EFFECTS OF METABOLIC FUELS ON FEEDING BEHAVIOR AND DRY MATTER INTAKE OF DAIRY COWS IN THE POSTPARTUM PERIOD

By

Laura Bibiana Gualdrón-Duarte

A DISSERTATION

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

Animal Science – Doctor of Philosophy

2017

ABSTRACT

EFFECTS OF METABOLIC FUELS ON FEEDING BEHAVIOR AND DRY MATTER INTAKE OF DAIRY COWS IN THE POSTPARTUM PERIOD

By

Laura Bibiana Gualdrón-Duarte

Feeding behavior (**FB**) and dry matter intake (**DMI**) of ruminants has been linked to hepatic oxidation of fuels. The type and temporal pattern of fuels absorbed from the gastrointestinal tract of ruminants and available for oxidation are affected by diet starch concentration and fermentability. However, research considering the fuels produced from starch digestion is lacking for cows in the postpartum (**PP**) period. Our overall hypothesis is that fuels produced during digestion affect hepatic metabolism and FB differently. To investigate the effect of anaplerosis of the tricarboxylic acid (**TCA**) cycle on FB, experiments were conducted using cows in PP period. Propionic acid (**PA**, 99.5%) and glycerol (**GL**, 99.7%) were infused into the abomasum (**AB**) in isoenergetic (193 kcal/h) and isomolar (~0.5 mol/h) solutions using a double crossover design. Infusion of PA decreased DMI compared with GL 17 and 23%, by decreasing meal size 13 and 15% with isoenergetic and isomolar infusions respectively, and PA decreased total metabolizable energy intake (**MEI**, diet plus treatment infusions) compared with GL. Compared with PA, GL increased plasma glucose and insulin concentrations for isomolar infusions. Pulse-dose (2 moles) of PA into the AB in a crossover design decreased hepatic acetyl CoA (**AcCoA**) content whereas GL increased 32%, resulting in lower content for PA compared with GL at 30 min (18.0 vs. 36.9 nmol/g), which persisted at 60 min after dosing (21.9 vs. 32.8 nmol/g). These results are consistent with anaplerosis from PA stimulating oxidation of AcCoA in the liver and satiety compared with GL.

To investigate the effect of fuels from starch digestion on FB, continuous isoenergetic

AB infusions (150 kcal/h) of PA (99.5%, 0.41 mol/h), lactic acid (**LA**, 88.0%, 0.46 mol/h) or glucose (**GLU**, 99.9%, 0.22 mol/h) were studied with cows in PP period in a duplicated 4 x 4 Latin square design. A control (**CON**, no infusion) treatment was also included. Treatment solutions were infused at 500 mL/h for 22 h/d and provided ~3.3 Mcal/d. Compared with CON, PA decreased DMI by 24% (14.3 vs. 18.9 kg/d) and MEI by 13% (34.8 vs. 40.2 Mcal/d) by a tendency to decrease meal frequency; and LA decreased DMI by 14% (16.3 vs. 18.9 kg/d) by decreasing meal size 20%, but did not affect MEI. Glucose infusion did not affect DMI or MEI.

While ruminal infusions of sodium acetate have little effect on DMI, infusion of acetic acid (**AA**) into the AB of cows in the PP period decreased DMI 64% compared with a sham control (Gualdrón-Duarte and Allen, unpublished). To understand the mechanism, we investigated the effects of continuous isomolar infusions (~0.75 mol/h; 0.5 L/h; 8 h) of AA, sodium acetate (NA) or CON (NaCl) into the rumen (**RU**) or into the AB on FB, DMI, and metabolic responses of cows in the PP period in a 6 x 6 Latin square design. Treatments were infused for the first 8 h following feeding with a rest day between infusion days. During infusions treatments decreased DMI 30% (4.3 kg vs. 6.1 kg) compared with CON by decreasing meal frequency but their effects differed; NA-RU did not reduce DMI (7.0 kg), whereas AA-RU (2.6 kg), AA-AB (3.7 kg) and NA-AB (4.0 kg) decreased DMI compared with CON. Contrary to the other treatments, NA-RU did not decrease DMI compared with CON, but the reason cannot be determined from the results available from this study.

Collectively, these experiments provide strong evidence that anaplerotic metabolites for the TCA cycle decreased DMI and MEI on cows in the PP period consistent with their expected effects on hepatic oxidation. Depression of feed intake by diets including highly fermentable starch is likely because of the ability of propionate to stimulate hepatic oxidation.

A mis padres Elsa y Luis, y mis hermanos
José Luis y Jorge Eduardo por ser todo en mi vida.
Y a mi abuelita y Lina, las dos nuevas estrellas
guiándome desde el cielo.

ACKNOWLEDGEMENTS

I would like to say “thank you”:

To my advisor Dr. Michael Allen for his guidance, patience and all the support that has helped me to improve during the past five years to become a better researcher and scientist. I will always be grateful for the opportunity he has given me to work in his team which allowed me to work with wonderful and knowledgeable people especially the technicians, graduate and undergraduate students.

To my graduate committee members, Dr. Thomas Herdt, Dr. Richard Ehrhardt, Dr. David Beede and Dr. Michael VandeHaar for their guidance and for helping me during my time at Michigan State University.

To everyone at the Dairy Cattle Teaching and Research Center, particularly Bob Kreft, Rob West and especially to Randy, Jake, Fred, Bob, Bruce. Thank you for being my farm-mates daily for almost one year and then a couple times again.

To Dave Main and Dewey Longuski, from Allen lab, for always having my back. To Jim Liesman for his time, patience and always lending a keen ear to hear me talk about my home country. To my lab and office members Paola P., Sarah S., Bill B., Sarah B., Jackie B., Sarah S., Katie K., Gabriela M., Rodrigo A., Roni Y and Danilo S who were always ready to help.

To K.C. Main and Dave Main (Main team) without whose help, I wouldn’t have been able to complete my longest and hardest experiments.

To Karen Beede and Dr. Beede for all your support, love and warmth, especially when I was new here. It was really meaningful to me.

To all my latino friends (including their significant others). My time here with all of you

is something that I will cherish forever. You all brought the Latino flavor that made life in Michigan truly wonderful.

To my roommates, Juan, Eduardo, Andrea, and especially Norma. And to my little sister Yan Sun. They became my new family.

To all those who don't speak English: Ustedes, mi familia y amigos de Colombia fueron lo más importante y lo que me mantuvo en pie y no me dejo desistir ante las muchas veces tristes y duras circunstancias que me rodearon. Todos ustedes son muy importantes en mi vida. Los quiero con todo mi corazón, "primates", todo el "Clan de las Duarte" y su descendencia, familia Gualdrón, familia Acero-Camelo y Bernal-Acero, familia Rueda-Uribe, Ximena, Nidia, Laila, Laurens y a mi Danielito y Samuel.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AA	Acetic acid treatment
AB	Abomasum
AcCoA	Acetyl CoA
BHB	ß-hydroxybutyrate
CON	Control treatment
CP	Crude protein
DE	Digestible energy
DEI	Digestible energy intake
DM	Dry matter
DMI	Dry matter intake
EB	Energy balance
FA	Fatty acids
FC	Fold change
FDR	False discovery rate
GE	Gross energy
GL	Glycerol treatment
GLU	Glucose treatment
GLP-1	Glucagon-like peptide
HOT	Hepatic oxidation theory
LA	Lactic acid treatment
ME	Metabolizable energy

MEI	Metabolizable energy intake
NA	Sodium acetate treatment
NDF	Neutral-detergent fiber
NEFA	Nonesterified fatty acids
NEB	Negative energy balance
OM	Organic matter
OXPHOS	Oxidative phosphorylation
PA	Propionic acid treatment
PP	Postpartum
RR	Reticulo-rumen
RU	Rumen
TCA	Tricarboxylic acid
TMR	Total-mixed ration
VFA	Volatile fatty acids

INTRODUCTION

The transition period from gestation to lactation in dairy cows causes homeostatic and homeorhetic changes highly related with the decrease in dry matter intake (**DMI**) plus increased energy output with the resulting negative energy balance. The postpartum (**PP**) period is characterized by a high incidence of metabolic disorders associated with the severe energy deficit and excessive lipid mobilization (Bell, 1995; Overton and Waldron, 2004). The decrease in plasma insulin concentration and insulin sensitivity in adipose tissue close to parturition promotes lipolysis leading to a lipolytic state (Drackley, 1999). Increased hepatic uptake and ß-oxidation of nonesterified fatty acids (**NEFA**) likely suppress feed intake by providing a continuous supply of acetyl CoA (**AcCoA**) for oxidation in the tricarboxylic acid (**TCA**) cycle, increasing ATP production and energy status and thus, signaling for satiety. Energy status is associated to phosphorylation potential and is measured by calculation of adenylate energy charge ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]) (Allen et al., 2009).

Starch is the principal energy source used in diets for dairy cows to increase energy intake and meet the high demand for glucose precursors. However, it has been shown that increasing ruminal starch fermentability can depress feed intake (Nelson et al., 2011; Albornoz and Allen, 2016). Diets with high moisture corn decreased DMI 7.6% for mid-lactation cows (Oba and Allen, 2003a) and 11.4% for cows in the PP period (Albornoz and Allen, 2016), compared with diets containing the less fermentable dry ground corn. Ruminal starch fermentability varies greatly depending upon particle size, gelatinization, solubility of endosperm proteins, type of processing method, and storage method (Kotarski et al., 1992; Larson and Hoffman, 2008). Ruminal starch fermentation ranged from less than 30% to more than 85% (Allen, 2000) thus, altering the type and temporal pattern of fuels absorbed. The ability of these

fuels for stimulating hepatic oxidation varies and therefore, their effect on DMI varies. According to the hepatic oxidation theory (**HOT**), oxidation of fuels in peripheral tissues is implicated in the control of feed intake as a result of the transmission of signals to brain feeding centers by hepatic vagal afferents. Fuels absorbed by the liver that anaplerotic of the TCA cycle may promote oxidation of AcCoA, increase hepatic energy charge signaling for satiety, thus decreasing intake (Allen et al., 2009).

Substituting cereal grains for forage increases dietary starch content and the production of propionic acid (Bauman et al., 1971; Sutton et al., 2003). Propionate decreased DMI by decreasing meal size compared with acetate when infused intraruminally in lactating cows (Choi and Allen, 1999, Oba and Allen, 2003a), and the hypophagic effect is greater in cows in the early PP period than in mid lactation (Oba and Allen, 2003b), likely because cows in the PP period are in a lipolytic state with both elevated plasma NEFA concentration and hepatic AcCoA content (Allen et al., 2009). Ruminal infusion of propionic acid was more hypophagic for cows in the PP period with greater hepatic AcCoA concentration (Stocks and Allen, 2012, 2013). Whereas propionate is hypophagic compared to acetate, comparisons of propionate to other fuels absorbed and its effect on energy intake of cows during the immediate PP is lacking. Objectives of this research were to evaluate the effects of fuels resulting from ruminal fermentation and digestion of starch on DMI and feeding behavior of cows in the PP period. Our central hypothesis is that these fuels differ in their effects on DMI and metabolizable energy intake consistent with their capacity to be metabolized by the liver and stimulate hepatic oxidation.

