1	13.2	0.9	0.07	0.78
Ruminating (min/12h)	104	94	95	71	10	0.34	0.47
(min/kg DMI)	7.0	7.1	8.2	8.2	1.3	0.34	0.99
Total (min/12h)	282	255	248	181	17	< 0.01	0.25
(min/kg DMI)	19.0	18.9	21.2	21.4	1.8	0.11	0.90
Drinking behavior							
Water intake[3] (L/12h)	103.9	97.0	90.7	82.6	3.7	< 0.001	0.87
Drinking bouts[3] (/12h)	14.6	13.6	11.8	11.6	0.8	< 0.01	0.59
Drinking interval[3] (min)	47.8	50.3	60.9	57.1	2.9	< 0.01	0.31
Drinking size (L/bout)	8.2	8.0	8.5	7.7	0.5	0.68	0.52

TABLE 3 (cont'd).

	Propionate : Acetate Ratio					*P* value	
	0 : 3	1 : 2	2 : 1	3 : 0	SE	L[1]	Q[2]
Milk production							
Milk yield (kg/d)	31.2	30.9	30.5	29.0	1.1	0.22	0.50
Milk fat (%)	4.81	4.49	4.80	4.38	0.26	0.46	0.81
Milk protein (%)	3.17	3.10	3.05	3.07	0.03	< 0.01	0.13
Milk lactose (%)	4.71	4.83	4.64	4.87	0.07	0.35	0.47
Milk SNF (%)	8.80	8.85	8.60	8.84	0.06	0.75	0.18
Milk urea nitrogen (mg/dl)	6.2	6.3	6.3	6.3	0.2	0.74	0.71
Milk energy (Mcal/d)	25.0	24.0	24.7	22.3	2.4	0.24	0.58
Milk energy (Mcal/d) : ME intake[4] (Mcal/12h)	0.61	0.60	0.71	0.81	0.09	< 0.01	0.25

[1] Linear effect of treatments

[2] Quadratic effect of treatments

[3] Period x treatment interaction was significant ($P < 0.10$).

[4] ME intake: total metabolizable energy intake from infusates and diets

intermeal interval was not significantly affected by treatment. Total ME intake decreased linearly by propionate dose from 44.5 Mcal/12h for 0% propionate to 29.1 Mcal/12h for 100% propionate ($P < 0.0001$). Infusion of propionate decreased water intake linearly from 103.9 L/12h for 0% propionate to 82.6 L/12h for 100% propionate treatment. This reduction of water intake was because of less frequent water intake ($P < 0.01$) because water consumed per bout was not affected by treatment. Milk yield, milk fat concentration and milk lactose concentration were not affected by treatment. However, milk protein concentration decreased linearly as infusion rate of propionate increased ($P < 0.01$). Quadratic effect of treatment was not observed for any response variable.

DISCUSSION

Feed Intake

In both experiments, infusion of propionate decreased DMI in a dose dependent manner. Treatment effect was attributed to the specific effect of propionate relative to acetate because infusates were similar in osmolarity and pH. Our observation provides strong evidence for hypophagic effect of propionate, in agreement with previous studies. Infusion of propionate into the portal vein of sheep decreased feed intake to a greater extent compared to infusion of acetate or butyrate (Anil and Forbes, 1980) and compared to infusion of acetate, mannitol, or saline (Farningham and Whyte, 1993). Infusion of propionate into the mesenteric vein of steers reduced feed intake while infusion of acetate did not (Elliot et al., 1985). Although the hypophagic effect of propionate has been investigated extensively, most experiments in the literature have monitored feed intake

over very short periods ranging from 30 minutes to 3 hours, and essentially investigated the effect of propionate on meal size only. Evaluation of feeding behavior helps to understand the regulation mechanism of DMI by propionate because DMI is a function of both meal size and intermeal interval, which are determined by satiety and hunger, respectively. In the present study, feeding behavior was monitored for 12 h, and effects of propionate on intermeal interval and meal frequency were evaluated as well as meal size. Infusion of propionate tended to decrease meal size and increase intermeal interval in experiment 1, and decreased meal size and tended to decrease meal frequency in experiment 2. Our observations indicated that propionate decreased feed intake by affecting both satiety and hunger.

Infusates contained more energy as the proportion of propionate increased because of the greater energy concentration for propionate compared to acetate, but total ME intake also decreased linearly for both experiments as propionate increased. Reduction in ME intake from the diet exceeded that supplied from infusion as proportion of propionate increased. Previous reports have shown that propionate decreased DMI compared to iso-caloric infusion of VFA mixture (Hurtaud et al., 1993) or acetate in lactating dairy cows (Sheperd and Combs, 1998). Wu et al. (1994) reported lower DMI for cows infused with propionate into the duodenum compared to iso-caloric infusion of glucose into the rumen. That is consistent with our results because glucose ferments to acetate and butyrate as well as propionate. These studies along with the present study suggest that hypophagic effect of propionate cannot be explained simply by the additional

energy supplied as propionate. Animals do not consume to meet their energy requirements per se but have specific mechanisms regulating satiety and hunger.

Increased ruminal fermentation has been related to reduced energy intake for lactating cows (McCarthy et al., 1989; Overton et al., 1995). McCarthy et al. (1989) compared ground shelled corn and steam rolled barley in high grain diets containing more than 45% grain, and found that starch digestibility in the rumen was 77% for cows fed barley-based diets and 48.5% for cows fed corn-based diets. In their experiment, cows fed ground corn had a DMI of 23.8 kg/d while cows fed steam rolled barley had a DMI of 20.7 kg/d. Although starch digestibility was lower for corn treatments, amount of DM and OM digested in the total tract appear to be greater for cows fed ground corn because of greater DMI. Similarly, Overton et al. (1995) fed steam rolled barley and ground shelled corn at five different ratios (100:0, 75:25, 50:50, 25:75, and 0:100, for ground shelled corn starch : steam rolled barley starch) in low forage diets (45% dietary DM). They found a linear increase in starch digestibility ($P < 0.01$) and linear decrease in DMI ($P < 0.0001$) as the ratio of steam rolled barley increased in the diet. In addition, amount of DM and OM apparently digested in the total tract linearly decreased as the fraction of steam rolled barley increased in diets. Excess propionate production in the rumen might have limited energy intake as well as DMI when cows were fed very fermentable grains for both experiments.

Quadratic effects were not significant for any response variable in either experiment, providing no evidence for a threshold response to infused propionate in

feeding behavior and energy intake in this study. Additionally, the breakpoint for response in DMI to treatments, estimated as -b/2a for the regression equation of ax^2+bx+c (SAS, 1990), was not identified within the range of rate for propionate infusion for both experiments, indicating a linear hypophagic effect of propionate only. In agreement with our results, Anil et al. (1993) and Farningham and Whyte (1993) reported that infusion of propionate linearly decreased feed intake in a dose dependent manner without a threshold. Leuvenink et al. (1997) showed that propionate infusion into the mesenteric vein of mature sheep at a rate of 1 mmol/min did not decrease feed intake, but the infusion at a rate of 2 mmol/min significantly decreased feed intake. However, their data cannot be used to support a threshold response of propionate in feed intake because the effect of propionate was evaluated at only two levels of infusion and feed intake was numerically decreased for the lower dose although it was not statistically different from control.

Water Intake

Water intake was nearly twice as high for experiment 2 compared to experiment 1 (93.6 vs. 48.7 L/12h). This difference is attributed to infusion of acids in experiment 1 and sodium salts in experiment 2. Murphy (1983) suggested that a gram of sodium intake increase water intake by 0.05 L. Using this relationship, infusion of 483 g of sodium over 14 h in experiment 2 would be expected to explain 24.2 L of the 44.9 L difference in water intake between experiment 1 and experiment 2. Infusion of sodium might have increased osmolarity of ruminal fluid drawing water into the rumen from the blood, resulting in thirst and increasing water intake. In addition, greater water intake might be

because of increased urine volume to excrete excess sodium. Although infusion treatment did not affect water intake in experiment 1, water intake decreased linearly as propionate infusion increased for experiment 2. This might be explained by the greater effect of propionate treatment on feed intake in experiment 2 compared to experiment 1 because infusates were iso-osmotic within in each experiment. Both feed intake and salt intake stimulate water intake in lactating dairy cows (Murphy et al., 1983).

Milk Production

In experiment 2, milk yield was not significantly affected by infusion of propionate despite the linear reduction in energy intake. This is probably because the short duration (14 h) of the infusions was not adequate to affect milk yield significantly. However, total ME intake decreased by 35% for 100% propionate treatment compared to 0% propionate treatment. If energy intake was affected by propionate for a longer period, milk yield would be expected to decrease. Previous experiments that reported lower digested OM intake for more fermentable diets also reported reductions in milk yield (McCarthy et al., 1989; Overton et al., 1995). Oba and Allen (2001) showed that DMI and fat corrected milk yield were decreased by feeding high moisture corn compared to dry ground corn for cows fed high grain diets. Reduction in milk protein concentration for cows infused with more propionate might be explained by lower microbial protein production. Infused acetate and propionate provided additional energy for animals but not for microorganisms in the rumen. Therefore, infused propionate reduced fermentable energy to synthesize microbial protein by decreasing DMI. Although propionate can increase availability of amino acids for the mammary gland by sparing them from being

utilized for gluconeogenesis, the linear reduction in milk protein concentration by increased propionate infusion indicates that milk protein synthesis is limited by microbial protein production to a greater extent (Wu et al., 1994). In addition, when metabolizable protein limits maximum milk yield, infusion of glucogenic energy as propionate without additional amino acid supply may have diluted milk protein by relatively greater lactose synthesis and milk yield.

CONCLUSION

Intra-ruminal infusion of propionate decreased DMI and ME intake in a dose-dependent manner. This indicates that the reduction in dietary energy intake from propionate infusion was greater than the energy supplied from propionate infusion and that excess propionate production in the rumen can decrease energy intake in lactating dairy cows consuming highly fermentable diets. However, quadratic effects of propionate infusion were not significant for DMI and ME intake, providing no evidence for a threshold response to infused propionate in feeding behavior and energy intake. As proportion of propionate in infused VFA increased, meal size tended to decrease and intermeal interval increased in experiment 1, and meal size decreased and meal frequency tended to decrease in experiment 2. These observations suggest that propionate plays an important role in feed intake regulation by affecting both satiety and hunger.

CHAPTER 4

Effects of intra-ruminal infusion of sodium, potassium and ammonium on hypophagic effect of propionate in lactating dairy cows

ABSTRACT

The objective of this experiment was to evaluate effects of salt type on hypophagic effects of intra-ruminal infusion of propionate in lactating dairy cows. Our working hypothesis is that oxidative metabolism of propionate causes satiety by increasing hepatic ATP concentration and decreasing the discharge rate of the hepatic vagus. We hypothesized that infusion of ammonium would reduce the hypophagic effects of propionate because of increased utilization of ATP for urea synthesis. We also hypothesized that infusion of potassium would decrease hypophagic effects of propionate compared to sodium by increasing the discharge rate of the hepatic vagus. Eight ruminally cannulated Holstein cows in mid-lactation were used in a duplicated 4 x 4 Latin square design, and treatments were intra-ruminal infusion of propionic acid, ammonium propionate, sodium propionate, and potassium propionate. Treatment solutions were 0.93 M for propionate among treatments and 0.67 M for salts among the treatments except for control (propionic acid). Treatment solutions were infused over 14 h starting 2 h before feeding at 17.9 ml/min, which is equivalent to 16.7 and 11.9

mmol/min for propionate and salts, respectively. Infusion of ammonium propionate decreased DMI compared to sodium propionate and potassium propionate ($P < 0.04$; 11.0 vs. 14.0 and 13.9 kg/12 h), and DMI was not different between sodium propionate and potassium propionate infusions. Contrary to our hypothesis, ammonium infusion did not reduce hypophagic effects of propionate possibly because the urea cycle indirectly stimulated oxidative metabolism in the liver by generating oxidizable carbon from amino acid catabolism.

(**Key words:** propionate infusion, ammonium, urea synthesis, feed intake, feeding behavior)

Abbreviation Key: MUN = Milk Urea Nitrogen

INTRODUCTION

Increasing ruminal fermentation is desirable to maximize microbial protein production and energy intake in high producing dairy cows. However, greater ruminal fermentation sometimes decreases feed intake and excess propionate production in the rumen is considered to have a direct hypophagic effect (Allen, 2000). Choi and Allen (1999) showed that meal size, intermeal interval, and DMI were not affected by infusion of acids compared to salts, indicating that ruminal pH *per se* does not have direct hypophagic effects. In that experiment, infusion of acetate resulted in similar meal size as iso-osmotic infusions of NaCl. It was concluded that acetate affects satiety by mechanisms related to osmotic effects in the rumen. However, infusion of propionate

decreased meal size and DMI, and increased intermeal interval compared to infusion of acetate, providing the evidence for a specific hypophagic effect of propionate. Anil and Forbes (1980, 1988) suggested that propionate decreases feed intake by a mechanism via the hepatic vagus but the mechanism for regulation of feed intake by propionate has not been elucidated.

Allen (2000) proposed that propionate decreases feed intake of ruminants by stimulating oxidative metabolism in the liver. Oxidative metabolism in the liver has been shown to affect satiety in rats (Langhans et al., 1983, 1984, 1985a) and a temporal relationship between feeding behavior and hepatic ATP concentration has been demonstrated (Koch et al., 1998). Langhans et al. (1985a) proposed that oxidative metabolism in the liver affects feed intake by hyperpolarizing cell membrane potentials. The sodium pump inhibitor, ouabain, increased feed intake in rats when injected intraperitoneally (Langhans and Scharrer, 1987b). Satiety signals originating in the liver are mediated by hepatic vagal afferents (Langhans et al., 1985c; Anil and Forbes, 1988), and Niijima (1983) reported that discharge rates of hepatic vagal afferents were reduced by glucose infusion in a dose-dependent manner in guinea pigs. These observations suggest that oxidative metabolism within hepatocytes generates ATP, increases sodium pump activity, hyperpolarizes hepatocyte membrane potential, and decreases the discharge rate of hepatic vagal afferents, resulting in satiety.

Our long-term goal is to alleviate hypophagic effects of propionate by a diet formulation that modulates a metabolic or neural pathway by which propionate causes

satiety. If propionate metabolism in the liver generates satiety signals by increasing hepatic ATP concentration, hypopagic effects of propionate can be alleviated by reducing hepatic ATP concentration. We hypothesized that infusion of ammonium reduces the hypophagic effects of propionate because urea synthesis consumes ATP in the liver. In addition, if decreased discharge rate of hepatic vagal afferents sends a satiety signal to the brain, increasing their discharge rate can alleviate hypophagic effects of propionate. We hypothesized that infusion of potassium increases feed intake by increasing discharge rate of vagal affernets compared to infusion of sodium. We expected infusion of potassium to decrease the potassium gradient across the membrane of vagal afferents, reducing potassium efflux, depolarizing resting transmembrane potential of vagal affernets, and increasing their discharge rate.

The objective of this experiment was to evaluate effects of salt type on hypophagic effects of intra-ruminal infusion of propionate in lactating dairy cows.

MATERIALS AND METHODS

Experimental procedures were approved by All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (143 ± 26 DIM; Mean ± SD) cannulated ruminally for previous experiments were selected from the Michigan State University Dairy Cattle Teaching and Research Center. Experimental diets contained dry cracked corn (mean particle size of 3.6 mm), corn silage, alfalfa

silage, 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 29.0, 15.9, and 30.8%, respectively. Means for BW, BCS [(Wildman, 1982); a five-point scale where 1 = thin to 5 = fat], DMI and milk yield before experiment were 623 kg, 2.6, 25.4 kg/d, and 36.4 kg/d, respectively. Cows were assigned to duplicated 4 x 4 Latin squares balanced for carry over effects.

Treatments were continuous intra-ruminal infusion of mixtures of propionic acid and bicarbonate salts (control, NH_3, Na, or K). Treatment solutions were prepared by diluting 16.8 moles of propionic acid and 12.0 moles of bicarbonate salts (NH_3, Na, or K) to 18 L with de-ionized water. Bicarbonate salts were used instead of hydroxide salts to maximize the purity of treatment salts; the purity of commercially available potassium hydroxide was approximately 85% whereas the purity of potassium bicarbonate was 99.9%. Control treatment (Acid) was propionic acid without any bicarbonate salts. The concentration of propionate was 0.93 M across treatments, and concentration of salts was 0.67 M across the treatments except for control. Solutions were infused at a rate of 15 L over 14 h. This infusion rate of 17.9 ml/min is equivalent to 16.7 and 11.9 mmol/min for propionate and salts, respectively. This rate of propionate infusion was shown to decrease DMI significantly in a previous experiment (Chapter 3), and was selected to evaluate how salt type affects hypophagic effects of propionate in this experiment. The concentration of salts was less than propionate to avoid the risk of ammonia toxicity for ammonium treatment; total amount of ammonium infused into the rumen was equivalent to 875 g of CP over 14 h. Solutions were infused using 4-channel peristaltic pumps

TABLE 1. Ingredients and nutrient composition of experimental diets (% of dietary DM except for DM).

Diet Ingredients	
Corn silage	27.0
Alfalfa silage	25.4
Dry cracked corn	25.9
Whole linted cottonseed	6.8
Protein mix[1]	9.9
Vitamin & mineral mix[2]	5.0
Nutrient Composition	
DM	49.5
OM	93.0
Starch	30.8
NDF	29.0
ADF	20.8
CP	15.9
Ether extract	3.8
Forage NDF	21.2
Metabolizable energy (Mcal/kg)[3]	2.72

[1] Protein mix contained 75% soybean meal, 20% distillers grain, and 5% blood meal.

[2] Vitamin & mineral mix contains 66.4% dry ground corn, 20.4% dicalcium phosphate, 7.8% salt, 2.4% magnesium oxide, 1.9% trace mineral premix, 0.34% vitamin A, 0.29% vitamin D, and 0.08% vitamin E.

[3] Metabolizable energy was calculated from book values according to NRC (1989).

(#78016-30, Cole-Parmer Instrument, IL) and Tygon® tubing (7.5 m x 1.6mm I.D.). Treatment periods were 2 d with 14 h of infusion followed by 34 h of recovery.

Throughout the experiment, cows were housed in tie-stalls, and fed once daily (1030 h) at 110% of expected intake. Cows were not allowed access to feeds between 0830 h to 1030 h. The amount of feed offered and orts were weighed for each cow daily. On every infusion day, samples of all dietary ingredients (0.5 kg) were collected, and cows were infused treatment solutions from 0830 h to 2230 h. Cows were milked twice daily in the milking parlor except for the evening milking on infusion days, for which cows were milked in their stalls. Feeding behavior was monitored from 1030 h to 2230 h on each infusion day by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, and water consumption were recorded for each cow every 5 sec, and meal bouts, interval between meals, meal size, eating time, ruminating time, and total chewing time were calculated. Milk yield was recorded and milk samples were taken at both milking on each infusion day for experiment 2. Milk samples were analyzed for fat, true protein, lactose, solids-non-fat, milk urea nitrogen (MUN) concentration with infrared spectroscopy by Michigan DHIA (East Lansing).

Diet ingredients 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 (1mm screen; Authur H. Thomas, Philadelphia, PA), and analyzed for ash, NDF, ADF, CP, and starch. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. The NDF and ADF concentrations were determined [(VanSoest et al., 1991); method A for

NDF]. Crude protein was analyzed according to Hach et al. (1985). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, and glucose concentration was measured using a commercial kit (Glucose kit #510; Sigma Chemical Co., St. Louis, MO). Concentrations of all nutrients except for DM were expressed as percentages of DM determined by drying at 105° C in a forced-air oven. Corn grain was dry sieved through 8 sieves (Sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan), using a sieve shaker (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant, and mean particle size of corn grain was calculated (ASAE, 1968).

All data were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijkl} = \mu + S_i + C(S)_{j(i)} + P_k + T_l + Cov_{INF} + e_{ijklm}$$

where

μ = overall mean,

S_i = fixed effect of square (i = 1 to 2)

$C(S)_{j(i)}$ = random effect of cow nested in a square (j = 1 to 8),

P_k = fixed effect of period (k = 1 to 4),

T_l = fixed effect of treatment (l = 1 to 4),

Cov_{INF} = effect of actual amount of solution infused into the rumen, and

e_{ijkl} = residual, assumed to be normally distributed

One pump was used for each square of 4 cows, and random effect of cow was nested in a square that shared the same infusion pump. Interactions of square x treatment and period x treatment were evaluated, but they were removed from the statistical model because interactions were not significant for response variables of interest. Volume of solution infused into the rumen was included in the statistical model as a covariate. Orthogonal contrasts were made for the effect of acid (acid vs. NH_3, Na, and K), the effect of ammonium (NH_3 vs. Na and K), and the effect of cation type (Na vs. K). Treatment effects was declared significant at $P < 0.05$, and tendency for treatment effects was declared at $P < 0.10$.

RESULTS AND DISCUSSION

Volume of solution infused into the rumen was slightly greater for propionate salt compared to propionic acid treatment and for sodium and potassium propionate compared to ammonium propionate treatment ($P < 0.01$; Table 2). This was unexpected because a 4-channel peristaltic pump was used for each square with identical tubing and fittings, but a difference in viscosity of infusates might have affected flow rate. Therefore, volume of solution infused into the rumen was included in the statistical model as a covariate.

