TEMPORAL EFFECTS OF RUMINAL INFUSION OF PROPIONIC ACID ON FEEDING BEHAVIOR AND HEPATIC METABOLISM OF DAIRY COWS IN THE POSTPARTUM PERIOD

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science – Doctor of Philosophy

2018

ABSTRACT

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The control of feed intake during the transition period of dairy cows is not completely understood, but research has shown that greater fermentability of starch and ruminal infusions of propionic acid (PA) can decrease intake of cows in early, mid- and late-lactation by decreasing size or frequency of meals. Evidence suggests that changes in feeding behavior (FB) are from the ability of PA to stimulate the oxidation of fuels likely increasing the energy status of the liver and stimulating satiety. Although the hypophagic effects of PA are well documented, the temporal effects of PA supply to the liver within the time frame of a meal is still unknown.

To determine the temporal effects of PA infusion at the initiation of meals on the FB of cows in the postpartum (PP) period, we infused 1.25 mol of PA over 5 min (FST) or 15 min (SLW) into the rumen at each meal. Infusion of PA decreased DMI 45% compared with control with similar effects for FST and SLW treatments (10.4 vs. 10.0 kg DM). SLW decreased meal size 29% compared with FST (1.23 vs. 0.87 kg DM) and FST decreased meal frequency 24% compared with SLW (8.5 vs. 11.2 meals/day). Meal length was not affected by treatment (23.0 min), but PA increased intermeal interval compared with control (88.9 vs. 120.9 min) and FST increased intermeal interval 35% compared with SLW (139 vs. 103 min). To investigate if the temporal effects of PA infusion vary according to amount of PA infused, we conducted an experiment infusing 2.5 L of either 0.5M (HI) or 0.2M (LO) solutions of PA at initiation of meals over FST or SLW for 12 h following feeding. No interactions of treatments were detected for any FB parameter measured. FST did not affect DMI but tended to increase meal length (28.1

vs. 22.7) and decrease total eating time (108 vs. 122 min/12 h) compared with SLW. HI decreased DMI (7.4 vs. 11.5 kg/12 h) compared with LO by decreasing meal frequency (5.8 vs. 7.5 meals/12 h). The lack of effect of infusion rate on meal size, along with the reduction in DMI by HI compared with LO, by decreasing meal frequency rather than meal size, suggested that propionate flux to the liver might have exceeded the hepatic capacity for PA uptake from the blood, likely extending hepatic oxidation longer after meals. However, we could not sample liver and blood during meals without interfering with FB. Therefore, to determine the temporal effects of PA infusion on hepatic metabolism, we infused 1.25 mol of PA either FST or SLW, before feeding and after feeding. Blood and liver samples were collected before, and after 5, 15 and 30 minutes following the start of infusions. Our results indicated that FST did not reduce the efficiency of extraction of propionate by the liver compared with SLW. However, FST increased plasma glucose and insulin concentrations and decreased plasma NEFA concentration compared with SLW. Decreased NEFA concentration during infusion likely decreased the supply of acetyl CoA for oxidation in the liver. In addition, propionate from FST likely did not result in greater TCA cycling because it entered the gluconeogenesis pathway increasing glucose concentration faster than SLW, so less malate was likely available to be converted to oxaloacetate and continue the cycle. Reduced oxidation of acetyl CoA within a meal likely explains the greater meal size for FST compared with SLW, consistent with the hepatic oxidation theory and our FB results.

Although rate of infusion can affect FB, these experiments show that the hypophagic effects of PA during the PP period are primarily from the amount of PA infused. Further, the reduction in DMI by increasing fermentability of starch is likely from greater, rather than faster PA production. We expect that this research will help nutritionists to better understand the complex mechanisms that control feed intake in the PP period.

ACKNOWLEDGEMENTS

The path to this dissertation completion wasn't always easy. I was tremendously fortunate to have people to support me along the way. To you my most sincere thank you.

To my advisor Dr. Michael Allen: thank you for trusting me and giving me the opportunity to join your team. We didn't know each other when this journey started, but with your support, encouragement, and guidance, we built a not just a great work relationship but a solid friendship. I will always be grateful for all the times you helped me improve, challenged me to be a better researcher and showed me I was capable of enduring even the most difficult situations. I learned a lot from your actions, from the way you treat your students and you inspired me to be a better professional and a better person. I could not have made a better decision than to come work with you. Thank you.

To my graduate committee members, Dr. Thomas Herdt, Dr. Richard Ehrhardt, Dr. David Beede and Dr. Adam Lock: thank you for your time, your guidance and for helping me during my time at Michigan State University. I cannot express what an honor it was to work and to learn from all of you. Special thanks to Dr. Beede for mentoring me on my teaching experience and for always been available.

To Katie Kennedy, Rodrigo Albornoz and Laura Gualdrón-Duarte, my academic brother and sisters: thank you for being great partners in this journey. For all the help with my experiments at the farm, the nights baby-sitting cows but most importantly, for all the good moments outside the lab. You are amazing people, and I'm sure you are all off to great things.

To Dave Main and Dewey Longuski: thank you for all the patience and time, especially when I was a new student with a bad accent. Without your training and help everything in these experimental trials would be harder and not nearly as fun.

To my friends Crystal, Paola, Roni, Paige, Enhong, Jonas, Rodrigo, JP and all the ASGSA, LAC and Judo crew: thank you for making my time in Michigan truly wonderful. Matheus and Martin, thank you for always having my back and for being like family to me. Joe, thank you for believing and supporting me in all my dreams.

To the Dairy Cattle Teaching and Research Center team, particularly Randy, Jake, Rob, and Fred, and to Dr. Steve Bursian and Barb Sweeney: thank you for your help and the many encouraging words throughout these years.

To my Brazilian friends and family, thanks for proving that there is no distance when there's love. Finally, to my mom, dad and brother: thank you for understanding that I needed to go and for always loving me. You are the reason why I made it so far.

To the USDA National Research Initiative Competitive Grants Program, thanks for the financial support of this research as well as CAPES - Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil, for supporting me while conducting this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
BHBA	ß-hydroxybutyrate
CON	Control treatment
CoA	Coenzyme A
CP	Crude protein
DE	Digestible energy
DEI	Digestible energy intake
DGC	Dry ground corn
DM	Dry matter
DMI	Dry matter intake
EB	Energy balance
FB	Feeding behavior
FAD	Flavin adenine dinucleotide
GE	Gross energy
GTP	Guanosine-5'-triphosphate
HMC	High moisture corn
HMG	β-Hydroxy β-methylglutaryl
HOT	Hepatic oxidation theory
ME	Metabolizable energy
MEI	Metabolizable energy intake

NAD	Nicotinamide adenine dinucleotide
NDF	Neutral-detergent fiber
NEFA	Nonesterified fatty acids
NEB	Negative energy balance
OM	Organic matter
PA	Propionic acid
PP	Postpartum
PVN	Paraventricular nucleus of the hypothalamus
TCA	Tricarboxylic acid
TMR	Total-mixed ration
VFA	Volatile fatty acids

INTRODUCTION

The suppression of appetite of cows in the postpartum (PP) period extends and amplifies the negative energy (NEB) in transition cows. Sustained depression in feed intake can lead to metabolic disorders and decreased milk production, decreasing profitability of dairy farms. Increasing energy density in the diet by increasing the amount of starch in the ration is a common strategy to alleviate the NEB in fresh cows. Also, increased dietary starch can provide glucose precursors needed for milk lactose production. However, research has shown that increasing ruminal starch fermentability can depress feed intake during the PP period and more so in diets with higher inclusion of starch (Albornoz and Allen, 2018). Of the fuels produced by digestion and fermentation of starch, including propionate, lactate and glucose, only propionic acid (PA) decreased metabolizable energy intake of cows in the PP period compared with no infusion when all were infused abomasally as isoenergetic solutions (Gualdron-Duarte and Allen, 2018).

According to the hepatic oxidation theory (HOT), the hypophagic effects of PA are likely due to its ability to stimulate the oxidation of fuels (Allen *et al*., 2009). Propionate is an obligatory anaplerotic metabolite to the tricarboxylic acid (TCA) cycle in hepatocytes, replenishing TCA intermediates. Therefore, it can stimulate hepatic oxidation of acetyl-CoA, enhance ATP production and stimulate satiety. Several studies demonstrated hypophagic effects of propionate infusions in ruminants, but to our knowledge no research has evaluated how different rates of PA infusion affect the feeding behavior of cows in the PP period. Increasing starch fermentability of the diet increases PA production in the rumen, and increased propionate supply to the liver can reduce intake, so it is crucial that we understand the mechanisms that PA affects the metabolic control of feed intake of dairy cows.

The objectives of this research were to evaluate the temporal effects of PA supply on DMI and feeding behavior of cows in the PP period. Our central hypothesis was that differences in the temporal supply of PA affect feeding behavior according to the hepatic capacity to extract and metabolize this metabolite. Furthermore, our goal was to identify possible bottlenecks for hepatic propionate metabolism and understand how they could affect the oxidation of fuels and feed intake.

This dissertation includes results of three experiments conducted at the Michigan State University Dairy Cattle Teaching and Research Center. Experiments were performed to 1) determine temporal effects of PA infusion at the initiation of meals on the feeding behavior of cows in the PP period; 2) investigate how the temporal effects of PA infusion vary according to amount of PA infused at the initiation of meals; and, 3) determine the metabolic response to rate of ruminal infusion of propionic acid before and after feeding by cows in the PP period and how it relates to hepatic oxidation of fuels and feed intake.

Our rationale was that the results of this research would provide valuable new information related to regulation of energy intake of dairy cows in the PP period. Conceivably, it would unravel some of the complex mechanisms by which hepatic oxidation of fuels regulate energy intake in dairy cows and would allow for better diet formulation to increase DMI and improve energy balance in the critical PP period.

Chapter 1 from this dissertation has been published as "Temporal effects of ruminal propionic acid infusion on feeding behavior of Holstein cows in the postpartum period" in Gabriela Maldini and Michael S. Allen. 2018 J. Dairy Sci. 101:3077-3084.

CHAPTER 1. LITERATURE REVIEW

THE TRANSITION PERIOD

The transition period is a critical and vulnerable period of a cow's lifetime. Commonly, it is characterized as the period of 2-3 weeks before parturition and 3 weeks after parturition where significant homeorhetic changes in metabolism occur to support fetal development and milk synthesis. During this time, appetite is often suppressed and due to the increased demand in energy and glucose precursors, nutrient supply lags behind, resulting in negative energy balance (NEB).

Poor management of the transition period usually results in increased risk for metabolic disorders that aggravate the already suppressed DMI and exacerbate the NEB of transition cows (Grummer, 2008). According to Wallace *et al*. (1996), a poor transition from pregnancy to lactation can result in losses of 10-20 lbs (4.54-9.07 kg) of peak milk yield, which could equal to 2000-4000 lbs (907.18-1814.37 kg) through lactation (Wankhade *et al*., 2017). Losses in production, reproduction and extra expenses related to disease treatment can erase the entire profit potential for an individual cow (Drackley, 1999), therefore a careful and effective management of the transition period could be the key to a profitable dairy activity.

Over the past years, research attention has focused on understanding the physiological adaptations underpinning the transition to lactation, and on developing nutritional strategies to alleviate NEB, and improve productivity and health of dairy herds. Depression in appetite has been linked to several causes, but it is important that we understand how nutritional strategies can affect feed intake in the transition period, and most importantly, how they can be used to facilitate a successful transition from pregnancy to lactation.

This research aims to understand how propionic acid supply from the diet can affect feed intake and how diet can alter the pattern of oxidation of fuels in the liver to maximize glucose production and energy intake during this period, when feed intake is suppressed.

CONSTRAINTS TO FEED INTAKE IN THE TRANSITION PERIOD

By the end of pregnancy, energetic demands of gestating cows can be 1.3 to 1.5 times higher than the maintenance requirements (Quigley and Drewry, 1998). Bell (1995) estimated that the daily demands for fetal and placental growth in the last three weeks of gestation increase by 3-5 Mcal of net energy. However, despite the increase in energy demand, many studies have reported decreases in DMI in cows during the prepartum period. According to VandeHaar and Donkin, (1999) decreases in DMI in the first weeks of the transition period can vary widely, but as parturition approaches, suppression of DMI appears to occur very rapidly (Bertics *et al*., 1992; Hayirli *et al*., 1998), with the greatest reduction on the day of parturition. Goff and Horst (1997) and Bertics *et al*. (1992) observed that DMI decreases by 30% on day 1 or 2 before calving. Vasquez-Anon *et al*. (1994) reported a decline of approximately 40% during the same period.

The mechanisms of feed intake reduction have not been completely elucidated, but different factors appear to interact to cause this change pre and postpartum. According to Forbes (1977), in the prepartum period, the growing fetus might induce space constraints and restrict rumen volume, therefore physically limiting feed intake. Although fetal growth and prepartum DMI have been correlated, the growth of the fetus is more steady during the final trimester of gestation, whereas the drop in DMI does not occur until the last few days before parturition, indicating that factors other than rumen fill must be involved in the regulation of feed intake in the prepartum period.

Increases in blood estrogen have also been associated with suppression of appetite before parturition (Grummer *et al*., 2004). Green *et al*. (1994) reported a significant negative correlation (r =0.46) between feed intake and serum estradiol-17β concentrations in the blood of late gestation ewes. Estrogen can act in the paraventricular nucleus of the hypothalamus (PVN), a brain region involved in the control of feeding behavior, stimulating the release of anti-orexigenic molecules that trigger satiety and decrease feed intake. Butera (2010) studied the interactions between estradiol and cholecystokinin (CCK, a peptide hormone released by the small intestine during a meal, where it acts on gut receptors whose stimulation activates afferent fibers of the vagus nerve) and observed that peripheral treatment with estradiol decrease intake and also increase the suppressive effects of CCK on DMI.

Insulin also plays an important role as a regulator of intake and body weight in ruminants pre and postpartum. In the prepartum period, plasma insulin concentration decreases substantially (Doepel *et al*., 2002) continually declining until calving, while responsiveness of adipose tissue to insulin decreases (Bell and Bauman, 1997). Increased insulin resistance diminishes the uptake of glucose by skeletal muscle and increases the lipid mobilization from adipose tissue to provide energy for the body while sparing glucose for milk synthesis in the mammary gland, or to the fetus in late gestation. In the short-term, it is likely that higher relative to lower insulin concentrations contribute to the removal of fuels from the blood during meals (Oba and Allen, 2000) indirectly allowing for greater intakes by reducing the oxidation of acetyl CoA in the liver.

Close to calving, growth hormone concentration increases (Bauman, 1992) and can further increase insulin resistance in the adipose tissue. The surge in fatty acid (FA) mobilization, caused by the decrease in insulin concentration and the increased insulin resistance, increase the

supply of NEFA to the liver increasing ß-oxidation and the production of acetyl CoA. The continuous supply of acetyl CoA for oxidation in the TCA cycle is likely related to satiety during this period (Stocks and Allen, 2013).

In summary, all the above homeostatic and homeorhetic changes are related to a decrease in DMI. With the onset of lactation, more factors can contribute to the suppression of appetite. Cortisol and catecholamines released during calving are lipolytic and can intensify body fat mobilization. More acetyl CoA is then available for hepatic oxidation, and greater is the potential reduction in DMI due to metabolic control.

Diet factors can also affect intake by providing different fuels for oxidation. Oba and Allen (2003c) demonstrated that high moisture corn (HMC) reduced DMI (20.8 vs. 22.5 kg/d) compared with dry ground corn (DG) treatment in high-starch diets. Dann and Nelson (2011) showed that a low starch (21%) diet tended to increase DMI by 1.5 kg/d, milk fat yield by 0.2 kg/d and milk yield by 3.7 kg/d for the first 91 d PP compared with a high-starch (26%) diet. In both cases, the decrease in DMI was likely due to the ability of propionic acid from starch fermentation to stimulate oxidation of acetyl CoA in the liver, stimulating satiety. Gualdron-Duarte and Allen (2018) infused different fuels from the digestion of starch, including PA, lactate and glucose and reported that fuels had different effects on DMI that were consistent with their ability to stimulate hepatic oxidation of acetyl CoA. When compared with control, PA decreased total MEI while glucose and lactate did not.

According to Van Soest (1994) and Forbes (1995), there is a multifactorial control of feed intake. The multifactorial theory states that the initiation and termination of a meal seem more likely to be controlled by a combination of signals rather than a single signal. This

dissertation will focus primarily on the metabolic control of feed intake, more so concerning the liver role in the coordination of nutrient metabolism.

THE HEPATIC OXIDATION THEORY

According to the hepatic oxidation theory (HOT), the oxidation of different fuels in liver tissue is involved in the control of feeding behavior and feed intake (Allen *et al*., 2009). This control is exerted through signals from the liver, transmitted via hepatic vagal afferents to brain feeding centers depending on the firing rate of the vagal nerve (Friedman, 1995; Langhans and Sharrer, 1992). Increased hepatic oxidation of fuels likely decreases the firing rate of the nerve, inhibiting feeding while decreased hepatic oxidation likely increase hunger signaling.

Langhans (1996) published an extensive review on the metabolic control of feed intake and stated that not only glucose but acetate, pyruvate and some other metabolites also seem to mediate feed intake in non-ruminants. Evidence that the vagus nerve mediates the response and that the hypophagic signals are not due to a local action of the metabolites in the brain was shown by Langhans (1985). Langhans injected different metabolites subcutaneously in rats and observed failure to suppress eating in rats with hepatic branch vagotomy. The same was observed by Anil and Forbes (1988) when splanchnic blockade with anesthetic, bilateral splanchnotomy, and hepatic vagotomy, as well as with total liver denervation in sheep eliminated the hypophagic effects of portal infusion of propionate.