Therefore, this dissertation includes results of five experiments (Exp.) on cows in the PP period that were conducted to: 1) investigate the effect of anaplerosis of the TCA cycle on feeding behavior (Exp. 1 to 3); 2) investigate the effects of different fuels derived from starch

digestion on feeding behavior (Exp. 4); and, 3) investigate the effects of infusions of acetic acid or sodium acetate into the rumen or abomasum on feeding behavior (Exp. 5). In the appendices are presented results of gene expression analyzed as part of Exp. 4; and a preliminary experiment to Exp. 5 conducted to investigate the effects of fuel derived from starch digestion on feeding behavior that included the infusion of acetic acid into the abomasum.

Chapter 2 from this dissertation was already published as "*Increased anaplerosis of the tricarboxylic acid cycle decreased meal size and energy intake of cows in the postpartum period*" in Laura B. Gualdrón-Duarte and Michael S. Allen. 2017 J. Dairy Sci. 100:4425-4434.

CHAPTER 1. LITERATURE REVIEW

FEED INTAKE AND FEEDING BEHAVIOR

Feed intake is a function of the quantity of food ingested during a meal and the number of meals per period of time. Feed intake is the most important parameter driving the acquirement of nutrients and energy required for living and productive processes.

Mechanisms regulating feed intake have a differential temporal effect depending on their magnitude of duration over time, and involve a combination of short-term and long-term factors. Short-term regulation is more closely related to body homeostatic regulation and is mostly influenced by dietary factors, whereas long-term control relies more on homeorhetic adaptations according to changes in animal factors (Allen, 2014).

Feeding behavior includes the number of meals, the length of meals, and the rate of eating; strategies that animals can adjust in order to change dry matter intake (**DMI**). It is known that some aspects, for instance level of milk production, can modify feeding behavior. High-producing cows with free access to total mixed rations ate 9 to 14 meals/d compared with lower-producing cows who ate 7 to 9 meals/d (Grant and Albright, 1995). This behavior can be modified if other behavioral or management situations are involved in the studies or production systems.

Dado and Allen (1994) suggested that in dairy cows the control of individual meals and total DMI within a day are related to different mechanisms depending not only on level of production but on parity, ruminal capacity and body weight variability among animals as well. A meal initiates when the animal is hungry and stops when it is satiated. Hence, daily intake is the

sum of the mass ingested in several meals regulated, on an individual basis, by hunger and satiety mechanisms.

CONTROL OF FEED INTAKE

Animal and dietary factors affect mechanisms controlling feed intake. The importance of the different factors varies over time both within and across days and can be highly influenced by metabolic state (Allen, 2014).

Physical regulation

Regulation of DMI by physical factors occurs when feed intake is constrained by distention signals from the gastrointestinal tract and when the time required for chewing is increased. Physical regulation becomes a major factor regulating DMI with the increase in energy needs and filling effect of the diets (Allen, 2000).

The filling effect is one of the main dietary factors that regulates feed intake, and is determined by the quantity and initial bulk density, the forage source, and the digestibility of forage NDF present in the diet. The pressure tension and mechanoreceptors cause distention. Distention in the reticulo-rumen (**RR**) is determined by volume and weight of digesta (Schettini et al., 1999), the initial water content of the diet, as well as the water retention capacity of digesta over time (Allen, 2014). For ruminants, the RR has been considered as the major regulatory site for DMI induced distention (Allen, 1996; Forbes, 2007). Stretch receptors located in the muscle layer in the wall of the RR and cranial sac of the rumen are stimulated, and signals are sent to the brain satiety centers via vagal afferent nerves triggering the meal cessation. Despite the importance of distention in controlling feed intake, the differences in response among cows and

physiological state provide evidence that feed intake is controlled through the integration of various signals converging in satiety centers of the brain (Mbanya et al., 1993).

Osmotic regulation

Osmolality of the RR may range from 250 to 300 mOsm/kg during pre-feeding to as high as 500 mOsm/kg following the hours after a large meal (Forbes, 2007). It is affected by DM and water intake and is highly variable depending on the content of mineral salts and fermentability of OM in the diet (Allen, 2000; Forbes, 2007). Osmolality is decreased water entering the RR and by absorption from the RR (Scott, 1975). Additionally, Warner and Stacy (1972) reported that increasing ruminal osmolality by infusion of solutes into the rumen decreased salivary flow contrary to the increase in salivary secretion when hypotonic solutions are infused, so saliva flow is involved in the maintenance of ruminal osmolality.

In experiments conducted by Ternouth and Beattie (1971) where ruminal osmolality was increased by infusing inorganic salts or salts of volatile fatty acids (**VFA**), DMI was reduced. They concluded that the observed decrease in feed intake was related to increased osmolality, but not to the energy content of infusions. This effect can be associated with excitation of epithelial receptors in the RR (Leek and Harding, 1975). However the same authors indicated that even though epithelial receptors in the RR can be stimulated by acids, alkali, and hypo- and hyper-osmotic solutions, the threshold to cause excitation of those receptors is very rare under normal physiological conditions. Only undissociated VFA were found to excite those receptors with thresholds in the range of 40 to 100 mM (Harding and Leek, 1972).

The ruminal infusion of iso-osmotic solutions of NaCl or VFA as Na-salts or acids as spontaneous meals (Choi and Allen, 1999) showed a reduction in meal size, implying that

termination of meal was regulated by osmolality. However, sodium chloride infusions did not decrease total DMI because the time between meals also was reduced compared with Na salts of VFA and non-infused cows. Additionally there was a greater extent in the reduction of meal length, meal size, and DMI with propionate infusion indicating that the type of VFA in addition to osmolality affected the termination of a meal.

Despite lack of identification of osmoreceptors that are sensitive to the physiological range of osmolality in the RR, evidence indicates that hyperosmotic solutions administered into the RR reduced the amount consumed of single meals (Ternouth and Beattie, 1971). Ruminal osmolality artificially increased greater than 400 mOsm can terminate a meal and markedly decrease overall intake; but, the local administration of an anesthetic to the RR stops this effect (Bergen, 1972). However, the big increase in osmolality in the RR could activate a physiological mechanism different from the direct stimulation of osmoreceptors. When RR osmolality is high there is an influx of plasma fluids into the RR causing signs of systemic dehydration and stimulating vasopressin release from the posterior pituitary gland. It is possible that this hormone plays a role in the depression of intake when salt infusions are administered intra-ruminally (Forbes et al., 1992).

Metabolic regulation

Metabolic control of feed intake occurs as a result of concentrations of nutrients, metabolites and (or) hormones that signal the nervous system to cause the animal to start or stop feeding (McDonald et al., 2002). Metabolic regulation of intake assumes that some factors related to the metabolism of the feed control feed intake through feedback signals sent to the brain (Fisher, 2002). Factors, such as the concentrations and flows of nutrients and energy, including VFA produced via ruminal fermentation (Illius and Jessop, 1996) and mobilization of

tissues and subsequent utilization as fuels, may contribute to the signaling to brain feeding centers (Fisher, 2002). Physical and metabolic controls of intake are likely not mutually exclusive, but additive (Choi and Allen, 1999).

Endocrine regulation

Ghrelin is a hormone secreted by abomasal tissue that acts on the hypothalamus (Forbes, 2007). Currently, ghrelin is the only known gut peptide that stimulates feed intake, likely by promoting emptying and increasing passage rate from the rumen (Allen and Piantoni, 2013). However, the influence of ghrelin on feed intake has been inconsistent (Roche et al., 2008). Cholecystokinin and the incretin glucagon-like peptide 1 (**GLP-1**), which also may stimulate insulin secretion, are both gut peptides involved in short-term regulation of feed intake in ruminants (Bradford et al., 2008; Relling and Reynolds, 2008). These peptides are secreted by the enterocytes as a response to the presence of certain nutrients reaching the intestine, and are involved in decreasing gastrointestinal tract motility (Holst, 1997; Relling and Reynolds, 2007), thus, decreasing gastric emptying.

Insulin and glucagon, the principal pancreatic hormones controlling fat and carbohydrate metabolism in nonruminants, have inconsistent effects on regulation of feed intake in ruminants (Forbes, 2007). Insulin plays a role in long-term dietary intake and body weight regulation in ruminants. High plasma concentration of insulin indicates energy sufficiency and affects mobilization of body reserves and oxidation of fuels, and likely provides a negative feedback on gluconeogenesis (Vandermeerschen-Doize et al., 1983; McCann et al., 1992). However, within a day changes in insulin and glucagon can play important roles in the partitioning of fuels, and likely also have an indirect effect on feed intake. Short-term effects of insulin at meals seems to affect differently intake. Increased plasma insulin concentration after meals might remove fuels

from the blood more quickly (Oba and Allen 2000) increasing DMI of cows in the PP period and in late lactation. Pulsatile insulin secretion within a day probably reduces lipolysis causing a reduction in plasma NEFA concentration (Oba and Allen, 2003a) reducing hepatic AcCoA content available for oxidation in the liver decreasing hepatic energy charge and increasing intake (Piantoni et al., 2015), consistent with the hepatic oxidation theory (**HOT**). The increase in plasma insulin concentration within a day also may contribute to increased hunger, although promoting oxidation of fuels in the liver likely causes satiety (Allen et al., 2005).

Little research has been done on the effect of glucagon and GLP-1 on feed intake. Studies conducted with sheep (Deetz and Wangsness, 1981; Faulkner and Martin, 1998) showed that glucagon administered intravenously at physiological rates reduced intake, and that GLP-1 concentration is greater during lactation compared with the dry period; however, cause and effect is not clear for GLP-1. In brief, the effects of these hormones on feed intake are likely dependent on their effects on metabolism of fuels available in accordance with the stages of lactation (Allen et al., 2009).

Leptin is a hormone secreted mainly by adipose tissue and its secretion is proportional to the increase in adipocyte size (Ehrhardt et al., 2000) and according to Bradford et al. (2006), the secretion of leptin has a positive relationship ($r = 0.61$) with body condition score in lactating cows. Leptin stimulates satiety and may be related to the homeorhetic control of body weight (Houseknecht et al., 1998).

Cortisol and catecholamines (epinephrine and norepinephrine, principally) are hormones that have effects in multiple processes. They are produced and released mainly during stressing conditions such as weaning, loading, transport and calving. These hormones are lipolytic agents;

therefore, the stress response results in lipolysis and elevated plasma nonesterified fatty acids (**NEFA**) concentrations. Even if adrenergic mechanisms can decrease DMI (Brandt et al., 2007), the availability of NEFA provides an oxidative substrate for the liver, increasing energy charge and decreasing DMI through metabolic control (Allen et al., 2009).

Hepatic control of feed intake

The concept that animals eat to balance energy intake with energy expenditure fails to explain the suppression of appetite and extended negative energy balance (**NEB**) during the periparturient period. Instead, the possibility of a common integrated mechanism among peripheral tissues sensing the energy status based on the metabolism of fuels has gained acceptance during the last few decades. The liver has been considered the primary sensor organ integrating all the long- and short-term mechanisms affecting the initiation and termination of meals (Allen, 2014).

Metabolites derived from the diet or mobilized from body reserves with the ability to be oxidized in the liver may affect feed intake of animals including ruminants (Allen et al., 2009; Allen and Bradford, 2012). Research conducted on laboratory species suggests that control of feeding behavior is related to energy charge in the liver and is integrated synergistically with other metabolic inhibitory mechanisms by a common signal related to the hepatic energy status derived from the oxidation of different fuels. These signals are sent to the brain centers that control feed intake through afferent fibers of the vagus nerve (Anil and Forbes, 1988). Although enterocytes were proposed as the primary sensors for fuel oxidation and also are innervated by the vagus nerve (Langhans, 2008), research performed across species, including ruminants, provides evidence that the liver instead of the enterocytes could be the key organ sensing the oxidation of fuels (Allen and Bradford, 2012). Hepatic oxidation is the only proposed

mechanism that accounts for both differences in fuels absorbed and sites of absorption among species, and is consistent with feed intake responses to diet. Even if multiple mechanisms are involved in the control of feed intake, the HOT implicates a peripheral feedback coordination integrated with the central nervous system via hepatic vagal afferents (Allen and Bradford, 2012).