DMI and Feeding Behavior

Dry matter intake, number of meal bouts, intermeal interval, and meal size were similar between infusion of propionic acid and propionate salts, indicating pH per se does

not have a direct effect on feeding behavior of lactating dairy cows. These results agree with previous observations by Choi and Allen (1999). However, infusion of ammonium propionate decreased DMI compared to infusion of sodium propionate and potassium propionate ($P < 0.04$; Table 2). Contrary to our hypothesis, infusion of ammonium did not decrease, but increased the hypophagic effect of propionate. This reduction in DMI was not likely from toxic effects of ammonia because MUN concentrations for the ammonium propionate treatment were within the range of normal values for lactating cows (Butler, 1996). Ammonium infusion increased intermeal interval ($P < 0.01$) and decreased number of meal bouts ($P < 0.02$) compared to sodium propionate and potassium propionate treatments, but did not affect meal size. These observations indicate that the reduction in feed intake from ammonium infusion was from delaying the sense of hunger after a previous meal. No difference in DMI and feeding behavior was observed between infusion of sodium and potassium propionate. We infused 230 g of sodium or 391 g of potassium over 14 h, which are far greater than animal requirements (NRC, 2001). Any possible effects of potassium ions on alleviation of hypophagic effects of propionate are not likely from dietary manipulation.

Prior to the experiment, we hypothesized that infusion of ammonium propionate would increase DMI compared to other propionate salts because cows infused with ammonium need to synthesize additional urea, which consumes 3 moles of ATP per mole of urea synthesis. Greater MUN values for ammonium propionate treatment compared to other treatments ($P < 0.001$) support that additional urea synthesis occurred for cows infused with ammonium propionate. Observed hypophagic effect of ammonium may be

TABLE 2. Effects of intra-ruminal infusion of propionate salts on feeding behavior and milk production.

	Treatments					*P* value		
	Acid	Ammonium	Sodium	Potassium	SE	A[1]	B[2]	C[3]
Actual volume infused (L)	14.9	15.0	15.2	15.2	0.2	< 0.01	< 0.01	0.90
Feeding behavior								
DMI (kg/12h)	11.8	11.0	14.0	13.9	1.2	0.37	0.04	0.97
Meal bouts (/12h)	5.7	5.6	8.0	7.2	0.8	0.10	0.02	0.39
Intermeal interval (min)	94.5	105.0	62.8	73.9	12.1	0.24	< 0.01	0.42
Meal size (kg DM)	2.3	2.1	1.8	2.2	0.3	0.33	0.73	0.33
Chewing time								
Eating (min/12h)	133	124	157	161	14	0.32	0.03	0.82
(min/kg DMI)	11.5	11.7	11.2	11.6	0.6	0.98	0.52	0.54
Ruminating (min/12h)	228	165	163	175	15	< 0.001	0.80	0.54
(min/kg DMI)	19.6	15.0	11.7	12.9	1.2	< 0.001	0.06	0.43
Total (min/12h)	361	289	321	337	26	0.11	0.18	0.62
(min/kg DMI)	30.9	26.7	23.0	24.5	1.5	< 0.001	0.07	0.35
Drinking behavior								
Water intake (L/12h)	43.9	47.6	79.7	74.5	3.5	< 0.001	< 0.001	0.30
Drinking bouts (/12h)	9.5	9.1	14.7	13.2	1.5	0.02	< 0.001	0.24
Drinking interval[4] (min)	67.9	80.2	42.1	46.7	10.4	0.35	< 0.01	0.75
Drink size (L/bout)	5.1	6.7	5.7	6.0	0.7	0.09	0.19	0.66

TABLE 2 (cont'd).

	Treatments					*P* value		
	Acid	Ammonium	Sodium	Potassium	SE	A[1]	B[2]	C[3]
Milk production								
Milk yield (kg/d)	36.7	31.4	31.3	33.1	2.0	< 0.01	0.65	0.33
Milk fat (%)	4.33	4.29	4.33	4.55	0.28	0.82	0.58	0.47
Milk protein[4] (%)	2.98	3.00	3.00	2.86	0.07	0.57	0.17	0.02
Milk lactose (%)	4.61	4.64	4.81	4.52	0.12	0.59	0.77	< 0.01
Milk SNF[4] (%)	8.44	8.49	8.67	8.21	0.12	0.88	0.70	< 0.01
Milk urea nitrogen (mg/dl)	8.8	16.7	7.7	8.2	0.5	< 0.001	< 0.001	0.43
Milk energy (Mcal/d)	27.4	23.3	23.7	24.6	1.6	0.06	0.62	0.63
Milk energy (Mcal/d) : ME intake[5] (Mcal/12h)	0.80	0.80	0.58	0.61	0.09	0.20	0.08	0.80

[1] Comparison between Acid vs. Ammonium, Sodium, and Potassium

[2] Comparison between Ammonium vs. Sodium and Potassium

[3] Comparison between Sodium vs. Potassium

[4] Period x treatment interaction was significant ($P < 0.10$).

[5] ME intake: total metabolizable energy intake from infusates and diets

explained by increased oxidation of amino acids in the liver. One of the two amino groups of urea is from ammonia but the other is from amino acids via aspartate; urea production in the liver is associated with α-amino nitrogen removal in the liver (Reynolds, 1992; Parker et al., 1995). Infusion of NH_4Cl into the mesenteric vein of sheep increased oxidation of leucine in splanchnic tissues (Lobley et al., 1995). Therefore, urea synthesis might increase net ATP production in the liver by increasing hepatic amino acid oxidation because each turn of TCA cycle generates 12 ATP while each turn of urea cycle consumes 3 ATP.

Chewing and Drinking Activities

Infusion of ammonium propionate decreased eating time compared to infusion of sodium and potassium propionate ($P < 0.03$), which is in agreement with lower DMI for the ammonium treatment. Ruminating time ($P < 0.001$) was lower for infusion of propionate salts compared to propionic acid. Ruminating time per kg of DMI was also decreased by infusion of propionate salts compared to propionic acid ($P < 0.001$), but reduction in rumination per kg of DMI tended to be greater for sodium and potassium propionate compared to ammonium propionate treatment ($P < 0.06$). However, ruminating time per kg of DMI was similar for sodium and potassium treatments. These observations might be attributed to the expected greater osmolarity of ruminal fluid for propionate salt treatments compared to propionic acid treatment and for sodium and potassium treatments compared to ammonium treatment because greater osmotic pressure in ruminal fluid is related to decreased rumination (Welch, 1982). Iso-osmotic infusion of ammonium did not have as great of an effect at reducing ruminating time as infusion

of sodium and potassium possibly because of potential incorporation of ammonia into microbial protein, and osmolarity of rumen fluid was increased to a less extent compared to sodium and potassium treatments.

Consistent with treatment effects on ruminating time, infusion of propionate salts increased water intake compared to infusion of propionic acid. Sodium and potassium treatments increased frequency of drinking compared to ammonium treatment, indicated by a greater number of drinking bouts ($P < 0.001$) and decreased interval between bouts ($P < 0.01$). Drink size per bout tended to be greater for infusion of propionate salts compared to propionic acid ($P < 0.09$). Drinking behavior was similar for sodium and potassium treatments. Increased water intake from the infusions is probably because greater osmotic pressure in ruminal fluid results in translocation of water into the rumen from the blood resulting in thirst and from greater urine volume (not measured) to excrete sodium and potassium.

Milk Production

Infusion of propionate salts decreased milk yield drastically compared to infusion of propionic acid ($P < 0.01$). Reduction in milk yield is partially attributed to lower DMI for ammonium treatment but not for sodium and potassium treatments because DMI was numerically greater for sodium and potassium treatments compared to the acid treatment. Milk energy output per ME intake tended to be lower for sodium and potassium treatments compared to ammonium treatment ($P < 0.08$) which was similar to the acid treatment. Infused solutions were iso-energetic across treatments, and the difference in

energy intake cannot explain our observation. Reduction in milk yield for sodium and potassium treatments can be attributed to greater energy expenditures to maintain homeostasis of blood for osmolarity and ion balance but not attributed to a specific effect of sodium or potassium ion because milk energy output per Mcal of ME intake was similar for sodium and potassium treatments.

CONCLUSION

Contrary to our hypothesis, hypophagic effects of propionate were greater when infused as ammonium salt compared to sodium and potassium salts. Infusion of ammonium decreased DMI by decreasing meal frequency without affecting meal size, indicating that ammonium delayed the sense of hunger. Urea synthesis requires N from amino acids, and deamination of amino acids might stimulate oxidative metabolism in the liver by increasing amino acid carbon available for oxidation. No difference in DMI and feeding behavior was observed between infusion of sodium and potassium propionate at concentrations greater than practical for diet formulation, indicating that any possible effects of sodium or potassium ions on feeding behavior are not likely from dietary manipulation.

CHAPTER 5

Hypophagic effects of ammonium were greater when infused with propionate compared to acetate in lactating dairy cows

ABSTRACT

The objective of this experiment was to determine if hypophagic effects of ammonium are greater when infused with propionate compared to acetate in lactating dairy cows. Urea synthesis generates amino acid carbon available for gluconeogenesis or oxidation in the liver. We hypothesized that ammonium infusion stimulates oxidative metabolism in the liver causing greater hypophagia when infused with propionate compared to acetate because propionate is a primary substrate for gluconeogenesis while acetate is not metabolized in the liver. Eight ruminally cannulated Holstein cows in mid-lactation were used in a duplicated 4 x 4 Latin square design with a 2 x 2 factorial arrangement of treatments. Factors evaluated were type of VFA (acetate vs. propionate) and type of salt (sodium vs. ammonium). Concentration of VFA salts in infusates was 0.93 M across treatments, and infusion rate of 17.9 ml/min is equivalent to 16.7 mmol of VFA salts/min. Treatment solutions were infused continuously into the rumen starting 2 h before feeding and ending 12 h after feeding. Dry matter intake was lower for propionate compared to acetate treatment ($P < 0.0001$) and for ammonium compared to sodium treatment ($P < 0.001$). Hypophagic effects of ammonium were significantly

greater for cows infused with propionate (4.3 vs. 12.1 kg/12 h) compared to acetate (13.5 vs. 15.3 kg/12 h; interaction $P < 0.01$). This interaction is attributed to greater reduction in meal frequency for ammonium treatment compared to sodium treatment when infused with propionate (3.9 vs. 7.2 /12 h) compared to when infused with acetate (6.6 vs. 7.0 /12 h), indicating that infusion of ammonium propionate delayed the sense of hunger. Meal size was decreased by infusion of propionate compared to acetate ($P < 0.01$), but was not affected by salt type. Greater hypophagic effects of ammonium propionate might be because the urea cycle generates substrate for oxidation in the liver, increasing hepatic ATP concentration.

(**Key words:** propionate infusion, ammonium, urea synthesis, feed intake, feeding behavior)

Abbreviation Key: MUN = Milk Urea Nitrogen

INTRODUCTION

Greater ruminal fermentation sometimes decreases feed intake and excess propionate production in the rumen might have a direct hypophagic effect by stimulating oxidative metabolism in the liver (Allen, 2000). Oxidative metabolism in the liver has been shown to affect satiety in rats (Langhans et al., 1983, 1984, 1985a) and a temporal relationship between feeding behavior and hepatic ATP concentration has been demonstrated (Koch et al., 1998). Langhans et al. (1985a) proposed that metabolic fuels that are extensively metabolized in the liver have hypophagic effects. In previous

experiment (Chapter 4), infusion of ammonium decreased DMI compared to sodium and potassium propionate by decreasing meal frequency. Hypophagic effects of ammonium infusion were not likely from ammonia toxicity because observed milk urea nitrogen (MUN) concentration for the ammonium propionate treatment was within the range of normal variation for lactating cows (Butler, 1996).

Hypophagic effect of ammonium may be explained by increased oxidative metabolism in the liver. One of the two amino groups of urea is from ammonia but the other is from amino acids via aspartate; urea production in the liver is associated with α-amino nitrogen removal in the liver (Reynolds, 1992; Parker et al., 1995). Infusion of NH_4Cl into the mesenteric vein of sheep increased oxidation of leucine by splanchnic tissues (Lobley et al., 1995). Therefore, urea synthesis might increase net ATP production in the liver by increasing hepatic amino acid oxidation despite utilization of ATP by the urea cycle. Although carbon from some amino acids can be utilized for gluconeogenesis and consume ATP in the liver, they might be oxidized to a greater extent when glucose demand of peripheral tissues is low or when the liver has plenty of other substrates for gluconeogenesis. If enhanced oxidative metabolism in the liver decreases DMI, hypophagic effects of ammonium are expected to be greater when infused with propionate compared to acetate because propionate is the primary substrate for gluconeogenesis while acetate is not metabolized in the liver (Ricks and Cook, 1981). When ammonium is infused with acetate, carbon from amino acids would be utilized for gluconeogenesis to a greater extent. We hypothesized that hypophagic effects of

ammonium infusion vary depending on its stimulatory effect on oxidative metabolism in the liver and increase when infused with propionate compared to acetate.

The objective of this experiment was to determine if hypophagic effects of ammonium are greater when infusion with propionate compared to acetate in lactating dairy cows.

MATERIALS AND METHODS

Experimental procedures were approved by All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (151 ± 26 DIM; mean ± SD) cannulated ruminally for previous experiments were selected from the Michigan State University Dairy Cattle Teaching and Research Center. Experimental diets contained dry cracked corn (mean particle size of 3.6 mm), corn silage, alfalfa silage, 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 29.0, 15.9, and 30.8%, respectively. Means for BW, BCS [(Wildman, 1982); a five-point scale where 1 = thin to 5 = fat], DMI and milk yield before experiment were 623 kg, 2.6, 25.4 kg/d, and 36.4 kg/d, respectively.

TABLE 1. Ingredients and nutrient composition of experimental diets (% of dietary DM except for DM).

Diet Ingredients	
Corn silage	27.0
Alfalfa silage	25.4
Dry cracked corn	25.9
Whole linted cottonseed	6.8
Protein mix[1]	9.9
Vitamin & mineral mix[2]	5.0
Nutrient Composition	
DM	49.5
OM	93.0
Starch	30.8
NDF	29.0
ADF	20.8
CP	15.9
Ether extract	3.8
Forage NDF	21.2
Metabolizable energy (Mcal/kg)[3]	2.72

[1] Protein mix contained 75% soybean meal, 20% distillers grain, and 5% blood meal.

[2] Vitamin & mineral mix contains 66.4% dry ground corn, 20.4% dicalcium phosphate, 7.8% salt, 2.4% magnesium oxide, 1.9% trace mineral premix, 0.34% vitamin A, 0.29% vitamin D, and 0.08% vitamin E.

[3] Metabolizable energy was calculated from book values according to NRC (1989).

Cows were assigned to duplicated 4 x 4 Latin squares balanced for carry-over effects with a 2 x 2 factorial arrangement of treatments. Factors evaluated were type of VFA (acetate vs. propionate) and type of salt (sodium vs. ammonium). Treatments were continuous intra-ruminal infusion of sodium acetate, ammonium acetate, sodium propionate, or ammonium propionate. Treatment solutions were prepared by diluting 16.8 moles of VFA salts to 18 L with de-ionized water. Concentration of VFA salts were 0.93 M across treatments, and 15 L of each solution was infused over 14 h. Infusion rate of 17.9 ml/min is equivalent to 16.7 mmol of VFA salts/min. Solutions were infused using 4-channel peristaltic pumps (#78016-30, Cole-Parmer Instrument, IL) and Tygon® tubing (7.5 m x 1.6mm I.D.). Treatment periods were 2 d with 14 h of infusion followed by 34 h of recovery. We thought that the infusion rate of ammonium in a previous experiment (11.9 mmol/min; Chapter 4) could be increased without risk of ammonia toxicity based on MUN data from that experiment. In addition, we expected to see a greater treatment effect on feeding behavior and DMI for this experiment compared to the experiment in Chapter 4 by increasing the infusion rate for salts. Total amount of ammonium infused into the rumen in this experiment was equivalent to 1227g of CP over 14h.

Total metabolizable energy (ME) intake was calculated by adding ME from infusates to ME of the diet because energy concentration of infusates differed. The experimental diet was assumed to contain 2.72 Mcal/kg of ME based on book values from NRC (1989). Acetate and propionate were assumed to contain 0.2094 and 0.3672 Mcal/mol of ME, respectively (Sheperd and Combs, 1998). Infusates were weighed

before and after infusion, and actual amount of solutions infused into the rumen was calculated. The ME from infusates was calculated by multiplying ME concentration of infusates by the amount that actually infused into the rumen for 14 h or 12 h. Total ME intake was calculated using ME intake from infusion of 14 h and 12 h, but results were similar with same statistical significance. Therefore, ME intake for the 12 h period for which feeding behavior was monitored is discussed.

Throughout the experiment, cows were housed in tie-stalls, and fed once daily (1030 h) at 110% of expected intake. Cows were not allowed access to feed between 0830 h to 1030 h. The amount of feed offered and orts were weighed for each cow daily. On every infusion day, samples of all dietary ingredients (0.5 kg) were collected, and cows were infused treatment solutions from 0830 h to 2230 h. Cows were milked twice daily in the milking parlor except for the evening milking on infusion days, for which cows were milked in their stalls. Feeding behavior was monitored from 1030 h to 2230 h on each infusion day by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, and water consumption were recorded for each cow every 5 sec, and meal bouts, interval between meals, meal size, eating time, ruminating time, and total chewing time were calculated. Milk yield was recorded and milk samples were taken at both milking on each infusion day. Milk samples were analyzed for fat, true protein, lactose, solids-non-fat, milk urea nitrogen (MUN) concentration with infrared spectroscopy by Michigan DHIA (East Lansing).

Diet ingredients 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 (1mm screen; Authur H. Thomas, Philadelphia, PA), and analyzed for ash, NDF, ADF, CP, and starch. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. The NDF and ADF concentrations were determined [(VanSoest et al., 1991); method A for NDF]. Crude protein was analyzed according to Hach et al. (1985). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, and glucose concentration was measured using a commercial kit (Glucose kit #510; Sigma Chemical Co., St. Louis, MO). Concentrations of all nutrients except for DM were expressed as percentages of DM determined by drying at 105° C in a forced-air oven. Corn grain was dry sieved through 8 sieves (Sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan), using a sieve shaker (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant, and mean particle size of corn grain was calculated (ASAE, 1968).

All data except for MUN were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijkl} = \mu + S_i + C(S)_{j(i)} + P_k + T_l + Cov_{INF} + e_{ijklm}$$

where

μ = overall mean,

S_i = fixed effect of square (i = 1 to 2)

$C(S)_{j(i)}$ = random effect of cow nested in a square (j = 1 to 8),

P_k = fixed effect of period (k = 1 to 4),

T_l = fixed effect of treatment (l = 1 to 4),

Cov_{INF} = effect of actual amount of solution infused into the rumen, and

e_{ijkl} = residual, assumed to be normally distributed

One pump was used for a square of 4 cows, and random effect of cow was nested in a square that shared the same infusion pump. Interactions of square x treatment and period x treatment were originally evaluated, but they were removed from the statistical model because interactions were not significant for response variables of interest. Volume of solution infused into the rumen was included in the statistical model as covariate. Orthogonal contrasts were made for the effect of VFA type (acetate vs. propionate), salt type (sodium vs. ammonium), and interaction of VFA and salt. Treatment effects and their interaction were declared significant at $P < 0.05$ and $P < 0.10$, respectively, and tendency for treatment effects and their interaction were declared at $P < 0.10$ and $P < 0.15$, respectively.

RESULTS AND DISCUSSION

Volume of solution infused into the rumen with sodium treatment was greater than ammonium treatment ($P < 0.001$; Table 2). This was unexpected because a 4-channel peristaltic pump was used for each square with identical tubing and fittings, but a

TABLE 2. Effects of intra-ruminal infusion of VFA salts on feeding behavior and milk production.

	Treatments					*P* value		
	Acetate		Propionate					
	Sodium	Ammonium	Sodium	Ammonium	SE	VFA[1]	Salt[2]	INT[3]
Actual volume infused (L)	15.4	14.9	15.3	15.1	0.1	0.47	< 0.001	0.13
Feeding behavior								
DMI (kg/12h)	15.3	13.1	12.1	4.3	1.3	< 0.0001	<0.001	< 0.01
Meal bouts (/12h)	7.0	6.6	7.2	3.9	0.8	0.03	< 0.01	< 0.01
Intermeal interval (min)	64.6	80.8	70.5	96.1	14.0	0.45	0.19	0.73
Meal size (kg DM)	2.3	2.1	1.7	1.3	0.3	< 0.01	0.35	0.72
Metabolizable energy intake								
Diet (Mcal/12h)	41.7	35.5	32.8	11.8	3.5	< 0.0001	< 0.001	< 0.01
Infusion (Mcal/12h)	2.5	2.6	4.5	4.4	0.01	< 0.0001	0.66	0.06
Total (Mcal/12h)	44.2	38.1	37.3	16.3	3.5	< 0.0001	< 0.001	< 0.01
Chewing time								
Eating (min/12h)	172	150	153	55	20	< 0.001	< 0.01	< 0.01
(min/kg DMI)	11.1	11.3	12.6	12.3	0.8	0.04	0.97	0.63
Ruminating (min/12h)	137	211	124	75	22	< 0.001	0.58	< 0.01
(min/kg DMI)	9.0	15.9	10.5	12.5	1.9	0.61	0.04	0.19
Total (min/12h)	311	362	277	130	39	< 0.001	0.22	< 0.01
(min/kg DMI)	20.2	27.3	23.0	24.8	2.2	0.94	0.08	0.23
Drinking behavior								
Water intake (L/12h)	86.0	50.4	88.1	24.8	4.0	< 0.01	< 0.0001	< 0.01
Drinking bouts[4] (/12h)	13.1	11.7	12.5	6.9	1.9	< 0.01	0.02	0.02
Drinking interval[4] (min)	53.3	71.0	59.5	94.0	13.1	< 0.05	0.03	0.25
Drink size[4] (L/bout)	7.1	5.1	7.8	4.1	0.9	0.81	< 0.01	0.13

TABLE 2 (cont'd).