Allen *et al*. (2005) showed evidence that same mechanism might apply to ruminants. However, it is important to note that the because of the low activity of glucokinase (Emmanuel, 1981), hepatic uptake of glucose is negligible in adult ruminants (Stangassinger and Giesecke, 1986) and therefore fatty acids (dietary or mobilized from body reserves), propionate, lactate and amino acids would be more relevant fuels for the hepatic oxidation and control of feed intake.

Consistent with the HOT, glucose does not appear to have a hypophagic effect in ruminants (Baile and Forbes, 1974; Frobish and Davis, 1977). Although acetate is an abundant fuel from ruminal fermentation, hepatic uptake and utilization of plasma acetate is negligible (Seal and Reynolds, 1993) because acetyl CoA synthetase activity is very low. Propionyl-CoA synthetase (Demigne *et al*. 1986) however is very high in the ruminant hepatocyte and propionate is very efficiently extracted from the blood.

Because there is an increased requirement for glucose utilization for milk production in the PP period, nutritionists commonly increase starch concentration in diets of fresh cows. Therefore, propionic acid becomes the primary fuel from the diet stimulating hepatic oxidation and triggering satiety in this period. Propionate is the principal gluconeogenic precursor for dairy cows, however, feeding diets with highly fermentable starch may not be the best strategy to maximize glucose production and energy intake. Although propionate is gluconeogenic, it must be metabolized to succinyl CoA and enter the TCA cycle. Anaplerosis of the TCA cycle promotes the oxidation of acetyl CoA (Gualdron and Allen, 2017). According to the HOT, by increasing propionate uptake in the liver, and therefore the oxidation of the hepatic pool of acetyl CoA, which is elevated in transition cows in a lipolytic state, the hepatic energy charge increases and meal size decreases, ultimately decreasing total DMI intake (Allen and Piantoni, 2013).

Choi and Allen (1999) reported that propionate infusions into the rumen of lactating dairy cows at the onset of spontaneous meals reduced meal length and meal size to a greater extent than equimolar amounts of NaCl or acetate. In PP cows, continuous isoenergetic infusions of propionic acid (PA) into the abomasum decreased DMI 24.3% compared with control (Gualdrón-Duarte and Allen, 2018). When infused at spontaneous meals, PA consistently decreased feed intake in PP (Maldini and Allen, 2018) and mid-lactation cows (Choi and Allen,

1999). In conclusion, research has shown that the hypophagic effects of propionate are well established, however, the mechanism by which it affects feeding is still unclear.

Balancing the gluconeogenic and hypophagic effects of PA is a challenge and research evaluating effects of the temporal supply of PA on feeding behavior of cows during this period is lacking. There is evidence that hepatic oxidation of fuels is involved in the control of feed intake in the PP period and our goal with this research is to help unravel the underlying mechanisms driving the reduction in DMI associated with the supply of PA from the diet in order to better support cows during the PP period.

STARCH FERMENTABILITY AND PROPIONIC ACID

Numerous dietary strategies have been examined for transition cows. Recommendations in the literature vary greatly for both the pre and postpartum periods. Feeding strategies for the prepartum period include restricting feed intake by increasing the fiber content of the diet (Rabelo *et al*., 2003), limiting feed offered to attenuate the drastic reduction in feed intake PP; and to increase energy intake by increasing energy and protein density of diets (VandeHaar *et al*., 1999). In the PP period, strategies consist of increasing dietary energy density and intake, in an effort to alleviate NEB and also to provide adequate substrates to support lactation. However, the range in concentration and ruminal fermentability of starch varies widely. Starch concentration can exceed 30% of the dietary DM for lactating cows, and starch fermentability can vary from 30% to more than 85% depending on the grain, storage type, and processing method when fed to dairy cows (Allen, 2000). In a recent meta-analysis, Moharrery *et al*. (2014) showed that rumen digestibility of starch ranged from 224 to 942 g/kg, while intestinal digestibility ranged from 114 to 901g/kg and hindgut ranged from 81 to 951g/kg depending on the starch source for dairy cows.

Oba and Allen (2003c) compared high moisture corn (HMC), a more fermentable starch source, and dry ground corn (DGC), a less fermentable starch source, and found that HMC nearly doubled the fractional rate of starch fermentation in the rumen, increasing the contribution of short-chain fatty acids as fuels at the expense of glucose from starch digestion in the small intestine. They also observed an 8% reduction in feed intake by the HMC treatment when compared with DGC. Decreased DMI when feeding HMC vs. DGC was also reported by Albornoz and Allen (2018) and the reduction in DMI was more pronounced (3.9 kg/d vs. 0.9 kg/d) when starch was included at 28% compared with 22% of diet DM. Of the ruminal fermentation products, PA comprises 15 to 40% of total ruminally produced organic acids and it is the predominant substrate for gluconeogenesis (Lemosquet *et al*., 2009). Propionate provides from ~50% (Bergmann, 1990) to as high as 90% (Cridland, 1984) of the glucose requirements in ruminants. Lactate is also a product from starch digestion and it can supply 10 to 26% of gluconeogenic substrates (Reynolds *et al*., 2003; Larsen and Kristensen, 2009) in dairy cows.

Both PA and lactate can enter the TCA cycle as anaplerotic metabolites stimulating oxidation of acetyl CoA. However, lactate is expected to have less effect on DMI than propionate because a greater amount of PA is produced in the rumen and because PA extraction by the liver is much more efficient (Reynolds *et al*., 2003). Gualdron-Duarte and Allen (2018) infused both lactate and PA into the abomasum of dairy cows to evaluate the effects of both gluconeogenic precursors on feeding behavior. PA decreased metabolizable energy intake (MEI) by 13% (34.8 vs. 40.2 Mcal/d) whereas lactate did not affect MEI, consistent with their expected effects on hepatic oxidation. Odd-carbon organic acids (e.g. valerate) are also glucogenic (Hall and Eastridge, 2014), however, particularly in high yielding cows, hepatic gluconeogenesis relies heavily on propionate.

The production and absorption of propionate are greater after feeding and gluconeogenesis in ruminants is also greater after periods of high-energy intake (Aschenbach *et al*., 2010). However, when supplied in excess, as it could happen in highly fermentable diets, PA has deleterious effects on feed intake (Baile, 1971; Anil and Forbes, 1980). Propionate metabolism by the ruminant occurs nearly exclusively in the liver (Brockman, 2005). Once trapped in the liver as propionyl-CoA and entered the TCA cycle as succinyl-CoA, propionate carbons can be utilized for gluconeogenesis immediately or remain in the TCA cycle to oxidize acetyl CoA until it exits the TCA cycle. Therefore, even if propionate follows gluconeogenic pathways the increased propionate flux to the liver during meals likely stimulates the oxidation of acetyl-CoA produced by β-oxidation of NEFA, stimulating satiety (Allen *et al*., 2009).

Intraruminal isomolar infusions of propionate compared with acetate in lactating dairy cows decreased total MEI, despite having a higher energy concentration than acetate (Oba and Allen, 2003b). Feed intake decreased linearly from 15.0 to 8.3 kg DM/12 h primarily through reduction in meal size, as propionate increased from 0% to 100% of the infusate. Similarly, propionate infusions at spontaneous meals decreased meal size compared with acetate (Choi and Allen, 1999), and the degree of hypophagia from PA increased with greater hepatic acetyl CoA concentration (Stocks and Allen, 2012; 2013). Propionate also decreased feed intake of dairy cows compared with mixtures of SCFA (Hurtaud *et al*., 1993) and acetate (Sheperd and Combs, 1998). These studies indicate that hypophagic effects of propionate cannot be explained merely by the additional energy supplied.

Although the control of feed intake is multifactorial, the literature reviewed in this dissertation indicates that there are fuel-specific mechanisms regulating satiety and hunger and playing an important role on the regulation of intake of PP cows. As reviewed earlier,

fermentability of dietary starch is related to the reduction in DMI; however, there is a lack of research that evaluates how different fluxes of propionate to the liver affect hepatic oxidation, appetite and intake within each meal. It is logical that less fermentable starch sources will provide a slower PA production and flux of propionate to the liver and that more fermentable starch sources will increase hepatic propionate supply. However, the effects on meal size, meal frequency, hunger, and satiety may be different and need evaluation. Our objective with the experiments in this dissertation is to understand how the temporal effects of PA production in the rumen affects feeding behavior and DMI of cows in the PP period.

TRICARBOXILIC ACID CYCLE: REGULATION AND PROPIONATE ANAPLEROTIC REACTIONS

The tricarboxylic acid (TCA) cycle is a series of biochemical reactions involved in the release of stored energy through the oxidation of acetyl-CoA. It is a common pathway for the oxidation of fuels, such as amino acids, fatty acids, and carbohydrates, but the cycle also provides precursors for specific amino acids, nucleotide bases, and cholesterol, as well as reducing equivalents necessary in other biochemical reactions. The TCA cycle takes place in the mitochondrial matrix. Except for succinate dehydrogenase, which is embedded in the inner membrane of the mitochondria, the enzymes involved in the cycle are soluble in the matrix.

The overall pattern of the TCA cycle and how propionate enters the TCA cycle is shown in Figure 1. In summary, a full spin of the cycle consists of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one guanosine triphosphate (GTP) and reduced forms of nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD). NADH and $FADH_2$ are reducing equivalents that enter the electron

transport chain to undergo oxidative phosphorylation, creating a proton gradient that is used to drive ATP synthesis.

Propionate is an obligatory anaplerotic metabolite and must enter the TCA cycle to be metabolized (Gualdrón-Duarte and Allen, 2017). Anaplerosis of the TCA cycle can stimulate oxidation of the existing pool of acetyl CoA, therefore increasing hepatic energy charge. According to the HOT (Allen, 2014) increased energy charge in the hepatocyte stimulates the vagus nerve generating a satiety signal to the brain feeding centers, potentially reducing feed intake or terminating a meal.

The TCA cycle is conserved among mammals. However, in contrast to monogastric species, NEFA, instead of glucose, are the principal fuels oxidized in the hepatocytes of dairy cows. Hepatic glucokinase, which is responsible for the activation of glucose, has not been detected in the liver of dairy cows (Panserat *et al*., 2014). The liver is also limited in its ability to utilize acetoacetic acid (McCann,1957), which may explain why these fuels generally do not induce hypophagia in ruminants

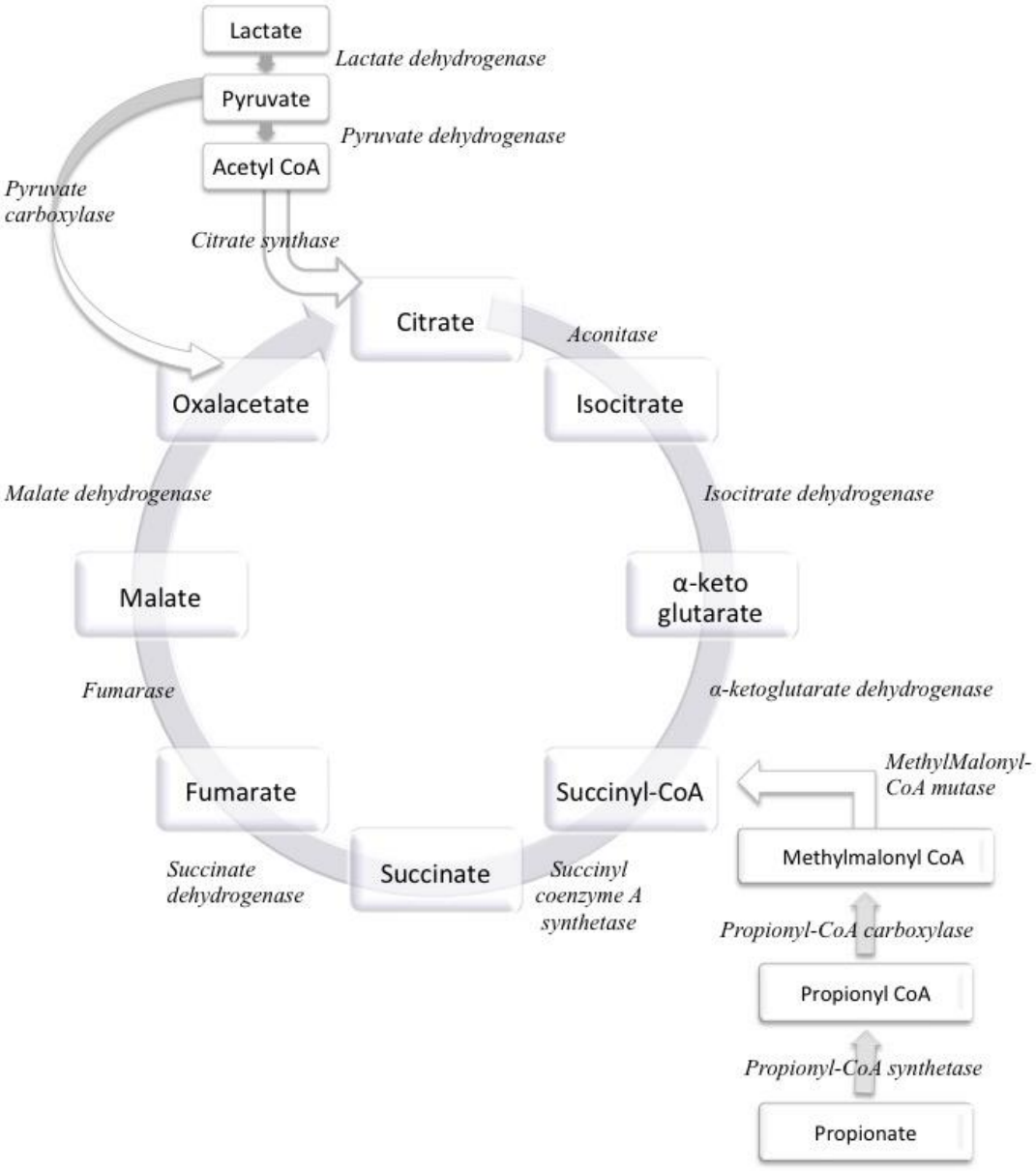

Figure 1.1 - TCA cycle and pathway for anaplerosis from propionate

Regulation of the TCA cycle

Carbon flow and oxidation in the TCA cycle are tightly regulated. The conversion of pyruvate into acetyl-CoA, mediated by the pyruvate dehydrogenase complex, is allosterically inhibited at high [ATP]/[ADP], [NADH]/[NAD+], and [acetyl CoA]/(CoA] ratios, all of which indicate the energy sufficient metabolic state. The reversible phosphorylation of the E1 unit of pyruvate dehydrogenase complex regulation complements this regulation. When ATP concentration increases, a kinase phosphorylates E1, deactivating the complex. On the other hand, the complex is activated by increased AMP, CoA, and NAD+, when the higher flux of acetyl-CoA into the citric acid cycle is required. Because pyruvate is not the only source of acetyl CoA in the liver, the availability of intermediates from other pathways is also important in the regulation of pyruvate oxidation and of the TCA cycle. In the PP period, specifically, cows with more intensive body fat mobilization had increased plasma NEFA concentration, increased anaplerosis, TCA cycling, and mitochondrial oxidation of acetyl CoA (Schäff *et al*., 2012).

Within the TCA cycle, there are three main points of regulation. These reactions are exergonic steps in the cycle and can become the rate-limiting step under some circumstances. The first is the citrate synthase reaction, which conjugates acetyl CoA and oxaloacetate forming citrate. Accumulation of citrate, succinyl CoA, NADH, and ATP inhibits the reaction. The inhibition of citrate synthase by ATP is relieved by ADP, an allosteric activator of this enzyme. The second regulation point is at the isocitrate dehydrogenase reaction, where α-ketoglutarate is formed. Increases in the [NADH]/[NAD+] ratio and in ATP inhibits the reaction, while ADP and low levels of α-ketoglutarate stimulate the reaction flow. Finally, the α-ketoglutarate dehydrogenase that forms succinyl CoA, is inhibited by the accumulation of the product of the reaction: succinyl CoA, NADH and by ATP.

Another reaction that plays a significant role in the TCA cycle is the malate dehydrogenase reaction. When [NADH]/[NAD+] is elevated the malate dehydrogenase reaction is inhibited resulting in an accumulation of malate and upstream metabolites and increased export of malate from the mitochondria to the cytosol.

Anaplerotic reactions

One important anaplerotic reaction in the liver of animals is the reversible carboxylation of pyruvate to form oxaloacetate. The reaction is catalyzed by pyruvate carboxylase. It plays an important role when the TCA cycle is deficient in oxaloacetate. The addition of a carboxyl group to the pyruvate, however, requires energy and biotin. The activity of pyruvate carboxylase is virtually inactive in the absence of acetyl-CoA, but whenever acetyl-CoA accumulates, it stimulates the pyruvate carboxylase reaction to produce more oxaloacetate, enabling the cycle to use more acetyl-CoA in the citrate synthase reaction. McClure and Lardy (1970) showed that pyruvate carboxylase activity is not regulated in an "on-off" manner, as are enzymes that participate solely in gluconeogenesis, for it participates also in lipogenesis and TCA cycle activity. Zeczycki *et al*. (2010) in a complete review described the several factors that affect pyruvate carboxylase activity, including the accumulation of oxaloacetate that seems to compete for the binding site, reducing the enzyme activity. Both succinyl CoA and methylmalonyl CoA have also been shown to inhibit this enzyme in other species (Barritt *et al*., 1976).

There are different anaplerotic reactions to the TCA cycle that allow for the use of different carbon sources to produce energy and also to provide necessary molecules to the metabolism. This dissertation will focus on the propionate anaplerotic reactions.

In the PP period, cows experience a great increase in glucose and energy demands, and to accommodate them, the liver increase its metabolic activity (Reynolds *et al*., 2003, 2004).