Friedman and coworkers (Tordoff et al., 1991; Rawson et al., 1994; Horn et al., 2004) showed that feeding behavior was related to hepatic energy status rather than fuel oxidation and ATP production *per se*. Prevention of ATP production in the liver by sequestering of inorganic phosphate by infusing the fructose analogue 2,5-anhydromannitol, or by inhibiting fatty acid (**FA**) oxidation by using methyl palmoxirate and etomoxir stimulated feeding behavior. Additionally, these experiments confirmed that the response tending to reduce intake likely originates in the liver for the following reasons: 1) the effect was faster when the infusion was into the portal vein compared with the jugular vein; 2) radiolabelled 2,5-anhydromannitol increased in the liver but not in brain tissue; and, 3) vagotomy blocked the response.

Energy status is measured by adenylate energy charge and considers liver ATP content, ATP-to -ADP ratio, and phosphorylation potential ([ATP]/[ADP][Pi]) (Allen et al., 2009). The equation to measure energy charge, ([ATP] + 0.5[ADP])/([ATP]+[ADP]+[AMP]) takes into account the balance between the rate of production of high-energy phosphate bonds from oxidation of fuels and their rate of utilization by energy consuming reactions. When hepatic energy charge is reduced, firing rate of hepatic vagal afferents increases, resulting in hunger and meal initiation. As energy charge increases after a meal, the firing rate decreases, thus signaling satiety. Albeit hepatic energy charge is likely responsible for changes in the firing rate of the

hepatic vagal afferents that control intake, the exact mechanism by which the signal is transmitted is not fully understood (Allen and Piantoni, 2013).

HEPATIC METABOLISM OF FUELS

Fuels extracted from the blood and oxidized in the ruminant liver include glycerol, amino acids, lactate and NEFA, most of them mobilized from body reserves through catabolic processes or from dietary sources; as well as propionate and butyrate produced by fermentation in the rumen. Plasma NEFA are extracted by the liver from the blood in proportion to their concentration (Bell, 1979) and generally are oxidized or stored as triacylglycerides for later oxidation. Glycerol results mostly from lipolysis in adipose tissue, and lactate can be produced from the Cori cycle or via the absorption of glucose in the small intestine. All the fuels extracted by the liver have the potential to affect feed intake by inducing satiety (Langhans, 1996; Friedman, 1998; Allen et al., 2009).

One of the most important differences between ruminants and non-ruminants is that ruminant liver does not extract glucose from the blood. Hepatic uptake of glucose is negligible in adult ruminants (Stangassinger and Giesecke, 1986) likely because of the low activity of glucokinase (Emmanuel, 1981). Consistent with HOT, glucose also does not appear to have a hypophagic effect in ruminants (Baile and Forbes, 1974; Frobish and Davis, 1977) contrary to the effect in non-ruminant species. Additionally, hepatic uptake and utilization of plasma acetate is negligible in ruminants (Seal and Reynolds, 1993) because AcCoA synthetase activity is very low. Acetate, which is the most abundant fuel resulting from ruminal fermentation, is used principally as an energy source for peripheral tissues and can spare the utilization of glucose. The effect of acetate on feed intake in ruminants is likely related to osmotic effect in the rumen (Allen et al., 2009).

For ruminant species, NEFA instead of glucose are the principal fuels oxidized in the hepatocytes and the primary energy source for the liver. Medium-chain FA and unsaturated C_{18} FA are delivered and oxidized faster and have a greater hypophagic effect than long-chain saturated FA (Langhans, 1996). In general, NEFA supply varies both within the day (negatively related with plasma insulin concentration; Nielsen et al., 2003) as well as throughout lactation, as the physiological state of cows change (Ospina et al., 2010).

Propionate, glycerol, lactate and some amino acids can either be oxidized in the liver or used as gluconeogenic precursors. Mobilization, uptake, oxidation in the tricarboxylic acid (**TCA**) cycle or gluconeogenesis of these fuels are regulated according to the concentration of insulin in plasma over the long-term, determined primarily by stage of lactation or energy balance (**EB**, Allen and Piantoni, 2013).

HEPATIC CONTROL OF INTAKE IN DAIRY COWS

Concentration of hormones and metabolites in blood, metabolic processes, responsiveness of tissues to hormones, and mobilization of fuels vary greatly according to physiological state. These differences may modify the importance of the several factors controlling feed intake. Control of feed intake in dairy cows by hepatic oxidation likely becomes more relevant as nutrient requirements and the filling effect of diets decrease, reducing the signal from gut distension (Allen, 2000). Hepatic oxidation likely controls feed intake to a greater extent for animals with low nutrient requirements, animals consuming a high-starch diet, or for animals in a lipolytic state caused by stress or physiological conditions surrounding the periparturient period. Intake in ruminants fed a high-forage diet or with high nutrient and energy demands, such as cows at peak lactation, are more likely to be regulated through other mechanisms related to gut distention signaling than to oxidation of fuels (Allen et al., 2009).

According to the HOT,control of feed intake at the dry period may be regulated initially by the filling effect of the diet, but, as calving approaches changes in hormonal augmenting lipolysis and consequently shifting the control of feed intake toward hepatic oxidation mechanisms (Allen et al., 2009). Hormonal changes are mainly directed to reduce insulin concentration in plasma and insulin sensitivity of adipose tissue and muscle several weeks before parturition (De Koster and Opsomer, 2013), and to increase growth hormone concentration in plasma close to calving (Bauman, 1992), Growth hormone is involved in the reduction of lipogenesis, the decrease in glucose uptake and oxidation, and decreases insulin sensitivity of adipose tissue and increases nutrient utilization by the mammary gland. Growth hormone alters the synthesis of lipids in adipose tissue but has no effect on rates of lipid mobilization (Simmons et al., 1994). During the last weeks before parturition, the aim for nutritionists is to formulate diets to increase DMI, milk yield, and reduce the incidences of metabolic disorders after calving. Feeding high-grain diets at the end of lactation and at the beginning of the dry period has been proposed as a strategy to stimulate insulin production and limit lipolysis after calving. However, those studies have shown inconsistent results (Grummer, 2008). In fact, feeding low-fiber diets when plasma insulin concentration and insulin responsiveness in tissues is still high decreases the potential benefit of the filling effect that may constrain excessive accumulation and mobilization of fat prepartum and postpartum, respectively. Reducing lipolysis postpartum likely decreases the pool of hepatic AcCoA available to be oxidized and so increases DM and energy intake post calving (Allen and Bradford, 2012). Moreover, the filling effect helps to improve DMI after parturition and prevent the harmful effect of shifting to a more fermentable diet during the early PP period (Murondoti et al., 2004; Douglas et al., 2006).

At the onset of lactation, energy demands are not met by energy intake and fat reserves

are mobilized to provide energy. There is an increased requirement for glucose utilization for milk production, and propionate is the principal gluconeogenic precursor in ruminants. However, feeding diets rich in rapidly fermentable starch may exacerbate the problem instead of solve the imbalance. Diets with high starch concentration and readily fermentable starch sources increase propionic acid production (Allen, 2000), and increase propionate absorption (Benson et al., 2002) and liver uptake (Kristensen, 2005). Although propionate follows the gluconeogenic pathway, it is first metabolized to succinyl CoA, which allows it to enter the TCA cycle. Its entrance into the TCA cycle likely promotes the oxidation of AcCoA, and thus, increases energy charge and decreases meal size that may ultimately decrease total DMI and energy intake (Allen and Piantoni, 2013).

As lactation progresses, milk yield increases and part of the NEFA mobilized from adipose tissue are redirected to the mammary gland for milk fat synthesis and as such reduce the NEFA supply to the liver. Metabolic signals controlling intake start to decrease and diets with high fiber content limit DMI and the supply of gluconeogenic precursors from dietary sources. During this period, animals respond well to diets high in fermentable starch sources and with lower forage NDF content (Allen et al., 2009). As lactation continues and milk yield decreases, metabolic control through the HOT likely begins to dominate intake again as a result of negative feedback on gluconeogenesis due to increased plasma glucose and insulin concentrations. Increased insulin sensitivity allows the animal to replenish the body reserves lost during early and peak lactation (Allen and Piantoni, 2013). Recombinant bovine somatotropin (rbST) favors partitioning of energy to milk by increasing nutrient uptake for milk synthesis, decreasing the loss of secretory cells in the mammary gland, increasing gluconeogenesis in the liver, decreasing uptake of glucose in muscle, and decreasing lipogenesis in positive-EB and increasing lipolysis

in negative EB in adipose tissue.

POSTPARTUM COWS

During the last 3 weeks of gestation there are a tremendous increase in nutrient demands (Bell, 1995), and significant changes in hormone concentrations and general metabolism adjusting for the new lactation cycle. The insulin concentration of plasma decreases and the insulin sensitivity of adipose tissue and muscle is reduced (Holtenius et al., 2003). The decrease in insulin sensitivity is likely caused by several factors interacting synergistically; for instance, the increase in growth hormone, the release of proinflammatory cytokines from different tissues (Bell and Bauman, 1997) causing an increase in lipolysis with the consequent elevation of plasma NEFA concentration . Nonesterified FA are used as fuels for the liver and extrahepatic tissues in order to spare glucose for fetal development, thus maintaining euglycemia, and as precursor for FA synthesis in the mammary gland close to parturition (Allen and Piantoni, 2014). However, excessive lipolysis during the periparturient period can result in increased risk for metabolic disorders, such as clinical ketosis, fatty liver and a chronic inflammatory reaction, with the concomitant decrease in DMI aggravating the NEB (Grummer, 2008).

Fatty acids oxidation

Fatty acids are the primary fuels oxidized in the liver. Uptake of NEFA by the liver increases greatly after calving (Reynolds et al., 2003) as a result of their proportional increase in concentration in circulation. Fatty acids are oxidized through β-oxidation in the mitochondria or in the cytosol by peroxisomes or microsomes. Fatty acids also can be re-esterified for later oxidation or exported as very low-density lipoproteins. However, ruminant liver has less capacity to export triacylglycerides compare with other species, and excessive lipid mobilization can cause lipid accumulation that compromises liver function; increasing the risk of metabolic

disorders (Kleppe et al., 1988; Zhu et al., 2000).

To undergo β-oxidation, long-chain FA are transported into mitochondria bound to carnitine acyl transferase. The activity of this enzyme is controlled by various factors such as the availability of the enzyme and the presence of inhibitory metabolites like malonyl CoA and methylmalonyl CoA, one of the intermediates in FA synthesis and an intermediate in propionate metabolism, respectively. The end products of β-oxidation are mostly AcCoA and reduced cofactors (NADH and $FADH_2$) that are re-oxidized through oxidative phosphorylation (**OXPHOS**) for the generation of ATP.