	Treatments					*P* value		
	Acetate		Propionate					
	Sodium	Ammonium	Sodium	Ammonium	SE	VFA[1]	Salt[2]	INT[3]
Milk production								
Milk yield (kg/d)	34.0	32.1	32.9	27.4	1.7	< 0.01	0.02	0.06
Milk fat (%)	3.99	3.42	3.53	3.79	0.20	0.73	0.45	0.01
Milk protein (%)	3.16	3.10	2.98	3.13	0.09	0.27	0.64	0.15
Milk lactose (%)	4.83	4.68	4.89	4.72	0.01	0.25	0.05	0.86
Milk SNF (%)	8.87	8.56	8.72	8.66	0.17	0.72	0.17	0.08
Milk urea nitrogen (mg/dl)	7.5	21.8	7.4	21.4	1.2	0.75	< 0.01	0.78
Milk energy (Mcal/d)	25.5	21.2	22.8	18.3	1.2	< 0.01	< 0.01	0.88
Milk energy (Mcal/d) : ME intake[5] (Mcal/12h)	0.57	0.67	0.63	1.45	0.16	< 0.01	0.04	0.03

[1] Effects of VFA type: Acetate vs. Propionate

[2] Effects of salt type: Sodium vs. Ammonium

[3] Effects of interaction between VFA type and salt type

[4] Period x treatment interaction was significant ($P < 0.10$).

[5] ME intake: total metabolizable energy intake from infusates and diets

difference in viscosity of infusates might have affected flow rate. Therefore, volume of solution infused into the rumen was included in the statistical model as a covariate.

DMI

Interactions of main effects were observed for DMI ($P < 0.01$). Infusion of ammonium propionate decreased DMI by 65% compared to sodium propionate (4.3 vs. 12.1 kg/12h), but reduction in DMI for ammonium acetate compared to sodium acetate treatment was only 14% (13.1 vs. 15.3 kg/12h). Although propionate treatments contain more energy in infusates compared to acetate treatments, total ME intake was decreased by infusion of propionate compared to acetate. In addition, the total ME intake reduction by ammonium infusion was much greater when infused with propionate compared to when infused with acetate.

The hypophagia caused by infusion of ammonium propionate cannot be attributed to ammonium toxicity in this experiment because the same amount of ammonium did not decrease DMI to a similar extent when infused with acetate. It is more logical to speculate that propionate exacerbated the hypophagic effects of ammonium by stimulating oxidative metabolism in the liver. As discussed previously, urea synthesis requires amino acid catabolism for a source of N and increase availability of amino acid carbon to be either oxidized or utilized for gluconeogenesis in the liver. However, maximum rate of gluconeogenesis at any point in time is affected by enzyme activity regulated by hormones such as insulin and glucagon. When ammonium acetate was infused, carbon from amino acids that provided N for urea synthesis might have been

utilized for gluconeogenesis to a greater extent compared to oxidation in the liver because of a relative lack of glucose precursors. However, when ammonium propionate was infused, amino acid carbon was probably oxidized extensively in the liver because propionate also serves as substrate for gluconeogenesis in the ruminant liver. Concentration of ATP in the liver is expected to be greatest for ammonium propionate treatment compared to other treatments. Our working hypothesis that ATP production in the liver affects DMI and feeding behavior may explain the strong interaction between ammonia and propionate observed for DMI in this experiment.

Our speculation is also supported by observations of Dhiman et al., (1993). Dhiman et al. (1993) reported abomasal infusion of glucose at 1 kg/d decreased DMI by 18% compared to control for cows fed alfalfa silage at 98% of dietary DM (18.5% dietary CP) and that blood urea nitrogen concentration was greater than 30 mg/dl regardless of treatments. It is contrary to observation of Frobish and Davis (1977) in which abomasal infusion of glucose at 2.15 kg/d did not decrease DMI for cows fed a diet containing 60% concentrate. Although dietary CP and blood urea nitrogen concentrations were not reported by Frobish and Davis (1977), ammonium flux to the liver is expected to be significantly lower for their experiment because of greater fermentability of the diet compared to that in the experiment of Dhiman et al. (1993). Inconsistent hypophagic effects of glucose can be attributed to the interaction of glucose infusion with ammonium flux to the liver because glucose *per se* does not have hypophagic effects in ruminants (Allen, 2000). Enhanced urea synthesis might have stimulated oxidative metabolism to a

greater extent with abomasal infusion of glucose because glucose infusion is expected to decrease gluconeogenesis in the liver.

Dhiman et al. (1993) also reported that infusion of glucose with soy protein did not decrease DMI and this observation appeared to be inconsistent with the discussion above. However, lack of hypophagic effect of glucose when infused with soy protein can be explained by the difference in glucose demand of the mammary gland and subsequent gluconeogenesis in the liver. Infusion of glucose with soy protein increased milk yield by 6.1 kg/d compared to infusion of glucose alone. Milk production might have been limited by availability of essential amino acids when cows were infused with glucose only, but not when infused with glucose and soy protein. This difference in milk production increases glucose demand by 435 g/d according to the calculation method of Amaral-Phillips et al. (1993), and this is equivalent to 44% of the infused glucose. Thus, the gluconeogenesis is expected to be greater for cows infused with glucose and soy protein compared to cows infused with glucose only, decreasing oxidative metabolism of amino acid carbon generated by the urea cycle.

The explanation for the interaction of main effects observed in our experiment might be challenged because propionate can decrease hepatic capacity to detoxify ammonia by inhibiting the synthesis of N-acetyl glutamate, the activator of carbamoyl phosphate synthetase (Choung and Chamberlain, 1995). Choung and Chamberlain (1995) showed that intra-ruminal infusion of propionate and urea markedly increased plasma ammonia concentration compared to infusion of urea alone in dairy cows.

Mutsvangwa et al. (1997) evaluated the effect of propionate on urea production with isolated hepatocytes prepared from sheep, and found that urea production was decreased by half when hepatocytes were incubated with 1.25 mM of propionate and 1.25 mM of NH_4Cl compared to when they were incubated with 1.25 mM of NH_4Cl alone. However, in our experiments, the ammonium propionate treatment increased MUN to the same extent as the ammonium acetate treatment. Because urea equilibrates within body fluids, MUN is highly correlated with blood urea nitrogen and is an indicator of urea production by the liver (Bulter et al., 1996). Our data show that infusion of ammonium increased MUN synthesis similarly for each VFA type infused, indicating that propionate did not inhibit urea production in the liver substantially. Furthermore, although hepatocytes decreased urea production in vitro when incubated with propionate (Mutsvangwa et al., 1997), urea production increased four fold when hepatocytes were incubated with 1.25 mM of propionate and 1.25 mM of NH_4Cl compared to control (no NH_4Cl), indicating that propionate did not inhibit the urea cycle completely.

Feeding Behavior

Infusion of ammonium decreased meal frequency and this effect was greater when infused with propionate compared to acetate. Ammonium propionate decreased number of meal bouts by 46% compared to sodium propionate treatment (3.9 vs. 7.2 /12h), but ammonium acetate treatment decreased number of meal bouts by only 6% compared to sodium acetate treatment (6.6 vs. 7.0 /12h). However, meal size was not affected by ammonium treatment. This is consistent with observations from the previous experiment (Chapter 4), in which infusion of ammonium propionate decreased meal

frequency without affecting meal size compared to sodium and potassium propionate. These observations indicate that ammonium infusion does not result in satiety sooner, but that hunger is delayed. Our data did not provide conclusive evidence that ammonium exerts its hypophagic effect by oxidative metabolism of carbon from amino acids in the liver because ATP concentration in the liver was not determined in this experiment. However, sustained satiety or delayed hunger observed for ammonium treatments is consistent with our theory.

A possible explanation for the lack of ammonium effect on meal size is that urea synthesis occurs over time. The liver is a heterogeneous organ varying in enzyme activity between periportal and perivenous regions. Urea synthesis occurs in periportal hepatocytes and at physiological portal concentrations of ammonia, about two thirds of ammonia are incorporated in urea while glutamine synthesis in perivenous hepatocytes scavenges the remaining ammonia (Haussinger et al., 1992). Glutamine synthesis minimizes ammonia escaping hepatic detoxification when ammonium flux to the liver exceeds rate of urea synthesis; Rodriguez et al. (1997) showed that a diurnal variation in ruminal ammonia concentration is 10 times greater than that for plasma urea nitrogen concentration. Ammonia incorporated in glutamine is available for later urea synthesis because of significant activity of glutaminase in periportal hepatocytes (Haussinger et al., 1992). Nitrogen in carbamoyl phosphate comes from either ammonia absorbed from the gut or ammonia from glutamine. Generation of ammonia from glutamine in periportal hepatocytes allows urea synthesis to continue over time after meals. Ammonium infusion decreased DMI by delaying the sense of hunger without affecting satiety

possibly because urea synthesis occurs over time after meals providing carbon from amino acids for oxidative metabolism in the liver for an extended period. Total urea synthesis per day, estimated from MUN concentration, was similar for ammonium acetate and ammonium propionate treatments. Thus, decreased rate of urea synthesis by propionate (Choung and Chamberlain, 1995) following meals probably extended urea synthesis for propionate compared to acetate treatment over a longer period of time increasing intermeal interval.

Chewing Behavior

Eating time was decreased by infusion of propionate ($P < 0.001$) and ammonium ($P < 0.01$) compared to acetate and sodium, respectively. In addition, reduction in eating time was greater when ammonium was infused with propionate compared to acetate. Ruminating time was shorter for ammonium treatment compared to sodium treatment when infused with propionate (75 vs. 124 min), and this might be explained by decreased stimuli for chewing by the decreased digesta mass in the rumen from lower DMI. However, sodium treatment decreased ruminating time compared to ammonium treatment when infused with acetate (137 vs. 211 min), and ruminating time per kg DMI was lower for sodium treatment compared to ammonium treatment ($P < 0.04$). This might be attributed to the expected greater osmolarity of ruminal fluid for sodium treatment compared to ammonium treatment because of ammonium incorporation into microbial N. Greater osmotic pressure in ruminal fluid can decrease rumination (Welch, 1982). Water intake ($P < 0.0001$) and number of drinking bouts ($P < 0.02$) were greater for sodium treatments compared to ammonium treatments consistent with treatment

effects on ruminating time per kg DMI. An interaction of main effects was significant for water intake ($P < 0.01$), indicating that water intake was lower for ammonium propionate compared to ammonium acetate treatment while VFA type did not affect water intake for sodium treatments. This interaction can be attributed to lower DMI for ammonium propionate treatment compared to the others.

Milk Production

An interaction of main effects was observed for milk yield ($P < 0.06$) and milk fat concentration ($P < 0.01$). Ammonium treatment decreased milk yield compared to sodium treatment to a greater extent when infused with propionate (27.4 vs. 32.9 kg/d) compared to when infused with acetate (32.1 vs. 34.0 kg/d). Ammonium treatment decreased milk fat concentration (3.42 vs. 3.99 %) compared to sodium treatment when infused with acetate, but infusion of ammonium increased milk fat concentration (3.79 vs. 3.53 %) compared to sodium treatment when infused with propionate. Milk energy output was lower for propionate compared to acetate treatment ($P < 0.01$) and for ammonium compared to sodium treatment ($P < 0.01$), and these reductions in milk energy output can be attributed to lower ME intake. Milk lactose concentration was greater for sodium treatment compared to ammonium treatment ($P < 0.05$) regardless of VFA treatments. Milk lactose concentration is usually constant because milk fluid is synthesized by osmotic pressure of lactose in the Golgi apparatus, and osmolarity of milk is similar to that of blood (Halt, 1983). Increased milk lactose concentration might indicate that osmolarity of blood was increased (Wheelock et al., 1965) for sodium

treatments compared to ammonium treatments although blood osmolarity was not measured for this experiment.

CONCLUSION

Infusion of ammonium decreased DMI compared to sodium to a greater extent when infused with propionate compared to when it was infused with acetate. Reduction in DMI from ammonium infusion is attributed to decreased meal frequency not smaller meal size, indicating that ammonium delayed the sense of hunger. Urea synthesis requires N from amino acids, and deamination of amino acids increases carbon available for oxidation or gluconeogenesis in the liver. When ammonium was infused with propionate, increased oxidative metabolism and production of ATP in the liver are expected because rate of substrate supply for gluconeogenesis is more likely to exceed its rate of utilization. These results are consistent with the hypothesis that ATP production in the liver can affect feeding behavior and DMI. However, hepatic ATP concentration was not analyzed, and further research is needed to investigate the mechanism of feed intake regulation by propionate.

CHAPTER 6

Dose-response effects of intra-ruminal infusion of propionate on feeding behavior of lactating cows fed diets differing in fermentability

ABSTRACT

Two experiments were conducted to evaluate how dose-response effects of intra-ruminal infusion of propionate on feeding behavior and DMI are altered by diets differing in fermentability. Twelve ruminally-cannulated Holstein cows (99 ± 25 and 53 ± 21 days in milk, respectively for experiment 1 and 2; mean ± SD) were used in each experiment. Cows were fed diets containing either steam flaked corn or dry cracked corn (30% of dietary DM) in experiment 1, and cows were fed diets differing in forage to concentrate ratio (66:34 vs. 36:64) in experiment 2. For both experiments, experimental design was a crossover for dietary treatment, and a 6 x 6 Latin square for infusion treatment within a diet for each period. Infusion treatments were mixtures of sodium propionate and sodium acetate, at ratios of 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0, infused into the rumen continuously for 18 h starting 6 h before feeding at a rate of 23.1 mmol of sodium VFA/min. Propionate infusion decreased DMI for all dietary treatments. Although a difference in basal propionate production between diets was expected within each experiment, an interaction of main effects was not observed for DMI in both experiments. This indicates that propionate flux through the rumen per se does not generate satiety signals. Cows used in

experiment 1 decreased DMI and increased plasma glucose concentration linearly as propionate infusion increased. However, cows used in experiment 2 did not decrease DMI by lower rates of propionate infusion which were much more effective at increasing plasma glucose concentration (quadratic effect $P < 0.01$). It is speculated that propionate had less hypophagic effects when infused propionate was extensively utilized for gluconeogenesis but decreased DMI when the marginal effect of infused propionate on plasma glucose concentration decreased because propionate is oxidized in the liver unless it is utilized for gluconeogenesis. Propionate decreases feed intake in lactating dairy cows possibly by stimulating oxidative metabolism in the liver.

(**Key words:** propionate infusion, threshold response, diet fermentability, fill)

Abbreviation Key: SF = Steam Flaked Corn; **DC** = Dry Cracked Corn; **HF** = high forage diet; **LF** = low forage diet

INTRODUCTION

Feeding fermentable grains in diets sometimes decreases DMI in lactating dairy cows (Allen, 2000), and hypophagic effects of propionate have been reported (Choi and Allen, 1999; Chapter 3). However, some experiments in the literature have reported no effects of propionate infusion on feed intake (Deetz and Wangsness, 1981; Quigley and Heitmann, 1991; De Jong et al., 1981; Anil et al., 1993). Inconsistent hypophagic effects of propionate might be explained by a threshold response of propionate in feed intake regulation; infusion of propionate might not affect feeding behavior and DMI unless the

amount of infused propionate exceeds a threshold. Dose response effects of propionate on feed intake were previously investigated for lactating dairy cows (Anil et al., 1993; Chapter 3) and sheep (Farningham and Whyte, 1993), and infusion of propionate linearly decreased feed intake as infusion rate of propionate increased. A threshold response in DMI was not observed for those experiments.

Fermentability of experimental diets may affect the threshold for infused propionate to decrease DMI. In a review of the literature (Allen, 1997), amount of ruminally fermented organic matter and total VFA production were reported to range from 5.7 to 15.4 kg/d and 42 to 115 moles/d for lactating dairy cows, respectively. Because propionate concentration in the rumen can increase from 15 to 45% of total fermentation acids as amount of ruminally fermented OM increases (Davis, 1967), propionate production can range from 6.3 to 52 moles/d. Lack of a threshold response for infused propionate on DMI in the experiment reported by Farningham and Whyte (1993) might be because sheep were fed a very fermentable diet *ad libitum* containing 50% hay, 30% barley, and 10% molasses, and propionate production from diets might have already exceeded the threshold. However, Leuvenink et al. (1997) fed sheep a pelleted grass, and reported that propionate infusion into the mesenteric vein of mature sheep at a rate of 2 mmol/min decreased intake but the infusion at a rate of 1 mmol/min had no effect. Fermentability of diets can be altered by feeding grains differing in fermentability in the rumen or by feeding diets differing in forage to concentrate ratio. We hypothesized that fermentability of diets affects animal responses to intra-ruminal infusion of propionate in feeding behavior and DMI and that cows fed more fermentable

diets decreases DMI at lower doses of propionate compared to cows fed less fermentable diets.

In a previous study (Chapter 3), in which dose-response effect of propionate on DMI was evaluated, a threshold response was not observed although we tried to minimize fermentability of the experimental diet by feeding dry cracked corn that is poorly fermented in the rumen. In that study, propionate was dosed at 8 different rates of infusion from 0 to 16.7 mmol/min in experiment 1 or at 4 different rates of infusion from 0 to 25.0 mmol/min in experiment 2. Marginal reduction in DMI was numerically greater as infusion rate of propionate increased in both experiments, but quadratic effects of propionate infusion were not detected. Range of propionate infusion might not have been great enough for experiment 1 (Chapter 3) and increments of treatments might not have been sufficient for experiment 2 (Chapter 3) to detect the quadratic effects of propionate infusion on DMI. We expected to observe a threshold response to infused propionate on DMI using an appropriate experimental design with a wider range and sufficient treatment increments of infusion rates.

The objective of this experiment was to evaluate how dose-response effects of intra-ruminal infusion of propionate on feeding behavior and DMI are altered by diets differing in fermentability.

MATERIALS AND METHODS

Experimental procedures were approved by All University Committee on Animal Use and Care at Michigan State University.

Experiment 1

Twelve multiparous Holstein cows (99 ± 25 DIM; mean ± SD) cannulated ruminally for previous experiments were selected from the Michigan State University Dairy Cattle Teaching and Research Center. Experimental design was a crossover for dietary treatments and a 6 x 6 Latin square within a diet for each period. Experimental diets contained either steam flaked corn (SF) or dry cracked corn (DC) at 30% of dietary DM. Both corn grains were obtained from Pennfield Feeds (Lancaster, PA). Flake density of SF was 0.36 kg/L, and the mean particle size of DC was 3.7 mm. Both experimental diets contained corn silage (50% of forage DM), alfalfa silage (50% of forage DM), cottonseeds, a premix of protein supplements (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins (Table 1). Dietary NDF and CP concentrations were approximately 27.8 and 16.7%, respectively for both diets, and fed as total mixed rations. Throughout the experiment, cows were housed in tie-stalls, and fed once daily at 110% of expected intake. Periods were 34 d in length, and each period consisted of 20 d for diet adaptation, 3 d for data and sample collection to determine effects of dietary treatments, and 11 d for data and sample collection to determine effects of infusion treatments.

TABLE 1. Ingredients and nutrient composition of experimental diets in experiment 1 (% of dietary DM except for DM).

	SF[1]	DC[2]
Diet Ingredients		
Steam flaked corn	29.7	...
Dry cracked corn	...	29.6
Corn silage	23.5	23.6
Alfalfa silage	22.4	22.4
Whole linted cottonseed	6.7	6.7
Protein mix[3]	12.6	12.7
Vitamin & mineral mix[4]	5.0	5.1
Nutrient Composition		
DM	50.5	50.7
OM	94.3	94.1
Starch	32.1	29.9
NDF	27.6	27.9
ADF	19.5	19.5
CP	16.5	16.9
Ether extract	4.7	5.4
Forage NDF	19.9	20.0

[1] Diet containing steam flaked corn

[2] Diet containing dry cracked corn

[3] Protein mix contained 75% soybean meal, 20% distillers grain, and 5% blood meal.

[4] Vitamin & mineral mix contains 66.4% dry ground corn, 20.4% dicalcium phosphate, 7.8% salt, 2.4% magnesium oxide, 1.9% trace mineral premix, 0.34% vitamin A, 0.29% vitamin D, and 0.08% vitamin E.