Propionate from ruminal fermentation is quantitatively the greatest contributor to gluconeogenesis during the periparturient period (Reynolds *et al*., 2003) and therefore increases in PA supply are likely associated to increases in hepatic metabolic activity. After absorption in the rumen, propionate is transported to the liver via the portal circulation. There, it is activated to propionyl CoA, trapping it in the liver. Propionyl CoA is then carboxylated in a reaction catalyzed by propionyl CoA carboxylase producing methylmalonyl CoA. Methylmalonyl CoA mutase rearrange the structure of the molecule, yielding succinyl CoA, a TCA cycle intermediate. Vitamin B_{12} is required as an essential cofactor in the conversion of methylmalonyl CoA to succinyl CoA, catalysed by methylmalonyl CoA mutase (Weidemann *et al*, 1970). Daugherty *et al*. (1986) showed a high correlation between liver B_{12} and endogenous propionate metabolizing activity in lambs, but CoA availability can also be a limitation, possibly slowing down the reaction.

Although propionyl CoA synthetase activity is high in ruminant hepatocytes, plasma propionate concentration increased linearly from 0.19 to 0.34 mM when propionate was infused intraruminally from 0 to 1.3 moles/h for 18 h for cows in the PP period (Oba and Allen, 2003a) indicating limitations to the activation of propionate. Mesenteric (Baird *et al*., 1980) and ruminal (Berthelot *et al*., 2002) infusion of propionate decreased the efficiency of propionate extraction by the liver in dairy cows and sheep, respectively, demonstrating a limitation in the hepatic capacity to extract PA from the blood. However, specific bottlenecks for propionate metabolism in the liver have not been identified for cows in the PP period.

Experiment 3 of this dissertation aims to help unravel some of these complex mechanisms and reaction that can potentially limit hepatic oxidation and feed intake in dairy cows.

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CHAPTER 2. TEMPORAL EFFECTS OF RUMINAL PROPIONIC ACID INFUSION ON FEEDING BEHAVIOR OF HOLSTEIN COWS IN THE POSTPARTUM PERIOD

Gabriela Maldini and Michael S. Allen. 2018. J. Dairy Sci. 101(4), pp.3077-3084.

ABSTRACT

The objective of this study was to determine the temporal effects of intraruminal infusion of propionic acid at the initiation of meals on feeding behavior of cows in the postpartum period. Propionic acid derived from ruminal fermentation can reduce energy intake of dairy cows. The suppression of appetite by propionic acid is likely caused by a signal related to the hepatic oxidation of fuels. Greater propionate flux to the liver is expected to result in faster oxidation of acetyl coenzyme A, which can stimulate satiety and reduce feed intake. Therefore, the rate of propionate supply to the liver, within the timeframe of meals, might be an important limitation to feed intake. Our hypothesis was that faster rate of propionate infusion during meals would decrease meal size and feed intake by decreasing the time required to stimulate satiety within a meal. Six ruminally-cannulated, multiparous Holstein cows in the postpartum period were used in a duplicated 3 x 3 Latin square design experiment balanced for carryover effects. Treatments included control (no infusion) or 1.25 mol of propionic acid infused over 5 min (FAST) or 15 min (SLOW) at each meal. Infusions were initiated at the conditioned meal at feeding (1200 h) and were triggered at each spontaneous meal for 22 h. Contrary to our hypothesis, SLOW decreased meal size 29% (0.87 vs. 1.23 kg of dry matter) compared with FAST, and FAST decreased meal frequency 27% (8.5 vs. 11.2 per d) compared with SLOW. Dry matter intake was similar between FAST and SLOW, but propionic acid decreased dry matter intake 45% compared with control. A potential explanation is that FAST resulted in greater liver bypass of propionate compared with SLOW, extending anaplerosis of the tricarboxylic acid cycle, hepatic oxidation of acetyl coenzyme A, and satiety over a longer time after meals.

INTRODUCTION

Maximizing intake in the postpartum (PP) period is critically important to the health, production, and profitability of dairy cows. However, the mechanisms controlling feed intake during the transition period are not completely understood. According to the hepatic oxidation theory (HOT), feeding behavior and feed intake are controlled by signals from the liver that are transmitted via hepatic vagal afferents to brain feeding centers (Allen *et al*., 2009). Increased hepatic oxidation of fuels likely decreases the firing rate of vagus, inhibiting feeding, while decreased hepatic oxidation increases its firing rate stimulating feeding.

The hypophagic effects of propionate are well-established but the mechanism by which it affects feeding is unclear (Allen, 2000). Previous research showed that infusion of propionic acid (PA) decreased feed intake of cows (Stocks and Allen, 2012; Choi and Allen, 1999; Gualdrón-Duarte and Allen, 2017), and that dry matter intake (DMI) and net energy intake decreased linearly in response to increasing amounts of propionic acid infused (Oba and Allen, 2003b). The rate of PA production in the rumen increases with diet starch content (Bauman *et al*., 1971, Sutton *et al*., 2003) and the starch content of diets fed to cows in the PP period varies widely. Furthermore, rumen digestibility of starch varies greatly, ranging from 224 to 942 g/kg, depending on the starch source (Moharrery *et al*., 2014). Variation in diet starch content and ruminal digestibility will result in very different rates of PA production, absorption and flux to the liver. We hypothesized that a greater rate of propionate absorption within meals would stimulate hepatic oxidation and satiety sooner compared to a slower rate of absorption. Our objective was to determine the temporal effects of intraruminal infusion of PA on the feeding behavior of cows in the PP period. We expected that a faster rate of infusion would decrease meal size and the time between meals compared with a slower rate of infusion.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Michigan State University approved all experimental procedures in this study.

Animals, Housing, and Diets

Multiparous Holstein cows (n = 8) from the Michigan State University Dairy Cattle Teaching and Research Center were ruminally cannulated at least 40 d prepartum and used in this experiment. Our goal was to evaluate the effects of treatment on cows during the PP period that are in a lipolytic state. Feed intake and health were monitored following parturition. Six healthy cows with the lowest range in days PP were selected for the experiment and moved to feeding behavior stalls for an adaptation period of 3 d before the first infusion day. Cows averaged 13.8 ± 2.9 DIM on first day of infusion, and all cows completed the experiment by 22 d PP.

The experimental diet (Table 2.1) was fed immediately following parturition and contained 16.0% CP, 32.9 % NDF, 26.4% forage NDF, and 25.0% starch and consisted of corn silage, alfalfa silage, chopped alfalfa hay, ground corn, soybean meal, soy hulls and a vitamin and mineral mix formulated to meet requirements according to NRC (2001). Cows were fed once a day (1200 h) at 110% of expected intake and feed offered was adjusted daily, if needed. Cows were not allowed access to feed between 1000 h and 1200 h each day while orts and the amount offered were weighed for each cow.

Table 2.1 Ingredients and nutrient composition of experimental diet

Item	%
Ingredient (% DM)	
Corn silage	33.5
Ground corn	17.1
Soybean meal	16.8
Alfalfa silage	13.2
Alfalfa hay	9.5
Soy hulls	5.6
Vitamin and mineral mix[1]	2.0
Sodium bicarbonate	1.1
Limestone	1.2
Nutrient composition	
DM	56.8
OM	91.3
Starch	25.0
NDF	32.9
Forage NDF	26.4
CP	16.0

[1]Vitamin and mineral mix contained 25.6% NaCl, 10.0% Ca, 2.0 Mg, 2.0% P, 30 ppm Co, 506 ppm Cu, 20 ppm I, 2220 ppm Fe, 2,080 ppm Mn, 15 ppm Se, 2,030 ppm Zn, 300 KUI/kg vitamin A, 50 KIU/kg vitamin D, 1500 KIU/kg vitamin E.

Experimental Design and Treatments

The experimental design was a duplicated 3x3 Latin square. Cows were blocked by parturition date and randomly assigned to a treatment sequence within a square. Treatments included no infusion (Control) and infusion of 1.25 mol of PA (99.5%, 0.5 *M*, Kemin Industries, Inc.) over 5 (FAST) or 15 minutes (SLOW) into the rumen at the conditioned meal and at each spontaneous meal for 22 h. Infusion rates were selected in an attempt to maximize treatment differences while constraining the slow treatment to the expected length of a typical spontaneous meal. Past research in our lab with intraruminal infusions of PA used twice this rate at initiation of meals with no adverse effects (Choi and Allen, 1999). Solutions were infused into the rumen

using peristaltic pumps (FPU401, Omegaflex® Peristaltic Pump, Norwalk, CT) and Tygon® tubing that passed through a hole in the cannula plug and was kept in place by stainless steel hose clamp fixed in each side of the cannula plug. All cows were infused on the same day and a 24-h recovery period following infusion periods was allowed to reduce the potential for carryover effects.

Feeding Behavior

Feeding behavior was monitored using a computerized data acquisition system (Dado and Allen, 1993) programmed to trigger infusion pumps at the initiation of each new meal according to Choi and Allen (1999) and Bradford and Allen (2007b). Feed manger weights were monitored every 5 s, and when the running standard deviation reached threshold (average running standard deviation greater than 0.9 kg), an "eating flag" was triggered. Infusions were initiated when at least 5 eating flags were triggered within 100 s to avoid false meals. To prevent mid-meal infusions, the eating flags were required to be triggered less than 13 times in the preceding 7.5 m, otherwise it would be counted as the part of the previous meal. Consecutive infusions began at least 15 min apart under both treatment protocols to prevent potential treatment bias.

Feed disappearance and water consumption were recorded for each cow every 5 seconds. Feeding behavior data were analyzed to quantify the number, size, length, and time between meals. Hunger ratio was calculated as the weight of meal divided by pre-meal interval and satiety ratio was calculated as the weight of meal divided by post-meal interval (Forbes, 2007). Triggering infusions based on real-time monitoring of feeding behavior resulted in reasonably good relationship between infusion events and meals, identified by post-hoc analysis. On average, there was a mean of 3 false-positives (infusions without meals) out of a total of 10.5 infusions/cow/d, and 0 false-negatives (meals without infusions)/cow/d with no differences

between FAST and SLOW treatments.

Sample Collection

All dietary ingredients were sampled daily. Forage samples were analyzed separately for each day and concentrate ingredients were composited across days of the experiment. Orts were weighed and sampled for each cow (~0.5 kg) at the end of each infusion day. All samples were stored in plastic bags at -20°C until processed. Cows were milked twice daily at 0530 h and 1730 h in their stalls. Milk yield was recorded at each milking, and milk samples were collected from each milking and stored with preservative (Bronopol tablet; D&F Control Systems, San Ramon, CA) at 4°C. Milk samples were analyzed by Flow Cytometer (FCM), and Fourier Transform Spectrometer (FTS) for fat, true protein, lactose, somatic cell count, and milk urea nitrogen by Michigan DHIA.

Blood samples were collected before and after each infusion period. Blood was sampled from coccygeal vessels and collected into 4 evacuated tubes, 3 containing K_2EDTA and 1 containing potassium oxalate with NaF as a glycolytic inhibitor, and immediately centrifuged at 3,000 x *g* for 15 min at 5°C. Plasma was harvested and 200 μL of benzamidine (0.25 M) was added to 1000 μL of plasma as a protease inhibitor for glucagon analysis. All samples were immediately frozen and kept at -20^{o} C until analyzed. Ruminal contents were sampled from 5 sites throughout the rumen and squeezed through a nylon screen (1-mm pore size) to collect the liquid phase at the beginning and end of each infusion period. Ruminal fluid pH was measured immediately using a portable pH meter (ATI Orion model 230A, Boston, MA), and samples were stored at −20°C until analysis. Fecal samples were collected on rest days (days after infusions) every 8 h in a 24 h period (1400 h, 2200 h and 0600 h) and frozen at -20°C for later

analysis to determine digestibility and energy concentration of the diet using methods described by Harvatine and Allen (2006).

Analysis of Samples

Diet ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h and grounded with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, indigestible NDF, crude protein and starch. Feces were composited by cow by day on an equal DM basis before analysis. Ash content was determined after 6 h of oxidation at 500°C in a muffle furnace. NDF was analyzed according to Mertens (2002) with the inclusion of amylase and Na sulfite. Crude protein was determined according to Hach *et al*. (1987). Starch was analyzed using an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured by a glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Indigestible NDF was determined as NDF residue after 240 h *in vitro* fermentation (Goering, 1970). Ruminal fluid was collected from a non-pregnant fistulated dry cow and flasks were re-inoculated at 120 h to ensure microbes were viable. Indigestible NDF was used to estimate fecal output and apparent total-tract digestibility (Cochran *et al*., 1986). All samples were analyzed for gross energy (GE) by bomb calorimeter according to the manufacturer's instructions (Parr Instrument Inc., Moline, IL). Digestible energy (DE) intake was calculated as GE intake minus GE output in feces. Metabolizable energy (ME) from the diet was calculated according to NRC (2001) as ME (Mcal/kg) = 1.01 × DE (Mcal/kg) − 0.45, based on measured DE. ME infusion was calculated assuming yields of 365.03 kcal/mol of propionate (Lebedeva, 1964). ME intake (MEI) was calculated as MEI (Mcal/d) = ME x DMI and total MEI= ME intake diet + ME infusion.

Plasma samples were analyzed colorimetrically with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp., Sunnyvale, CA) to determine concentrations of metabolites. Commercial kits were used to analyze for plasma concentrations of NEFA (NEFA HR kit, Wako Chemicals USA), BHBA (Stanbio Laboratory, Boerne, TX), glucose (Glucose oxidase -Sigma Chemical. Co.) and glucagon (Glucagon kit #GL-32K, Linco Research). Insulin was determined using an ELISA kit (Mercodia, Uppsala, Sweden). Plasma propionate, acetate, butyrate and organic acid concentrations were analyzed by HPLC (Waters Corp., Milford, MA) as described by Gualdrón-Duarte and Allen (2017).

Statistical Analysis

Data were analyzed as a replicated Latin Square design using JMP 13.0 software (SAS Institute, Inc. Cary, NC). Feeding behavior, intake, milk production, milk components, plasma and rumen metabolites were analyzed using the following linear model:

$$Y_{ijkl} = \mu + B_i + B_i(C_j) + P_k + T_l + P_k * T_l + e_{ijkl}$$

where Y_{ijkl} = the response variable; μ = overall mean; B_i = random effect of block i; $B_i(C_j)$ = random effect of cow within block; P_k = fixed effect of period j; T_l = fixed effect of treatment; $P_k * T_l$ = interaction between period and treatment; and e_{ijkl} = residual. Orthogonal contrasts were determined for infusions (FAST and SLOW) vs. control and for FAST vs. SLOW infusions. Significance was declared at $P \leq 0.05$ and tendency for significance at $P \leq 0.10$.

RESULTS

Feed Intake and Feeding Behavior

Results for feed intake and feeding behavior are reported in Table 2.2. The PA treatments decreased meal size compared with control (1.59 vs. 1.05 kg DM, $P < 0.01$), and SLOW

decreased meal size 29% compared with FAST (1.23 vs. 0.87 kg DM, $P = 0.01$). The PA treatments also decreased meal frequency compared with control (12.0 vs. 9.8 meals/day, $P = 0.01$) and FAST decreased meal frequency 24% compared with SLOW (8.5 vs. 11.2 meals/day, $P = 0.01$). Meal length was not affected by treatment (23.0 m), however PA treatments increased intermeal interval compared with control (88.9 vs. 120.9 m, $P < 0.01$) and FAST increased intermeal interval 35% compared with SLOW (139 vs. 103 m, $P = 0.01$). Both PA treatments decreased DMI 45% compared with control (18.6 vs. 10.2 kg/d, $P < 0.01$) with similar effects for FAST and SLOW treatments (10.4 vs. 10.0 kg DM, $P = 0.74$).

The amount of PA infused (~14.5 mol/d) provided 5.29 Mcal ME/d and did not differ ($P = 0.35$) between FAST and SLOW. When the ME from the treatment infusions was added to the ME intake from the diet, PA decreased total ME intake 30.6% per day (41.7 vs. 28.9 Mcal/d, $P < 0.01$) and effects of FAST and SLOW were similar (28.3 vs. 29.6 Mcal/d, $P = 0.68$). PA infusion decreased hunger ratio (0.037 vs. 0.013, $P <0.01$) and tended to decrease satiety ratio (0.045 vs. 0.022, $P = 0.07$) compared with control but no differences were observed for FAST vs. SLOW infusion treatments ($P > 0.60$).

The PA treatments decreased water intake compared with control (84.4 vs. 26.8 L/d; $P < 0.01$) by decreasing drinking frequency (12.0 vs. 4.8 bouts, $P < 0.01$) but effects of FAST and SLOW were similar for both water intake (29.3 vs. 24.3 L/day, $P = 0.7$) and drinking frequency (5.0 vs. 4.7 bouts, $P = 0.9$). When the amount of infusate from the treatments was added to the daily water intake, PA decreased overall liquid daily intake 34% when compared with control (84.4 vs. 55.8 L/day, $P = 0.02$).

Milk Production and Components

Treatments did not affect yields of milk (39.1 kg/d), protein (0.55 kg/d), fat (1.03 kg/d), lactose (0.93 kg/d), non-fat solids (1.09 kg/d), SSC (43.0 *1000/ml) or MUN (11.47 mg/dl), all $P > 0.2$ (data not shown).