Acetyl CoA is a compound shared in the metabolic pathway for most of the fuels with the ability to be oxidized, entering the TCA cycle in the citrate synthase reaction to complete oxidation to CO_2 and water. ATP and reducing equivalents also are produced with the latter being converted to ATP through OXPHOS. The production of CO_2 stimulates OXPHOS by bicarbonate production (Acin-Perez et al., 2009). During the postpartum period, when cows are in a lipolytic state, liver increases the uptake of NEFA increasing hepatic energy charge as a result of the complete FA metabolism through β-oxidation, TCA cycle and OXPHOS. According to the HOT, when feeding behavior is mostly regulated by metabolic control, temporal pattern of absorption of fuels, mobilization of body reserves and oxidation in the liver can alter meal size and meal frequency affecting feeding behavior and feed intake. All metabolic processes stimulating oxidative pathways in the liver promote the increase in hepatic energy charge and decrease intake (Allen et al., 2009). Excess of AcCoA that cannot be oxidized in the TCA cycle can be metabolized to ketone bodies and exported from the liver and be used as an alternative energy source for some extrahepatic tissues.

The capacity to oxidize AcCoA in the TCA cycle is regulated by the availability of intermediates of the cycle. Because the liver is a key organ in the control of metabolism, the TCA cycle not only contributes to oxidation of metabolites, but also in other biosynthetic pathways in which intermediates leaving the cycle are mostly converted to glucose or non-essential amino acids. This process is called cataplerosis, and all reactions tending to replace the TCA cycle intermediates, allowing for its continued function, are called anaplerotic reactions (Owen et al., 2002). Hence, the regulation of the entrance and exit of intermediates to the TCA cycle depends upon the metabolic and physiologic state, and the specific tissue or organ involved. Therefore, the capacity for hepatocytes to oxidize AcCoA is affected by the concentrations and flux of TCA cycle intermediates as wells as by the concentrations and activity of the enzymes required for oxidation.

Gluconeogenic precursors and effect on DMI

Availability and partitioning of gluconeogenic precursors and their effects on DMI depend on the physiological state and glucose requirements of the animal. Quantitatively important substrates for hepatic gluconeogenesis in the order of hepatic uptake are propionate (60 to 74%), L-lactate (16 to 26%), valerate and isobutyrate (5 to 6%), alanine (3 to 5%), glycerol (0.5 to 3%), and other amino acids (8 to 11%) (Aschenbach et al., 2010).

Amino acids

Although skeletal muscle is a reservoir of amino acids, amino acids released after fasting or during severe periods of NEB, do not reflect the amino acid composition of the skeletal muscle proteins. Local metabolism in muscle appears to interconvert amino acids specifically to alanine and glutamine, which therefore represent a disproportional increase in the fraction of amino acids in blood circulation (Owen et al., 2002). There is an important contribution of

alanine (3 to 5%) to the total hepatic uptake of gluconeogenic precursors by the liver in the postpartum period (Reynolds et al., 2003; Larsen and Kristensen, 2009).

Amino acids are used mainly for protein synthesis, however, when protein synthesis is limited by deficiency of individual amino acids or amino acids are supplied in excess, the amino acid surplus is deaminated and the carbon chains can enter the TCA cycle via anaplerotic reactions and promote oxidation or be oxidized as fuels and likely affect intake. Ammonia and urea were hypophagic (Mugerwa and Conrad, 1971) and intraruminal infusion of ammonium salts of VFA compared with potassium salts decreased DMI by decreasing the number of meals without affecting meal size (Oba and Allen, 2003d); furthermore, the effects were increased when propionate salts were infused compared with acetate salts (Oba and Allen, 2003b). A possible explanation of this effect consistent with the HOT is that gluconeogenesis from propionate is decreased by ammonia, thus increasing the availability of propionate to be oxidized. In addition, ammonia detoxification through urea synthesis generates additional anaplerotic carbon sources to be oxidized (Oba and Allen, 2003b). In sum, both explanations increase hepatic energy charge and decrease intake (Allen et al., 2009).

Glycerol

Glycerol as a feed ingredient was used mostly to replace starch sources or as a possible alternative treatment for metabolic disorders in periods of NEB. The inclusion of glycerol in diets ranges from less than 1 up to 15% of DM with responses related to the level of inclusion and stage of lactation. Inclusion at 3.6% of the DM in mid-lactation cows had no effect on milk production or composition but it altered concentration of ruminal fermentation end products, increasing propionate and butyrate at the expense of acetate (Khalili et al., 1997). In a more recent study on mid-lactation cows, Donkin et al. (2009), fed diets containing 0, 5, 10, or 15% of

glycerol for 56 days with no difference in production responses except for a slight reduction in feed intake during the first week of the study for the 15% glycerol inclusion treatment (treatment x time effect). In a transition period study, DeFrain et al. (2004) reported a 17% decrease in DMI in the prepartum period and no effect on milk production or DMI after calving when glycerol was fed at 5.4% of the ration DM from 2 weeks before calving to 3 weeks after calving. However, Carvalho et al. (2011), replaced high moisture corn with glycerol at ~11% of the dietary DM from 4 weeks before calving to 8 weeks after calving with no effect on intake. On the other hand, Bodarski et al. (2005), reported increased milk yield and milk protein content when feeding glycerol at 3.1% of DM from 3 weeks before calving to 70 days after calving.

It is important to clarify that glycerol is mostly fermented in the rumen when added as a feed ingredient and has little or no opportunity to reach the liver through portal circulation. When glycerol is administered as a drench or infused for treatment of ketosis, it ideally should be absorbed mostly intact from the rumen or escape ruminal fermentation to be absorbed from the small intestine. Glycerol is metabolized in the liver and enters the cytosolic gluconeogenic pathway as triose-phosphate without the need of entering the TCA cycle, bypassing the metabolic steps with limiting enzymes (Johnson, 1954). When glycerol and propylene glycol were infused into the abomasum, glycerol was more effective at increasing glucose and insulin concentrations; however, when they were infused into the rumen, propylene glycol was more effective at increasing insulin and glucose concentrations in plasma. Abomasal infusions of both precursors tended to decrease ß-hydroxybutyrate concentration in plasma without an effect on DMI (Piantoni and Allen, 2015). In a second experiment where propylene glycol and glycerol were dosed into the cranial rumen, ~30 % more glycerol than propylene glycol (5.2 mol vs. 3.8

mol) was required to observe similar effects on plasma concentrations of glucose and insulin with both glucose precursors (Piantoni and Allen, 2015).

Glycerol, an endogenous gluconeogenic source, is mobilized from adipose tissue along with NEFA during ketosis, starvation or other periods of body fat mobilization. Glycerol turnover rate is highly increased during ketosis and starvation compared with the fed state (Aschenbach et al., 2010).

The effect of glycerol on feed intake is consistent with the control of feed intake by the HOT. At high dietary levels of inclusion, glycerol is fermented to propionate causing hypophagia, and when it is administered as a drench or infused, some of it likely escapes ruminal fermentation and is used directly as a glucose precursor in the liver, thus with less hypophagic effect.

Lactate

Some lactate is consumed via diets containing silages and fermented products, or may be produced by ruminal fermentation of starch or anaerobic metabolism of glucose by splanchnic tissues and erythrocytes (Aschenbach et al., 2010). In starvation periods or during the periparturient period, when cows are in a low glucose status, the release of gluconeogenic carbon from peripheral tissues is increased and lactate becomes the main endogenous gluconeogenic substrate (De Koster and Opsomer, 2013; Aschenbach et al., 2010). At the end of gestation the uterus and placenta are important sources of lactate. In skeletal muscle the expression of lactate dehydrogenase, the enzyme which catalyze the conversion of pyruvate into lactate is upregulated, whereas the expression of the main enzymes of the TCA cycle are downregulated (De Koster and Opsomer, 2013).

Lactate is a fuel extracted by the liver at a rate related to the ratio of NAD to NADH in the hepatic tissue, with less absorption when the ratio is decreased (Allen et al., 2009). During the postpartum period and starvation periods, when insulin concentrations in circulation are low, liver uptake of lactate is increased (Reynolds et al., 2003; Brockman and Laarveld, 1986), with the highest contribution around the second week after calving (Reynolds et al., 2003). Rate of uptake of lactate decreases as lactation progresses and as intake increases, and gluconeogenesis becomes more dependent on the flux of propionate to the liver rather than endogenous sources (Reynolds et al., 2003; Aschenbach et al., 2010).

Lactic acid can supply between 10 to 26% of gluconeogenic substrates (Reynolds et al., 2003; Larsen and Kristensen, 2009) and lactate enters the TCA cycle through conversion to pyruvate and then to oxaloacetic acid in an anaplerotic reaction promoting oxidation of AcCoA, increasing energy charge in the liver and decreasing feed intake (Allen et al., 2009). Oxaloacetic acid also can leave the TCA cycle through conversion to phosphoenol pyruvate and follow the gluconeogenic pathway. Lactate is expected to have a smaller effect on decreasing feed intake than propionate (Allen et al., 2009), considering that extraction of lactate by the liver is less efficient than propionate (Reynolds et al., 2003).

Propionic acid

Of the VFA produced by ruminal fermentation, only propionate, valerate, and isobutyrate can serve as glucogenic precursors for net synthesis of glucose. Propionate is the most abundant of the three-glucogenic acids (15 to 40% of total ruminally released organic acids) and by far the predominant substrate for gluconeogenesis in ruminants. The production and absorption of propionate are greatest after feeding; gluconeogenesis in ruminants also is greatest after periods of high energy intake (Aschenbach et al., 2010).

Of the fuels produced during fermentation of the diet, propionate is most likely to stimulate satiety. Propionate can be produced and absorbed rapidly (Benson et al., 2002) within a time frame of a meal. Activation with CoA is necessary for metabolism of FA. Propionate is readily extracted from the blood by the liver as a result of the high activity of propionyl CoA synthetase in ruminants, and the low activity of hepatic acetyl CoA synthetase could explain, in part, the differences in hypophagic effects of infusions of propionate and acetate in ruminants (Ricks and Cook, 1981). Even though butyrate can be oxidized in the liver, its net metabolism is usually less than propionate or acetate because its ruminal production is lower and it is mostly oxidized by ruminal epithelia (Weigand et al., 1975).

Propionate is an anaplerotic compound and promotes AcCoA oxidation in the TCA cycle in the liver unlike acetate (Allen and Piantoni, 2013). Propionate enters the TCA cycle as succinyl CoA, promoting oxidation of AcCoA and increasing hepatic energy charge, and likely decreasing the firing rate of the vagus nerve, thus signaling satiety. Total liver denervation in sheep and splachnic blockade using anesthetic, bilateral splanchnotomy, and hepatic vagotomy eliminates the hypophagic effect of portal infusion of propionate (Anil and Forbes, 1988).

Isosmotic mixtures of propionate infused continuously into the rumen in lactating cows linearly decreased metabolizable energy intake by decreasing meal size compared with acetate (Oba and Allen, 2003e). Similarly propionate infusions at spontaneous meals decreased meal size compared with acetate (Choi and Allen, 1999), and the degree of hypophagia from propionic acid increased with increased hepatic AcCoA content (Stocks and Allen, 2012; 2013).

When ruminal propionate production exceeds hepatic gluconeogenic capacity it is likely that some propionate could be oxidized. When glucose demands are increased (peak of

lactation), propionate is mainly used for gluconeogenesis, reducing the possibility for propionate oxidation within meals, and extending the length and size of meals. In the contrary, when glucose demand is low (e.g., past peak of lactation), propionate could be oxidized within or during actual meals, resulting in satiety and smaller meal size (Oba and Allen, 2003c). However, propionate oxidation during the PP period is less likely because of the high availability of hepatic AcCoA for oxidation (Allen, 2014) and the consequent down regulation of pyruvate dehydrogenase complex activity.