Experiment 2

Twelve multiparous Holstein cows (53 ± 21 DIM; mean ± SD) cannulated ruminally for previous experiments were selected from the Michigan State University Dairy Cattle Teaching and Research Center. Experimental design was a crossover for dietary treatments and a 6 x 6 Latin square within a diet for each period. Experimental diets differed in forage to concentrate ratio; 66:34 for high forage diet (HF; Table 2) and 36:64 for low forage diet (LF). Both diets contained corn silage, alfalfa silage, cottonseeds, a premix of protein supplements (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins). The primary difference in diets was substitution of corn silage and distillers grain in the HF diet for dry ground corn in the LF diets. Distillers grain was used to compensate the lower CP concentration for corn silage compared to dry ground corn so that diet contained similar CP concentration with similar amino acid profiles. Dietary NDF and starch concentrations were 34.0 and 21.3 % for HF and 25.2 and 35.1 % for LF, respectively. Throughout the experiment, cows were housed in tie-stalls, and diets were fed as total mixed rations once daily at 110% of expected intake. Periods were 35 d in length, and each period consisted of 21 d for a diet adaptation, 3 d for data and sample collection to determine effects of dietary treatments, and 11 d for data and sample collection to determine effect of infusion treatments.

Common Infusion Protocol

Infusion treatments were continuous intra-ruminal infusion of mixtures of sodium propionate and sodium acetate at 6 different ratios. Cows were assigned to a 6 x 6 Latin square balanced for carry-over effects. Treatment solutions were prepared by diluting

TABLE 2. Ingredients and nutrient composition of experimental diets in experiment 2 (% of dietary DM except for DM).

	HF[1]	LF[2]
Diet Ingredients		
Corn silage	53.5	24.4
Alfalfa silage	12.1	12.0
Dry ground corn	...	34.3
Protein mix[3]	18.0	17.7
Distillers grain	4.7	...
Whole linted cottonseed	6.7	6.6
Vitamin & mineral mix[4]	5.0	5.0
Nutrient Composition		
DM	44.1	56.0
OM	94.0	94.8
Starch	21.3	35.1
NDF	34.0	25.2
ADF	23.0	16.8
CP	17.6	17.0
Ether extract	6.0	4.9
Forage NDF	26.3	15.4

[1] High forage diet
[2] Low forage diet
[3] Protein mix contained 75% soybean meal, 20% distillers grain, and 5% blood meal.
[4] Vitamin & mineral mix contains 66.4% dry ground corn, 20.4% dicalcium phosphate, 7.8% salt, 2.4% magnesium oxide, 1.9% trace mineral premix, 0.34% vitamin A, 0.29% vitamin D, and 0.08% vitamin E.

28.1 moles of sodium VFA (sodium propionate and sodium acetate at ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0) to 18 L with de-ionized water. Sodium acetate was added to keep the osmolarity and pH of infusates constant across the treatments to isolate the specific effects of propionate relative to acetate on feeding behavior of dairy cows. Concentration of total VFA was 1.56 M across treatments, and 16 L of each solution was infused over 18 h beginning 6 h prior to feeding. Infusion rate was 14.8 ml/min, which is equivalent to infusion of 23.1 mmol of VFA/min. The solutions were infused using 4-channel peristaltic pumps (#78016-30, Cole-Parmer Instrument, IL) and Tygon® tubing (7.5 m x 1.6mm I.D.). Infusion started 6 h prior to feeding so that VFA concentrations in the rumen reached steady state (assuming absorption rate and passage rate of 20 %/h and 15 %/h, respectively) by feeding time, at which monitoring for feeding behavior began. Sub-periods for infusion treatment were 2 d with 18 h of infusion followed by 30 h of recovery.

Data and Sample Collection

The amount of feed offered and orts were weighed for each cow daily during the collection period. Samples of all dietary ingredients (0.5 kg) were collected daily during each 3 d collection period and on feeding behavior monitoring days during each infusion period (d 1, 3, 5, 7, 9, and 11) and composited to one sample per diet period. Samples of orts (12.5%) were collected daily during the 3d-collection period and composited into one sample per cow per period. Body weight and BCS were measured [(Wildman, 1982); five-point scale where 1 = thin to 5 = fat] on d 23 of each period. Cows were milked twice daily in the milking parlor except for the evening milking for days in which

feeding behavior was monitored (d 1, 3, 5, 7, 9, and 11 of each infusion period) when cows were milked in their stall. Milk yield was measured daily during the 3 d collection period and was averaged to determine effects of dietary treatments. Milk yield was also measured on feeding behavior monitoring days to determine effects of infusion treatments. Milk was sampled at every milking during the 3 d collection period and d 1, 3, 5, 7, 9, and 11 of the infusion period, and analyzed for fat, true protein, lactose, solids-non-fat with infrared spectroscopy by Michigan DHIA (East Lansing).

Samples of feces, ruminal fluid, and blood were collected every 9 h during the 3 d collection period. Ruminal fluid samples were collected from 5 different sites in the rumen and 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 floride as a glycolytic inhibitor. Both were centrifuged at 2,000 x g for 15 minutes immediately after sample collection, and plasma was harvested and frozen at -20° C until analysis.

On feeding behavior monitoring days (d 1, 3, 5, 7, 9, and 11 of infusion period), infusion started at 0800 h, 6 h prior to feeding, and continued for 18 h. Cows were not allowed access to feed between 1000 h to 1400 h to minimize the confounding effects of ruminal fermentation from previous feeding. Feeding behavior was monitored for 12 h (1400 h to 0200 h) by a computerized data acquisition system (Dado and Allen, 1993).

Data of chewing activities, feed disappearance, and water consumption were recorded for each cow every 5 sec, and meal bouts, interval between meals, meal size, eating time, ruminating time, and total chewing time were calculated. At the end of the feeding behavior monitoring period (0200 h), samples of ruminal fluid were collected from 5 different sites in the rumen for each cow. Blood samples were collected from coccygeal vessels, centrifuged at 2,000 x g for 15 minutes, and plasma was harvested and frozen at -20° C until analysis.

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 (1mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, ADF, CP, and starch. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. Concentrations of NDF and ADF were determined [(VanSoest et al., 1991); method A for NDF]. Crude protein was analyzed according to Hach et al. (1985). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, and glucose concentration was measured using a commercial kit (Glucose kit #510; Sigma Chemical Co., St. Louis, MO). Indigestible NDF was estimated as NDF residue after 120 h in vitro fermentation (Goering and Van Soest, 1970) and used as an internal marker to calculate apparent total tract digestibility (Cochran et al., 1986). Concentrations of all nutrients except for DM were expressed as percentages of DM determined from drying at 105° C in a forced-air oven. Corn grain was dry sieved through 8 sieves (Sieve apertures: 4750, 2360, 1180,

600, 300, 150, 75 μm and bottom pan), using a sieve shaker (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant, and mean particle size of corn grain was calculated (ASAE, 1968).

Ruminal fluid samples were analyzed for VFA concentrations according to the method described previously (Chapter 2). Plasma samples were processed to determine concentrations of acetate and propionate in the similar manner as described for ruminal fluid (Chapter 2). Commercial kits were used to determine plasma concentration of glucose (Glucose kit #510; Sigma Chemical Co., St. Louis, MO) and insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

For both experiments, all data for dietary effects from 3 d of collection period were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijklm} = \mu + S_i + C(S)_{i(j)} + P_k + D_l + e_{ijklm}$$

where

μ = overall mean,

S_i = fixed effect of diet sequences (i = 1 to 2)

$C(S)_{i(j)}$ = random effect of cow nested in a diet sequence (j = 1 to 12),

P_k = fixed effect of periods (k = 1 to 2),

D_l = fixed effect of diets (l = 1 to 2), and

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

Orthogonal contrasts were made for the effect of diets (SF vs. DC or HF vs. LF). Treatment effects were declared significant at $P < 0.05$, and tendency for treatment effects was declared at $P < 0.10$.

All data for infusion effects from 11 d of infusion period were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijklmnop} = \mu + S_i + C(S)_{i(j)} + P_k + SP(P)_{l(k)} + D_m + L_n + Q_o + DL_{mn} + DQ_{mo} + e_{ijklmnop}$$

where

μ = overall mean,

S_i = fixed effect of diet sequences (i = 1 to 2)

$C(S)_{i(j)}$ = random effect of cows nested in a diet sequence (j = 1 to 12),

P_k = fixed effect of periods (k = 1 to 2),

$SP(P)_{l(k)}$ = fixed effect of sub-periods nested in a period (l = 1 to 6),

D_m = fixed effect of diets (l = 1 to 2),

L_n = linear effect of infusion

Q_o = quadratic effect of infusion

DL_{mn} = interaction between effect of diet and linear effect of infusion

DQ_{mo} = interaction between effect of diet and quadratic effect of infusion, and

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

Orthogonal contrasts were made for the effect of diet (SF vs. DC or HF vs. LF), linear effect of infusion, quadratic effect of infusion, interaction between effect of diet

and linear effect of infusion, and interaction between effect of diet and quadratic effect of infusion. Main treatment effects were declared significant at $P < 0.05$, and tendency for treatment effects was declared at $P < 0.10$. Interaction effects were declared significant at $P < 0.10$. Total expected number of observations (n) was 144, but 7 observations for experiment 1 and 3 observations for experiment 2 were missing. Our infusion protocol caused temporary metabolic alkalosis, and some cows experienced hypocalcemia and hypokalemia. Administration of infusates was cancelled when cows had adverse effects of previous infusion treatment.

Data from 3 d of collection period before infusion were analyzed to characterize the animals used in experiment 1 and experiment 2, using ANOVA procedure of JMP® according to the following model:

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

where

μ = overall mean,

E_i = fixed effect of experiment (i = 1 to 2; experiment 1 or experiment 2), and

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

The effect of experiment was declared significant at $P < 0.05$, and tendency was declared at $P < 0.10$.

RESULTS AND DISCUSSION

Experiment 1

Dry matter intake was not affected by dietary treatment (19.1 vs. 19.3 kg/12 h, respectively for SF and DC; Table 3), but milk yield was greater for cows fed SF compared to cows fed DC (37.4 vs. 35.8 kg/d; $P < 0.01$). Cows fed SF had higher apparent total tract digestibility of starch (95.2 vs. 77.3 %; $P < 0.001$), but lower total tract NDF digestibility (16.7 vs. 28.3 %; $P < 0.001$) compared to cows fed DC. Ruminal propionate concentration was greater (27.3 vs. 22.6 %; $P < 0.001$) and ruminal acetate concentration was less (55.8 vs. 61.1 %; $P < 0.001$) for SF compared to DC treatment although ruminal pH was not affected by treatments. Plasma insulin concentration was greater for SF compared to DC treatment (12.3 vs. 9.3 μIU/ml; $P < 0.001$) while plasma glucose concentration was not affected by dietary treatment. Cows fed SF increased milk protein (3.07 vs. 2.91%; $P < 0.001$) and milk lactose concentration (4.71 vs. 4.64%; $P < 0.01$), but decreased milk fat concentration (3.25 vs. 3.64%; $P < 0.05$) compared to cows fed DC. These observations are consistent with expected greater ruminal fermentation and greater ruminal propionate production for the SF diet compared to the DC diet although they were not directly measured.

Dry matter intake, meal size, and total ME intake were decreased linearly by increasing rate of intra-ruminal infusion of propionate ($P < 0.001$; Table 4). Interactions between diet and infusion effects were not significant for feeding behavior and DMI, indicating that diet did not affect the response to propionate infusion contrary to our

TABLE 3. Effects of processing method of corn grain on feeding behavior, ruminal fermentation, blood metabolites, nutrient digestibility, and milk production.

	SF[1]	DC[2]	SE	*P*-value
Feeding behavior				
DMI (kg/12h)	19.1	19.3	0.4	0.68
Meal bouts (/12h)	6.2	6.3	0.2	0.59
Intermeal interval (min)	86.0	81.2	4.6	0.48
Meal size (kg DM)	3.3	3.2	0.1	0.67
ME intake (Mcal/12h)[3]	43.3	40.8	1.4	0.23
Chewing time				
Eating (min/12h)	187	207	5	**
Ruminating (min/12h)	240	223	5	0.06
Total (min/12h)	426	430	8	0.74
Water intake (L/12h)	69.6	64.7	1.9	0.10
Ruminal fermentation				
pH	6.14	6.15	0.02	0.71
Total VFA (mM)	121.0	121.9	1.9	0.73
Acetate (%)	55.8	61.1	0.4	***
Propionate (%)	27.3	22.6	0.5	***
Iso-butyrate (%)	0.68	0.92	0.03	***
Butyrate (%)	12.7	11.9	0.4	0.17
Iso-valerate (%)	1.18	1.58	0.05	***
Valerate (%)	2.31	1.83	0.17	0.07
A:P ratio[4]	2.09	2.73	0.05	***
Plasma				
Acetate (mM)	1.07	1.21	0.05	0.09
Propionate (mM)	0.21	0.20	0.01	0.53
Glucose (mg/dl)	56.1	55.5	0.4	0.32
Insulin (μIU/ml)	12.3	9.3	0.6	**

TABLE 3 (cont'd)

	SF[1]	DC[2]	SE	*P*-value
Apparent total tract digestibility (%)				
DM	58.7	54.4	0.9	**
OM	60.4	55.9	0.9	**
NDF	16.7	28.3	1.3	***
Starch	95.2	77.3	1.3	***
CP	59.3	58.2	0.8	0.37
EE	72.8	68.8	1.2	*
Milk production				
Milk yield (kg/d)	37.4	35.8	0.4	**
Milk fat (%)	3.25	3.64	0.12	*
Milk protein (%)	3.07	2.91	0.02	***
Milk lactose (%)	4.71	4.64	0.02	**
Milk SNF (%)	8.64	8.40	0.02	***
Milk energy (Mcal/d)	24.5	24.5	1.5	0.93
Milk energy (Mcal/d) : MEI[5] (Mcal/12h)	0.56	0.61	0.03	0.10
Body Weight (kg)	703	693	6	0.28
BCS	2.73	2.63	0.07	0.29

[1] Diet containing steam flaked corn;
[2] Diet containing dry cracked corn
[3] Metabolizable energy was calculated according to NRC (2001) based on actual digestibility of diets.
[4] Acetate to propionate ratio
[5] MEI: metabolizable energy intake
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

TABLE 4. Dose-response effects of intra-ruminal infusion of sodium propionate relative to sodium acetate on feeding behavior and milk production for cows fed steam flaked corn or dry cracked corn.

	SF[1]						DC[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Actual volume infused (L)	16.3	16.2	16.4	16.2	16.3	16.3	16.0	15.9	16.2	16.3	16.0	16.1	0.2	0.18	0.62	0.38
Feeding behavior																
DMI (kg/12h)	14.8	13.5	13.7	12.9	11.7	10.3	13.2	12.9	12.1	11.7	11.6	9.7	0.8	0.14	***	0.21
Meal bouts (/12h)	6.7	7.3	6.7	6.8	7.0	6.7	7.1	7.3	6.3	7.7	7.4	6.3	0.5	0.55	0.68	0.48
Intermeal interval (min)	95.2	80.5	97.5	91.1	90.1	91.6	91.5	87.1	93.4	79.6	76.1	98.1	8.0	0.25	0.90	0.33
Meal size (kg DM)	2.6	2.0	2.1	2.1	1.9	1.7	2.0	2.0	2.1	1.7	1.6	1.6	0.2	0.21	***	0.51
ME intake (Mcal/12h)																
Diet[7]	34.0	31.1	31.7	29.4	27.0	23.5	27.9	27.3	25.6	25.0	24.4	20.3	1.7	**	***	0.19
Infusion[8]	3.5	4.0	4.6	5.2	5.6	6.2	3.5	4.1	4.7	5.2	5.7	6.2	0.04	0.22	***	0.06
Total	37.5	35.1	36.3	34.6	32.5	29.6	31.5	31.4	30.2	30.2	30.1	26.5	1.7	**	***	0.17
Chewing time (min/12h)																
Eating	140	129	134	127	120	108	144	139	131	131	128	111	8	0.74	***	0.35
Ruminating	85	97	92	105	86	92	94	95	95	86	96	76	9	0.68	0.47	0.19
Total	224	225	228	230	207	192	239	234	225	217	223	186	14	0.88	**	0.09
Water intake (L/12h)	101	102	90	95	87	87	93	92	89	93	88	84	4	0.49	***	0.79

TABLE 4 (cont'd).

	SF[1]						DC[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Milk production																
Milk yield (kg/d)	35.4	35.4	35.2	36.7	34.2	35.2	32.3	33.9	33.1	33.1	34.2	32.7	0.7	**	0.89	0.20
Milk fat (%)	3.98	3.94	4.16	4.73	3.83	3.73	4.13	4.38	4.18	4.01	4.00	4.07	0.23	0.52	0.20	0.08
Milk protein (%)	3.25	3.13	3.14	3.01	3.11	3.07	3.06	3.02	3.01	2.96	2.71	2.91	0.06	*	***	0.29
Milk lactose (%)	4.84	4.77	4.81	4.75	4.87	4.85	4.78	4.73	4.77	4.82	4.52	4.88	0.09	0.30	0.99	0.36
Milk SNF (%)	9.00	8.80	8.87	8.64	8.87	8.81	8.74	8.63	8.67	8.65	8.06	8.66	0.17	0.08	0.08	0.35
Milk energy (Mcal/d)	26.3	26.3	26.2	29.2	24.8	25.1	23.9	26.0	24.6	24.0	24.0	23.9	1.8	*	0.23	0.20
Milk energy (Mcal/d): MEI[9](Mcal/12h)	0.71	0.80	0.74	0.92	0.78	0.89	0.79	0.83	0.87	0.81	0.87	0.92	0.01	0.60	*	0.97

[1] Diet containing steam flaked corn
[2] Diet containing dry cracked corn
[3] Propionate content in infusates (%)
[4] Effect of diet
[5] Linear effect of intra-ruminal infusion of propionate
[6] Quadratic effect of intra-ruminal infusion of propionate
[7] Metabolizable energy intake from diet was calculated according to NRC (2001) based on actual digestibility of diets.
[8] Metabolizable energy intake from infusates assumed energy density of 0.2094 and 0.3672 Mcal/mol for acetate and propionate, respectively.
[9] MEI: total metabolizable energy intake from infusates and diets
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

hypothesis. Milk yield was not affected by infusion treatments although milk protein concentration decreased linearly as rate of propionate infusion increased ($P < 0.001$). This is consistent with previous observations (Chapter 3), and might be because decreased DMI compromised microbial protein production in the rumen while additional energy supplied in infusates sustained milk yield. Infusion of glucogenic energy as propionate without additional amino acid supply may have diluted milk protein by relatively greater lactose synthesis and milk yield when metabolizable protein limited maximum milk yield. A significant interaction was observed between dietary treatment and linear effect of infusion for ruminal propionate concentration at the end of infusion. As rate of propionate infusion increased, ruminal propionate concentration increased linearly ($P < 0.001$) for both diets. However, ruminal propionate concentration was greater for SF compared to DC treatment at lower rates of propionate infusion while it was similar for both treatments at higher rates of propionate infusion. This might be because DMI decreased to a greater extent and ruminal fermentation of diets contributed to ruminal propionate concentration to a lesser extent at higher rates of propionate infusion. However, similar propionate concentration in ruminal fluid does not necessarily indicate similar propionate production in the rumen because concentration is determined by the rate of removal (absorption and passage) as well as rate of production.

Propionate infusion linearly increased plasma concentration of propionate, glucose, and insulin, but decreased plasma acetate concentration ($P < 0.001$; Table 5). Interaction for main effects was significant for plasma concentrations of propionate and

TABLE 5. Dose-response effects of intra-ruminal infusion of sodium propionate relative to sodium acetate on metabolites in the rumen and the blood for cows fed steam flaked corn or dry cracked corn.

	SF[1]						DC[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Ruminal fermentation																
Total VFA (mM)	122	128	125	125	123	126	123	119	119	118	127	122	5	0.19	0.68	0.69
Acetate (%)[DL]	69.8	65.7	60.3	56.3	50.9	44.5	72.7	67.9	62.8	57.5	50.3	44.2	0.7	*	***	**
Propionate (%)[DL]	19.3	24.1	28.5	33.1	38.4	45.1	16.2	21.2	26.3	31.2	38.6	45.0	0.8	*	***	**
Iso-butyrate (%)	0.60	0.56	0.62	0.66	0.83	0.78	0.58	0.66	0.66	0.76	0.91	0.92	0.06	0.23	***	0.47
Butyrate (%)	7.9	7.1	7.7	7.0	6.8	6.6	8.0	7.5	7.5	7.5	7.3	6.8	0.3	0.32	***	0.77
Iso-valerate (%)	1.24	1.27	1.46	1.41	1.57	1.49	1.48	1.56	1.44	1.67	1.58	1.68	0.07	0.07	***	0.63
Valerate (%)	1.18	1.24	1.40	1.45	1.55	1.54	1.11	1.17	1.26	1.41	1.41	1.45	0.06	*	***	0.14
A:P ratio[6, DL, DQ]	3.75	2.78	2.17	1.76	1.35	1.02	4.59	3.22	2.39	1.84	1.33	1.00	0.09	0.12	***	***

TABLE 5 (cont'd).