Plasma Metabolites and Rumen Fluid

Results for plasma metabolites are reported in Table 2.3. The PA infusions increased blood glucose concentration compared with control (11.5 vs. -0.29 mg/dl, $P = 0.03$) but effects of rate of infusion did not differ ($P = 0.35$). Plasma concentrations of insulin, glucagon, NEFA and propionate were not affected by treatment (all $P > 0.24$), but PA decreased BHBA concentration compared with control (2.4 vs. -12.4 mg/dL, $P < 0.01$) and the reduction tended to be greater for FAST than SLOW (-14.1 vs. -10.6 mg/dL, $P = 0.08$). The PA treatments decreased plasma acetate (0.02 vs. -0.56 mM, $P < 0.01$) compared with control, but the effects of rate of infusion were similar ($P = 0.25$).

Results for rumen fluid pH and organic acids are reported in Table 2.4. The PA treatments increased PA concentration (2.1 vs. 14.6 mM, $P = 0.04$) and pH (-0.21 vs. 0.26, $P = 0.04$) compared with control with no difference detected between FAST and SLOW treatments ($P \geq 0.20$). The PA treatments decreased concentrations of acetic acid (10.0 vs. -16.4 mM, $P < 0.01$) and butyric acid (3.10 vs. -3.99 mM, $P < 0.01$) compared with control and effects were similar for FAST and SLOW treatments ($P > 0.3$). Concentration of lactic acid was not affected by treatment ($P = 0.44$).

DISCUSSION

Compared with control, both infusion treatments reduced the size and frequency of meals resulting in a 45% reduction in DMI. It is unlikely that the decrease in DMI by PA infusion was from additional energy supplied by the infusions because total ME intake was reduced by PA treatments. The reduction in DMI and ME intake by PA infusion was expected and consistent with studies previously reviewed (Allen 2000; Allen *et al*., 2009). Suppression of DMI by PA was likely due to hypophagic signals from increased hepatic oxidation of fuels (Friedman, 1995; Langhans and Scharrer, 1992). The decreased hunger ratio for PA infusions indicates that PA treatments decreased hunger compared with control. Although the infusion treatments affected both the size and frequency of meals, the effects for FAST and SLOW were opposite and compensated for each other decreasing DMI equally.

We hypothesized that faster PA infusion within meals would decrease meal size and increase meal frequency. The FAST treatment was expected to increase anaplerosis and stimulate oxidation of acetyl CoA in the tricarboxylic acid (TCA) cycle sooner, resulting in smaller meal size compared with SLOW. Because meals were expected to be smaller, we also expected the intermeal interval to decrease for FAST compared with SLOW, increasing meal frequency. However, contrary to our hypothesis, FAST increased meal size and decreased meal frequency compared with SLOW.

The greater flux of propionate supplied by FAST might have saturated the pathway for propionate metabolism sooner, resulting in a lower first-pass extraction by the liver compared with SLOW. Propionate is rapidly (Reynolds *et al*., 2003) and very efficiently (Bell and Bauman, 1997) extracted from the blood by the liver. However, propionate metabolism in hepatocytes can be saturated (Ali and Jois, 2007) and propionate not taken up by the liver would enter the general

circulation. Because propionate metabolism by other organs in the ruminant is minimal (Brockman, 2005), propionate bypassing the liver would be expected to extend anaplerosis of TCA cycle and hepatic oxidation of acetyl CoA over time, increasing intermeal interval and decreasing meal frequency. Propionate is a very effective TCA anaplerotic precursor, even at low concentrations (Brunengraber and Roe, 2006). Accordingly, FAST increased intermeal interval 35% and decreased meal frequency 24% compared with SLOW.

Hepatic extraction of propionate is by activation to propionyl CoA, which is then trapped in the mitochondria (Ricks and Cook, 1981). Although propionyl-CoA synthetase activity is high in ruminant hepatocytes (Demigné *et al*., 1986; Ricks and Cook, 1981), plasma propionate concentration increased linearly from 0.19 to 0.34 mM when propionate was infused intraruminally from 0 to 1.3 moles/h for 18 h for cows in the PP period (Oba and Allen, 2003a) indicating limitations to activation to propionyl-CoA. However, bottlenecks can also occur at other reactions for entry into, or within the TCA cycle. For instance, the malate dehydrogenase reaction is inhibited when NADH is elevated, potentially causing an accumulation and release of malate into the blood and allowing for extended anaplerosis if metabolized by the liver later.

Both infusion treatments reduced meal size compared with control but the reduction in meal size for SLOW compared with FAST is likely because saturation of the propionate metabolism pathway was reduced for SLOW compared with FAST, allowing greater first-pass propionate extraction with less propionate bypass during the infusion. Greater anaplerosis potentially increased oxaloacetate available for the citrate synthase reaction, facilitating the entry and oxidation of acetyl CoA in the TCA cycle causing satiety sooner.

Meal size and frequency were not affected by rate of PA infusion in a similar experiment in our laboratory (Bradford and Allen, 2007a) likely because cows were later in lactation (51

DIM) and in a different physiological state. Propionate was more hypophagic for cows in the PP period than later in lactation (Oba and Allen, 2003b), likely because cows in the PP period are in a lipolytic state with greater availability of acetyl CoA for entry into the TCA cycle (Piantoni *et al*., 2015).

The increased plasma glucose concentration by PA treatments was expected because propionate is the major glucose precursor in ruminants (Lemosquet *et al*., 2009) and is consistent with previous experiments (Oba and Allen, 2003a; Stocks and Allen, 2012). Increased PA concentration in the rumen by PA treatments was expected while no effect of treatment on plasma propionate concentration was likely because of extraction by the liver and because blood samples were taken at the beginning and end of the infusion period, and not over time following infusions. Whereas plasma propionate concentration likely changed during infusions, possibly affecting plasma insulin, glucagon, and NEFA concentrations within a day, lack of treatment effects on changes in their plasma concentrations were likely due to the long time (22h) between sampling and the time between the last infusion and sampling at the end of the infusion period. We chose not to sample throughout the infusion period to prevent interference with the natural feeding behavior of the cows. The reductions in ruminal acetate and butyrate concentrations and plasma acetate concentration by the PA treatments were consistent with the reduction in DMI and substrate available for ruminal fermentation. Whereas PA infusion might be expected to decrease ruminal pH compared with control, the opposite occurred likely because PA reduced DMI. In addition, ruminal pH was only measured at the beginning and end of each infusion day and not during infusions.

The reduction in plasma BHBA concentration by the PA treatments is consistent with greater oxidation of acetyl CoA in the TCA cycle because plasma NEFA concentration was not

affected by treatment. In this study, FAST tended to decrease BHBA compared with SLOW, possibly because of extended anaplerosis and oxidation of acetyl CoA from bypassed propionate. Another possible explanation is that the PA treatments decreased ketogenesis because propionate decreases expression of HMG CoA synthetase (Bush and Milligan, 2011).

The lack of treatment effect for yields of milk and milk components is likely because of the short duration of this experiment. Although treatments increased the amount of propionate potentially available for gluconeogenesis, DMI was reduced and a longer period of evaluation would be needed for a change in milk yield or composition to be detected.

Rate and amount of PA production in the rumen is highly variable and easily manipulated by altering diet starch concentration and fermentability (Allen and Piantoni, 2014). However, diet starch concentration and fermentability affect both the rate of PA production and the amount of PA produced. Starch sources with greater rates of fermentation generally increase both rate and amount of PA produced. We conducted this experiment to try to determine the effects of rate of PA production independent of the amount produced per day.

Caution should be used for application of these results for diet formulation. Although rate of PA infusion did not affect DMI of cows in the PP period in this experiment, our cows were housed in tie-stalls with access to the ration throughout the day and did not compete with other cows for feed, allowing greater flexibility to compensate for reduced meal size. Altering the content and fermentability of starch in diets might have different effects for cows housed in groups compared with cows housed individually because competition among cows for feed might limit the opportunity to compensate for reduced meal size.

The amount of PA infused at each meal in this experiment might have affected our results. We chose to infuse 1.25 moles of PA at each meal for this experiment, which is nearly

half of the expected ruminal pool size of PA for a cow with 80 L of rumen liquid volume and a PA concentration of 30 mM. Production rates of PA in the rumen for cows consuming normal or low-roughage diets have been reported to range from 13-31 moles/d for cows consuming ~15 kg DM/d (Bauman *et al*., 1971) and 17-36 moles/d for cows consuming ~12 kg DM/d (Sutton *et al*., 2003). In previous experiments in our laboratory, cows in the PP period consumed ~20 kg DM/d with 10-12 meals per day. Therefore, the amount of PA produced during each meal is likely greater than the amount we infused per meal. The experiments that measured production rates of PA did so over much longer periods of time compared with the length of meals. What is not known is the variation in PA production within the timeframe of meals. Because the amount of PA infused at each meal had a large effect on DMI for both treatments, infusion of a lower dose of PA over 5 or 15 minutes might have different results and should be evaluated. In addition, evaluation of intermediates of propionate metabolism over time after the start of infusion will help identify bottlenecks to propionate metabolism and should be evaluated.

CONCLUSIONS

A greater rate of PA infusion into the rumen of cows in the PP period resulted in greater meal size and decreased intermeal interval, contrary to our hypothesis. A possible explanation is that a faster rate of propionate supply and absorption saturated the pathway for propionate metabolism in the liver, resulting in less stimulation of hepatic oxidation during meals, larger meals and longer time between meals. Diets for dairy cows vary greatly in the type and temporal pattern of fuels absorbed and while glucose precursors must be provided to support milk production, careful consideration must be given to the fermentability of the ingredients used when formulating rations to maximize energy intake. More research is needed to determine the mechanisms by which temporal absorption of fuels affect feeding behavior and energy intake of

cows, particularly during the PP period. Potential bottlenecks for propionate metabolism in the liver should be explored further.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support for this project by USDA National Research Initiative Competitive Grant no. 2014-67015-21708 to MSA, and from CAPES – Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil to GM. We also thank D. G. Main, R. Longuski, K. Kennedy, L.G. Duarte, R. Albornoz and R. West (all from Michigan State University), as well as the staff of the Michigan State University Dairy Cattle Teaching and Research Center for their assistance in this experiment, and Kemin Animal Nutrition and Health for supplying the propionic acid.

Table 2.2 Effects of intra-ruminal infusion (22 h/d) of propionic acid over 5 (FAST) or 15 (SLOW) minutes compared with control on feeding behavior and energy intake for cows in the immediate postpartum period.

	Infusion				*P*-value	
	Control	FAST	SLOW	SEM	Infusion vs. Control	FAST vs. SLOW
DMI, kg/d	18.6	10.4	10.0	0.62	<0.01	0.74
Meal frequency, meals/ d	12.0	8.5	11.2	0.56	0.01	0.01
Meal length, min/meal	25.0	23.0	20.8	1.42	0.16	0.35
Meal size, kg/meal	1.59	1.23	0.87	0.06	<0.01	0.01
Eating interval, min	88.9	138.8	102.9	8.05	0.01	0.01
Eating rate, kg/min	0.065	0.058	0.050	0.0005	<0.01	0.04
Hunger ratio	0.037	0.013	0.013	0.003	<0.01	0.90
Satiety ratio	0.045	0.019	0.025	0.009	0.07	0.61
Water intake, L/d	84.4	29.3	24.3	6	<0.01	0.66
Liquid intake, L/d	84.4	57.3	54.2	5.1	0.02	0.70
Drinking frequency, bouts/day	12.0	5.0	4.7	1.54	0.03	0.90
ME intake (Mcal/d)						
Diet[1]	41.7	23.2	24.1	2.09	<0.01	0.70
Infusion[2]	0	5.12	5.46	0.20	<0.01	0.35
Total[3]	41.7	28.3	29.6	1.96	<0.01	0.68

[1] ME intake from the diet was calculated according to NRC (2001). ME (Mcal/kg) = 1.01 × DE (Mcal/kg) − 0.45, based on actual digestibility of diets. ME intake (Mcal/d) = ME (Mcal/kg) x DMI
[2] ME intake from the infusion was based on energy density of 0.365 Mcal/mol ME (Lavedba, 1964) for propionic acid
[3] Total ME intake = ME intake diet + ME infusion

Table 2.3 Effects of intra-ruminal infusion (22 h) of propionic acid over 5 (FAST) or 15 (SLOW) minutes compared with control on plasma metabolites and hormones for cows in the immediate postpartum period.

	Infusion				*P*-value	
	Control	FAST	SLOW	SEM	Infusion vs. Control	FAST vs. SLOW
Glucose before, mg/dL	48.1	44.3	48.2	1.51	0.38	0.15
Glucose after, mg/dL	47.8	57.9	57.6	2.68	<0.01	0.88
Change in glucose	-0.29	13.6	9.42	2.77	0.03	0.35
Insulin before, µg/L	0.14	0.11	0.13	0.03	0.43	0.52
Insulin after, µg/L	0.13	0.14	0.19	0.03	0.33	0.28
Change in insulin	-0.01	0.03	0.06	0.02	0.23	0.55
Glucagon before, mg/dL	153	142	143	17.6	0.02	0.71
Glucagon after, mg/dL	147	129	125	16.3	0.04	0.58
Change in glucagon	-5.94	-12.8	-18.6	9.9	0.31	0.58
NEFA before, mEq/L	1053	997	1081	164	0.93	0.68
NEFA after, mEq/L	951	976	1300	107	0.29	0.54
Change in NEFA	-101.6	-20.9	219.4	176	0.23	0.21
BHBA before, mg/dL	13.4	20.3	15.7	2.05	0.11	0.15
BHBA after, mg/dL	15.8	6.20	5.07	0.72	<0.01	0.37
Change in BHBA	2.42	-14.1	-10.6	1.90	<0.01	0.08
Propionate before, mM	0.41	0.40	0.38	0.02	0.40	0.61
Propionate after, mM	0.36	0.34	0.35	0.01	0.45	0.65
Change in propionate[1]	-0.02	-0.05	-0.02	0.02	0.66	0.43
Acetate before, mM	1.28	1.28	1.21	0.18	0.90	0.80
Acetate after, mM	1.30	0.62	0.76	0.14	0.03	0.55
Change in acetate[1]	0.02	-0.67	-0.45	0.16	0.00	0.25

[1]Cow and block effect treated as fixed

Table 2.4 Effects of intra-ruminal infusion (22 h) of propionic acid over 5 (FAST) or 15 (SLOW) minutes compared with control on ruminal pH and organic acids for cows in the immediate postpartum period.

	Infusion				*P*-value	
	Control	FAST	SLOW	SEM	Infusion vs. Control	FAST vs. SLOW
Ruminal Fluid						
pH before infusions	6.35	6.35	6.38	0.10	0.87	0.79
pH after infusions	6.15	6.71	6.56	0.09	<0.01	0.09
Change in pH	-0.21	0.35	0.17	0.16	0.03	0.34
Propionic acid before, mM	22.2	19.5	21.7	2.73	0.07	0.04
Propionic acid after, mM	24.3	30.5	40.1	3.90	0.06	0.11
Change in propionic acid, mM	2.12	10.9	18.4	2.84	0.04	0.20
Lactic acid before, mM	1.72	0.03	0.1	0.91	0.22	0.96
Lactic acid after, mM	0.17	0.17	0.05	0.03	0.51	0.28
Change in lactic acid, mM	-0.9	-0.20	-0.5	0.05	0.31	0.53
Acetic acid before, mM	58.1	59.5	58.3	5.38	0.86	0.82
Acetic acid after , mM	68.1	41.1	44.0	1.70	<0.01	0.15
Change in acetic acid, mM	10.0	-18.4	-14.3	4.2	<0.01	0.40
Butyric acid before, mM	8.65	9.73	8.88	1.33	0.63	0.59
Butyric acid after, mM [1]	11.8	4.97	5.73	0.25	<0.01	0.13
Change in butyric acid, mM	3.10	-4.78	-3.20	1.26	<0.01	0.30

[1]Cow and block effect treated as fixed

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BIBLIOGRAPHY

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CHAPTER 3. EFFECTS OF RATE AND AMOUNT OF PROPIONIC ACID INFUSED INTO THE RUMEN ON FEEDING BEHAVIOR OF HOLSTEIN COWS IN THE POSTPARTUM PERIOD

ABSTRACT

Suppression of appetite of cows during the postpartum (PP) period is likely caused by a signal related to hepatic oxidation of fuels. We hypothesized that faster absorption and hepatic uptake of propionate will result in more rapid oxidation, stimulating satiety sooner and reducing meal size. However, excessive propionate delivery to the liver might reduce its efficiency of extraction, diminishing its effect on meal size and potentially extending hepatic oxidation following meals, decreasing meal frequency. The objective of this study was to determine the effects of two amounts of propionic acid (PA) infused intraruminally, at two rates, at the initiation of meals on the feeding behavior of Holstein cows in the PP period. Eight ruminally-cannulated, multiparous Holstein cows were used in a 4x4 Latin square design experiment. Cows were blocked by parturition date and randomly assigned to a treatment sequence within square. Treatments were infusion of 2.5 L of 0.5M (HI) or 0.2M (LO) PA at initiation of meals over 5 min (FST) or 15 min (SLW) over a 12 h period following feeding. FST did not affect DMI or metabolizable energy (ME) intake compared with SLW. FST tended to increase meal length compared with SLW (28.1 vs. 22.7; $P = 0.07$) but did not affect meal size ($P = 0.67$). FST tended to decrease total eating time (108 vs. 122 min/12 h; $P = 0.05$) but did not affect meal frequency ($P = 0.20$) compared with SLW. HI decreased DMI (7.4 vs. 11.5 kg/12 h, $P < 0.01$) and total ME intake (22.5 vs. 29.1 Mcal/12 h, $P = 0.01$) compared with LO by decreasing meal frequency (5.8 vs. 7.5 meals/12 h; $P = 0.03$). HI decreased eating time (103 vs. 127 min/12 h, $P < 0.01$) compared with LO but did not affect meal size ($P = 0.42$). No interactions of treatments were detected for any feeding behavior variables. The lack of effect of infusion rate on meal size along

with the reduction in DMI by HI compared with LO by decreasing meal frequency rather than meal size suggests that propionate flux to the liver might have exceeded the liver's capacity for first-pass extraction of propionate from the blood. An extended supply of propionate from peripheral circulation to the liver likely extended hepatic oxidation longer after meals for the higher propionate dose.