STARCH FERMENTABILITY AND EFFECT ON FEED INTAKE IN THE POSTPARTUM PERIOD

Because starch constitutes up to 35% of the dietary DM for lactating cows, its ruminal fermentability can have a great effect on the type and pattern of absorbed fuels. The ruminal fermentation and small intestine digestion of starch is affected by several factors, such as particle size, physical processing, and amount and solubility of endosperm proteins (Kotarski et al., 1992; Larson and Hoffman, 2008)). Different grain types and different hybrids from the same type of grain differ in proportion and solubility of endosperm proteins affecting the rate of fermentation and site of starch digestion (Allen, 2000). All physical processes (e.g., rolling and grinding) that increase surface area or chemical treatment (e.g., steaming that gelatinizes starch) that increase accessibility for ruminal microorganisms increase the rate of starch fermentation (Owens et al., 1986; Huntington, 1997). The ensiling process also increases fermentability by solubilizing endosperm proteins, and greater moisture content at the time of ensiling and longer periods of storage before use of ensiled material can further augment starch fermentability (Oba and Allen, 2003a).

In accordance with the rate of digestion and passage, dietary starch can be digested in

different sections of the gastrointestinal tract in ruminants. The site of digestion determines the principal end products of starch digestion. It can be fermented partially by microorganisms in the rumen and large intestine, and digested in the small intestine by pancreatic amylases. Ruminal fermentability of starch can range from ~25% to 90% (Allen, 2000), increasing the total production of VFA as fermentability increases. However, when modifying ruminal starch fermentation, propionic acid is the VFA that varies more when compared with acetic and butyric acid. Propionic acid production could increase from 6 to 52 moles/d (Allen, 1997) because its concentration in the rumen can range from 15 to 45% of total fermentation acids based on organic matter fermented in the rumen (Davis, 1967). Starch that passes through the rumen can be digested in the small intestine by the action of pancreatic and intestinal enzymes. Digestion at the level of the small intestine increases the absorption of glucose directly, although most is metabolized to lactate by intestinal tissues (Reynolds et al., 2003). Depending on the site of digestion and digestibility of starch, the type of fuel produced and the patterns of absorption are affected greatly.

Diets with greater ruminal starch fermentability can depress feed and energy intakes. Increasing the amount of a more fermentable starch source in the diet could reduce feed intake of cows nearly 3 kg/d, likely as a result of the increased flow of propionate to the liver (Allen, 2000). More fermentable starch sources increase ruminal propionate production compared with less fermentable starch sources.

Starch fermentability of the diet interacts with the physiological state of the cow and can affect energy intake differently. High-moisture corn, a more fermentable starch source, decreased feed intake by 7.6% (20.8 vs. 22.5 kg/d) in early lactation cows compared with dry ground corn, a less fermentable source, by reducing meal size in diets containing 32% of starch (Oba and Allen,

2003a). The decrease in meal size is likely because of a more rapid flux of propionate to the liver and the stimulation of oxidation in the hepatocyte signaling for satiety (Allen et al., 2009). However, propionate is more hypophagic for early postpartum cows than cows in mid-lactation. After parturition, cows are in a lipolytic state with high plasma NEFA concentration, increased glucose demand and low concentration of insulin in plasma causing an increase in glucogenic flux. The increase of propionate supply to the liver is likely used for gluconeogenic purposes more than its oxidation. However, the metabolic pathway of propionate makes it an obligatory anaplerotic compound and it likely promotes the oxidation of the increased pool of hepatic AcCoA derived from ß-oxidation, which in turn increases the energy charge in the liver and induces satiety; thus decreasing meal size and likely total DMI. In a recent study, Albornoz and Allen (2016) evaluated the effect of starch content (22 and 28%) and fermentability on cows during the first 3 weeks of lactation. Intake increased by 2.2 kg/d when diets formulated with dry ground corn (less fermentable starch source) compared with high-moisture corn (more fermentable starch source) diets (19.7 kg/d vs. 17.1 kg/d, P <0.01). They reported an interaction between starch content and starch fermentability, where high starch diets tended to increase DMI 3.4 kg/d (20.0 vs. 16.6 kg/d) compared with the low starch diets which increased DMI 1 kg/d (18.6 vs. 17.6 kg/d).

Therefore, this review presents support that the hepatic oxidation of fuels is involved in the control of feed intake in the postpartum period and shows that ruminal fermentability of starch should be an important consideration for diets formulated for cows in the immediate postpartum period.

Our long-term goal is to understand the metabolism of fuels in the liver and their integrating effects on feeding behavior to develop feeding strategies to achieve a transition from

gestation to lactation with a better EB and with less metabolic problems. Our main objective is to determine the effects of different fuels derived from ruminal fermentation and digestive processes during the postpartum period on feeding behavior and energy intake. The results of this new research will aid in the formulation of diets to increase energy intake during the postpartum period, reduce risk of metabolic disorders after calving, and improve the efficiency in nutrient utilization.

BIBLIOGRAPHY

BIBLIOGRAPHY

Acin-Perez, R., E. Salazar, M. Kamenetsky, J. Buck, L.R. Levin, and G. Manfredi. 2009. Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. Cell Metab. 9:265–276. doi:10.1016/j.cmet.2009.01.012.

Albornoz, R.I., and M.S. Allen. 2016. Diet starch content and fermentability affects feed intake and milk yield of cows in the postpartum period. J. Anim. Sci. 94:362. doi:10.2527/jam2016-0754.

Allen, M.S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74:3063-3075

Allen, M.S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447–1462. doi:10.3168/jds.S0022-0302(97)76074-0.

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. doi:10.3168/jds.S0022-0302(00)75030-2.

Allen, M.S. 2014. Drives and limits to feed intake in ruminants. Anim. Prod. Sci. 54:1513–1524. doi:10.1071/AN14478.

Allen, M.S., and B.J. Bradford. 2012. Control of food intake by metabolism of fuels: a comparison across species. Proc Nutr Soc. 71:401–409. doi:10.1017/S0029665112000572.

Allen, M.S., and P. Piantoni. 2013. Metabolic control of feed intake. Vet. Clin. North Am. Food Anim. Pract. 29:279–297. doi:10.1016/j.cvfa.2013.04.001.

Allen, M.S., and P. Piantoni. 2014. Carbohydrate nutrition. Vet. Clin. North Am. Food Anim. Pract. 30:577–597. doi:10.1016/j.cvfa.2014.07.004.

Allen, M.S., B.J. Bradford, and K.J. Harvatine. 2005. The cow as a model to study food intake regulation. Annu. Rev. Nutr. 25:523–547. doi:10.1146/annurev.nutr.25.050304.092704.

Allen, M.S., B.J. Bradford, and M. Oba. 2009. BOARD-INVITED REVIEW: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci.. 87:3317–3334. doi:10.2527/jas.2009-1779.

Anil, M.H., and J.M. Forbes. 1988. The roles of hepatic nerves in the reduction of food intake as a consequence of intraportal sodium propionate administration in sheep. Vet. Q. 73:539–546.

Anil, M.H, J.N. Mbanya, H.W. Symonds, and J.M. Forbes. 1993. Responses in the voluntary intake of hay or silage by lactating cows to intra-ruminal infusions of sodium acetate or sodium propionate, the tonicity of rumen fluid or rumen distension. Br. J. Nutr. 73:539-

546.

Aschenbach, J.R., N.B. Kristensen, S.S. Donkin, H.M. Hammon, and G.B. Penner. 2010. Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. IUBMB Life. 62:869–877. doi:10.1002/iub.400.

Baile, C.A., and J.M. Forbes. 1974. Control of feed intake and regulation of energy balance in ruminants. Physiol. Rev. 54:160–214.

Bauman, D.E. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75:3432-3451

Bell, A.A.W. 1979. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. Prog. Lipid Res. 18:117–164.

Bell, A.W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bell, A.W., and D.E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. Neoplasia. 2:265–278.

Benson, J.A., C.K. Reynolds, P.C. Aikman, B. Lupoli, and D.E. Beever. 2002. Effects of abomasal vegetable oil infusionon splanchnic nutrient metabolism in lactating dairy cows. J. Dairy Sci. 85:1804–1814. doi:10.3168/jds.S0022-0302(02)74255-0.

Bergen, W.G. 1972. Rumen osmolality as a factor in feed intake control of sheep. J. Anim. Sci. 34:1054–1060.

Bodarski, R., T. Wertelecki, F. Bommer, and S. Gosiewski. 2005. The changes of metabolic status and lactation performance in dairy cows under feeding TMR with glycerin (glycerol) supplement at periparturient period. EJPAU. 8:22.

Bradford, B.J., K.J. Harvatine, and M.S. Allen. 2008. Dietary Unsaturated Fatty Acids Increase Plasma Glucagon-Like Peptide-1 and Cholecystokinin and May Decrease Premeal Ghrelin in Lactating Dairy Cows. J. Dairy Sci. 91:1443–1450. doi:10.3168/jds.2007-0670.

Bradford, B.J., M. Oba, R.A. Ehrhardt, Y.R. Boisclair, and M.S. Allen. 2006. Propionate is not an important regulator of plasma leptin concentration in dairy cattle. Domest. Anim. Endocrinol. 30:65–75. doi:10.1016/j.domaniend.2005.05.007.

Brandt, K., M. Arnold, N. Geary, W. Langhans, and M. Leonhardt. 2007. Vagal afferents mediate the feeding response to mercaptoacetate but not to the beta (3) adrenergic receptor agonist CL 316,243. Neurosci. Lett. 411:104–107. doi:10.1016/j.neulet.2006.10.034.

Brockman, R., and B. Laarveld. 1986. Effect of insulin on gluconeogenesis and the metabolism of lactate in sheep. Can. J. Physiol. Pharmacol. 64:1055–1059.

Carvalho, E.R., N.S. Schmelz-Roberts, H.M. White, P.H. Doane, and S.S. Donkin. 2011.

Replacing corn with glycerol in diets for transition dairy cows. J. Dairy Sci. 94:908–916. doi:10.3168/jds.2010-3581.

Choi, B.R., and M.S. Allen. 1999. Intake regulation by volatile fatty acids and physical fill. S. Afr. J. Anim. Sci. 29:40–41(abstr.).

De Koster, J.D. and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 29:299-322. doi.org/10.1016/j.cvfa.2013.04.002

Dado, R.G., and M.S. Allen. 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. J. Dairy Sci. 77:132–144. doi:10.3168/jds.S0022-0302(94)76936-8.

Davis, C.L. 1967. Acetate production in the rumen of cows fed either control or low-fiber, high-grain diets. J. Dairy Sci. 50:1621–1625. doi:10.3168/jds.S0022-0302(67)87683-5.

Deetz, L.E., and P.J. Wangsness. 1981. Influence of intrajugular administration of insulin, glucagon and propionate on voluntary feed intake of sheep. J. Anim. Sci. 53:427–433.

DeFrain, J.M., A.R. Hippen, K.F. Kalscheur, and P.W. Jardon. 2004. Feeding glycerol to transition dairy cows: effects on bloodmetabolites and lactation performance. J. Dairy Sci. 87:4195–4206. doi:10.3168/jds.S0022-0302(04)73564-X.

Donkin, S.S., S.L. Koser, H.M. White, P.H. Doane, and M.J. Cecava. 2009. Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. J. Dairy Sci. 92:5111–5119. doi:10.3168/jds.2009-2201.

Douglas, G.N., T.R. Overton, H.G. Bateman II, H.M. Dann, and J.K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in holstein cows. J. Dairy Sci. 89:2141–2157. doi:10.3168/jds.S0022-0302(06)72285-8.