	SF[1]						DC[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Plasma																
Acetate (mM)	2.00	1.51	1.42	1.05	0.86	0.87	2.21	1.78	1.47	1.13	1.00	0.77	0.13	0.25	***	*
Propionate (mM)[DL, DQ]	0.21	0.21	0.22	0.22	0.28	0.34	0.20	0.22	0.22	0.23	0.26	0.27	0.02	0.50	***	**
Glucose (mg/dl)	61.9	62.9	62.9	65.0	63.7	65.9	59.7	60.8	61.4	62.3	62.0	65.3	0.8	**	***	0.41
Insulin (μIU/ml)[DL, DQ]	9.0	9.7	10.4	11.3	14.5	15.6	7.5	7.1	9.4	11.0	11.3	10.2	1.0	0.23	***	0.96
pH	7.55	7.55	7.55	7.55	7.53	7.54	7.53	7.54	7.54	7.54	7.53	7.54	0.01	0.35	0.20	0.88
Na^+ (mM)[DQ]	149	149	150	149	149	149	150	149	150	149	149	150	0.4	0.58	0.34	0.35
K^+ (mM)	3.47	3.45	3.42	3.40	3.48	3.26	3.68	3.48	3.54	3.51	3.51	3.28	0.09	0.47	**	0.40
Cl^- (mM)	93.7	93.0	94.1	93.4	92.4	93.7	94.5	94.3	95.0	94.3	94.3	94.2	0.6	*	0.54	0.98
Ca^{++} (mM)	1.06	1.07	1.03	1.06	1.04	1.01	1.05	1.04	1.06	1.03	1.03	0.98	0.02	0.71	***	0.09

[1] Diet containing steam flaked corn

[2] Diet containing dry cracked corn

[3] Propionate content in infusates (%)

[4] Effect of diet

[5] Linear effect of intra-ruminal infusion of propionate

[6] Quadratic effect of intra-ruminal infusion of propionate

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

[DL] Significant interaction of diet effect and linear effect of propionate ($P < 0.10$)

[DQ] Significant interaction of diet effect and quadratic effect of propionate ($P < 0.10$)

insulin; cows fed SF increased plasma concentrations of propionate and insulin to a greater extent at higher rates of propionate infusion compared to cows fed DC. Greater response to propionate infusion in plasma insulin concentration for SF compared to DC treatment might be due to greater propionate absorption for SF because propionate stimulates insulin secretion in ruminants (De Jong, 1982; Istasse et al., 1987). Propionate infusion at 100% of VFA increased plasma propionate concentration by 62% compared to propionate infusion at 0% of VFA for cows fed SF diet (0.21 and 0.34 mM, respectively for 0 and 100% propionate) and for cows fed DC diet by 35% (0.20 and 0.27 mM, respectively for 0 and 100% propionate). These observations indicate that some propionate escapes hepatic metabolism and reaches peripheral tissues although infused propionate is extensively metabolized in the liver.

In experiment 1, no interactions between diet and infusions were detected for feeding behavior or DMI, and we speculate that cows fed SF were more tolerant to hypophagic effects of propionate. Rate of glucose clearance from blood circulation was expected to be greater for SF treatment because of increased milk yield and greater plasma insulin concentration. However, plasma glucose concentration was not affected by dietary treatments during 3 d collection period before infusion, and was greater for SF compared to DC treatment during the infusion period. This suggests that rate of gluconeogenesis was greater for cows fed SF compared to cows fed DC possibly because greater starch digestion in the rumen increased availability of glucose precursors. Although propionate flux to the liver is expected to be greater for SF compared to DC treatment, greater rate of gluconeogensis might decrease the relative proportion of

infused propionate oxidized in the liver for the SF treatment. Our results suggest that the propionate flux to the liver does not relate directly to generation of satiety signals, but implies that hypophagic effects of propionate are altered by how propionate is utilized in the liver. Propionate may not exert hypophagic effects if it is utilized for gluconeogenesis, but decreases feed intake by stimulating oxidative metabolism in the liver.

Experiment 2

No effect of diet was observed on DMI and milk yield (Table 6). Although cows fed HF had greater apparent total tract digestibility of NDF (25.6 vs. 18.5 %; $P < 0.05$), CP (65.5 vs. 61.2 %; $P < 0.01$), and EE (84.7 vs. 67.9 %; $P < 0.001$) compared to cows fed LF, apparent total tract OM digestibility was not affected by dietary treatments. This is because HF treatment contained less starch, which is a highly digestible fraction of the diet, compared to LF treatment. The LF treatment increased ruminal propionate concentration (28.3 vs. 22.5%; $P < 0.01$) but decreased acetate concentration (55.0 vs. 60.2%; $P < 0.001$), and ruminal pH (5.87 vs. 6.04; $P < 0.01$) compared to HF. Plasma acetate concentration was greater for HF compared to LF treatment (1.23 vs. 0.94 mM; $P < 0.01$), but plasma concentrations of glucose and insulin were not affected by dietary treatments. The HF treatment increased milk fat concentration compared to LF (3.67 vs. 3.14%; $P < 0.01$). These observations are consistent with our expectation of greater ruminal fermentability and ruminal propionate production for LF compared to HF diets, although they were not directly measured.

TABLE 6. Effects of forage to concentrate ratio on feeding behavior, ruminal fermentation, blood metabolites, nutrient digestibility, and milk production.

	HF[1]	LF[2]	SE	*P*-value
Feeding behavior				
DMI (kg/12h)	14.7	15.8	0.6	0.26
Meal bouts (/12h)	7.1	7.2	0.2	0.91
Intermeal interval (min)	72.5	80.9	9.1	0.53
Meal size (kg DM)	2.1	2.4	0.1	0.28
ME intake (Mcal/12h)[3]	35.0	36.5	1.3	0.45
Chewing time				
Eating (min/12h)	198	173	8	*
Ruminating (min/12h)	243	187	11	**
Total (min/12h)	440	360	17	**
Water intake (L/12h)	65.1	63.2	4.9	0.80
Ruminal fermentation				
pH	6.04	5.87	0.03	**
Total VFA (mM)	114.6	117.0	2.2	0.47
Acetate (%)	60.2	55.0	0.9	**
Propionate (%)	22.5	28.3	1.2	**
Iso-butyrate (%)	0.98	0.84	0.03	*
Butyrate (%)	12.7	11.9	0.4	0.19
Iso-valerate (%)	1.87	1.63	0.10	0.12
Valerate (%)	1.72	2.30	0.18	*
A:P ratio[4]	2.71	2.07	0.15	*
Plasma				
Acetate (mM)	1.23	0.94	0.05	**
Propionate (mM)	0.11	0.13	0.01	0.35
Glucose (mg/dl)	51.4	50.8	1.1	0.72
Insulin (μIU/ml)	9.1	10.4	0.7	0.19

TABLE 6 (cont'd).

	HF[1]	LF[2]	SE	*P*-value
Apparent total tract digestibility (%)				
DM	58.0	59.4	0.9	0.28
OM	59.7	60.9	0.9	0.41
NDF	25.6	18.5	2.0	*
Starch	92.8	91.3	1.0	0.29
CP	65.5	61.2	0.9	**
EE	84.7	67.9	1.2	***
Milk production				
Milk yield (kg/d)	37.3	38.4	1.5	0.62
Milk fat (%)	3.67	3.14	0.12	**
Milk protein (%)	2.68	2.75	0.03	0.16
Milk lactose (%)	4.70	4.70	0.04	0.93
Milk SNF (%)	8.18	8.31	0.05	0.08
Milk energy (Mcal/d)	25.1	23.9	1.2	0.42
Milk energy (Mcal/d) : MEI[5] (Mcal/12h)	0.72	0.67	0.04	0.10
Body Weight (kg)	626	615	4	0.07
BCS	2.46	2.60	0.05	0.06

[1] High forage diet

[2] Low forage diet

[3] Metabolizable energy was calculated according to NRC (2001) based on actual digestibility of diets.

[4] Acetate to propionate ratio

[5] MEI: metabolizable energy intake

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

TABLE 7. Dose-response effects of intra-ruminal infusion of sodium propionate relative to sodium acetate on feeding behavior and milk production for cows fed diets differed in forage to concentrate ratio.

	HF[1]						LF[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Actual volume infused (L)	15.9	15.9	16.0	15.9	15.9	15.9	15.9	15.9	15.9	16.0	16.0	15.9	0.2	0.64	0.54	0.49
Feeding behavior																
DMI (kg/12h)	11.6	12.5	11.8	11.3	9.0	7.9	11.7	13.2	13.5	10.4	8.7	7.8	0.9	0.64	***	**
Meal bouts (/12h)	6.9	7.0	5.9	6.3	6.2	6.0	6.3	6.6	6.2	5.3	6.3	5.9	0.5	0.63	*	0.56
Intermeal interval (min)	92.7	86.4	95.8	90.4	90.2	102	90.8	92.1	93.4	83.0	72.8	96.2	9.0	0.51	0.91	0.31
Meal size (kg DM)	1.9	1.9	2.4	1.9	1.5	1.5	1.9	2.2	2.3	2.1	1.5	1.4	0.2	0.04	***	**
ME intake (Mcal/12h)																
Diet[7]	27.4	29.6	28.1	26.5	21.6	18.8	27.7	30.6	31.3	24.2	20.0	17.9	2.1	0.89	***	**
Infusion[8]	3.5	4.0	4.6	5.1	5.5	6.1	3.5	4.0	4.5	5.1	5.6	6.1	0.03	0.62	***	0.08
Total	30.9	33.6	32.7	31.6	27.1	24.8	31.2	34.5	35.8	29.3	25.6	24.0	2.0	0.89	***	**
Chewing time (min/12h)																
Eating	152	164	154	149	128	113	136	147	151	112	103	98	11	*	***	*
Ruminating	112	93	103	112	103	77	65	77	78	64	54	63	12	***	0.08	0.28
Total	264	258	258	261	228	190	196	224	235	176	157	160	19	**	***	*
Water intake (L/12h)	87	99	90	90	87	78	87	91	94	83	87	92	5	0.39	0.24	0.20

TABLE 7 (cont'd).

	HF[1]						LF[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Milk production																
Milk yield (kg/d)	30.9	34.2	32.5	34.1	32.3	32.5	33.4	37.2	36.3	35.4	34.6	35.3	1.2	*	0.73	0.07
Milk fat (%)	4.22	4.11	3.80	3.84	3.68	3.60	3.82	3.56	3.43	3.42	3.17	3.44	0.16	**	***	0.13
Milk protein (%)	2.87	2.77	2.71	2.69	2.65	2.63	2.96	2.81	2.78	2.70	2.66	2.70	0.03	0.32	***	***
Milk lactose (%)	4.89	4.95	5.00	4.98	5.06	5.08	4.99	5.02	5.06	5.05	5.10	5.08	0.04	*	***	0.60
Milk SNF (%)	8.68	8.64	8.62	8.60	8.60	8.61	8.88	8.72	8.76	8.65	8.67	8.69	0.05	0.07	**	0.06
Milk energy (Mcal/d)	22.9	24.9	22.5	23.8	22.1	22.1	23.9	25.3	24.3	23.6	22.2	23.6	1.2	0.55	*	0.57
Milk energy (Mcal/d): MEI[9](Mcal/12h)	0.78	0.78	0.73	0.78	0.91	1.00	1.00	0.80	0.73	1.06	0.91	1.08	0.11	0.36	*	*

[1] High forage diet
[2] Low forage diet
[3] Propionate content in infusates (%)
[4] Effect of diet
[5] Linear effect of intra-ruminal infusion of propionate
[6] Quadratic effect of intra-ruminal infusion of propionate
[7] Metabolizable energy intake from diet was calculated according to NRC (2001) based on actual digestibility of diets.
[8] Metabolizable energy intake from infusates assumed energy density of 0.2094 and 0.3672 Mcal/mol for acetate and propionate, respectively.
[9] MEI: total metabolizable energy intake from infusates and diets
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Quadratic effects of infusion were significant for DMI, meal size, and total ME intake ($P < 0.01$; Table 7). This observation indicates that a threshold for the effect of infused propionate on DMI exists and that infused propionate did not decrease DMI at lower rates of propionate infusion but decreased DMI after the threshold was reached. However, interactions between diet and infusion effects were not detected for feeding behavior and DMI, indicating that diet did not affect the response to propionate infusion, which is contrary to our hypothesis. We speculate that physical fill is a more dominant mechanism regulating feed intake for cows fed HF while satiety related to propionate metabolism is more dominant feed intake regulator for cows fed LF. A potential hypophagic effect of fill for HF treatment and that of greater basal propionate production for LF treatment might have had similar effects on DMI because integration of both physical fill and metabolic satiety signals contributes to the regulation of voluntary feed intake (Forbes, 1995). Mbanya et al. (1993) infused acetate, propionate, or both, with or without distention of the rumen by a balloon. Combination of VFA infusion and distention significantly depressed DMI while VFA infusion or distention alone did not. Their observation indicates that the threshold for infused propionate to decrease DMI can be altered by dietary fill.

Intra-ruminal infusion of propionate linearly increased milk lactose concentration ($P < 0.001$), but decreased concentrations of milk fat ($P < 0.001$), protein ($P < 0.001$), and SNF ($P < 0.01$). Propionate infusion linearly increased ruminal propionate concentration ($P < 0.001$; Table 8) and decreased ruminal acetate concentration ($P < 0.001$). Propionate infusion linearly increased plasma concentration of propionate ($P <$

TABLE 8. Dose-response effects of intra-ruminal infusion of sodium propionate relative to sodium acetate on metabolites in the rumen and the blood for cows fed diets differed in forage to concentrate ratio.

	HF[1]						LF[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Ruminal fermentation																
Total VFA (mM)	135	134	139	140	129	134	128	140	138	132	132	137	6	0.94	0.94	0.50
Acetate (%)	72.9	64.5	61.2	55.5	49.5	41.8	72.7	65.9	59.9	53.5	45.8	44.0	1.5	0.12	***	0.62
Propionate (%)	16.1	24.0	27.5	33.4	39.7	48.3	16.4	22.4	29.1	36.5	43.6	45.9	1.6	0.07	***	0.97
Iso-butyrate (%)	0.71	0.73	0.69	0.72	0.71	0.67	0.73	0.72	0.68	0.68	0.73	0.70	0.04	0.46	0.40	0.86
Butyrate (%)	8.0	8.1	7.9	7.4	7.0	6.4	7.8	8.3	7.6	6.6	6.8	6.5	0.4	0.21	***	0.31
Iso-valerate (%)	1.38	1.40	1.43	1.55	1.67	1.56	1.43	1.56	1.39	1.53	1.61	1.58	0.09	0.91	**	0.91
Valerate (%)	0.92	1.23	1.25	1.42	1.41	1.35	0.98	1.20	1.40	1.27	1.37	1.36	0.07	0.76	***	***
A:P ratio[6]	4.72	2.96	2.24	1.75	1.30	0.92	4.75	3.01	2.08	1.49	1.07	1.08	0.20	0.21	***	***

TABLE 8 (cont'd).

	HF[1]						LF[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		D[4]	L[5]	Q[6]
Plasma																
Acetate (mM)	3.4	2.1	1.4	1.2	0.9	0.8	2.9	1.7	1.4	1.0	0.9	0.7	0.2	0.67	***	***
Propionate (mM)	0.10	0.11	0.11	0.13	0.15	0.19	0.07	0.10	0.10	0.11	0.16	0.19	0.02	0.89	***	0.07
Glucose (mg/dl)	54.6	56.4	60.6	58.9	62.2	61.6	54.2	57.7	59.7	63.0	63.2	63.2	1.0	0.11	***	**
Insulin (μIU/ml)	7.8	9.5	10.9	11.6	9.8	10.5	7.9	10.3	11.2	11.5	12.2	13.2	1.0	0.55	***	0.06
pH [DL]	7.55	7.55	7.55	7.55	7.55	7.58	7.55	7.55	7.55	7.55	7.56	7.55	0.01	0.73	*	0.11
Na^+ (mM)	150	149	150	150	151	151	150	150	151	152	151	151	0.7	*	*	0.53
K^+ (mM)	3.39	3.37	3.32	3.12	3.10	2.97	3.22	3.24	3.19	3.19	3.07	2.99	0.08	0.52	***	0.23
Cl^- (mM)	91.8	91.7	92.9	92.9	91.9	91.0	91.8	92.7	92.6	93.4	91.5	92.0	0.7	0.85	0.52	*
Ca^{++} (mM)	1.08	1.03	1.03	1.04	1.00	0.99	1.02	1.02	1.02	0.97	0.96	0.97	0.02	*	***	0.74

[1] High forage diet

[2] Low forage diet

[3] Propionate content in infusates (%)

[4] Effect of diet

[5] Linear effect of intra-ruminal infusion of propionate

[6] Quadratic effect of intra-ruminal infusion of propionate

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

[DL] Significant interaction of diet effect and linear effect of propionate ($P < 0.10$)

0.001) and insulin ($P < 0.001$), but decreased quadratically plasma acetate concentration ($P < 0.001$). Linear reduction in plasma acetate concentration was expected because the proportion of acetate in the VFA infused decreases as the proportion of propionate in the VFA infused increases. However, a significant quadratic effect of infusion on plasma acetate concentration indicates that acetate concentration was decreased by propionate infusion to a greater extent at lower rates of infusion compared to higher rates of infusion. Similarly, quadratic effect of infusion treatment was significant for plasma glucose concentration ($P < 0.01$). This indicates that propionate infusion increased glucose concentration to a greater extent at lower dose of propionate infusion, but further increase in rates of propionate infusion increased plasma glucose concentration to a lesser extent. Interactions between diet and infusion effects were not observed for any ruminal fermentation and plasma metabolite response variables.

Threshold Response

In experiment 1, a threshold for effect of propionate on DMI did not exist and infused propionate decreased DMI linearly (Figure 1). However, in experiment 2, a threshold for propionate to affect DMI was observed; infused propionate did not decrease DMI at lower rates of propionate infusion and decreased DMI linearly after a threshold was reached (Figure 1). Inconsistent threshold responses observed between experiments 1 and 2 cannot be attributed to differences in dietary characteristics between experiments 1 and 2. Both experiments were designed to evaluate how dose-response effects of intra-ruminal infusion of propionate are affected by different fermentability of diets within each experiment. Apparent total tract starch digestibility was different by 17.9

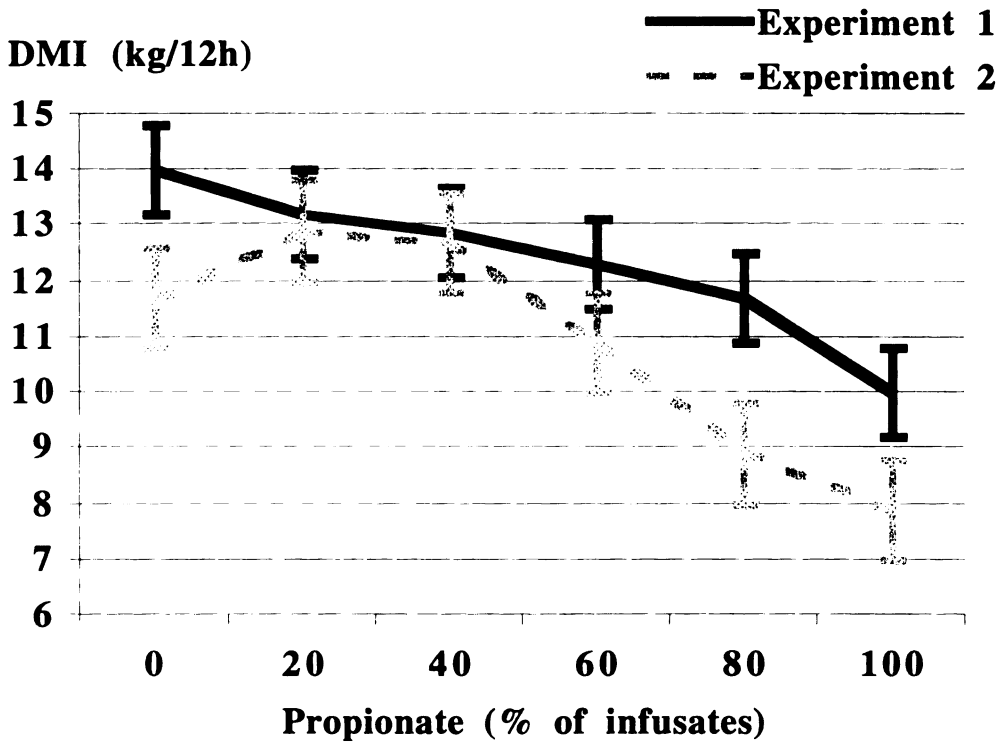

Figure 1. Dose-response effects of intra-ruminal propionate infusion on DMI (kg/12h).

Pooled means for DMI within an experiment at each infusion rate were used.

percentage units in experiment 1 (95.2 vs. 77.3 %), and dietary NDF concentration was different by 8.8 percentage units for experiment 2 (34.0 vs. 25.2 %DM). If diet affects DMI response to propionate infusion, dietary difference within each experiment should be great enough to detect the interaction between diet and infusion effects. Therefore, the threshold response observed in experiment 2 is more likely attributed to animal factors that differed from experiment 1 because different cows were used in each experiment.

Data from the 3 d collection period prior to infusion were analyzed to characterize the animals used in experiment 1 and experiment 2 (Table 9). Cows used in experiment 1 had greater DMI (19.2 vs. 15.3 kg/12h; $P < 0.001$) and meal size (3.3 vs. 2.2 kg; $P < 0.01$) compared to cows used in experiment 2 although milk yield was not different. Milk energy output per ME intake was greater for cows used in experiment 2 compared to cows used in experiment 1 (0.68 vs. 0.58 Mcal/ME intake). This indicates that efficiency of energy utilization for milk production was greater for cows used in experiment 2 compared to cows used in experiment 1. In addition, cows used in experiment 1 had greater BW than cows used in experiment 2 (698 vs. 620 kg; $P < 0.01$). Plasma concentrations of propionate (0.21 vs. 0.12 mM; $P < 0.001$) and glucose (55.8 vs. 51.1 mg/dl; $P < 0.001$) were greater for cows in experiment 1 compared to cows in experiment 2. The difference in plasma glucose concentration between cows used in each experiment might help to explain inconsistent threshold responses to propionate infusion in DMI and feeding behavior observed between the experiments because propionate is a primary substrate for gluconeogenesis in ruminants. We speculate that

TABLE 9. Characteristics of cows used in experiment 1 and experiment 2[1].