INTRODUCTION

Propionic acid (PA) plays an important role in controlling feed intake in ruminants fed cereal grains. Research has shown that that DMI depression prepartum is greater for cows consuming high-grain diets than for those consuming high-forage diets (Coppock *et al*. 1972). In the PP period, DMI depression was not only greater in cows fed more starch (Dann and Nelson, 2011) but also when cows were fed more fermentable starch sources at the same level of inclusion (Albornoz and Allen, 2018). Diet factors can affect intake by providing different fuels that affect hepatic oxidation.

Continuous intraruminal infusion of sodium propionate depressed total metabolizable energy intake (MEI) by cows in the postpartum (PP) period in a dose-dependent manner compared with isomolar solutions of sodium acetate (Oba and Allen, 2003). Continuous abomasal infusion of PA (0.41 mol/h) also decreased total MEI 13% compared with no infusion by decreasing meal frequency (Gualdrón-Duarte and Allen, 2018). When infused at spontaneous meals, PA decreased feed intake by decreasing both meal size and meal frequency for cows in the PP period (Maldini and Allen, 2018) and in mid-lactation (Choi and Allen, 1999). Langhans and Scharrer (1992) and Friedman (1995) demonstrated that hepatic oxidation of fuels signal satiety in rats and Koch *et al*. (1998) showed a temporal relationship between feed intake and hepatic energy status in rats, supporting the idea that oxidative metabolism in the liver is

involved in feed intake regulation. Allen (2000) presented evidence that same mechanism likely applies to ruminants.

Propionate is a major glucose precursor in ruminants and is an obligate anaplerotic metabolite, stimulating hepatic oxidation of acetyl CoA (Gualdron-Duarte and Allen, 2017) which is a likely mechanism for its hypophagic effects (Allen, 2014). We previously infused 1.25 mol of PA over 5 min (FST) and 15 min (SLW) at spontaneous meals to determine the temporal effects of intra-ruminal infusion of PA on feeding behavior of cows in the PP period (Maldini and Allen, 2018). We hypothesized that a greater flux of propionate to the liver during meals would stimulate hepatic oxidation and satiety sooner compared with a lower flux. Contrary to our hypothesis, FST increased meal size and increased the time between meals decreasing meal frequency compared with SLW.

Given our results, we speculated that faster supply of propionate might have saturated the pathway for propionate metabolism resulting in a lower first-pass extraction by the liver compared with SLW. Furthermore, that propionate that bypassed the liver likely extended anaplerosis of the TCA cycle and hepatic oxidation of acetyl-CoA over time, increasing intermeal interval and decreasing meal frequency.

Because both PA treatments resulted in a substantial and similar reduction of DMI, we reasoned that infusion of a lower dose of PA over FST or SLW might have different results. The objective of this experiment was to determine the effects of the temporal supply of PA on feeding behavior of cows in the PP period by evaluating the effects of the amount of PA infused and the length of infusion at meals. We hypothesized that amount and length of infusions would interact to affect feeding behavior; infusion of a higher dose of PA (1.25 mol) more quickly would result in greater propionate bypass, larger meal size and greater time between meals

compared with a slower rate; whereas infusion of a lower dose of PA (0.5 mol) would not saturate the propionate metabolism pathway and FST would reduce meal size and the time between meals compared with SLW.

MATERIALS AND METHODS

Animals, Housing, and Diets

To test our hypothesis, we used eight multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center. The Institutional Animal Care and Use Committee at Michigan State University approved all experimental procedures in this study. Cows were ruminally cannulated at least 40 d prepartum. Cows were fed a common experimental diet from the day of parturition until the end of the experiment. The experimental diet (Table 3.1) consisted of corn silage, ground corn, alfalfa silage, soybean meal, alfalfa hay, soy hulls and a vitamin and mineral mix. Diet was fed once a day (1200 h) at 110% of expected intake, and it was formulated to meet requirements according to NRC (2001). Each cow was housed in an individual tie-stall for the duration of the experiment. Cows were milked twice daily at 0530 and 1730 h, in their stalls. Cows were not allowed access to feed between 1000 h and 1200 h each day while orts and feed offered were weighed and sampled.

Experimental Design and Treatments

Four cows with the closest range in days PP were selected for each block of this experiment and adapted to the feeding behavior stalls for at least 2 days before the first infusion day. We used a duplicated 4x4 Latin square design experiment balanced for carryover effects. Cows within each block were randomly assigned to a treatment sequence within square. Cows

averaged 6.3 ± 2.0 DIM and 3.0 ± 0.46 BCS for the first block and 10.3 ± 5.7 DIM and 2.75 ±0.50 BCS for the second block, on the first day of infusion.

Table 3.1. Ingredients and nutrient composition of experimental diet (% of dietary DM except for DM).

Item	
Ingredient	
Corn silage	30.3
Ground corn	18.3
Alfalfa silage	14.9
Soybean meal	13.7
Alfalfa hay	11.9
Soy hulls	7.02
Vitamin and mineral mix[1]	3.93
Nutrient composition	
DM	56.1
OM	91.7
Starch	23.2
NDF	33.4
Forage NDF	25.6
CP	16.1

[1] Vitamin and mineral mix contained 12.8% Ca, 0.1% P, 11.4% NaCl,10.9% Na, 0.1% Mg, 14 ppm Co, 250.0 ppm Cu, 9.9 ppm I, 745.0 ppm Fe, 994.0 ppm Mn, 7.5 ppm Se, 1,093.0 ppm Zn, 148.7 KUI/kg vitamin A, 24.8 KIU/kg vitamin D_3, 737 IU/kg vitamin

Treatments were 2.5 L solutions at high (0.5 M, HI) or low (0.2 M, LO) concentration of PA (99.5%, 0.5 M, Kemin Industries, Inc.) infused into the rumen over 5 min (FST) or 15 min (SLW) at the conditioned meal (first meal after fresh feed was offered) and at each spontaneous meal for 12 h. The PA doses were chosen based on previous experiments, and infusion rates were selected in an attempt to maximize treatment differences, while constraining the slow treatment to the minimum expected length of a typical spontaneous meal. Solutions were infused into the rumen using peristaltic pumps (FPU401, Omegaflex® Peristaltic Pump, Norwalk, CT) and Tygon® tubing that passed through a hole in the cannula plug and was kept in place by

stainless steel hose clamp fixed in each side of the cannula plug. Infusion periods began at 1200 h and ended at 1000 h the following day. Pumps were programmed to infuse a total of 2.5 L of PA at each meal, independent of the concentration of PA or rate of infusion. The rate of PA infusion into the rumen was calculated to be 0.25 mol/min for FST and 0.084 mol/min for SLW for the HI treatment (1.25 mol per infusion) and 0.1 mol/min for FST and 0.034 mol/min for SLW for the LO treatment (0.5 mol per infusion). We included a rest day between infusion days to reduce carry-over effects of treatment. Each cow received one treatment combination (HI-FST, HI-SLW, LO-FST or LO-SLW) on each infusion day, and all the cows received all treatments by the end of the experiment. Each block was completed in 10 days: 2 days for adaptation, 4 infusion days and 4 rest days. All cows within a block were infused on the same days, and all cows completed the experiment by 20 d PP.

Data and Sample Collection

Diet ingredients were sampled daily and composited across days of the experiment within each block. Orts for each cow were taken at the end of each infusion period and stored in plastic bags at -20°C until processed. Fecal samples were collected every 8 h in a 24-h period (1400, 2200, and 0600 h) on rest days and frozen at −20° C for later analysis to determine digestibility and energy concentration of the diet using methods described by Harvatine and Allen (2006).

Feeding behavior (feed disappearance and water intake) was recorded continuously for 12 h daily for each cow during infusion days. A computerized data acquisition system recorded the manger weight and water dispensed every 5 s and triggered infusion at the initiation of each new meal according to according to Choi and Allen (1999) and Bradford and Allen (2007). Briefly, the system consisted of monitoring feed mangers that were attached to a load cell and a computer. Variations in manger weight were computed every 5 seconds and when the running

standard deviation exceeded the threshold of 0.9 kg an "eating flag" was triggered in the system. If at last five eating flags were triggered within 100 seconds, the peristaltic pumps would turn on and infusion would initiate. Infusions lasted for 5 or 15 minutes according to treatment. To prevent potential treatment bias consecutive infusions began at least 15 min apart under both treatment protocols.

Data and Sample Analysis

Diet ingredients, orts, and fecal samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. Ingredient samples were ground with a Wiley mill (1-mm screen; Authur H. Thomas, Philadelphia, PA) and analyzed for DM, ash, NDF, CP, and starch content as previously described Maldini and Allen (2018).

Feeding behavior data were analyzed to quantify the number of meals, meal size, meal length, intermeal interval, eating time, eating rate, hunger ratio, and satiety ratio. We calculated hunger ratio as the weight of meal divided by the pre-meal interval, and satiety ratio as the weight of meal divided by post-meal interval (Forbes, 2007). Drinking behavior data was analyzed to quantify drink frequency and total water intake. Total liquid intake was calculated by adding the total water intake to the amount of PA infused into the rumen. Moles infused were calculated based on the amount of liquid infused and the concentration of the solution of PA infused in each treatment.

Triggering infusions based on real-time monitoring of feeding behavior resulted in an average of 9.6 infusions per cow/12 h for LO and 7.9 infusions per cow/12 h for HI with 2.3 false-positive (infusions without meals) and 0.06 false-negatives (meals without infusions) per cow/day with no treatment differences. We chose to evaluate the effects of PA infusion during the first 12 hours after feeding because previous research in our laboratory demonstrated that PA

infusions decreased DMI in PP cows only over the first 4 h after feeding and not the remaining 20 h (Stocks and Allen, 2013). Therefore, by evaluating feeding behavior for 12h we expected to fully capture treatments effects.

All samples were analyzed for gross energy (GE) by bomb calorimetry according to the manufacturer's instructions (Parr Instrument Inc., Moline, IL). Digestible energy (DE) intake was calculated as GE intake minus GE output in feces. Metabolizable energy (ME) from the diet was calculated according to NRC (2001) as ME (Mcal/kg) = 1.01 x DE (Mcal/kg) − 0.45, based on measured digestible energy (DE). ME of the infusion was calculated according to the amount infused assuming 365.03 kcal/mol of propionate (Lebedeva, 1964). MEI was calculated as MEI (Mcal/d) = ME x DMI and total MEI = MEI diet + ME infusion.

Statistical Analysis

Statistical analyses were conducted using JMP Pro 14 software (SAS Institute Inc., Cary, NC), using the mixed model:

$$Y_{ijklm} = \mu + C_i(B_j) + P_k(B_j) + B_j + T_l + L_m + T_l L_m + e_{ijklm},$$

where Y_{ijklm} = the response variable; μ = overall mean; $C_i(B_j)$ = random effect of cow *i* nested within block *j*; $P_k(B_j)$ = fixed effect of period *k* nested within block *j*; B_j = fixed effect of block *j*; T_l = fixed effect of treatment concentration *l* (HI, LO); L_m = fixed effect length of infusion *m* (FST, SLW); $T_l L_m$ = interaction of treatment concentration and length of infusion and e_{ijklm} = residual. Treatment effects were declared significant at $P < 0.05$ and tendencies for treatment effects were declared at $P < 0.10$.

RESULTS

Contrary to our hypothesis no interaction of treatment was detected for any of the variables measured (Table 3.3). FST did not affect DMI or ME intake compared with SLW. FST tended to increase meal length compared with SLW (28.1 vs. 22.7; $P = 0.07$) but did not affect meal size (1.49 kg/meal, $P = 0.67$). FST tended to decrease total eating time (109 vs. 122 min/12 h; $P = 0.05$) but did not affect meal frequency ($P = 0.20$) compared with SLW. HI decreased DMI by 36% (7.4 vs. 11.5 kg/12 h, P <0.01) and total ME intake by 23% (22.5 vs. 29.1 Mcal/12 h, $P = 0.01$) compared with LO by decreasing eating time (103 vs. 127 min/12 h, P <0.01), increasing time between meals (70.4 vs. 104.9, P <0.01) and decreasing meal frequency (5.8 vs. 7.5 meals/12 h; $P = 0.03$). HI reduced eating rate (0.053 vs. 0.069 kg/min; P < 0.01) and decreased hunger ratio (0.015 vs. 0.027, P < 0.01) compared with LO but did not affect meal size ($P = 0.42$). The ME infused was greater for HI compared with LO (2.92 vs. 1.41 Mcal/d, P<0.01) despite the greater number of infusions for LO over 12 h. The additional energy provided by HI failed to compensate for the reduction in ME intake of the diet. Satiety ratio was not affected by treatment.

Drinking behavior of cows is reported in Table 3.3. HI tended to decrease water intake (19.5 vs. 30.5 L, P=0.07) and decreased liquid infused (15.4 vs. 19.7 L, P<0.01), decreasing total liquid intake 25.5% (39.3 vs. 52.8 L, P=0.03) compared with LO. Although no significant interaction of treatment was detected, SLW tended to have more liquid infused into the rumen than FST (18.2 vs. 16.6 L, P<0.10) because the number of meals was numerically greater for SLW in the LO treatment.

DISCUSSION

The results for HI treatments in this experiment differed from our previous experiment in which FST increased meal size and increased eating interval compared with SLW (Maldini and Allen, 2018) despite using the same dose of PA and length of infusions. Cows in the present experiment were earlier in lactation than the previous experiment (average 8.25 ± 4.49 vs. 13.8 ± 2.9 DMI) and might have been more sensitive to the PA treatments. The similar effects on meal size (1.42 kg) and eating interval (104 and 106 min) for FST and SLW respectively, within the HI treatment, is consistent with both treatments exceeding the capacity of the liver to extract propionate and extending the supply of PA after the infusion for both infusion rates.

The liver is very efficient in extracting PA from the blood because of the high activity of propionyl-CoA synthetase in ruminant hepatocytes (Ricks and Cook, 1981; Demigné *et al*., 1986). However, hepatic uptake of propionate is saturable, and a high flux of PA could limit the entry of propionate into the TCA cycle. Plasma propionate increased linearly with dose when propionate was infused intraruminally from 0 to 1.3 mol/h for 18 h for cows in the PP period (Oba and Allen, 2003). Mesenteric (Baird *et al*., 1980) and ruminal (Berthelot *et al*., 2002) infusion of propionate decreased the efficiency of propionate extraction by the liver in dairy cows and sheep, respectively, demonstrating a limitation in the hepatic capacity to extract PA from the blood.

The amount infused for LO was 40% of that infused in the previous experiment in an attempt to avoid bottlenecks for hepatic metabolism of propionate that might limit propionate extraction from the blood. We did not detect a significant treatment effect of rate of infusion, however, it is possible that the lower dose of PA for FST in this experiment was also able to saturate the propionate metabolism pathway, reducing the first-pass uptake by the liver and

extending anapleurosis of TCA cycle. The rate of infusion of PA for LO-FST was of 0.1 mol/min. The rate of infusion for HI treatments were 0.25 mol/min for FST and 0.084 mol/min for SLW. If in HI both rates overwhelmed the capacity of the liver to extract propionate from the blood during meals, resulting in similar effects on meal size and eating interval, then the intermediate infusion rate for LO-FST of 0.1 mol/min likely also exceeded the capacity of the liver to extract propionate.

Water intake is positively correlated with both DMI and the number of meals per day (Dado and Allen, 1994). Although LO had more meals and hence more infusions/12h, HI reduced DMI by 36% compared with LO, therefore, the reduction in water intake observed was expected.

Specific bottlenecks for propionate metabolism in the liver have not been identified for cows in the PP period. Possible bottlenecks include the propionyl CoA synthetase reaction, that can be inhibited by limited enzyme activity or a limited availability of CoA; and the methylmalonyl-CoA mutase reaction, that catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA, that can be inhibited by limited enzyme or cofactor (vitamin B_{12}) availability. Daugherty *et al*. (1986) showed a high correlation between liver B_{12} and endogenous propionate metabolizing activity in lambs, indicating that a limited availability of B_{12} could contribute to a bottleneck to propionate metabolism and hepatic uptake. In addition, elevated NADH/NAD ratio can inhibit the malate dehydrogenase reaction, which leads to an accumulation of malate and upstream metabolites, likely inhibiting propionate uptake or increasing the release of malate into the blood.

Different responses in feeding behavior are expected depending upon the amount and temporal supply of propionate to the liver, the capacity of the liver to metabolize propionate and

its metabolites, and the availability of acetyl CoA. For instance, anaplerosis from propionate stimulates acetyl-CoA oxidation, decreasing meal length and size. However, bottlenecks for propionate uptake and metabolism can attenuate the effects on meal length and size and increased intervals between meals would be expected. As previously mentioned, using similar protocols as this experiment and infusing 1.25 moles PA at each meal over 5 or 15 min, Maldini and Allen, (2018) observed that treatments decreased DMI and MEI similarly at 45% and 31% compared with control (no infusion). However, FST increased meal size by 29% and decreased meal frequency by 24% compared with SLW consistent with a greater bottleneck for propionate metabolism for FST compared with SLW. Bradford and Allen (2007) determined the effects of rate of PA infusion on feeding behavior of cows around peak lactation (51±19 d PP) and reported that PA treatments decreased DMI ~22% but had similar effects on meal size and frequency as observed in our experiment. The authors suggested that treatments might not have been adequately different to alter the rate of propionate metabolism. However, the current results suggest that it is also possible that both treatments exceeded the capacity of the liver for propionate metabolism resulting in propionate bypass.