Ehrhardt, R.A., R.M. Slepetis, J. Siegal-Willott, M.E. Van Amburgh, A.A.W. Bell, and Y.R. Boisclair. 2000. Development of a specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. J. Endocrinol. 166:519–528.

Emmanuel, B. 1981. Glucokinase, hexokinase, gluconeogenesis, glycogenesis and glycolysis in camel (*Camelus dromedarius*) and sheep (*Ovis aries*). Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 64:547–550.

Faulkner, A., and P. Martin. 1998. Changes in the concentrations of glucagon-like peptide-1(7–36)amide and gastric inhibitory polypeptide during the lactation cycle in goats. J. Dairy Res. 65:433–441.

Fisher, D.S. 2002. A review of a few key factors regulating voluntary feed intake in ruminants. Crop Sci. 42:1651–1655.

Forbes, J.M. 2007. Voluntary food intake and diet selection in farm animals. 2nd ed. C.

International, editor. Oxon, UK. 462 pp.

Forbes, J.M., J.N. Mbanya, and M.H. Anil. 1992. Effects of intraruminal infusions of sodium acetate and sodium chloride on silage intake by lactating cows. Appetite. 19:293–301.

Friedman, M. 1998. Fuel partitioning and food intake. the Am. J. Clin. Nut. 67(suppl):513–518.

Frobish, R.A., and C.L. Davis. 1977. Effect of abomasal infusions of glucose and proprionate on milk yield and composition. J. Dairy Sci. 60:204–209.

Grant, R.J., and J.L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. J. Anim. Sci. 73:2791–2803.

Grummer, R.R. 2008. Nutritional and management strategies for the prevention of fatty liver in dairy cattle. Vet. J. 176:10–20. doi:10.1016/j.tvjl.2007.12.033.

Harding, R.H., and B.F. LeeK. 1972. Rapidly adapting mechanoreceptors in the reticulo-rumen which also respond to chemicals. J. Physiol. 223:32P–33P.

Holst, J.J. 1997. Enteroglucagon. Annu. Rev. Physiol. 59:257–271.

Holtenius, K., S. Agenas, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J. Dairy Sci. 86:883-891.

Horn, C.C., H. Ji, and M.I. Friedman. 2004. Etomoxir, a fatty acid oxidation inhibitor, increases food intake and reduces hepatic energy status in rats. Physiol. Behav. 81:157–162. doi:10.1016/j.physbeh.2004.01.007.

Houseknecht, K.L., C.A. Baile, R.L. Matteri, and M.E. Spurlock. 1998. The biology of leptin: A review. J. Anim. Sci. 76:1405–1420.

Huntington, G.B. 1997. Starch utilization by ruminants: from basics to the bunk. J. Anim. Sci. 75:852–867.

Illius, A.W., and N. Jessop. 1996. Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci. 74:3052–3062.

Johnson, R.B. 1954. The treatment of ketosis with glycerol and propylene glycol. Cornell Vet. 44:6–21.

Khalili, H., T. Varvikko, V. Toivonen, and M. Suvitie. 1997. The effects of added glycerol or unprotected free fatty acids or a combination of the two on silage intake, milk production, rumen fermentation and diet digestibility in cows given grass silage based diets. Agric. Food Sci. 6:349–362.

Kleppe, B.B., R.J. Aiello, R.R. Grummer, and L.E. Armentano. 1988. Triglyceride accumulati and very low density lipoprotein secretion by rat and goat hepatocytes in vitro. J. Dairy Sci. 71:1813–1822.

Kotarski, S.F., R.D. Waniska, and K.K. Thurn. 1992. Starch hydrolysis by the ruminal microflora. J. Nutr. 122:178–190.

Kristensen, N.B. 2005. Splanchnic metabolism of volatile fatty acids in the dairy cow. J. Anim. Sci. 80:3–10. doi:10.1016/0026-0495(72)90028-5.

Langhans, W. 1996. Role of the liver in the metabolic control of eating: what we know-and what we do not know. Neurosci. Biobehav. Rev. 20:145–153.

Langhans, W. 2008. Fatty acid oxidation in the energostatic control of eating - A new idea. Appetite. 51:446–451. doi:10.1016/j.appet.2008.06.014.

Larsen, M., and N.B. Kristensen. 2009. Effect of abomasal glucose infusion on splanchnic amino acid metabolism in periparturient dairy cows. J. Dairy Sci. 92:3306–3318. doi:10.3168/jds.2008-1889.

Larson, J., and P.C. Hoffman. 2008. Technical note: a method to quantify prolamin proteins in corn that are negatively related to starch digestibility in ruminants. J. Dairy Sci. 91:4834–4839. doi:10.3168/jds.2008-1378.

Leek, B.F., and R.H. Harding. 1975. Sensory nervous receptors in the ruminant stomach and the reflex control of reticulo-ruminal motility. In Digestion and metabolism in the ruminant. I.W. McDonald and A.C.I. Warner, editors. University of New England Publishing Unit, Armidale, Australia. 60–76.

Mbanya, J.N., M.H. Anil, and J.M. Forbes. 1993. The voluntary intake of hay and silage by lactating cows in response to ruminal infusion of acetate or propionate, or both, with or without distension of the rumen by a balloon. British Journal of Nutrition. 69:713–720.

McCann, J.P., E.N. Bergman, and D.H. Beermann. 1992. Dynamic and static phases of severe dietaryobesity in sheep: food intakes, endocrinology and carcass and organ chemical composition. J. Nutr. 122:496–505.

McDonald, P., R.A. Edwards, J.F.D. Greeham, and C.A. Morgan. 2002. Animal nutrition. 5 ed. John Wiley and Sons Inc, New York.

Mugerwa, J., and H.R. Conrad. 1971. Relationship of dietary nonprotein nitrogen to urea kinetics in dairy cows. J. Nutr. 101:1331–1342.

Murondoti, A., R. Jorritsma, A.C. Beynen, T. Wensing, and M.J.H. Geelen. 2004. Unrestricted feed intake during the dry period impairs the postpartum oxidation and synthesis of fatty acids in the liver of dairy cows. J. Dairy Sci. 87:672–679. doi:10.3168/jds.S0022-0302(04)73210-5.

Oba, M., and M.S. Allen. 2003a. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174–183. doi:10.3168/jds.S0022-0302(03)73598-X.

Oba, M., and M.S. Allen. 2003b. Hypophagic effects of ammonium are greater when infused with propionate compared with acetate in lactating dairy cows. J. Nutr. 133:1100–1104.

Oba, M., and M.S. Allen. 2003c. Extent of hypophagia caused by propionate infusion is related to plasma glucose concentration in lactating dairy cows. J. Nutr.133:1094–1099.

Oba, M., and M.S. Allen. 2003d. Effects of intraruminal infusion of sodium, potassium, and ammoniumon hypophagia from propionate in lactating dairy cows. J. Dairy Sci. 86:1398–1404. doi:10.3168/jds.S0022-0302(03)73723-0.

Oba, M., and M.S. Allen. 2003e. Dose-response effects of intrauminal infusion of propionateon feeding behavior of lactating cows in early or midlactation. J. Dairy Sci. 86:2922–2931. doi:10.3168/jds.S0022-0302(03)73889-2.

Ospina, P.A., D.V. Nydam, T. Stokol, and T.R. Overton. 2010. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and β-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J. Dairy Sci. 93:3595–3601 doi: 1 0.3168/jds.2010-3074

Owen, O.E., S.C. Kalhan, and R.W. Hanson. 2002. The key role of anaplerosis and cataplerosis for citric acid cycle function. J. Biol. Chem. 277:30409–30412.doi:10.1074/jbc.R200006200.

Owens, F.N., R.A. Zinn, and Y.K. Kim. 1986. Limits to Starch Digestion in the Ruminant Small Intestine. J. Anim. Sci. 63:1634–1648.

Piantoni, P., and M.S. Allen. 2015. Evaluation of propylene glycol and glycerol infusions as treatments for ketosis in dairy cows. J. Dairy Sci. 98:5429–5439. doi:10.3168/jds.2015-9476.

Piantoni, P., C.M. Ylioja, and M.S. Allen. 2015. Feed intake is related to changes in plasma nonesterified fatty acid concentration and hepatic acetyl CoA content following feeding in lactating dairy cows. J. Dairy Sci. 98:6839–6847. doi:10.3168/jds.2014-9085.

Rawson, N., H. Blum, M.D. Osbakken, and M. Friedman. 1994. Phosphate loading prevents the decrease in ATP and increase in food intake produced by 2,5-anhydro-D-mannitol. Am. J. Physiol. 226:R112–R117.

Relling, A.E., and C.K. Reynolds. 2007. Plasma concentrations of gut peptides in dairy cattleincrease after calving. J. Dairy Sci. 90:325–330. doi:10.3168/jds.S0022-0302(07)72633-4.

Relling, A.E., and C.K. Reynolds. 2008. Abomasal infusion of casein, starch and soybean oil differentially affect plasma concentrations of gut peptides and feed intake in lactating dairy cows. Domest. Anim. Endocrinol. 35:35–45. doi:10.1016/j.domaniend.2008.01.005.

Reynolds, C. K. 2005. Glucose balance in cattle. Florida Ruminant Nutrition Symposium. http://dairy.ifas.ufl.edu/rns/2005/Reynolds.pdf Accessed Nov. 14, 2005.

Reynolds, C.K., P.C. Aikman, B. Lupoli, D.J. Humphries, and D.E. Beever. 2003. Splanchnic metabolism of dairy cows during the transitionfrom late gestation through early lactation. J. Dairy Sci. 86:1201–1217. doi:10.3168/jds.S0022-0302(03)73704-7.

Ricks, C.A., and R.M. Cook. 1981. Regulation of volatile fatty acid uptake by mitochondrial acyl CoA synthetases of bovine heart. J. Dairy Sci. 64:2336–2343. doi:10.3168/jds.S0022-0302(81)82855-X.

Roche, J.R., A.J. Sheahan, L.M. Chagas, and R.C. Boston. 2008. Short communication: change in plasma ghrelin in dairy cows following an intravenous glucose challenge. J. Dairy Sci. 91:1005–1010. doi:10.3168/jds.2007-0571.

Schettini, M.A., E.C. Prigge, and E.L. Nestor. 1999. Influence of mass and volume of ruminal contents on voluntary intake and digesta passage of a forage diet in steers. J. Anim. Sci. 77:1896–1904.

Schroder, A., and K.H. Sudekum. 1999. Glycerol as a by-product of biodiesel production in Diets for ruminants. M. Wratten and P.A. Salisbury, editors. Canberra, Australia. 1–7.

Scott, D. 1975. Changes in mineral, water and acid-base balance associeated with feeding and diet. I.W. McDonald and A.C.I. Warner, editors. University of New Enggland Publishing Unit, Armidale, NSW, Australia. 205–215.

Seal, C.J., and C.K. Reynolds. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutr. Res. Rev. 6:185–208. doi:10.1079/BJN19800116.

Simmons, C.R., W.G. Bergen, M.J. Vandehaar, D.J. Sprecher, C.J. Sniffen, E.P. Stanisiewski, and H.A. Tucker. 1994. Protein and fat metabolismo in cows given somavubove before parturition.

Stangassinger, M., and D. Giesecke. 1986. Splanchnic metabolism of glucose and related energy substrates. In Control of digestion and metabolism in ruminants. Milligan, L. P., W.L. Grovum, and A. Dobson, editors. Prentice Hall, Englewood Clifs, NJ. 347–366.