	Experiment 1	Experiment 2	SE	*P*-value
Days in milk[2]	98.5	53.3	6.7	***
Feeding behavior				
DMI (kg/12h)	19.2	15.3	0.6	***
Meal bouts (/12h)	6.3	7.2	0.5	0.18
Intermeal interval (min)	83.6	76.7	7.1	0.50
Meal size (kg DM)	3.3	2.2	0.2	**
ME intake (Mcal/12h)[3]	42.1	35.8	1.4	**
Chewing time				
Eating (min/12h)	197	185	10	0.39
Ruminating (min/12h)	231	215	8	0.19
Total (min/12h)	428	400	11	0.09
Water intake (L/12h)	67.1	64.3	2.8	0.49
Ruminal fermentation				
pH	6.15	5.96	0.04	**
Total VFA (mM)	121.5	115.8	2.2	0.09
Acetate (%)	58.5	57.7	0.9	0.52
Propionate (%)	25.0	25.4	0.8	0.72
Iso-butyrate (%)	0.83	0.93	0.03	*
Butyrate (%)	12.3	12.3	0.2	0.98
Iso-valerate (%)	1.43	1.78	0.06	***
Valerate (%)	2.09	2.03	0.12	0.74
A:P ratio[4]	2.42	2.39	0.11	0.88
Plasma				
Acetate (mM)	1.14	1.09	0.05	0.47
Propionate (mM)	0.21	0.12	0.01	***
Glucose (mg/dl)	55.8	51.1	0.9	***
Insulin (μIU/ml)	10.8	9.8	0.9	0.41

TABLE 9 (cont'd).

	Experiment 1	Experiment 2	SE	*P*-value
Apparent total tract digestibility (%)				
DM	56.6	58.7	0.7	*
OM	58.2	60.3	0.8	0.06
NDF	22.5	22.1	1.3	0.80
Starch	86.2	92.1	1.0	***
CP	58.8	63.4	0.7	***
EE	70.8	76.3	1.4	**
Milk production				
Milk yield (kg/d)	36.6	37.8	1.8	0.64
Milk fat (%)	3.45	3.40	0.12	0.79
Milk protein (%)	2.99	2.71	0.04	***
Milk lactose (%)	4.67	4.70	0.05	0.71
Milk SNF (%)	8.52	8.25	0.07	**
Milk energy (Mcal/d)	24.1	24.5	1.3	0.87
Milk energy (Mcal/d) : MEI [5](Mcal/12h)	0.58	0.68	0.03	*
Body Weight (kg)	698	620	20	**
BCS	2.68	2.53	0.21	0.62

[1] Responses during 3d-collection period are averaged for individual cow within a experiment, and statistically analyzed using ANOVA procedure of JMP.

[2] Days in milk at the beginning of experiment

[3] Metabolizable energy was calculated according to NRC (2001) based on actual digestibility of diets.

[4] Acetate to propionate ratio

[5] MEI: metabolizable energy intake

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

cows with low plasma glucose concentration are more tolerant to hypophagic effects of propionate.

Response of plasma glucose concentration to propionate infusion was different between cows used in each experiment; propionate infusion increased plasma glucose concentration linearly for cows in experiment 1, but quadratically for cows in experiment 2. In experiment 2, the marginal increase in plasma glucose concentration by propionate infusion was greater at lower rates of propionate infusion compared to higher rates of propionate infusion (Figure 2). Propionate infusion did not affect DMI while propionate infusion increased plasma glucose to a greater extent, but decreased DMI when propionate infusion increased plasma glucose to lesser extent. Infusion of propionate did not exert hypophagic effects while infused propionate was extensively utilized for glucose synthesis. In experiment 2, increases in plasma glucose concentration at lower rates of propionate infusion are attributed to a greater rate of gluconeogenesis. Although plasma glucose concentration is determined by rates of glucose supply from the liver and glucose utilization by tissues, milk yield and plasma insulin concentration increased at lower rates of propionate infusion. Therefore, greater plasma glucose concentration cannot be attributed to decreased glucose clearance from the blood circulation.

Marginal effect of infused propionate on plasma glucose concentration decreased at higher rates of propionate infusion in experiment 2, indicating that infused propionate might be extensively oxidized in the liver once glucose demand of body tissues is

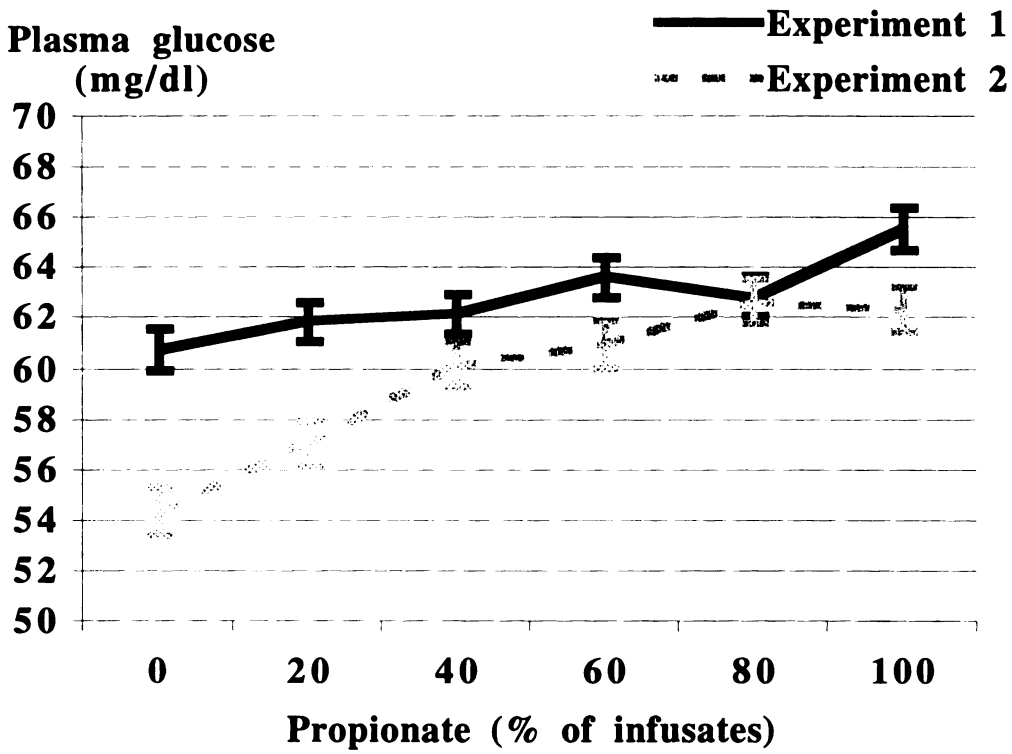

Figure 2. Dose-response effects of intra-ruminal propionate infusion on plasma glucose concentration (mg/dl). Pooled means for plasma glucose concentration within an experiment at each infusion rate were summarized.

satisfied. Enhanced oxidative metabolism in the liver might have caused reduction in DMI at higher rates of propionate infusion in experiment 2. Although propionate infusion increased plasma glucose concentration linearly in experiment 1, the response in glucose concentration to propionate infusion was less than that observed at lower rates of propionate infusion in experiment 2. Therefore, infused propionate might have been more extensively oxidized in the liver even at the lower rates of propionate infusion in experiment 1 compared to experiment 2, resulting in a linear decrease in DMI.

DMI, meal size, and plasma glucose concentration during 3 d collection period were lower, but efficiency of energy utilization for milk production was greater for cows used in experiment 2 compared to cows in experiment 1. In addition, cows used in experiment 2 did not decrease DMI at lower rates of propionate infusion. These observations suggest that something other than propionate dominated regulation of feed intake for cows used in experiment 2. Although distention in the rumen from physical fill is another important factor that can regulate feed intake, it is not possible to identify if ruminal distention limited maximum DMI for cows used in experiment 2 at lower rates of propionate infusion from the data obtained in this experiment.

Hypophagic Effects of Acetate

Infusion of acetate decreased DMI compared to 3 d collection period in the present studies. Dry matter intake during 3 d collection period before infusion was 19.1, 19.3, 14.7, and 15.8 kg/12h, respectively for SF, DC, HF, and LF treatments while it was

14.8, 13.2, 11.6, and 11.7 kg/12h, respectively for SF, DC, HF, and LF treatments during infusion of 100% acetate. Several studies in the literature reported hypophagic effect of acetate (Ulyatt, 1964; Eagan, 1966; Baile, and Pfander, 1966; Orskov et al., 1969; Bhattacharya and Alulu, 1975; Choi and Allen, 1999). Reduction in DMI by acetate infusion cannot be explained by extra energy supplied from acetate because total ME intake was lower when 100% acetate was infused compared to 3 d collection period. However, the liver does not utilize acetate, and the mechanism for hypophagic effects of acetate is not well understood. Hypophagic effects of acetate can be partially attributed to reduction in meal size due to increased osmolarity of ruminal fluid (Grovum, 1995; Choi and Allen, 1999), but Choi and Allen (1999) reported that acetate infusion decreased DMI by increasing intermeal interval compared to iso-osmotic infusion of saline.

Acetate might also exert hypophagic effects by sparing glucose utilization in tissues and decreasing glucose demand. Plasma glucose concentrations for 3 d collection period before infusion were 56.1, 55.5, 51.4, and 50.8 mg/dl, respectively for SF, DC, HF, and LF treatments. However, plasma glucose concentrations for the 100% acetate infusion (treatment 0) were 61.9, 59.7, 54.6, and 54.2 mg/dl, respectively for SF, DC, HF, and LF treatments. Plasma glucose concentration increased by infusion of 100% acetate solution for all dietary treatments although acetate is not a precursor for gluconeogenesis. This might be because acetate spared glucose in body tissues, decreasing rate of glucose clearance from the blood, increasing plasma glucose concentration, and increasing oxidative metabolism of organic acids in the liver.

CONCLUSION

Hypophagic effects of propionate were not affected by fermentability of dietary starch sources in experiment 1 or by forage to concentrate ratio in experiment 2. Our results indicate that propionate flux through the rumen *per se* did not generate satiety signals but that propionate metabolized in the liver might have affected satiety. A quadratic effect of propionate infusion on DMI was observed in experiment 2 but not in experiment 1 regardless of dietary treatments. Cows in experiment 2 did not decrease DMI at lower rates of propionate infusion while cows used in experiment 1 decreased DMI linearly. Different response to propionate infusion might be explained by the rate at which propionate is used for gluconeogenesis. Propionate infusion increased plasma glucose concentration to a greater extent at lower rates of infusion for cows used in experiment 2. Propionate may not exert hypophagic effects while infused propionate is extensively utilized for glucose synthesis, but decrease feed intake when marginal effect of propionate infusion on plasma glucose concentration becomes lower. We speculate that propionate decreases feed intake in lactating dairy cows by stimulating oxidative metabolism in the liver.

CHAPTER 7

Dose-response effects of intra-ruminal infusion of propionate on feeding behavior of lactating cows in early or mid lactation

ABSTRACT

The objective of this experiment was to evaluate if dose-response effects of intra-ruminal infusion of propionate on feeding behavior and DMI differ by stage of lactation. Five cows in early lactation (EL) and five cows in mid lactation (ML) were used in a duplicated 6 x 6 Latin square design (9 ± 6 and 192 ± 17 days in milk, respectively for EL and ML; mean ± SD). All cows were ruminally cannulated prior to the experiment. The experimental diet was formulated to contain 30% NDF, and dry cracked corn (mean particle size = 3.6 mm) was the major source of starch. Treatments were mixtures of sodium propionate and sodium acetate, at ratios of 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0, infused into the rumen continuously for 18 h starting 6 h before feeding at a rate of 21.7 mmol of sodium VFA/min. We hypothesized hypophagic effects of propionate infusion were greater for EL compared to ML because of greater plasma concentration of non-esterified fatty acids (275 vs. 76 μMeq/ml; $P < 0.001$) and expected greater basal oxidative metabolism in the liver for EL compared to ML. Propionate infusion decreased DMI for EL and ML, but quadratic effect of propionate infusion was observed for ML but not EL

(interaction $P < 0.10$), indicating greater marginal reduction in DMI at higher doses of propionate for ML compared to EL. Propionate infusion decreased meal size similarly for both stages of lactation, but linearly increased intermeal interval for ML but not EL. We speculate that greater milk yield for EL compared to ML (42.0 vs. 30.8 kg/d $P < 0.001$) increased glucose demand by the mammary gland and decreased the proportion of infused propionate oxidized in the liver for EL compared to ML. Future research needs to evaluate independent effects of glucose demand and rates of basal oxidative metabolism in the liver on hypophagic effects of propionate.

(**Key words:** propionate infusion, threshold response, stage of lactation, NEFA)

Abbreviation Key: EL = Cows in early lactation; **ML** = Cows in mid lactation; **NEFA** = Non-esterified fatty acids; **BHBA** = β-hydroxy butyrate)

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 not contribute in EL (Ingvartsen and Andersen, 2000). Low DMI in EL might be related to elevated concentration of plasma non-esterified fatty acids (NEFA; Ingvartsen and Andersen, 2000). The NEFA are

metabolic fuels extensively utilized by the liver, and extent of uptake by the liver is determined by plasma concentration of NEFA (Emery et al., 1992). Fatty acid oxidation in the liver provides a satiety signal in rats (Langhans et al., 1987a; Friedman et al., 1999)

Propionate has hypophagic effects in ruminants (Allen, 2000). 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). Langhans et al. (1985a) proposed that metabolic fuels that are extensively metabolized in the liver have hypophagic effects. 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. If propionate decreases feed intake by stimulating oxidative metabolism in the liver (Allen, 2000), hypophagic effect of propionate is expected to be greater for EL compared to cows in mid lactation (ML) because of greater basal oxidative metabolism in EL from mobilized NEFA.

Although hypophagic effects of propionate are well documented, some experiments in the literature report infusion of propionate does not affect feed intake (Deetz and Wangsness, 1981; Quigley and Heitmann, 1991; De Jong et al., 1981; Anil et al., 1993). Anil et al. (1993) reported that infusion of sodium propionate decreased feed intake linearly in one experiment, but not in two other experiments. They used cows

differing in age and stage of lactation in each experiment. Inconsistent hypophagic effects of propionate infusion in the literature might be because of unidentified differences in animal characteristics. Cows differing in physiological state might respond differently to intra-ruminal infusion of propionate by changing the threshold and marginal effects of propionate on DMI.

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

MATERIALS AND METHODS

Experimental Design and Treatments

Experimental procedures were approved by 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 mid lactation were used in this experiment. However, one cow in early lactation and another cow in mid lactation 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 ± SD) for five cows in early lactation and for five cows in mid lactation, respectively. Cows in early lactation were ruminally cannulated at least 30 d prior to calving, and cows in mid lactation 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 total mixed ration once daily at 110% of expected intake.

The experimental period 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 intra-ruminal infusion of mixtures of sodium propionate and sodium acetate at 6 different ratios. Cows were assigned to duplicated incomplete 6 x 6 Latin squares balanced for carry-over effects: one square for cows in early lactation and the other square for cows in mid lactation. Treatment solutions were prepared by diluting 28.1 moles of sodium VFA (sodium propionate and sodium acetate at the ratio of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0) to 18 L with de-ionized water. Sodium acetate was added to keep the osmolarity and pH of infusates constant across the treatments to isolate specific effect 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, which is equivalent to infusion of 21.7 mmol of VFA/min. Solutions were infused using

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 & 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

[1] Protein mix contained 75% soybean meal, 20% distillers grain, and 5% blood meal.
[2] Vitamin & mineral mix contains 66.4% dry ground corn, 20.4% dicalcium phosphate, 7.8% salt, 2.4% magnesium oxide, 1.9% trace mineral premix, 0.34% vitamin A, 0.29% vitamin D, and 0.08% vitamin E.

4-channel peristaltic pumps (#78016-30, Cole-Parmer Instrument, IL) and Tygon® tubing (7.5 m x 1.6mm 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%/h and 15%/h, respectively) by feeding time when feeding behavior monitoring started. Sub-periods for infusion treatment were 2 d with 18 h of infusion followed by 30 h of recovery.

Data and Sample Collection

The amount of feed offered and orts were weighed for each cow daily during the collection period. 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 1, 3, 5, 7, 9, and 11) and compositied to one sample per period. 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 per period. Body weight and BCS were measured [(Wildman, 1982); five-point scale where 1 = thin to 5 = fat] 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 1, 3, 5, 7, 9, and 11 of infusion period) 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 yield was measured on feeding behavior monitoring days to determine effects of infusion treatments. Milk was sampled at every milking during the 3 d collection period and on d 1, 3, 5, 7, 9, and 11 of the infusion period. Milk samples were analyzed for fat, true protein, lactose, solids-non-fat with infrared spectroscopy by Michigan DHIA (East Lansing).

Samples of feces, ruminal fluid, and blood were collected every 9 h on d 15 to 17. Ruminal fluid samples were collected from 5 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 floride as a glycolytic inhibitor. Both were centrifuged at 2,000 x g for 15 minutes immediately after sample collection, and plasma was harvested and frozen at -20° C until analysis.

On feeding behavior monitoring days (d 1, 3, 5, 7, 9, and 11 of infusion period), infusion started at 0800 h, 6 h prior to feeding, and continued for 18 h. Cows were not allowed access to feed between 1000 h to 1400 h to minimize confounding effects of ruminal fermentation from the previous feeding. Feeding behavior was monitored for 12 h (1400 h to 0200 h) by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, and water consumption were recorded for each cow every 5 sec, 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), samples of ruminal fluid were collected from 5 different sites in the rumen for each cow. Blood samples were collected from coccygeal vessels, centrifuged at 2,000 x g for 15 minutes, and plasma was harvested and frozen at -20° C until analysis.

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 (1mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, ADF, CP, and starch. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. Concentrations of NDF and ADF were determined [(VanSoest et al., 1991); method A for NDF]. Crude protein was analyzed according to Hach et al. (1985). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide, and glucose concentration was measured using a commercial kit (Glucose kit #510; Sigma Chemical Co., St. Louis, MO). Indigestible NDF was estimated as NDF residue after 120 h in vitro fermentation (Goering and Van Soest, 1970) and used as an internal marker to calculate apparent total tract digestibility (Cochran et al., 1986). Concentrations of all nutrients except for DM were expressed as percentages of DM determined from drying at 105° C in a forced-air oven. Corn grain was dry sieved through 8 sieves (Sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan), using a sieve shaker (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant, and mean particle size of corn grain was calculated (ASAE, 1968).

Ruminal fluid samples were analyzed for VFA concentrations according to the method described previously (Chapter 2). Plasma samples were processed to determine concentrations of acetate and propionate in a similar manner as described for ruminal fluid (Chapter 2). 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., St. Louis, MO).

All data from the 3 d collection period were analyzed to characterize cows at each stage of lactation using the ANOVA procedure of JMP® 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)

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 $P < 0.10$.

All data for infusion effects from 11 d of infusion period were analyzed using the fit model procedure of JMP® according to the following model:

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

where

μ = overall mean,

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

$C(S)_{i(j)}$ = random effect of cows nested in a lactation stage (j = 1 to 10),

P_k = fixed effect of periods (k = 1 to 2),

L_l = linear effect of infusion

Q_m = quadratic effect of infusion

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

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

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

Orthogonal contrasts were made for the effects of lactation stage (EL vs. ML), linear effect of infusion, quadratic effect of infusion, interaction between effect of lactation stage and linear effect of infusion, and interaction between effect of lactation stage and quadratic effect of infusion. Main treatment effects were declared significant at $P < 0.05$, and tendency for treatment effects was declared at $P < 0.10$. Interaction effects were declared significant at $P < 0.10$.

RESULTS AND DISCUSSION

DMI and Feeding Behavior

During the 3 d collection period before infusion, DMI tended to be lower for EL compared to ML (16.2 vs. 18.3 kg/12h; $P < 0.09$; Table 2) although milk yield was greater for EL compared to ML (42.0 vs. 30.8 kg/d; $P < 0.001$). Milk energy output per ME intake was greater for EL compared to ML (0.88 vs. 0.54 Mcal/ME intake; $P < 0.001$), indicating greater efficiency of dietary energy utilization for milk production for EL compared to ML. An interaction between effect of lactation stage and quadratic effect of infusion was significant on DMI ($P < 0.10$; Table 3). Propionate infusion decreased DMI linearly for EL ($P < 0.001$) but quadratically for ML ($P = 0.03$; Table 4; Figure 1). This indicates that there was less marginal reduction in DMI at lower doses of propionate but greater marginal reduction in DMI at higher doses of propionate for ML compared to EL. Propionate infusion linearly 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 was significant for intermeal interval ($P < 0.10$). Propionate infusion linearly increased intermeal interval for ML ($P < 0.01$) but not EL (Figure 3). The greater marginal reduction in DMI at higher rates of propionate infusion for ML compared to EL is because propionate infusion decreased DMI by both decreasing meal size and increasing intermeal interval in ML while decreasing meal size only for EL. Propionate infusion delayed the sense of hunger for ML but not EL.