Propionate is also a potent insulin secretagogue in ruminants (Leuvenink *et al*., 1997). Insulin is one of the principal pancreatic hormones controlling fat and carbohydrate metabolism in non-ruminants, and although effects of insulin on feed intake in ruminants are inconsistent, it is possible that temporal supply of propionate may affect hepatic metabolism mediated by an insulin action. Increased plasma insulin concentration during meals might contribute to the removal of fuels from the blood more quickly (Oba and Allen, 2000) consequently reducing hepatic acetyl CoA content available for oxidation in the liver (Allen, 2014). A greater reduction

of hepatic acetyl CoA concentration following feeding was related to increased feed intake (Piantoni *et al*., 2015), consistent with HOT.

The control of feed intake in dairy cows is complex. The initiation and termination of a meal is more likely to be controlled by a combination of signals rather than a single signal. However, it is clear that in ruminants, propionic acid plays a major role as the hypophagic fuel from the diet affecting feed intake. The current and previous results from our laboratory (Bradford and Allen, 2007; Maldini and Allen, 2018) are the first experiments to evaluate the effects of temporal supply of PA within a meal on feeding behavior on dairy cows. Although we continue to develop our understanding in this area, to fully comprehend what is driving the hunger and satiety within a meal, further research is needed. Evaluation of hepatic metabolism, and of the changes in plasma metabolites and hormones are essential to support the hypothesis presented in this chapter.

CONCLUSION

Our findings do not support the hypothesis that the amount of PA infused within a meal and the length of infusions interact to affect feeding behavior of dairy cows. However, the possibility of a bottleneck to hepatic metabolism of propionate still remains. The similar changes in feeding behavior for FST and SLW treatments observed within the HI treatment and the tendency of SLW to decrease meal length compared to FST despite the amount infused, suggests that treatments might have exceeded the capacity of the liver to extract propionate from the blood during meals. Because we evaluated feeding behavior, we could not sample blood or biopsy the liver over time during these experiments. Future identification of bottlenecks for propionate metabolism will improve our understanding of the complex mechanisms controlling

feed intake in dairy cows and might result in methods to overcome the challenges of feeding cows in the transition period.

ACKNOWLEDGEMENTS

We acknowledge funding for this project by USDA National Research Initiative Competitive Grant no. 2014-67015-21708 to M.S.A., and from CAPES–Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil to G.M. while conducting this research. We thank D. G. Main, R. Longuski, K. Kennedy, L. G. Duarte and R. Albornoz, for their assistance, and Kemin Animal Nutrition and Health (Des Moines, IA) for supplying the propionic acid.

Table 3.2. Effect of intra-ruminal infusion at the conditioned and spontaneous meals for 12 h after feeding of 2.5 L solutions of propionic acid (PA) at 0.5 M (HI) or 0.2M (LO) over 5 (FST) or 15 (SLW) minutes on feeding behavior and dry matter and energy intake for cows in the immediate postpartum period

	Infusion							
	0.2 M (LO)		0.5M (HI)			*P*-value		
	FST	SLW	FST	SLW	SEM	SLW vs. FST	HI vs. LO	Interaction
DMI, kg/d	10.8	12.3	6.48	8.26	1.15	0.13	<0.01	0.88
Meal frequency, meals/12h	6.75	8.25	5.57	6.00	0.64	0.20	<0.01	0.47
Meal length, min/meal	27.7	21.0	28.5	24.3	2.43	0.07	0.46	0.66
Meal size, kg/meal	1.66	1.49	1.42	1.42	0.17	0.67	0.42	0.67
Eating interval, min	73.6	67.2	104	106	10.0	0.85	<0.01	0.71
Eating Time, (min/12h)	124	130	92.9	114	6.91	0.05	<0.01	0.26
Eating rate	0.065	0.072	0.052	0.055	0.004	0.30	<0.01	0.74
Hunger ratio[1]	0.026	0.029	0.017	0.012	0.21	0.51	<0.01	0.24
Satiety ratio[1]	0.037	0.047	0.030	0.030	0.21	0.58	0.15	0.61
ME intake (Mcal/12h)								
Diet[2]	26.8	28.5	18.7	20.5	3.12	0.46	<0.01	0.99
Infusion[3]	1.29	1.53	2.94	2.90	0.13	0.42	<0.01	0.24
Total[4]	28.1	30.1	21.7	23.4	3.11	0.44	0.01	0.97

[1]Data analyzed after log transformation. SEM reported in the analyzed scale.
[2] ME intake from the diet was calculated according to NRC (2001). ME (Mcal/kg) = 1.01 × DE − 0.45, based on actual digestibility of diets. ME intake (Mcal/d) = ME x DMI
[3] ME intake from the infusion was based on energy density of 0.365 Mcal/mol ME (Lavedba, 1964) for PA
[4] Total ME intake = ME intake diet + ME infusion

Table 3.3. Effect of intra-ruminal infusion at the conditioned and spontaneous meals for 12 h after feeding of propionic acid (PA) at 0.5 M (HI) or 0.2M (LO) over 5 (FST) or 15 (SLW) minutes on drinking behavior of cows in the immediate postpartum period

	Infusion							
	0.2 M (LO)		0.5M (HI)			*P*-value		
	FST	SLW	FST	SLW	SEM	SLW vs. FST	HI vs. LO	Interaction
Water intake, L/12h[1]	31.1	29.9	14.2	24.7	0.29	0.31	0.07	0.24
Infusion, L/12h	17.9	21.4	15.3	15.5	1.95	<0.10	<0.01	0.14
Total liquid intake, L/12 h[1]	51.3	54.4	36.0	42.6	0.13	0.39	0.03	0.67
Drinking frequency, bouts/12h	5.13	7.00	4.29	4.25	0.95	0.35	0.08	0.33
Infusions, n/12h	8.75	10.38	7.84	7.88	0.60	0.15	<0.01	0.17
Moles infused/12h	3.53	4.18	8.07	7.94	0.34	0.42	<0.01	0.24

[1]Data analyzed after log transformation. SEM reported in the analyzed scale.

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CHAPTER 4. METABOLIC RESPONSE TO RATE OF RUMINAL INFUSION OF PROPIONIC ACID BEFORE AND AFTER FEEDING IN COWS IN THE POSTPARTUM PERIOD

ABSTRACT

A faster rate of infusion of propionic acid into the rumen of cows in the postpartum period increased meal size compared to a slower rate of infusion in a previous experiment. Because propionate is anaplerotic and stimulates oxidation of acetyl CoA in the liver, and hepatic oxidation has been linked to satiety, this result was opposite to the expected response. We hypothesized that the faster rate of infusion might have saturated the pathway for propionate metabolism in hepatocytes resulting in lower first-pass extraction by the liver, but because we were measuring feeding behavior we could not sample blood and liver tissue over time in that experiment. Therefore, to determine the temporal effects of propionic acid (PA) infusion on hepatic metabolism and plasma metabolites over the time course of a meal, we infused 1.25 mol of PA (2.5 L of 0.5M PA) over 5 min (FST) or 15 min (SLW) into the rumen. We evaluated response to PA infusions both before feeding, when ruminal PA production by rumen microbes is lower and hepatic acetyl-CoA concentration is greater; and 4 h after feeding, when PA production is greater and hepatic acetyl-CoA concentration is lower. Blood and liver samples were collected before, and after 5, 15 and 30 minutes of infusion. Contrary to our hypothesis, the rate of PA infusion into the rumen did not affect plasma propionate concentration indicating the FST effects on feeding behavior were not because of bottlenecks for propionate uptake by the liver. However, FST increased plasma glucose and insulin concentrations faster than SLW resulting in a reduction in plasma NEFA concentration during the timeframe of meals. Decreased plasma NEFA concentration during infusion likely decreased the supply of acetyl CoA for oxidation in the liver. The faster increase in plasma glucose concentration was likely

from greater initial flux of propionate to malate and shuttling of malate from the mitochondria to the cytosol, which might have been enhanced by a bottleneck at the malate dehydrogenase reaction. FST treatment also increased fumarate concentration at 5 min after the initiation of infusion but did not affect oxaloacetate concentration compared with SLW, consistent with a bottleneck. A bottleneck at malate for FST compared with SLW would further contribute to a reduction in hepatic oxidation within the timeframe of a meal, allowing greater meal size consistent with the hepatic oxidation theory and our previous results.

INTRODUCTION

Propionic acid (PA) produced from ruminal fermentation of starch and other organic matter is the primary glucose precursor for ruminants (Lemosquet *et al*., 2009). For this reason, diets for lactating dairy cows are frequently formulated to increase starch concentration in an effort to increase the supply of glucose precursors to the cow. However, feeding diets that contain highly fermentable starch can decrease dry matter intake (Allen, 2000; Albornoz and Allen, 2018; Sadri *et al*., 2009). Of the fuels produced during ruminal fermentation, PA is the fuel most likely to stimulate satiety and reduce feed intake in dairy cows (Allen, 2000).

Propionate is an obligatory anaplerotic metabolite for the tricarboxylic acid cycle (TCA), and its hypophagic effects are likely from its capacity to stimulate oxidation of hepatic acetyl CoA within the time frame of a meal (Allen *et al*., 2009). According to the hepatic oxidation theory (HOT), feeding behavior is controlled by signals transmitted to the brain feeding centers through hepatic vagal afferents. Increased oxidation of fuels decreases the firing rate of the nerve, which is associated with satiety, whereas decreased oxidation of fuels increases the firing rate, which is associated with hunger. The mechanism by which hepatic oxidation affects the firing rate of the hepatic vagal afferents is yet to be determined, but research has consistently

demonstrated the hypophagic effects of PA, that the signal is carried to brain feeding centers by hepatic vagal afferents (Allen *et al*., 2009), and that hypophagic effects of PA are related to its ability to stimulate hepatic oxidation (Gualdron-Duarte and Allen, 2017).

In a previous experiment (Maldini and Allen, 2018) we evaluated ruminal infusions of 1.25 mol of PA over 5 min (FST) and 15 min (SLW) at each meal. We expected that FST would decrease meal size compared with SLW by stimulating hepatic oxidation and resulting in satiety sooner. Contrary to our hypothesis, we observed that FST increased meal size and decreased meal frequency when compared with SLW. We then hypothesized that the rapid supply of PA in the FST treatment saturated the pathway for propionate metabolism in the liver resulting in a by-pass of propionate. The extended interval between meals for FST suggested that sustained propionate flux to the liver from the increased meal size and from propionate that escaped first-pass extraction, extended hepatic oxidation over time, delaying hunger. Because we were evaluating feeding behavior, we were unable to sample blood or biopsy the liver over time during that experiment.

The objective of this experiment was to evaluate metabolic responses, including concentrations of plasma metabolites and hormones, and liver metabolites, to infusions of PA into the rumen of cows in the postpartum (PP) period using the same protocol and treatments of our previous experiment, before feeding and after feeding. We chose to evaluate treatment effects before and after feeding because propionate flux from the rumen to the liver is expected to be lower before feeding and higher after feeding. In addition, concentrations of NEFA in plasma and acetyl CoA in the liver are higher before feeding and lower after feeding (Piantoni *et al*., 2015). We hypothesized that FST would increase plasma propionate concentration following the start of infusion compared with SLW, characterizing the limitation of hepatic uptake of

propionate and subsequent bypass. Elevated concentrations of intermediates of propionate metabolism for FST compared with SLW would indicate bottlenecks to propionate metabolism. Because SLW was expected to result in greater anaplerosis of the TCA over the timeframe of meals because the propionate metabolism pathway would be less saturated, we hypothesized that SLW would stimulate acetyl-CoA oxidation more quickly than FST, explaining the results in feeding behavior previously observed.

MATERIALS AND METHODS

Animals, Housing and Diets

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Cows were housed individually and each cow was housed in the same tie-stall for the duration of the experiment. Cows were fed once daily (1200 h) at 110% of expected intake and milked twice daily at 0530 and 1730 h. All cows received a common experimental diet from parturition through the end of the experiment. The experimental diet (Table 4.1) was formulated to meet or exceed cow's estimated requirements according to NRC (2001) and fed as a TMR. Before the initiation of the experiment, all animals were clipped over the 11, 12 and 13 intercostal areas from the backbone ventrally for approximately 30 cm to prepare for the liver biopsies.

Experimental Design and Treatments

Twelve multiparous Holstein cows (n =12, 6 ± 1.9 d PP) in the PP period from the Michigan State University Dairy Teaching and Research Center were used in a crossover design experiment with double repeated measures. Cows (n=2) with the closest range in days PP were selected for each block of this experiment. There was a total of 6 blocks, and each block was

conducted over a 2-d period with 2 infusions per day. Cows within a block were randomly assigned to treatment sequence.

Table 4.1 Ingredients and nutrient composition of the experimental diet (% of dietary DM except for DM).

Item	**%**
Ingredient	
Corn silage	31.9
Soybean meal	19.2
Ground corn	19.1
Alfalfa silage	12.4
Alfalfa hay	9.7
Soy hulls	4.0
Vitamin and mineral mix[1]	3.8
Nutrient composition	
DM	58.7
OM	91.3
Starch	24.9
NDF	29.5
Forage NDF	22.1
CP	17.2

[1]Vitamin and mineral mix contained 25.6% NaCl, 10.0% Ca, 2.0 Mg, 2.0% P, 30 ppm Co, 506 ppm Cu, 20 ppm I, 2220 ppm Fe, 2,080 ppm Mn, 15 ppm Se, 2,030 ppm Zn, 300 KUI/kg vitamin A, 50 KIU/kg vitamin D, 1500 KIU/kg vitamin E.

Treatments were infusion of 2.5 L of 0.5M PA (1.25 mol) into the rumen over 5 min (0.25 mol/min, FST) or over 15 min (0.08 mol/min, SLW). Cows were infused twice per day, at 1 h before feeding (BF) and 4 h after feeding (AF) through a nasogastric tube. The amount and length of time for infusions were selected based on our previous studies in which PA decreased DMI of cows in the PP period. Each cow within a block, received the same treatment BF and AF on the same day (e.g. FST-BF, FST-AF on day 1, followed by SLW-BF, SLW-AF on day 2 or the reverse). Feed access was blocked 4 hours before feeding when cows were cleaned in

preparation for the biopsies and the nasogastric tubes were placed. After the last sampling BF, cows were allowed access to feed for 4h. Before the initiation of the AF infusion, access to feed was prevented until the last collection at 30 minutes.

Before each infusion, cows were restrained in a chute to pass the nasogastric tube into the rumen. A clear long flexible veterinary stomach tube (12.7 mm x 274.3 cm) was placed in warm water for 5 min previous to insertion to increase flexibility. We verified that the tubing was in the rumen by blowing air in the tube and listening for burbling noises; and that rumen gas was escaping the rumen through the tube. Once placed, the tube was attached to the halter and cows returned to their stalls. In the stalls, the nasogastric tubing was connected to a peristaltic pump (FPU401, Omegaflex® Peristaltic Pump, Norwalk, CT). Tubes were removed immediately after infusions were finished.

Blood and liver were sampled prior to infusion and at 5, 15 and 30 minutes following the initiation of infusion.

Sample Collection and Analysis

Samples of all dietary ingredients were collected daily and composited to characterize the experimental diet. Feed ingredients were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. Samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for ash, NDF, crude protein and starch as described by Maldini and Allen (2018).

Blood was collected prior to infusion and at 5, 15 and 30 minutes following the initiation of infusion. Blood was sampled from coccygeal vessels and collected into 4 evacuated tubes, 3 containing K_2EDTA and 1 containing potassium oxalate with NaF as a glycolytic inhibitor and kept in ice until the last sample was harvested. Blood was then immediately centrifuged at 3,000

x g for 15 min at 5°C. Plasma was harvested, and 200 μL of benzamidine (0.25 M) was added to 1000 μL of plasma as a protease inhibitor for glucagon analysis. All samples were immediately frozen and kept at -20^0 C until analyzed.

Commercial kits were used to analyze for plasma concentrations of NEFA (NEFA HR kit, Wako Chemicals USA), BHBA (Stanbio Laboratory, Boerne, TX) and glucose (Glucose oxidase -Sigma Chemical. Co.); and samples were analyzed colorimetrically with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp., Sunnyvale, CA) to determine concentrations of metabolites. Plasma glucagon concentration was determined using RIA kit (kit no. GL-32K, Linco Research Inc., St. Charles, MO). Insulin was determined using an ELISA kit (Mercodia, Uppsala, Sweden). Plasma propionate, acetate, butyrate and organic acid concentrations were analyzed by HPLC (Waters Corp., Milford, MA) as described by Gualdrón-Duarte and Allen (2017).

Liver samples were obtained by biopsy. The liver tissue was biopsied as modified from Ferreira *et al*. (1996) and Bradford and Allen (2005). Briefly, the incision site was cleaned with 7.5% povidone-iodine followed by 70% ethanol and anesthetized locally with lidocaine 2% (20 cc). The effectiveness of the local anesthesia was tested before collection of tissue, and if the cow reacted to stimulus after 10 minutes of the first anesthesia, up to 10 cc of additional lidocaine were administered. At each sampling time, a 14-gauge biopsy needle was inserted into the intercostal space between the 11th and 12th ribs on a line between the olecranon and the tuber coxae on the right side. Eight samples of approximately 20 mg each were collected, snap-frozen in liquid nitrogen, and stored at -80°C until analyzed. Cows were monitored daily following biopsies; no health complications were observed during the experiment.