Stocks, S.E., and M.S. Allen. 2012. Hypophagic effects of propionate increase with elevated hepatic acetyl coenzyme A concentration for cows in the early postpartum period. J. Dairy Sci. 95:3259–3268. doi:10.3168/jds.2011-4991.

Stocks, S.E., and M.S. Allen. 2013. Hypophagic effects of propionic acid are not attenuated during a 3-day infusion in the early postpartum period in Holstein cows. J. Dairy Sci. 96:4615–4623. doi:10.3168/jds.2013-6653.

Ternouth, J.H., and A.W. Beattie. 1971. Studies of the food intake of sheep at a single meal. Br. J. Nutr. 25:153–171. doi:10.1079/BJN19710073.

Tordoff, M.G., N. Rawson, and M. Friedman. 1991. 2,5-anhydro-D-mannitol acts in liver to initiate feeding. Am. J. Physiol. 261:R283–R288.

Vandermeerschen-Doize, F., J.C. Bouchat, M.A. Bouckoms-Vandermeir, and R. Paquay. 1983. Effects of long-term ad libitum feeding on plasma lipid components and blood glucose, ß-hydroxybutyrate and insulin concentrations in lean adult sheep. Reprod. Nutr. Dev. 23:51–63.

Warner, A.C.I., and B.D. Stacy. 1972. Water, sodium and potassium movements across the rumen wall of sheep. Exp. Physiol. 57:103–119.

Weigand, E., J.W. Young, and A.D. McGilliard. 1975. Volatile fatty acid metabolism by rumen mucosa from cattle fed hay or grain. J. Dairy Sci. 58:1294–1300.

Zhu, L.H., L.E. Armentano, D.R. Bremmer, R.R. Grummer, and S.J. Bertics. 2000. Plasma concentration of urea, ammonia, glutamine around calving, and the relation of hepatic triglyceride, to plasma ammonia removal and blood acid-base balance. J. Dairy Sci. 83:734–740. doi:10.3168/jds.S0022-0302(00)74935-6.

CHAPTER 2. INCREASED ANAPLEROSIS OF THE TRICARBOXYLIC ACID CYCLE DECREASED MEAL SIZE AND ENERGY INTAKE OF COWS IN THE POSTPARTUM PERIOD

We evaluated effects of anaplerosis of the tricarboxylic acid cycle on feeding behavior and energy intake of cows in the postpartum period using abomasal infusions of glycerol and propionic acid. Both are three-carbon glucose precursors with similar energy contents but propionic acid is obligatorily anaplerotic while glycerol is not. Propionic acid decreased meal size and total energy intake compared with glycerol. For a full text of this work see Laura B. Gualdrón-Duarte and Michael S. Allen. 2017. J. Dairy Sci. 100:4425-4434.

CHAPTER 3. FUELS DERIVED FROM STARCH DIGESTION HAVE DIFFERENT EFFECTS ON ENERGY INTAKE AND METABOLIC RESPONSES OF COWS IN THE POSTPARTUM PERIOD

ABSTRACT

Absorbed fuels from the digestion of starch include propionic acid (PA) produced by ruminal fermentation and glucose (GLU) from intestinal digestion, which is partially metabolized to lactic acid (LA) by intestinal tissues. Our objective was to evaluate the effects of these fuels on dry matter intake (DMI) and feeding behavior of cows in the postpartum period. We hypothesized that these fuels affect feed intake differently and that their effects are related to their capacity to stimulate oxidation in the liver. Glucose was expected to have little effect on feed intake because little or no GLU is extracted from the blood by the liver. Whereas, both LA and PA are anaplerotic and can stimulate oxidation of acetyl CoA in hepatocytes, hepatic extraction of PA is greater than LA, which depends on cytosolic redox state. Continuous isoenergetic infusions (150 kcal/h) of PA, LA or GLU or no infusion were administered abomasally to eight ruminally cannulated multiparous Holstein cows (12.4 ± 6.2 d postpartum) in a duplicate 4 x 4 Latin square design experiment balanced for carry-over effects. Treatment sequences were assigned to cows randomly, and treatments included control (CON, no infusion), PA (99.5%, 0.41 moles/h), LA (88.0%, 0.46 moles/h) and GLU (99.9%, 0.22 moles/h) Solutions containing treatments were infused at 500 mL/h for 22 h/d and provided ~3.3 Mcal/d. Feeding behavior was recorded by a computerized data acquisition system. Gross energy digestibility of the diet was determined for each cow and used to calculate metabolizable energy intake (MEI) from the diet. Total MEI was calculated as the sum of MEI from the diet plus energy from infusions. Data were analyzed statistically with a mixed model including the fixed effect of

treatment and random effects of block and cow within block. Each treatment was compared with CON by preplanned contrasts. Compared with CON, PA decreased DMI by 24% (14.3 vs. 18.9 kg/d) and total MEI by 13% (34.8 vs. 40.2 Mcal/d) with a tendency to decrease meal frequency. Lactic acid decreased DMI by 14% (16.3 vs. 18.9 kg/d) compared with CON by decreasing meal size 20% but did not affect MEI. Glucose infusion did not affect DMI or MEI. Treatment effects on DMI and MEI were consistent with their expected effects on hepatic oxidation. Depression of feed intake in diets containing highly fermentable starch is likely because of the ability of propionic acid to stimulate hepatic oxidation.

INTRODUCTION

Cereal grains high in starch are included in rations to meet dietary energy requirements of high-producing dairy cows. However, highly fermentable starch sources can depress feed intake compared with less fermentable starch sources (Allen, 2000). High moisture corn decreased DMI 8% for mid-lactation cows (Oba and Allen, 2003a) and 11% for cows in the postpartum (**PP**) period (Albornoz and Allen, 2016), compared with the less fermentable dry ground corn. Ruminal fermentability of starch varies greatly from ~30% to ~90% (Allen, 2000) depending upon genetics, maturity, processing, and storage method of the starch source (Kotarski et al., 1992; Larson and Hoffman, 2008). This wide variation affects the type and supply of fuels available for absorption including propionic acid from ruminal fermentation, glucose from enzymatic hydrolysis of starch that passed from the rumen to the small intestine, and lactic acid from the partial metabolism of glucose by enterocytes. Once absorbed, metabolism of these fuels varies with different efficiencies of extraction from the blood by the liver and potential of stimulating hepatic oxidation. According to the hepatic oxidation theory (**HOT)** of the control of

feed intake, anaplerotic fuels extracted by the liver may promote oxidation of acetyl CoA (**AcCoA**) increasing hepatic energy charge contributing to satiety (Allen et al., 2009). Of the primary fuels absorbed from starch digestion in ruminants, propionate and lactate are anaplerotic whereas little glucose is extracted from the blood by the liver. However, hepatic extraction of propionate from the blood is greater than lactate (Reynolds et al., 2003), which is dependent upon cytosolic redox state in the hepatocyte (Allen et al., 2009) The objective of this experiment was to evaluate the effects of fuels derived from fermentation and digestion of starch on DMI and feeding behavior of cows in the PP period. We hypothesized that these fuels differ in their effects on DMI and ME intake (**MEI**) and that their effects are consistent with their ability to stimulate hepatic oxidation, with propionic acid causing the greatest hypophagic effect, followed by lactic acid, and little effect for glucose.

MATERIALS AND METHODS

Animals, Housing, and Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Cows were ruminally cannulated at least 45 d before expected calving date. Each cow was housed in the same individual tie-stall for the duration of the experiment. Cows were fed once daily (1200 h) at 125% of expected intake and milked in their stalls twice daily at 0530 and 1730 h.

Experimental Design, Diets and Treatments

Eight multiparous Holstein cows (n = 8, 12.4 ± 4.2 d PP) at the Michigan State University Dairy Teaching and Research Center were used in a repeated 4 x 4 Latin square

design with 2 blocks of cows containing 4 cows each. Cows were blocked by date of parturition; the first block was conducted during March, 2015 and the second during October, 2015. Cows within each block were randomly assigned to treatment sequence. Each block was conducted over an 8-d period with 4 infusion days separated by a washout day. All cows received a common experimental diet from parturition through the end of the experiment. The experimental diet (Table 3.1) was formulated to meet or exceed cows estimated requirements according to NRC (2001) and fed as a TMR.

Treatments were propionic acid (**PA**; food grade, 99.5%, Kemin Industries, Inc., Des Moines, IA), L-lactic acid (**LA**; food grade 88.0%, Sena International Inc., Polo, IL) and glucose (**GLU**; ≥ 99.5%, Sigma Aldrich, Chemical Co., St. Louis, MO) infused continuously into the abomasum, and control (**CON**; no infusion). Isoenergetic amounts of PA (0.41 mol/h), LA (0.46 mol/h) and GLU (0.22 mol/h) dissolved in distilled water were infused at a rate of 508 mL/h for 22 h/d, providing an average of 3.26 Mcal ME/d (148 kcal/h), approximately 10% of daily MEI by cows in the postpartum (**PP)** period. Rate of infusion was selected based on previous studies in which PA decreased DMI of cows in the PP period compared with acetic acid (Stocks and Allen, 2012; Oba and Allen, 2003b). Metabolizable energy content of treatments were assumed to equal their gross energy of 365.0 kcal/mol for PA (Labedeva, 1964), 321.2 kcal/mol for LA (Saville and Gundry, 1959) and 673.4 kcal/mol (Emery and Benedict, 1911) for GLU. Solutions were infused into the abomasum using Baxter Flo-Gard 6201 infusion pumps (Baxter Medical Products, Deerfield, IL) through vinyl tubing (0.95 cm OD, 0.71 cm ID) connected to a Nalgene bottle (3.8 cm diameter, 8.5 cm long) held in place with a rubber disk as described in Gualdrón-Duarte and Allen (2017).

Table 3.1. Ingredients and nutrient composition of diets fed during blocks 1 and 2

Item	Block 1	Block 2
Ingredient, % of DM		
Corn Silage	39.6	33.8
Soybean meal	16.1	15.9
Alfalfa silage	14.1	5.54
Ground corn	10.4	17.8
Alfalfa Hay	9.9	9.7
Soybean hulls	5.7	5.5
Grass silage	-	7.6
Vitamin and mineral mix[1]	4.2	4.1
Nutrient composition		
DM, %	51.0	55.0
Starch, % of DM	19.8	22.0
NDF, % of DM	35.1	34.2
Forage NDF, % of DM	29.5	27.5
CP, % of DM	16.6	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.

Data and Sample Collection

Cows were withheld from feed from 1000 to 1200 h daily, and the amount of feed offered and orts were weighed for each cow daily. Samples (0.5 kg) of all dietary ingredients and the TMR were collected daily throughout the experiment, and orts for each cow were collected at the end of each infusion day and stored in plastic bags at -20°C until processed. Feeding behavior data (feed disappearance and water intake) were recorded continuously for 22 h daily for each cow during infusions via computer every 5 s, and size, length, and frequency of meals, intermeal interval, total eating time, and water intake were calculated (Dado and Allen, 1995).

Milk yield was recorded daily 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 4ºC. Milk samples were analyzed for fat, true protein, lactose, somatic cell count, and milk urea nitrogen by Michigan DHIA (AOAC, 1997).

Fecal samples were collected every 6 h for 24 h (n = 4) during each day following infusion days and frozen at -20°C for later analysis to determine diet digestibility. Blood was sampled every day at the beginning and end of each infusion period 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 placed on ice until centrifugation. Tubes were centrifuged at 3,000 x *g* for 15 min at 5°C immediately after sample collection. A 1-mL aliquot of plasma from each potassium EDTA tube was stored with 0.05 M benzamidine (final concentration) to prevent enzymatic degradation of glucagon. Plasma was harvested and frozen at -20°C until analysis. Liver samples were collected by needle biopsy (Bradford and Allen, 2005) between 1100 h and 1200 h (before feeding) before each infusion and at the end of infusions for each period for analysis of hepatic AcCoA content. Samples were flash frozen in liquid nitrogen and stored on dry ice for transportation. Samples were stored at -80°C until further analysis.