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

	Early[1]	Mid[2]	SE	*P*-value
Feeding behavior				
DMI (kg/12h)	16.2	18.3	0.8	0.09
Meal bouts (/12h)	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/12h)[3]	34.0	41.8	3.2	0.11
Chewing time				
Eating (min/12h)	198	195	15	0.92
Ruminating (min/12h)	224	235	9	0.45
Total (min/12h)	422	430	20	0.78
Water intake (L/12h)	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

TABLE 2 (cont'd).

	Early[1]	Mid[2]	SE	*P*-value
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 (μIU/ml)	5.7	11.9	0.9	***
NEFA (μMeq/ml)	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
EE	61.5	62.7	3.1	0.79

TABLE 2 (cont'd).

	Early[1]	Mid[2]	SE	*P*-value
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	***
Milk energy (Mcal/d) : MEI[5] (Mcal/12h)	0.88	0.54	0.03	***
Body Weight (kg)	650	631	27	0.63
BCS	3.00	2.50	0.20	0.11

[1] Early stage of lactation
[2] Mid stage of lactation
[3] Metabolizable energy was calculated according to NRC (2001) based on actual digestibility of diets.
[4] Acetate to propionate ratio
[5] MEI: metabolizable energy intake
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

TABLE 3. Dose-response effects of intra-ruminal infusion of sodium propionate relative to sodium acetate on feeding behavior and milk production for cows in early and mid lactation

	Early[1]						Mid[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/12h) [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 (/12h)	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/12h)																
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/12h)																
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

TABLE 3 (cont'd).

	Early[1]						Mid[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		S[4]	L[5]	Q[6]
Milk production																
Milk yield (kg/d)	37.3	39.9	40.5	39.4	38.6	36.0	28.2	28.7	27.0	29.7	28.5	27.9	1.4	***	0.49	*
Milk fat (%)	4.74	4.31	4.43	4.42	4.12	4.61	4.41	3.93	3.89	4.05	4.01	4.02	0.22	0.29	0.23	0.06
Milk protein (%)	2.86	2.66	2.61	2.53	2.58	2.66	3.25	2.92	3.13	3.09	3.07	3.02	0.10	**	0.09	0.08
Milk lactose (%)	4.89	4.90	4.89	4.84	4.88	4.94	4.98	4.95	5.01	4.98	4.97	5.05	0.03	0.33	0.18	0.07
Milk SNF (%)	8.66	8.46	8.39	8.25	8.35	8.54	9.17	8.79	9.09	8.99	8.96	8.99	0.12	***	0.25	*
Milk energy (Mcal/d)	29.6	29.5	30.2	29.1	27.7	27.7	22.1	20.7	19.7	22.0	21.0	20.6	1.4	**	0.22	0.85
Milk energy (Mcal/d): MEI[9](Mcal/12h)[SQ]	0.92	1.05	1.12	1.08	1.10	1.19	0.63	0.72	0.65	0.66	0.93	1.07	0.07	**	***	0.20

[1] Early stage of lactation
[2] Mid stage of lactation
[3] Propionate content in infusates (%)
[4] Effect of stage of lactation
[5] Linear effect of intra-ruminal infusion of propionate
[6] Quadratic effect of intra-ruminal infusion of propionate
[7] Metabolizable energy intake from diet was calculated according to NRC (2001) based on actual digestibility of diets.
[8] Metabolizable energy intake from infusates assumed energy density of 0.2094 and 0.3672 Mcal/mol for acetate and propionate, respectively.
[9] MEI: metabolizable energy intake
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
[SL] Significant interaction of lactation stage and linear effect of propionate
[SQ] Significant interaction of lactation stage and quadratic effect of propionate

TABLE 4. Dose-response effects of intra-ruminal infusion of sodium propionate relative to sodium acetate on DMI and intermeal interval for cows in early and mid lactation

								P value	
	0[1]	20	40	60	80	100	SE	L[2]	Q[3]
DMI (kg/12h)									
Cows in early lactation	13.5	11.9	11.0	10.8	9.5	8.4	0.9	***	0.99
Cows in mid lactation	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 mid lactation	60.2	74.0	70.6	79.9	109.0	86.4	9.3	**	0.52

[1] Propionate content in infusates (%)
[2] Linear effect of intra-ruminal infusion of propionate
[3] Quadratic effect of intra-ruminal infusion of propionate
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

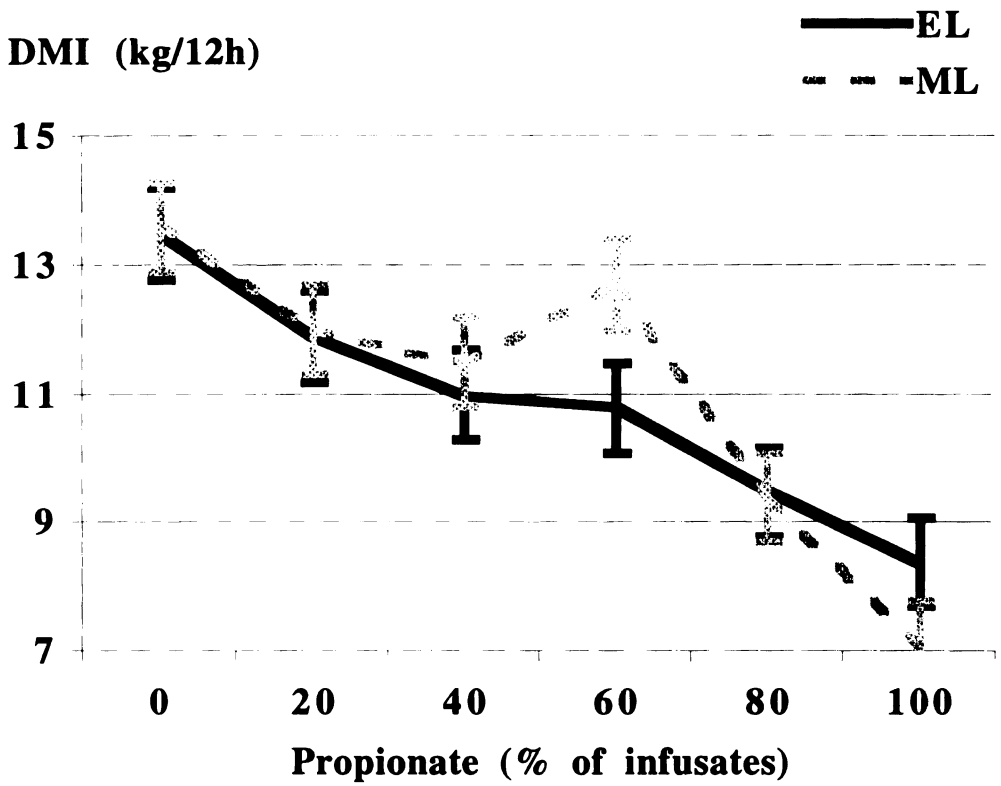

Figure 1. Dose-response effect of intra-ruminal propionate infusion on DMI (kg/12h) for cows in early lactation (EL) and mid lactation (ML).

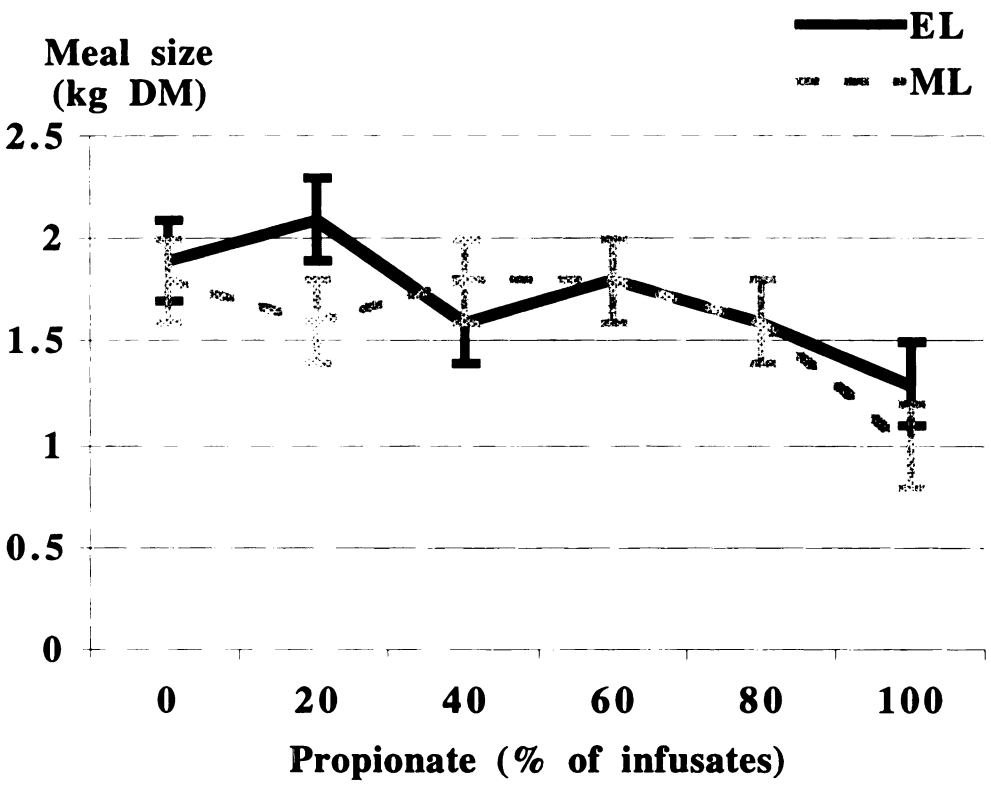

Figure 2. Dose-response effect of intra-ruminal propionate infusion on meal size (kg) for cows in early lactation (EL) and mid lactation (ML).

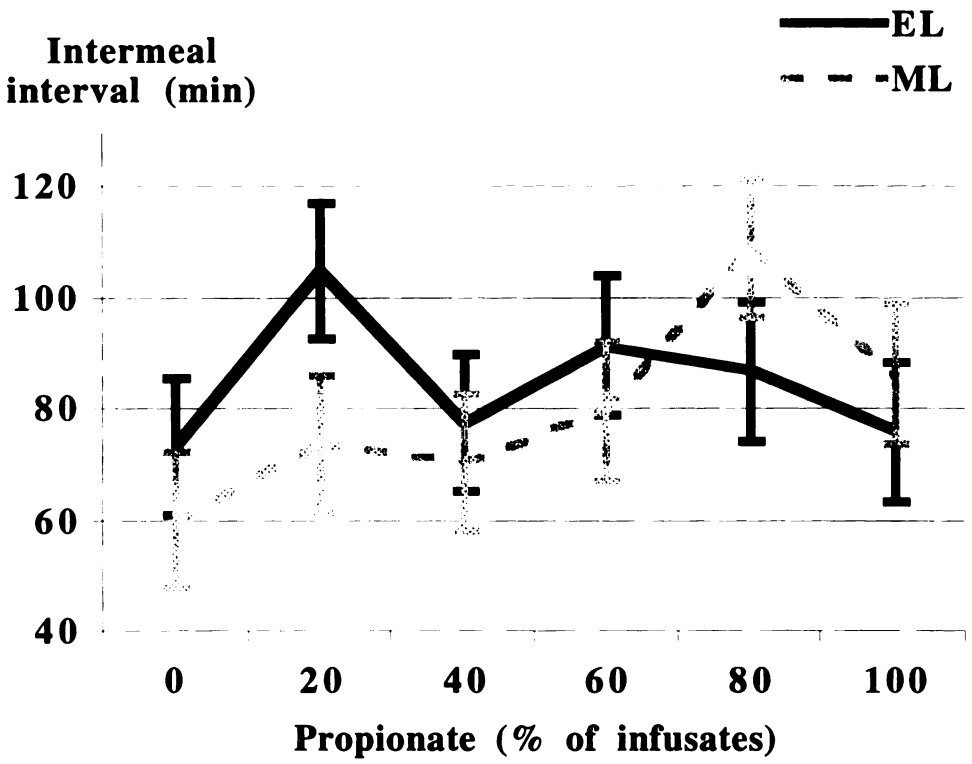

Figure 3. Dose-response effect of intra-ruminal propionate infusion on intermeal interval (min) for cows in early lactation (EL) and mid lactation (ML).

The different response in intermeal interval to propionate infusion might be related to the fate and rate of propionate utilization in the liver because post-meal propionate flux to the liver is expected to be similar for EL and ML at respective infusion treatments. The same diet was fed to both groups of cows and propionate infusion decreased meal size to a similar extent for EL and ML. Ruminal propionate concentration and apparent total tract OM digestibility were not affected by stage of lactation during the 3 d collection period before infusions. We speculate that glucose demand of body tissues 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 body tissues increases. 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 is available as TCA cycle intermediates for a longer period of time if glucose demand in peripheral tissues is relatively low, increasing oxidation of fuels in the liver and delaying the sense of hunger. Intermeal interval increased linearly by propionate infusion for ML possibly because of lower glucose demand related to lower milk yield and higher DMI compared to EL.

Contrary to our hypothesis, propionate infusion decreased meal size for EL and ML to a similar extent. We hypothesized that lower rates of propionate infusion would have a greater effect on meal size for EL compared to ML because basal oxidative metabolism in the liver is expected to be greater for EL compared to ML. In support of

this, plasma NEFA concentration was greater for EL compared to ML (275 vs. 76 μeq/ml; $P < 0.001$), and a greater extent of NEFA uptake by the liver was expected for EL (Emery et al., 1992). However, the similar response to propionate infusion in meal size for EL and ML indicates that greater plasma NEFA concentration did not decrease meal size synergistically when propionate was infused. This might be because uptake of NEFA by the liver is not directly related to rate of oxidation. Although extent of NEFA uptake by the liver is proportional to its concentration in plasma (Emery et al., 1992), NEFA can be used to form triglycerides that are stored in the liver or exported into the blood as VLDL. In addition, infused propionate can decrease mobilization of NEFA by increasing insulin secretion. In this experiment, propionate infusion linearly increased insulin secretion ($P < 0.05$; Table 4) and decreased plasma NEFA concentration ($P < 0.05$) for EL. In addition, propionate may have inhibited fatty acid oxidation in the liver by decreasing activity of fatty acyl-CoA dehydrogenase (Emery et al., 1992).

Another explanation for the similar effect of propionate infusion on meal size for both stages of lactation might be the greater glucose demand for EL compared to ML, which might have reduced the hypophagic effects of infused propionate that were expected to be greater for EL because of greater basal oxidative metabolism of NEFA in the liver. The relative proportion of infused propionate oxidized in the liver might have been lower for EL compared to ML because greater milk production and glucose demand in the mammary gland enhanced propionate utilization for gluconeogenesis in the liver. It is difficult to isolate specific hypophagic effects of mobilized NEFA from effects of

TABLE 5. Dose-response effects of intra-ruminal infusion of sodium propionate relative to sodium acetate on metabolites in the rumen and the blood for cows in early and mid lactation

	Early[1]						Mid[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[6, 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	***	***

TABLE 5 (cont'd)

	Early[1]						Mid[2]						SE	*P*-value		
	0[3]	20	40	60	80	100	0	20	40	60	80	100		S[4]	L[5]	Q[6]
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]	53.0	58.6	60.2	62.4	62.6	63.8	61.2	64.2	64.0	64.9	65.6	65.6	1.3	*	***	*
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/ml) [SL]	244	349	200	196	167	174	80	79	181	66	78	80	32	**	*	0.46
BHBA (mg/dl) [SL, SQ]	15.0	7.8	3.5	3.5	3.6	2.3	5.0	3.1	4.3	3.7	3.0	2.9	1.0	0.92	***	***
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

[1] Early stage of lactation
[2] Mid stage of lactation
[3] Propionate content in infusates (%)
[4] Effect of stage of lactation
[5] Linear effect of intra-ruminal infusion of propionate
[6] Quadratic effect of intra-ruminal infusion of propionate
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
[SL] Significant interaction of lactation stage and linear effect of propionate
[SQ] Significant interaction of lactation stage and Quadratic effect of propionate

greater milk production and glucose demand in peripheral tissues and to evaluate if infused propionate exerts more hypophagic effects for EL compared to ML. Future research needs to examine independent effects of different demand for gluconeogenesis and different basal oxidative metabolism in the liver on hypophagic effects of propionate.

Plasma Metabolites

Plasma concentrations of glucose (59.0 vs. 52.8 mg/dl; $P < 0.01$) and insulin (11.9 vs. 5.7 μIU/ml; $P < 0.001$) were greater for ML compared to EL during the 3 d collection period before infusion. Propionate infusion increased plasma glucose concentration linearly for ML but quadratically for EL; the effect of propionate infusion on plasma glucose concentration was greater for EL especially at lower rates of propionate infusion (Figure 4). In Chapter 6, it was discussed that hypophagic effects of propionate are related to the marginal effect of propionate infusion on plasma glucose concentration. However, in this experiment, propionate infusion decreased DMI linearly for EL even at lower rates of propionate infusion that increased plasma glucose concentration to a greater extent. This observation appears inconsistent with the discussion in Chapter 6. However, infused propionate at lower rates of infusion might have stimulated oxidative metabolism in the liver by increasing TCA cycle intermediates and oxidation of acetyl CoA. 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). This reduction in plasma BHBA concentration might indicate that acetyl CoA in the liver was completely oxidized as the proportion of propionate in infusates increased to 40%. Although infused propionate increased plasma glucose concentration,

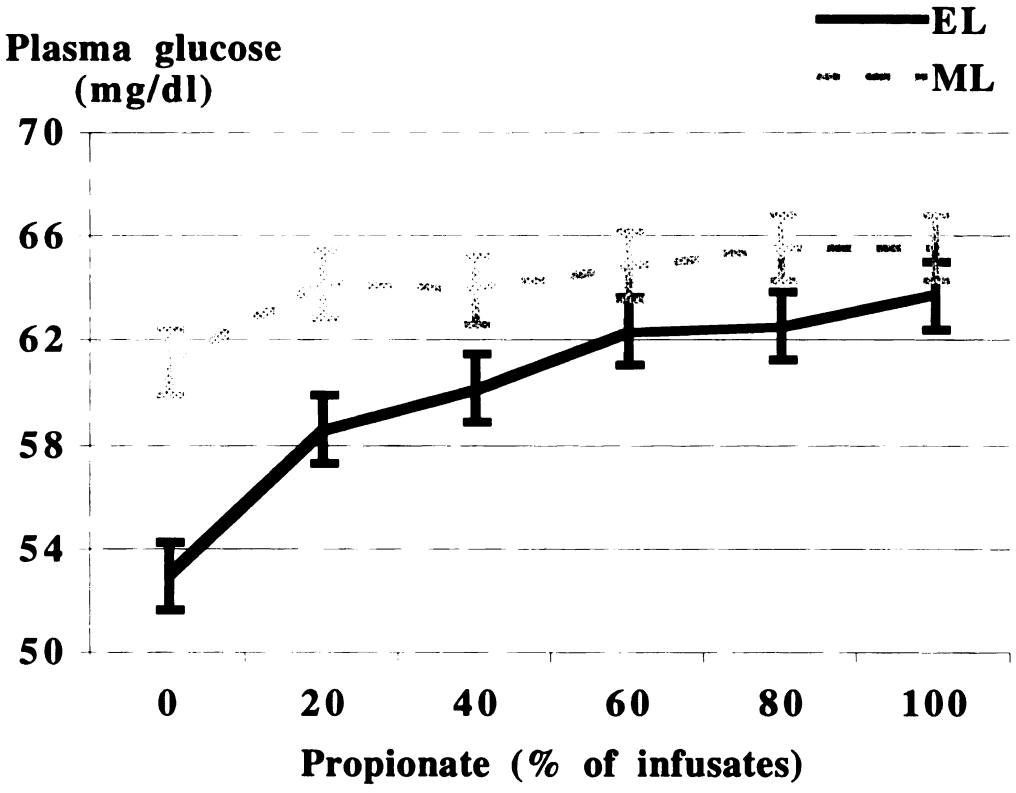

Figure 4. Dose-response effect of intra-ruminal propionate infusion on plasma glucose concentration (mg/dl) for cows in early lactation (EL) and mid lactation (ML).