Liver metabolites related to hepatic metabolism, including succinate, fumarate, malate, oxaloacetate, citrate, α-ketoglutarate, glutamate, pyruvate, lactate, aspartate, 3-hydroxybutyrate, glucose and γ-aminobutyric acid (GABA) were analyzed using GC-MS. CoA, acetyl CoA, propionyl CoA, succinyl CoA and methylmalonyl CoA were analyzed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Approximately 40 mg of frozen tissue was weighed into clean, flat bottom 2 mL centrifuge tubes and stored on dry ice. Cold 1% formic acid in 70% ACN: 30% H_2O was added (500 µL) to each tube and immediately homogenized using a Polytron® homogenizer. After homogenization, samples were centrifuged for 10 min at 4°C. Aliquots of supernatant were transferred to clean centrifuge tubes for analysis and stored at -80°C. All samples for GC-MS and LC-MS/MS were evaporated to dryness using a Savant SC110 SpeedVac Concentrator (Savant Instruments, Inc., Holbrook, NY).

Gas Chromatography – Mass Spectrometry: Samples evaporated to dryness were derivatized by methoximation and trimethylsilylation as modified by Kind *et al*. (2009). Briefly,

30 µL of O-methylhydroxylamine reagent solution (40mg/1 mL of pyridine; Sigma Aldrich, St. Louis, MO, USA) was added to each sample and vortex for 30 sec for homogenization. Samples were then shaken for 3 h at 60°C in a Forma Orbital Shaker Model 420 (Thermo Fisher Scientific, Waltham, MA). An additional 90 µL of either MTBSTFA (for pyruvate, citrate, succinate, fumarate, malate, BHBA, lactate, phosphate, aspartate and glutamate) or BSTFA (for keto-glutarate, oxaloacetate, glucose and GABA) was added to each sample, vortexed for 10 sec, and then shaken for an additional 30 min at 37°C. Samples were then centrifuged for 10 min each at 22 °C. The supernatant was transferred into 250 µL glass insert within a 2 mL amber autosampler vial (Thermo Fisher Scientific, Waltham, MA, USA).

Vials were stored in a desiccator in the dark until use for GC-MS within 24 h of derivatization. Peaks were identified and quantified by comparison with standards.

Liquid Chromatography – tandem Mass Spectrometry: Samples were analyzed according to Gotoh *et al*. (2015), with modifications by Kennedy and Allen (2018). Peaks were identified and quantified by comparison with standards. Samples evaporated to dryness were re-suspended in 400 mM ammonium formate with 10 mM TCEP HCl (Sigma Aldrich, St. Louis, MO, USA). Samples were centrifuged at for 10 min at 4°C, transferred to a new clean vial a centrifuged a second time each for 10 min at 4°C. The supernatant was then transferred into 250 uL glass insert within a 2 mL amber autosampler vial (Thermo Fisher Scientific, Waltham, MA, USA) for analysis on LC-MS/MS.

Statistical Analysis

Prior to statistical analysis, Box-Cox transformation analyses were applied to each response variable to determine necessary transformations on the data using JMP Pro (version 13.2, 2016; SAS Institute Inc., Cary, NC). Data was then analyzed using the MIXED procedure of SAS (version 9.4, 2013; SAS Institute Inc., Cary, NC) with double repeated measures. Hepatic and plasma metabolites and hormones were analyzed using the following model:

$$Y_{ijklmn} = \mu + C_i(B_j) + P_k(B_j) + B_j + T_l + S_m + F_n + T_lS_m + T_lF_n + F_nS_m + T_lF_nS_m + e_{ijklmn},$$

where Y_{ijklmn} = the response variable; μ = overall mean; $C_i(B_j)$ = random effect of cow *i* nested within block *j*; $P_k(B_j)$ = random effect of period *k* nested within block *j*; B_j = random effect block, T_l = fixed effect of treatment *l* (rate of infusion: FST, SLW); S_m = fixed effect of sampling time (before infusion, 5, 15 and 30 minutes after infusion); F_n = fixed effect of time of infusion relative to feeding (BF, AF) ; T_lS_m = interaction of treatment (rate of infusion) and

sampling time; $T_l F_n$ = interaction of treatment (rate of infusion) and time of infusion relative to feeding; $F_n S_m$ = interaction of time of infusion relative to feeding and sampling time; $T_l F_n S_m$ = interaction of treatment (rate of infusion), time of infusion relative to feeding and sampling time; and e_{ijklmn} = residual. Time of infusion relative to feeding was the macro repeated measure and sampling time was the micro repeated measure. The covariance structure was unstructured @ compound Symmetry for all variables. Paired differences were determined using the either the *diff* or *slice* option in PROC MIXED.

Treatment effects were declared at $P \leq 0.05$ and tendencies for treatment effects were declared at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies for interactions were declared at $P \leq 0.15$. Data transformed are noted in the tables and figures along with the transformation used for statistical analysis. All transformed data has been back-transformed for interpretation purposes.

RESULTS

Blood Metabolites and Hormones

Rate of infusion and time of infusion relative to feeding interacted with time to affect plasma propionate concentration ($P = 0.06$; Table 4.2, Figure 4.1). Plasma propionate increased for SLW before feeding, peaking at 15 minutes of infusion ($P<0.05$) and tended to be higher at 30 minutes ($P=0.06$) compared with the pre-infusion sample. In contrast, plasma propionate did not increase for FST before feeding, and when compared with the pre-infusion sample FST-BF decreased plasma propionate by 30 minutes after the start of infusion ($P<0.05$). Plasma propionate concentration did not differ from the pre-infusion sample for either treatment when PA was infused after feeding.

Rate of infusion interacted with sampling time to affect plasma glucose concentration (P = 0.03; Table 4.2) with FST increasing glucose concentration until 15 min but SLW decreasing glucose concentration at 5 min with similar concentrations for both treatments by 30 min (Figure 4.2a). Plasma glucose concentration also tended to be affected by an interaction of rate of infusion and time of infusion relative to feeding (P = 0.15) with a greater concentration for FST before feeding than FST after feeding or SLW before or after feeding.

Rate of infusion and time of infusion relative to feeding tended to interact with sampling time to affect plasma glucagon concentration (P = 0.12, Figure 4.2b). There were no treatment differences for plasma glucagon concentration at any individual sampling time (P > 0.15) either BF or AF, however when compared with the pre-infusion sample, SLW increased glucagon concentration at 15 minutes BF, while there was no change in plasma glucagon for FST. After feeding, plasma glucagon concentration did not differ from the pre-infusion sample for either treatment.

The FST treatment increased plasma insulin concentration compared with SLW until 15 min after the start of infusion, but concentrations were similar by 30 min (P = 0.02; Figure 4.2c). Plasma insulin concentration was greater AF than BF (0.21 vs. 0.28 μg/L, P = 0.04). The FST treatment tended to decrease plasma NEFA concentration (P = 0.06) and the decrease was more rapid compared with SLW (P = 0.06; Figure 4.2d). Plasma NEFA concentration was lower AF compared with BF (544 vs. 849 mEq/L; P < 0.01). Compared with SLOW, FST treatment increased plasma lactate concentration BF (1.27 vs. 1.18 mM) but decreased it AF (1.20 vs. 1.44 mM; P = 0.07). Treatments tended to interact with time to affect plasma lactate concentration (P = 0.15; Figure 4.2e). There were no treatment differences from baseline until 15 minutes,

however at 30 minutes SLW tended to increase plasma lactate compared with FST (P=0.08). Plasma acetate and BHBA were not affected by treatment or time of infusion relative to feeding.

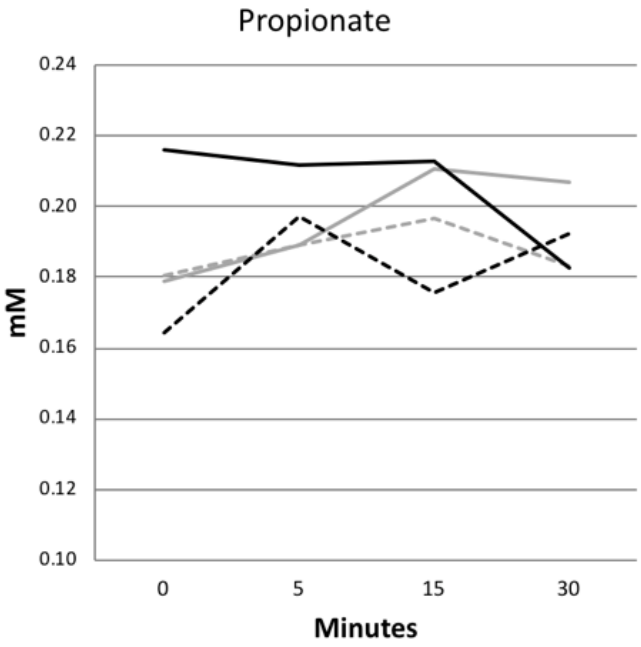

Figure 4.1 - Propionate plasma concentration for early postpartum cows over the first 30 minutes post-infusion of 1.25 mol of propionic acid over 5 min before feeding (black solid line) or 15 minutes before feeding (grey solid line) and over 5 min after feeding (black dashed line) or 15 minutes after feeding (grey dashed line).

Hepatic Metabolites

No main effects of treatment were detected for any of the metabolites measured and no treatment by sampling time interactions were detected with the exception of fumarate and lactate (Table 4.3). Treatments tended to interact with time to affect fumarate concentration (P = 0.12); FST significantly increased hepatic fumarate concentration at 5 minutes compared with SLW (P = 0.05; Figure 4.3a). Fumarate concentrations were higher AF compared with BF (37.7 vs. 31.2 μmoles/mg, P = 0.05).

Lactate concentrations were higher AF compared with BF ($P < 0.01$) but treatments tended to interact with time of infusion relative to feeding and sampling time ($P = 0.12$; Figure 4.3b).

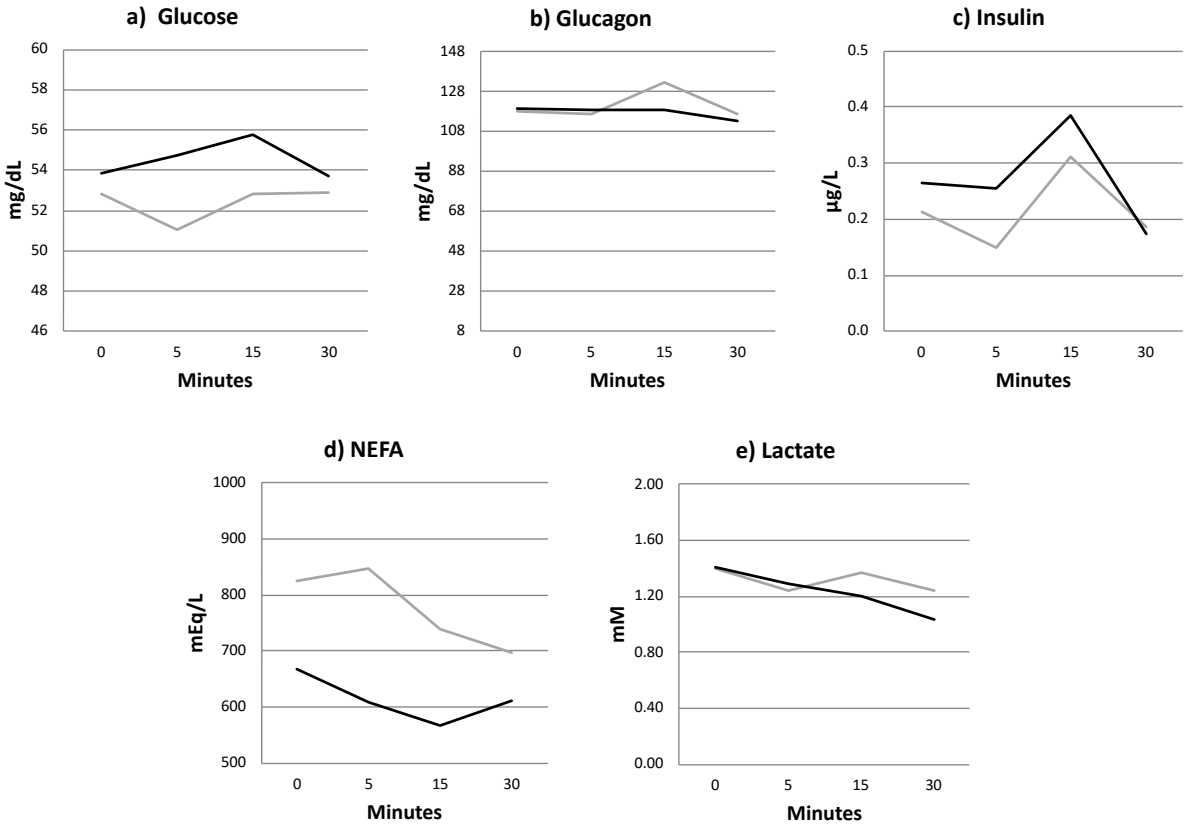

Figure 4.2 - Plasma glucose (a), glucagon (b); insulin (c), NEFA (d), and lactate (e) from early postpartum cows over the first 30 minutes post-infusion of 1.25 mol of propionic acid over 5 (black line) or 15 minutes (gray line).

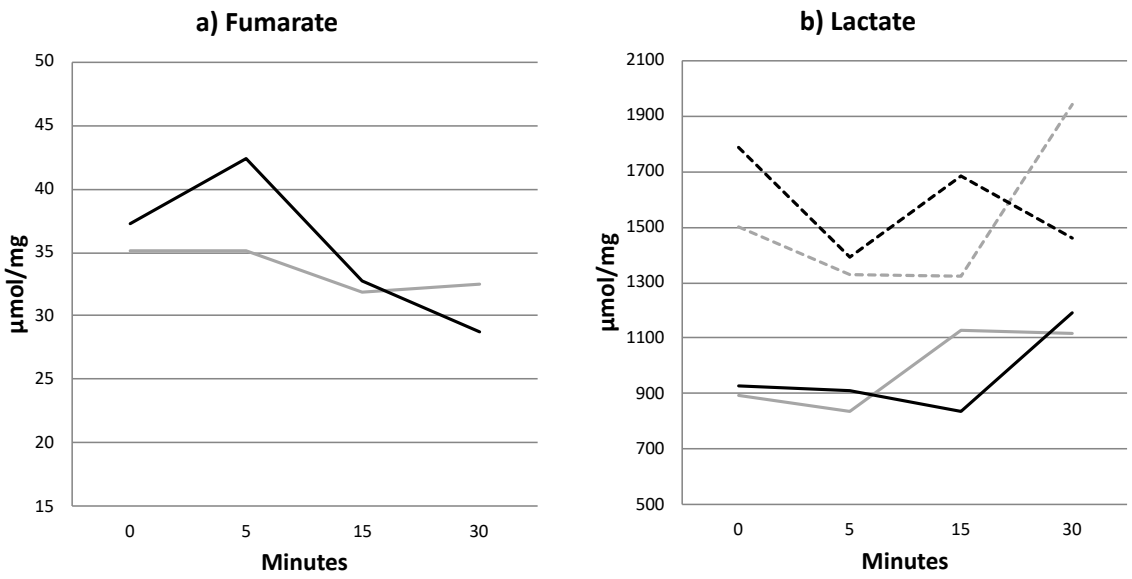

Figure 4.3 - Fumarate (a) and lactate (b) concentration in liver tissue for early postpartum cows over the first 30 minutes post-infusion of 1.25 mol of propionic acid over 5 min before feeding (black solid line) or 15 minutes before feeding (grey solid line) and over 5 min after feeding (black dashed line) or 15 minutes after feeding (grey dashed line).

Most metabolites decreased or tended to decrease AF compared with BF including propionyl CoA (0.53 vs. 1.27 µmoles/mg; $P < 0.01$), methylmalonyl CoA (0.22 vs. 0.37 µmoles/mg; $P = 0.05$), succinyl CoA (3.36 vs. 5.53 µmoles/mg; $P = 0.02$), succinate (35 vs. 58 µmoles/mg; $P = 0.01$), fumarate (31 vs. 38 µmoles/mg; $P = 0.05$), malate (72 vs. 114 µmoles/mg; $P = 0.02$), GABA (337 vs. 284 µmoles/mg, $P = 0.06$) glutamate (1501 vs. 1061 µmoles/mg, $P = 0.03$), and CoA (3.68 vs. 6.32 µmoles/mg; $P = 0.03$). However, pyruvate (69 vs. 79 µmoles/mg; $P = 0.03$) and lactate (911 vs. 1501 µmoles/mg; $P < 0.01$) increased AF compared with BF (Table 4.3).

Rate of infusion interacted with time of infusion relative to feeding to affect acetyl CoA ($P = 0.08$), glucose ($P = 0.11$), and phosphate ($P = 0.09$). The FST treatment decreased acetyl CoA BF (4.46 vs. 4.16 µmoles/mg) and increased acetyl CoA AF (5.71 vs. 3.45 µmoles/mg)

compared with SLW. The SLW treatment increased glucose (1877 vs. 1762 μmoles/mg) and phosphate (5658 vs. 5308 μmoles/mg) BF but decreased glucose (1304 vs. 1725 μmoles/mg) and phosphate (4555 vs. 5421 μmoles/mg) AF compared with FST.

Time of infusion relative to feeding and sampling time interacted to affect BHBA (P = 0.03), glutamate (P =0.08), and aspartate (P = 0.06, Figure 4.4). BHBA increased from 994 to 1115 μmoles/mg from baseline to 30 minutes BF whereas it decreased from 1303 to 1009 μmoles/mg AF. Glutamate decreased from 1749 to 1455 μmoles/mg BF and from 1533 to 670 μmoles/mg AF. Aspartate varied from 256 to 265 μmoles/mg BF and 271 to 198 μmoles/mg AF.

Sampling time effects for malate (P = 0.01), inorganic phosphate (P = 0.03) and GABA (P = 0.02) are presented in Figure 4.5. Malate concentration increased slightly at 5 min and decreased at 15 and 30 min after infusion independent of treatment, while phosphate and GABA decreased over time.