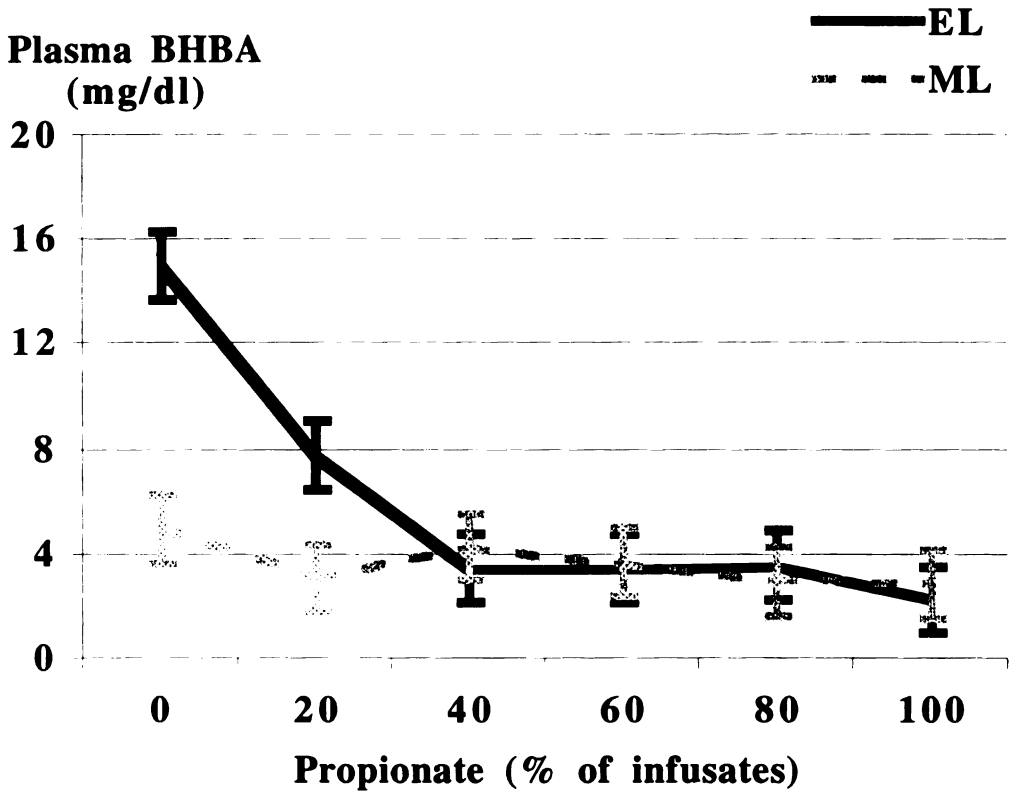

Figure 5. Dose-response effect of intra-ruminal propionate infusion on plasma β-hydroxy butyrate (BHBA; mg/dl) for cows in early lactation (EL) and mid lactation (ML).

it probably also increased oxidation of acetyl CoA in the liver, decreasing DMI. Discussion in Chapter 6 indicated that gluconeogenesis is the major metabolic pathway for propionate in the liver, but accumulation of acetyl CoA in the liver might increase the relative proportion of infused propionate used for driving TCA cycles. Accumulation of acetyl CoA stimulates pyruvate carboxylase and increases oxaloacetate formation from pyruvate (Ballard et al., 1969). Therefore, infused propionate might be sufficient to stimulate gluconeogenesis and oxidative metabolism in the liver simultaneously. Although BHBA reduction at lower rates of propionate infusion indicates enhanced oxidative metabolism in the liver, it does not exclusively support this explanation. Propionate may decrease ketogenesis by increasing insulin secretion and decreasing lipolysis in the adipose tissues or by decreasing β-oxidation of NEFA (Shaw and Engel, 1985).

Plasma glucose concentration for ML was increased by infusion of 100% acetate solution (0% propionate treatment) compared to that of the 3 d collection period before infusions (61.2 vs. 59.0 mg/dl), which is in agreement with the observation in Chapter 6. This observation suggests that acetate spared glucose in some tissues for ML and decreased clearance rate of glucose from the blood circulation. However, plasma glucose concentration was not affected by infusion of the 100% acetate solution for EL (53.0 vs. 52.8 mg/dl), 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 carboxlylase 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 incorporation of acetate into fatty acids to a lesser extent for ewes in early lactation compared to non-lactating ewes in mid-stage 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 to 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. Greater milk production relative to ME intake for EL might increase demand for gluconeogenesis and decrease the proportion of infused propionate oxidized in the liver at higher rates of propionate infusion compared to ML. 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. This might be attributed to confounding effects of greater glucose demand and subsequent 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 propionate might have caused hypophagia by stimulating oxidation of acetyl CoA in the liver despite greater glucose demand. Future research needs to examine independent effects of different glucose demand and different rates of basal oxidative metabolism in the liver on hypophagic effects of propionate.

CHAPTER 8

IMPLICATIONS

Shifting primary site of starch digestion from the rumen to intestines by replacing dry ground corn for ground high moisture corn increased DMI of lactating dairy cows fed a high starch diet (Chapter 2). Excess propionate production in the rumen was considered to limit maximum voluntary feed intake in lactating dairy cows. Lactating dairy cows linearly decreased DMI, meal size and meal frequency as the rate of intra-ruminal infusion of propionate increased (Chapter 3). Infusion of propionate decreased DMI and total metabolizable energy (ME) intake. This means that hypophagic effects of propionate are not explained by additional energy supplied as propionate, but that feed intake was regulated specifically by propionate. Hepatic ATP concentration has been suggested as a regulator of satiety and hunger in rats. Because propionate is one of the major metabolic fuels for the ruminant liver, the extent and rate of propionate production in the rumen may influence hepatic ATP concentration and feeding behavior. Although hepatic ATP was not directly measured in experiments for this dissertation, the series of experiments showed that oxidative metabolism in the liver is involved in regulatory mechanism of feeding behavior by affecting satiety and hunger in lactating dairy cows.

Infusion of ammonium decreased DMI (Chapter 4), and hypophagic effects of ammonium are facilitated by intra-ruminal infusion of propionate but not by acetate (Chapter 5). It is speculated that infusion of ammonium stimulated urea synthesis that

requires N from amino acids and that deamination of amino acids increases carbon available for oxidation or gluconeogenesis in the liver. When ammonium was infused with acetate, DMI was similar compared to infusion of sodium acetate possibly because the liver does not utilize acetate, and carbon from amino acids were utilized for gluconeogenesis without extensively stimulating oxidative metabolism. However, when ammonium was infused with propionate, oxidative metabolism in the liver might have increased because the liver utilizes propionate extensively. Propionate and amino acid carbon generated from urea synthesis are substrates for gluconeogenesis. Substrate supplied above that needed to meet glucose demand of body tissues increases oxidation in the liver. Our observations in this experiment support the theory that oxidative metabolism in the liver affects DMI and feeding behavior.

Two experiments in Chapter 6 were designed to determine if a threshold for infused propionate to affect DMI exists and if it exists how the threshold is affected by fermentability of diets. The extent of hypophagic effects of propionate on feeding behavior and DMI were not affected by fermentability of dietary starch or by forage to concentrate ratio, although response in feeding behavior to propionate infusion differed between cows used in each trial. Cows used in trial 2 did not decrease DMI at lower doses of propionate while cows used in trial 1 decreased DMI linearly. Different responses to propionate infusion might be explained by the marginal effect of propionate on plasma glucose concentration; propionate infusion at lower doses increased plasma glucose concentration to a greater extent for cows used in trial 2 compared to cows in trial 1. These observations suggest that propionate does not exert hypophagic effects if

infused propionate is extensively used for glucose synthesis, but propionate infusion decreases feed intake when marginal effects of propionate infusion on plasma glucose concentration decrease. Cows in early lactation decreased DMI linearly at lower doses of propionate while infused propionate greatly increased plasma glucose concentrations (Chapter 7). Although this appears to be inconsistent with observations described in Chapter 6, propionate infusion decreased plasma concentration of β-hydroxy butyrate. Infusion of propionate might have stimulated oxidative metabolism of acetyl CoA in the liver for cows in early lactation, and resulted in a linear reduction in DMI.

Experiments in chapter 6 and 7 showed that propionate flux to the liver does not directly decrease feed intake but hepatic oxidative metabolism stimulated by infusion of propionate has direct hypophagic effects. Treatment means from the experiments described in Chapter 6 and Chapter 7 were plotted to determine the relationship between marginal reduction in DMI (kg) per mmol/min of propionate infusion and plasma glucose concentration (Figure 1). As plasma glucose concentration increases, DMI decreased to a greater extent by propionate infusion. It is probably because glucose demand of body tissues is satisfied at greater concentration of plasma glucose and infused propionate is not extensively utilized for gluconeogenesis but oxidized in the liver. Our observations imply that the threshold for propionate to affect DMI becomes greater for cows with lower plasma glucose concentration and that plasma glucose concentration might be useful to predict DMI responses to diet change. Cows with low plasma glucose concentration might be more tolerant to hypophagic effects of propionate, thus it is likely to increase their productivity by feeding more fermentable

diets. However, cows with high plasma glucose concentration might have greater risk to decrease DMI by a similar diet change. This knowledge can be utilized to make management decisions on how to group cows within a herd.

Propionate infusion decreased DMI linearly for cows in early lactation (EL), but quadratic effect of propionate infusion was observed for cows in mid lactation (ML), indicating greater marginal reduction in DMI for ML compared to EL at higher rates of propionate infusion (Chapter 7). Propionate infusion linearly increased intermeal interval for ML, but not EL. Greater milk production for EL might have increased gluconeogenesis and decreased the proportion of infused propionate oxidized in the liver at higher rates of propionate infusion compared to ML.

The series of experiments in this dissertation showed that the effects of propionate on DMI in lactating dairy cows is consistent with its effects at stimulating oxidative metabolism in the liver. This knowledge can be utilized to improve profitability of dairy operations. Maximizing energy intake is an important goal in nutritional management for high producing dairy herds, and future research should focus on how to increase energy intake by optimum diet formulation. Replacing high moisture corn with dry ground corn is a possible approach to decrease rate of propionate production in the rumen (Allen, 2000). It is also necessary to investigate how splanchnic tissues metabolize glucose that is absorbed in the small intestine. If oxidative metabolism in the liver affects DMI and feeding behavior as proposed, it is important to understand how to formulate diets that

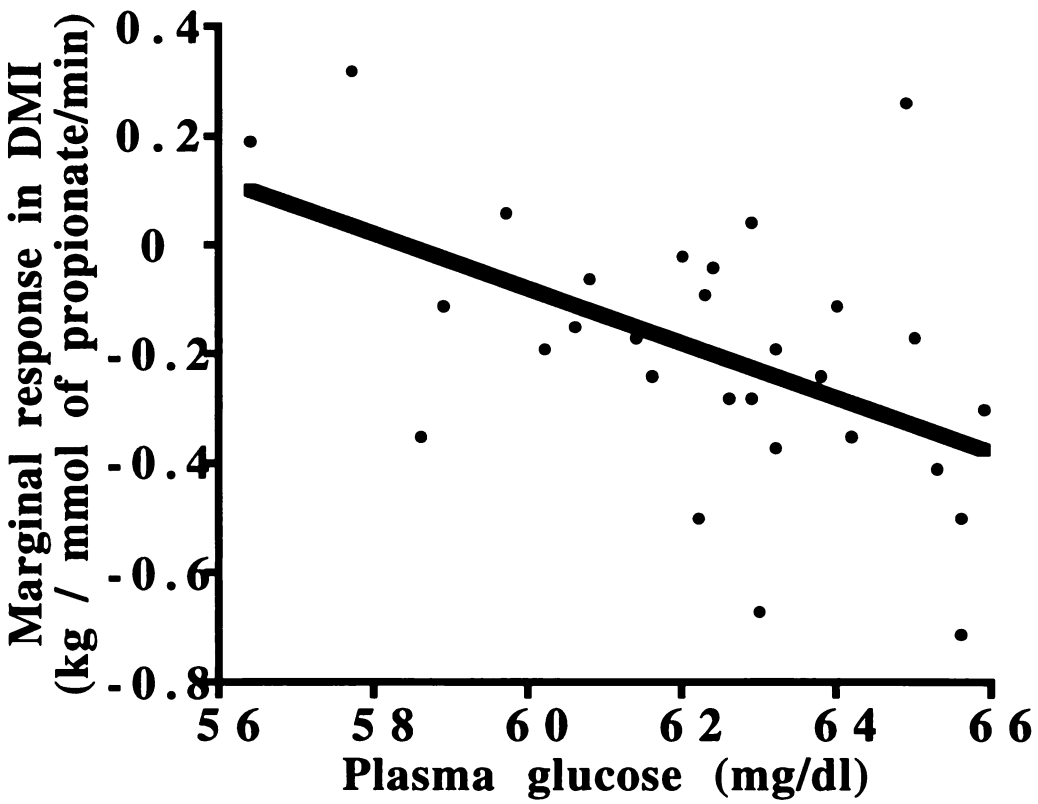

Figure 1. Relationship between marginal response in DMI (kg) per mmol/min of propionate infusion and plasma glucose concentration (mg/dl). Marginal response in DMI = 3.0 - 0.05 x plasma glucose concentration ($r^2 = 0.26$; $P < 0.01$).

will minimize oxidative metabolism of propionate. Interactions with other dietary components such as lipid and protein need to be investigated further because both fatty acids and amino acids are metabolic fuels utilized extensively in the ruminant liver. In addition, if hypophagic effects of propionate are altered by glucose demand of body tissues, the potential for dietary treatments to alter the metabolic fate of propionate in the liver (i.e. increasing gluconeogenesis and decreasing oxidative metabolism) should be investigated. These are only a few examples of possible research that can be done in the future. Elucidating regulation mechanisms for feed intake is a fertile and exciting area of research.

APPENDIX

The Effect of Pulse Dose of Sodium VFA

ABSTRACT

Dose-response effects of intra-ruminal infusion of propionate on feeding behavior of cows that differed in stage of lactation were to be evaluated, but the experiment was aborted due to the severe adverse effects of the infusion protocol. Treatment solutions were prepared by diluting 33.8 moles of sodium VFA (sodium propionate and sodium acetate at ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0) to 18 L with de-ionized water. Concentrations of total VFA were 1.88 M across the treatments. A priming dose of 2.29 L of each solution was administered ruminally 2 h prior to feeding and 13.4 L of each solution was infused over 14 h starting immediately after the priming dose. Infusion rate was 16 ml/min, which is equivalent to infusion of 30.0 mmol of VFA/min. Infusion started 2 h prior to feeding with priming dose so that VFA concentrations in the rumen reached steady state concentrations prior to feeding. However, 5 cows out of 12 were unable to stand or walk properly 6 h after the end of infusion. Common symptoms were dehydration, hypocalcemia, hypokalemia, and metabolic alkalosis. It is speculated that the priming dose of VFA salt increased osmolarity of ruminal fluid causing systemic dehydration. Decreased gastric emptying from hyper-osmotic duodenal digesta probably increased the severity of dehydration by limiting water intake because of ruminal

distention. In addition, infusion of sodium with VFA caused metabolic alkalosis and decreased serum concentration of potassium. Metabolic alkalosis might also induce conformational change in PTH receptors, developing hypocalcemia. It is concluded that systemic dehydration was caused by a priming dose of hyper-osmotic solutions, and infusion of sodium VFA solution affected acid-base balance in serum, resulting in metabolic alkalosis, hypokalemia, and hypocalcemina.

BACKGROUND

Six multiparous Holstein cows in early lactation (11 ± 6 DIM; mean ± SD) and six multiparous Holstein cows in mid lactation (100 ± 17 DIM; mean ± SD) were used for this experiment. Cows in early lactation (ruminally cannulated at least 30 d prior to calving), and cows in mid lactation (ruminally cannulated for previous experiments) were selected from the herd of Michigan State University Dairy Cattle Teaching and Research Center. Mean BW and BCS for the cows were 666 kg and 2.8, respectively. Experimental diet and research protocols were similar to those described in Chapter 7 except for the infusion protocol. The experiment described in Chapter 7 originally planned to use total of 24 cows (12 cows in early lactation and 12 cows in mid lactation) with two blocks in time using 12 cows each because an automated system for feeding behavior monitoring was available for 12 stalls. The experiment described in this appendix was the first part of the experiment that evaluated dose-response effects of

intra-ruminal infusion of propionate on feeding behavior of lactating cows in early or mid lactation

Experimental periods were 28 d consisting of 14 d for a diet adaptation, 3 d for data and sample collection to determine effects of stage of lactation, and 11 d for data and sample collection to determine effect of infusion treatments. Infusion treatments were continuous intra-ruminal infusion of mixtures of sodium propionate and sodium acetate at 6 different ratios. Treatment solutions were prepared by diluting 33.8 moles of sodium VFA (sodium propionate and sodium acetate at ratios of 0:5, 1:4, 2:3, 3:2, 4:1, and 5:0) to 18 L with de-ionized water. Sodium acetate was added to keep the osmolarity and pH of infusates constant across the treatments to isolate specific effect of propionate relative to acetate on feeding behavior of dairy cows. Concentrations of total VFA were 1.88 M across the treatments, and 2.29 L of each solution was prime-dosed 2 h prior to feeding and 13.4 L of each solution was infused over 14 h starting immediately after administration of the priming dose. The priming dose was administered to shorten the infusion period required to reach steady state concentrations of VFA prior to feeding; it would take 6 h of infusion to reach steady state concentrations of VFA by continuous infusion without the priming dose, assuming absorption and passage rates of 20%/h and 15%/h, respectively. Infusion rate was 16.0 ml/min, which is equivalent to infusion of 30.0 mmol of VFA/min. The solutions were infused using peristaltic pumps (#78016-30, Cole-Parmer Instrument, IL) and Tygon® tubing (7.5 m x 1.6mm I.D.). At the end of infusion, blood samples were collected from coccygeal vessels, and serum was harvested and analyzed for the concentrations of Na and K. Water intake and DMI were recorded.

RESULTS

After the first infusion period, 5 cows (4 cows in early lactation and 1 cow in mid lactation) were unable to stand or walk properly within 6 h following cessation of infusion. Common symptoms were dehydration, hypocalcemia, hypokalemia, and metabolic alkalosis. Two cows showed abnormally high blood $_{P}CO_{2}$ (70.2 and 92.0 mmHg) and died within two days while three cows recovered within a week. Necropsy revealed that abomasums were impacted with extremely dry digesta although epithelial cells were not damaged.

DISCUSSION

Continuous infusion without a priming dose did not cause adverse effects in previous experiments (Chapter 3 and 4). In addition, no relationship was observed between type of VFA infused and occurrence of metabolic disorders in this experiment. Therefore, the priming dose of hyper-osmotic solutions likely caused the metabolic disorders observed. Dry matter intake, water intake, and serum concentrations of sodium and potassium for cows downed by infusion treatment (DOWN) were compared to those of cows that appeared to be normal (NORM) to identify the factors characterizing the adverse effect of the infusion protocol. Serum concentrations of sodium and potassium were determined by atomic absorption spectrometry after digestion with hydrogen

peroxide. Dry matter intake was lower for DOWN compared to NORM (4.0 vs. 10.1 kg/12h; Table 1). Although infusion of sodium VFA increased water intake, water intake was increased less by DOWN than by NORM. Compared to the average for 3 d prior to the infusion period, infusion of sodium VFA increased water intake by 24% for DOWN (111.3 vs. 88.2 L/12h), but by 53% for NORM (126.0 vs. 85.2 L/12h).

These observations lead us to speculate that a priming dose of hyper-osmotic solution of sodium VFA resulted in systemic dehydration great enough to affect subsequent drinking behavior. Although cows are tolerant to sodium chloride at maximum concentration of 9.0% of dietary DM (Meyer et al., 1955), the total amount of sodium infused over 14 h was expected to be less than 4% of dietary DM in this experiment. A pulse dose may result in greater adverse effects compared to continuous infusion or feeding because cows are given less time to respond to infusion of hyper-osmotic solutions. The priming dose of VFA salt increased osmolarity of ruminal fluid, but cows might not have been able to drink enough water to adjust for hyper-osmolarity of the ruminal fluid, and water flux from blood to the rumen caused systemic dehydration for all cows. Hyper-osmolarity in duodenal digesta decreases gastric emptying (Ruckebusch, 1993) and might further worsen dehydration status by limiting water intake because of ruminal distention. Necropsy revealed abomasums impacted by dry digesta for 2 DOWN cows, indicating that the decrease in gastric emptying might have been more severe for DOWN.

TABLE 1. Characteristics of cows that downed by the infusion protocol (DOWN) and those appeared to be normal (NORM).

	NORM	DOWN
Days in milk	97.3	43.8
BW	664	670
BCS	2.79	2.80
DMI (kg/12h)		
3d-collection period before infusion	13.1	11.6
Infusion day	10.1	4.0
Water intake (L/12h)		
3d-collection period before infusion	85.2	88.2
Infusion day	126.0	111.3
Serum Na (mM)		
3d-collection period before infusion	129.6	124.6
Infusion day	136.2	138.1
Serum K (mM)		
3d-collection period before infusion	4.59	4.75
Infusion day	3.55	3.10

Infusion of sodium with VFA can cause metabolic alkalosis, and decrease serum concentration of potassium by activating a potassium/ H^+ pump: intracellular protons are exchanged with potassium in blood to maintain blood pH (NRC, 2001). Although infusion of sodium VFA decreased serum concentration of potassium for all cows, potassium concentration to greater extent for DOWN compared to NORM. Compared to the average of 3 d prior to the infusion period, infusion of sodium VFA decreased serum potassium concentration by 35% for DOWN (4.75 vs. 3.10 mM), but only by 23% for NORM (4.59 vs. 3.55 mM). DOWN cows experienced more severe hypokalemia possibly because of less water intake and DMI compared to NORM. Metabolic alkalosis might also induce conformational change in PTH receptors (Goff et al. 1991), and some cows could not stand or walk properly due to hypocalcemia (less than 0.7 mM of ionized Ca concentration). In addition, cows were observed to slow down respiration rate to correct metabolic alkalosis. Two cows increased $_PCO_2$ drastically (possibly resulting in permanent cell damage) and died.

CONCLUSION

A priming dose of hyper-osmotic solutions resulted in dehydration. In addition, infusion of sodium VFA solution affected acid-base balance in serum, resulting in metabolic alkalosis, hypokalemia, and hypocalcemina. A priming dose of hyper-osmotic solution should not be administered for future experiments.

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