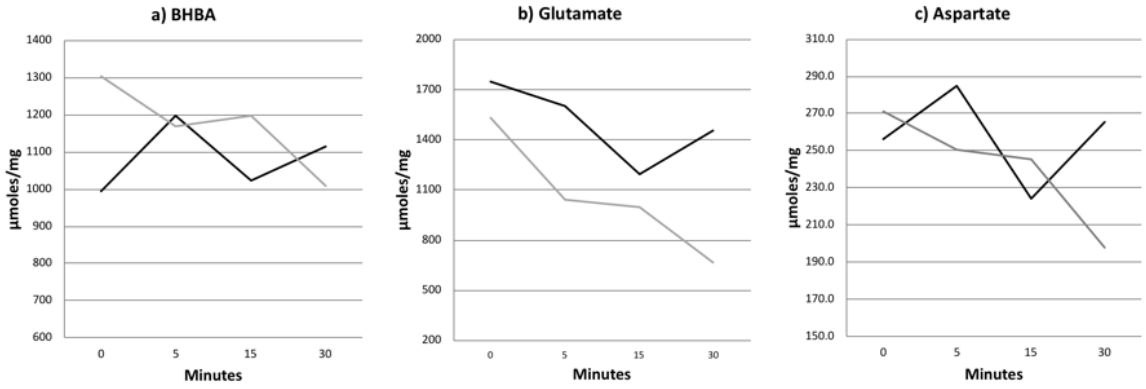

Figure 4.4 - Hepatic BHBA (a), glutamate (b), and aspartate (c) concentration before and after 5, 15 and 30 minutes of of 1.25 mol of propionic acid infusion before feeding (black solid line) or after feeding (grey solid line).

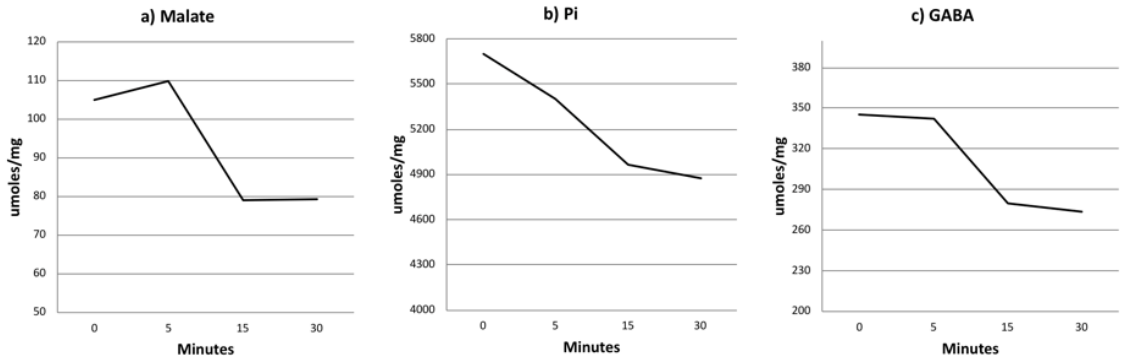

Figure 4.5 - Malate (a), inorganic phosphate (Pi, b) and gamma aminobutyric acid (GABA, c) concentration in liver tissue over the first 30 minutes post-infusion of 1.25 mol of propionic acid over 5 or 15 min and before or after feeding.

DISCUSSION

Propionate extracted from the blood by the liver can stimulate oxidation of acetyl CoA in the TCA cycle contributing to satiety according to the HOT (Allen *et al*., 2009). However, fractional hepatic extraction of propionate can be decreased when PA is infused intraruminally in sheep (1.78 mmol/h/kg BW for 4 h; Berthelot *et al*. 2012) and mesenterically in cows (1.2 mmol/h/kg BW; Baird *et al*., 1980). As a result, propionate would be expected to increase in the peripheral circulation. Oba and Allen (2003) reported that plasma propionate plasma concentration increased linearly from 0.19 to 0.34 mM when propionate was infused intraruminally from 0 to 1.3 moles/h for cows in the PP period.

In a previous experiment, we observed an increase in meal size and extended time between meals by FST, reducing meal frequency of cows in the PP period (Maldini and Allen, 2018) and we hypothesized that FST saturated the pathway for propionate metabolism resulting in a lower first-pass extraction by the liver compared with SLW. However, the results of this experiment do not support our hypothesis. On the contrary, SLW increased, and FST decreased plasma propionate concentration over time BF with no consistent difference AF. We infused PA

for a maximum of 15 minutes while most of the research cited infused PA continuously for at least 4h (up to 18h). The lack of treatment effects in our experiment suggests that a more prolonged and more continuous infusion of PA may be required to saturate hepatic propionate metabolism. Because our goal was to evaluate the metabolic changes within a meal, we restricted our infusions and sampling to an average meal length, and therefore the cumulative effects of PA continuous infusion on metabolism were probably not captured.

The lack of treatment effects and their interactions with sampling time for plasma propionate concentration as well as concentrations of propionyl CoA, methylmalonyl CoA, succinyl CoA, and succinate in the liver are consistent with the idea that efficiency of extraction of propionate by the liver from the blood was not affected by treatment. However, whereas significant differences between treatments were not detected overall, plotting concentrations of metabolites over time for individual cows indicated that the site of potential bottlenecks at which individual metabolites of propionate metabolism were elevated varied by cow. The only potential bottleneck identified among cows was fumarate, which increased over time for FST compared with SLW. We expected that the malate dehydrogenase reaction might be a bottleneck in the TCA cycle when NADH is elevated, resulting in elevated malate concentration in the mitochondria. Whereas malate concentration was not affected by treatment, we measured metabolite concentrations in whole liver tissue. Mitochondrial malate concentration might have increased causing the increase observed for fumarate, its upstream precursor, for FST compared with SLW with no overall increase for malate concentration in whole liver tissue. The more rapid increase in plasma glucose for FST compared with SLW with no effect of treatment on oxaloacetate or citrate is consistent with the increased flow of propionate carbon from FST being exported to the cytosol rather than stimulating oxidation of acetyl CoA. The rapid increase in

plasma glucose concentration for FST compared with SLW was not related to changes in plasma glucagon concentration, which was increased by SLW but not FST.

Rate of oxidation in hepatocytes is dependent upon the number of TCA intermediates which is determined by the balance between anaplerosis and cataplerosis, the availability of acetyl CoA for the citrate synthase reaction, and the speed at which the cycle spins, affected by enzyme concentration and activity (Allen, 2014). The primary source of acetyl CoA is from mitochondrial ß-oxidation of NEFA extracted from the blood by the liver (Allen *et al*., 2009). The hypophagic effects of propionate infusions were related to hepatic acetyl-CoA concentration among early-lactation cows (Stocks and Allen, 2012, 2013). The more rapid reduction in plasma NEFA concentration for FST compared with SLW, likely reduced the supply of acetyl CoA for oxidation in the TCA cycle. Concentration of acetyl CoA is dependent upon the balance between its supply and its disappearance from oxidation in the TCA cycle or export as ketone bodies and acetate. There were no temporal effects of treatment within infusions on hepatic acetyl CoA or BHBA concentrations; or on plasma BHBA or acetate concentrations. If the faster reduction in plasma NEFA concentration resulted in a decreased supply of acetyl CoA, hepatic oxidation might have been slower for FST compared with SLW allowing greater meal size because acetyl CoA concentration was not reduced by FST during the timeframe of meals. Consistent with this, Piantoni *et al*. (2015) showed a positive relationship between the extent of reduction in plasma NEFA concentration and DMI during the first 4 h after feeding for cows in the PP period and that the reduction in plasma NEFA concentration and the reduction in hepatic acetyl CoA concentration were linearly related.

The reduction in concentrations of hepatic metabolites in the pathway from propionate to malate AF compared with BF was not expected because of the greater anaplerosis from

propionate and other metabolites to the liver AF compared with BF. Because concentration is a function of the balance between anaplerosis and cataplerosis, and anaplerosis was likely increased AF, their reduction in concentration indicates that cataplerosis was also increased AF, likely from increased gluconeogenesis. Whereas plasma glucose concentration was not greater AF compared with BF, propionate is a potent insulin secretagogue in ruminants (Leuveninck at al, 1997) and greater insulin concentration likely increased glucose clearance from the blood.

Effects of treatment on neurotransmitters produced and potentially released by hepatocytes were investigated as a potential mechanism for the both the communication between hepatocytes and hepatic vagal afferents as well as differences between treatments on feeding behavior. According to the HOT, inhibition of hepatic vagal afferents causes satiety and excitation stimulates hunger (Allen *et al*., 2009). GABA is an inhibitory neurotransmitter whereas glutamate and aspartate are excitatory neurotransmitters and all are produced by hepatic metabolism. However, there were no effects of treatment or interactions with sampling time on concentration of any of these neurotransmitters and both glutamate and GABA decreased over time similarly across treatments with little change in aspartate.

CONCLUSION

Whereas we cannot discern the pattern of oxidation of acetyl CoA from our data, the more rapid increase in plasma glucose and insulin concentrations, and more rapid reduction in plasma NEFA concentration for FST compared with SLW likely decreased supply of acetyl CoA for oxidation in the TCA cycle which might have allowed greater meal size for the FST treatment compared with SLW observed in our previous experiments. Increased meal size would be expected to extend the time between meals, decreasing meal frequency.

To our knowledge, the current experiment is the first to attempt to evaluate the effects of temporal supply of propionate within a meal on hepatic metabolism to explain feeding behavior. These data will help our understanding of the complex mechanisms controlling feed intake in dairy cows. Further research involving the metabolism of propionate relative to meals and its relation to hepatic energy status is needed to better understand metabolic control of intake in ruminants.

ACKNOWLEDGMENTS

We acknowledge funding for this project by USDA National Research Initiative Competitive Grant no. 2014-67015-21708 to MSA, and from CAPES–Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil to GM while conducting this research. We thank K. Kennedy, D. G. Main, L. G. Duarte and R. Albornoz, for their assistance, and Kemin Animal Nutrition and Health (Des Moines, IA) for supplying the propionic acid.

Table 4.2 Blood from early post partum cows over the first 30 minutes post infusion of 1.25 mol of propionic acid over 5 (FST) or 15 minutes (SLW) before feeding (AM) and 4 hours after feeding (PM). Metabolite values reported in μmoles/mg

	AM		PM		*P value*						
	FST	SLW	FST	SLW	T[1]	F[1]	TxF	S[1]	TxS	SxF	TxSxF
Propionate, mM [3]	0.21	0.20	0.18	0.19	0.92	0.34	0.67	0.42	0.47	0.46	0.06
Acetate, mM [2]	0.55	0.55	0.66	0.51	0.22	0.59	0.19	0.73	0.77	0.40	0.88
NEFA, mEq/L[2]	725	972	504	584	0.06	<0.01	0.44	<0.01	0.06	0.23	0.84
BHBA, mg/dL [3]	10.0	11.3	10.6	12.4	0.18	0.20	0.73	<0.01	0.39	0.76	0.67
Glucagon, mg/dL [3]	118	121	117	125	0.47	0.76	0.71	<0.01	0.54	0.89	0.12
Insulin, μg/L [2]	0.24	0.17	0.30	0.26	0.13	0.04	0.45	<0.01	0.02	0.07	0.68
Lactate, mM	1.27	1.18	1.20	1.44	0.40	0.27	0.07	0.00	0.15	0.22	0.60
Glucose, mg/dL	56.1	52.2	52.9	52.6	0.14	0.27	0.15	0.10	0.03	0.56	0.40

[1]T = treatment, rate of infusion, F = time of infusion relative to feeding (BF/AF), S = sampling time
[2] Data analyzed after log transformation
[3] Data analyzed after square root transformation

Table 4.3 Hepatic metabolites of propionate metabolism and TCA cycle intermediates from early post partum cows over the first 30 minutes post infusion of 1.25 mol of propionic acid over 5 (FST) or 15 minutes (SLW) before feeding (AM) and 4 hours after feeding (PM). Metabolite values reported in μmoles/mg

	BF		AF		*P value*						
	FST	SLW	FST	SLW	T[1]	F[1]	T*F	S[1]	TxS	S*F	T*S*F
Propionyl-CoA [2]	1.12	1.42	0.64	0.43	0.83	0.01	0.26	0.37	0.97	0.13	0.30
Methylmalonyl-CoA [2]	0.37	0.37	0.30	0.14	0.28	0.05	0.22	0.38	0.98	0.57	0.35
Succinyl-CoA [2]	5.35	5.70	4.12	2.60	0.32	0.02	0.27	0.54	0.94	0.66	0.93
Succinate [3]	55.7	59.6	37.8	33.1	0.46	0.01	0.34	0.20	0.30	0.45	0.27
Fumarate [3]	37.5	37.8	33.0	29.4	0.47	0.05	0.54	<0.01	0.12	0.61	0.60
Malate [2]	112	116	79.0	65.1	0.74	0.02	0.52	0.01	0.76	0.36	0.54
OAA	348	358	363	359	0.32	0.56	0.67	0.66	0.66	0.38	0.55
Citrate [2]	70.3	54.1	64.0	52.9	0.18	0.66	0.88	0.33	0.73	0.46	0.20
Acetyl-coA[3]	4.16	4.46	5.71	3.45	0.27	<0.01	0.08	0.59	0.62	0.61	0.73
CoA [2]	5.66	6.98	4.39	2.98	0.72	0.03	0.23	0.72	0.64	0.28	0.74
Ketoglutaric acid [3]	118	115	118	106	0.46	0.59	0.57	0.53	0.29	0.71	0.97
Lactate [3]	967	994	1579	1521	0.95	<0.01	0.78	0.17	0.69	0.75	0.12
Glucose [2]	1762	1877	1715	1304	0.41	0.08	0.11	0.91	0.63	0.17	0.89
Pyruvate [3]	69.3	68.8	82.8	74.3	0.33	0.03	0.37	0.29	0.50	0.91	0.47
GABA [3]	354	320	319	249	0.14	0.06	0.44	0.02	0.68	0.42	0.71
Glutamate [3]	1628	1373	1170	952	0.23	0.03	0.98	<0.01	0.86	0.08	0.81
Aspartate [2]	274	242	247	236	0.46	0.53	0.73	0.16	0.53	0.06	0.56
Phosphate [3]	5308	5658	5421	4555	0.43	0.16	0.09	0.03	0.75	0.22	0.36
BHBA [3]	956	1209	1115	1226	0.25	0.55	0.63	0.47	0.90	0.03	0.59

[1]T = treatment, rate of infusion, F = time of infusion relative to feeding (BF/AF), S = sampling time
[2] Data analyzed after log transformation
[3] Data analyzed after square root transformation

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CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

This research shows that alteration of the temporal supply of propionate can alter feeding behavior of cows in the postpartum period. Changes in metabolism occurred within 5 min following the initiation of infusion into the rumen showing the ability of propionate to affect feeding behavior within meals. Although we could not discern the pattern of oxidation of acetyl CoA from our experimental data, the hyphophagic effects of propionate appear to be related to the balance between anaplerosis and cataplerosis of TCA intermediates as well as availability of acetyl CoA for oxidation and not from the rate of extraction of propionate from the blood alone. In addition, it does not appear that alteration of the production of neurotransmitters (glutamate, aspartate, or GABA) by hepatocytes are involved in the hypophagic effects of propionate.

Previous research has shown a positive relationship between the extent of reduction in plasma NEFA concentration and DMI for cows in the PP period and that the reduction in plasma NEFA concentration was correlated with a reduction in hepatic acetyl CoA concentration over 4 h following feeding. The present research indicates that this is likely true over a shorter timeframe, within meals. More rapid infusion of propionic acid increased plasma insulin concentration and decreased plasma NEFA concentration, likely reducing the supply of acetyl CoA for hepatic oxidation and possibly reducing the activity of the TCA cycle by partitioning more malate to support gluconeogenesis rather than conversion to oxaloacetate in the TCA cycle.

Although the rate of infusion can affect feeding behavior, these experiments show that the hypophagic effects of PA during the PP period are primarily from the amount of PA infused at meals. When different amounts were infused into the rumen at different rates, only the amount of propionic acid infused at each meal affected DMI. The inconsistent effects of rate of infusion

of propionic acid on feeding behavior might be explained by a threshold response of propionate uptake and ability to stimulate oxidation of acetyl-CoA in the liver.

The links between the effects of propionate on metabolic response and feeding behavior based on hepatic oxidation were indirectly inferred from the observations among these and other experiments. A limitation of this research is that feeding behavior and metabolic response was measured in different experiments with different cows. Also, we measured concentrations of metabolites in whole liver tissue whereas measurement of metabolite concentrations in the mitochondria only would help identify if the malate dehydrogenase reaction was a bottleneck resulting in greater export of malate to the cytosol. Further, measurement of carbon flux through the pathway using labeled propionate might yield more conclusive results regarding the rate of partitioning of anaplerotic carbon to OAA or glucose as well as rate of oxidation. Evaluating ATP to ADP ratio, phosphorylation potential, and release of neurotransmitters in different rates and amount of fuels provided to the hepatocyte can elucidate by which mechanisms the changes in hepatic oxidation stimulate the firing rate of the vagal nerve and indicate opportunities to diet manipulation to increase feed intake.

The experiments conducted for this dissertation are consistent with the control of feed intake through hepatic oxidation. However, this is only the beginning of a process to decipher the complex mechanisms that control feed intake in the PP period. More research evaluating the interaction among starch fermentability and starch concentration in diets fed to cows in the early PP period on feeding behavior, DMI, milk yield and yield components are also needed, to better understand the animal response to diets and to provide nutritionist with practical information about feeding strategies to increase DMI in the postpartum period.