Analysis of Samples

Feed ingredients, orts and fecal samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1-mm screen; 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. All nutrients are expressed as percentages of DM, determined by drying at 105ºC in a forced-air oven for more than 8 h. Ash content was determined after 6 h of oxidation at 500°C in a muffle furnace. . Crude protein was determined according to Hach et al. (1987). Starch was

analyzed using an enzymatic method to hydrolyze it to glucose (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured by a glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Content of NDF was analyzed according to Mertens (2002) with the inclusion of amylase and Na sulfite. Indigestible NDF was determined as NDF residue after 240 h *in vitro* fermentation (Goering and Van Soest, 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, MEI from the diet and total MEI was calculated following the procedure described in Gualdrón-Duarte and Allen (2017).

Plasma samples were analyzed using commercial kits for concentration of nonesterified fatty acids (**NEFA**; NEFA HR kit, Wako Chemicals USA, Richmond, VA), β-hydroxybutyrate (**BHB**; kit no. 2240, Stanbio Laboratory, Boerne, TX). Triglycerides were analyzed with a commercial kit (L-type Triglyceride M, Wako Chemicals USA, Richmond, VA) modifying the quantity of sample used (40 µL). Insulin was determined using an ELISA kit (kit no. 10-1201-01, Mercodia, Uppsala, Sweden). Plasma concentration of metabolites were analyzed colorimetrically with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp.) Plasma glucagon concentration was determined using a RIA kit (kit no. GL-32K, Linco Research Inc., St. Charles, MO). Plasma propionate and lactate concentrations were analyzed by HPLC (Waters Corp., Milford, MA). Plasma (600 μL) was mixed with 600 μL $Ca(OH)_2$ and 300 *m*L of $CuSO_4$ containing crotonic acid as an internal marker in 1.7 ml microcentrifuge tubes. Samples

were frozen, thawed and centrifuged at 12,000 × *g* for 10 min, and supernatant (1000 μL) was mixed with 42 μL of H_2SO_4 in 1.5-mL microcentrifuge tubes. Samples were frozen and thawed twice, and centrifuged at 12,000 × *g* for 10 min to precipitate and remove protein. Supernatant was transferred to HPLC vials. The column used was Aminex HPX-87H (catalog number 125-0140; 300 x 7.8 mm; Bio-Rad Laboratories, Richmond, CA). Column temperature was 50°C, and the solvent was 0.015N H_2S0_4. Detection was by refractive index (Waters 410; Millipore Corp., Milford, MA). Liver acetyl CoA (**AcCoA**) was determined by HPLC following the King and Reiss (1985) procedure with modifications by Stocks and Allen (2012). Liver AcCoA content was expressed per g of wet liver.

RNA Extraction and Real-Time Quantitative PCR

Treatment effects on expression of genes associated with lipid and carbohydrate metabolism were analyzed. Methods, primers, and results are reported in the Appendix.

Statistical Analysis

Feeding behavior, intake, metabolite and hormone responses, yield of milk and milk components, and concentration of milk components were analyzed using the Fit Model procedure of JMP (version 12.0, 2015; SAS Institute Inc., Cary, NC) with the following model:

$$Y_{ijkl} = \mu + S_i + S(C_j)_i + S(P_k)_i + T_l + e_{ijk}$$

where Y_{ijkl} = the dependent response variable of interest, μ = overall mean, S_i = random effect of square (i = 1 to 2), $S(C_j)_i$ = random effect of cow within square (j = 4), $S(P_k)_i$ = fixed effect of period within square (k = 1 to 4), T_l = fixed effect of treatment (l = 1 to 4), and e_{ijkl} = residual, normally distributed. Treatment effects were declared significant at $P \leq 0.05$ and

tendencies for treatment effects at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies for interactions at $P \leq 0.15$. All data are expressed as least squares means (**LSM**) and standard error of the means (**SEM**), unless otherwise specified. Contrasts were conducted between each treatment and the control as pre-planned comparisons.

Eight cows started the experiment; however, one cow from the second block was removed from the study due to displaced abomasum during the first period and all data from this animal were excluded before statistical analysis. That cow was replaced with a spare, which followed the subsequent treatments within its sequence beginning with the second period and skipping the GLU infusion.

RESULTS

Feed Intake and Feeding Behavior

Results for feed intake and feeding behavior are reported in Table 3.2. Propionic acid and LA decreased DMI compared with CON whereas GLU had no effect. Lactic acid decreased DMI by 14% (16.3 vs. 18.9 kg/d, $P = 0.02$) by decreasing meal size by 20% (1.38 vs. 1.72 kg, $P = 0.04$) whereas PA decreased DMI by 24.3% (14.3 vs. 18.9 kg/d, $P < 0.001$) by tending to decrease the number of meals per day (10.6 vs. 12.3 meals; $P = 0.09$). Similarly, LA and PA decreased DE intake by 15% and 22%, respectively compared with CON and decreased ME intake from the diet by 15% and 21%, respectively compared with CON. However, only PA infusion decreased total ME intake including ME from the infusions and the ME from the diet compared with CON; PA decreased ME intake 13.2% compared with CON (34.8 vs. 40.1 Mcal/d; $P = 0.04$).

Table 3.2. Effects of isoenergetic[1] intra-abomasal infusion of glucose (GLU), lactic acid (LA) and propionic acid (PA) relative to a no infusion control (CON) on feeding behavior and energy intake for cows in the immediate postpartum period

						Significance, *P*		
	CON	GLU	LA	PA	SEM	GL vs. CON	LA vs. CON	PA vs. CON
DMI, kg/d	18.9	17.3	16.3	14.3	0.72	0.14	0.02	< 0.001
Feeding behavior (22h/d)								
Meal frequency, meals/d	12.3	12.4	11.9	10.6	0.61	0.91	0.68	0.09
Meal length, min/meal	23.7	23.6	21.7	24.7	1.81	0.96	0.32	0.59
Meal Size, kg/meal	1.72	1.46	1.38	1.54	1.53	0.12	0.04	0.26
Eating interval, min	95.6	89.3	91.4	109.6	6.0	0.51	0.63	0.13
Hunger ratio[2]	0.029	0.025	0.026	0.025	0.004	0.284	0.30	0.16
Satiety ratio[3]	0.036	0.036	0.029	0.042	0.012	0.928	0.59	0.69
Water intake, L/d	82.6	72.3	69.0	52.9	3.3	0.07	0.02	< 0.0001
Drink frequency, bouts/day	15.5	13.2	14.3	10.9	1.94	0.10	0.33	< 0.01
DM digestibility, %	60.6	59.4	60.6	62.7	2.22	0.42	0.99	0.17
DE intake (Mcal/d)[4]	48.2	43.6	41.1	37.7	2.85	0.12	0.02	<0.01
ME intake (Mcal/d)[5]								
Diet	40.1	36.2	34.2	31.6	2.6	0.12	0.02	<0.01
Infusion[6]	0	3.36	3.33	3.25	0.06			
Total[7]	40.1	39.6	37.5	34.8	2.65	0.80	0.28	0.04

[1]Isoenergetic infusion, 151 kcal/h.
[2]Hunger ratio = weight of meal/pre-meal interval
[3]Satiety ratio = weight of meal/post-meal interval
[4]Digestible energy (DE) intake (Mcal/d) = gross energy (GE) intake – GE feces.
[5]ME intake from the diet was calculate according to NRC (2001). ME (Mcal/kg) = 1.01 x (DE – 0.45). ME intake (Mcal/d) ME x DMI.
[6]ME from the infusion was based on energy density of 0.673 Mcal/mol ME (Emery and Benedict, 1911) for GLU; 0.321 Mcal/mol (Saville and Gundry, 1959) for LA; and 0.365 Mcal/mol (Lavedeva, 1964) for PA.
[7]Total ME intake = ME intake diet + ME infusion.

Infusion treatments did not affect any other feeding behavior variable compared with CON (Table 3.2). Glucose infusion tended ($P = 0.07$) to decrease water intake and LA and PA decreased ($P = 0.02$ and $P = < 0.0001$, respectively) water intake compared with CON. Propionic acid decreased ($P = < 0.01$) the number of drinking bouts per day compared with CON.

Milk Production and Components

Milk yield averaged 37.5 kg/d and no differences were observed between infusion and CON treatments ($P > 0.50$; Table 3.3). There was a tendency for PA to increase the true protein content in milk ($P = 0.07$, 3.11% vs. 2.87%), true protein yield ($P = 0.10$, 1.19 kg/d vs. 1.08 kg/d), and milk urea nitrogen concentration in milk (9.68 mg/dL vs. 8.38 mg/dL, $P = 0.10$) compared with CON. Lactic acid tended to decrease lactose yield ($P = 0.08$, 1.56 kg/d vs. 1.70 kg/d) compared with CON.

Table 3.3. Effects of intra-abomasal infusion of propionic acid (PA), lactic acid (LA) and glucose (GLU) relative to a control (CON) with no infusion on milk yield and milk components over the 22 h/d infusion for cows in the immediate postpartum period for isoenergetic infusions[1]

	Infusion					Significance, *P*		
	CON	GLU	LA	PA	SEM	GL vs. CON	LA vs. CON	PA vs. CON
Milk yield kg/d	37.9	38.1	36.3	37.8	2.16	0.92	0.50	0.96
Fat, %	4.58	4.43	4.09	4.63	0.321	0.76	0.30	0.91
Fat yield, kg/d	1.68	1.59	1.47	1.77	0.109	0.61	0.21	0.61
True protein, %	2.87	2.70	2.70	3.11	0.145	0.42	0.56	0.07
True protein yield,	1.08	1.13	0.99	1.19	0.043	0.55	0.16	0.10
Lactose, %	4.50	4.50	4.38	4.72	0.043	0.67	0.56	0.28
Lactose yield, kg/d	1.70	1.71	1.56	1.78	0.044	0.77	0.08	0.32
Solids, %	5.45	5.38	5.21	5.50	0.258	0.81	0.37	0.58
Total solids, kg/d	1.98	1.97	1.97	2.00	0.010	0.90	0.92	0.86
MUN[2], mg/dL	8.38	9.40	8.87	9.68	2.02	0.22	0.52	0.10

[1]Isoenergetic infusion, 151 kcal/h.
[2]MUN = milk urea nitrogen

Hepatic Acetyl CoA content and Plasma Hormones and Metabolites

Results for hepatic AcCoA content and plasma concentration of hormones and metabolites before and after infusion, and the differences between them are shown in Table 3.4. After 22 h of infusion, GLU tended to decrease ($P = 0.10$, 8.5 nmol/g) and LA decreased ($P =$

0.04, 11.1 nmol/g) liver AcCoA content compared with CON, but PA had no effect compared with CON.

All three infusion treatments increased plasma insulin concentration compared with control. Infusion of GLU had the greatest effect, more than doubling plasma insulin concentration by 0.22 µg/ L ($P < 0.0001$) whereas, LA and PA increased plasma insulin concentration by 0.05 µg/L ($P = 0.03$) and 0.06 µg/L ($P = 0.02$), respectively. Plasma glucagon concentration decreased by 9.7 pg/mL after GLU infusion which was greater than CON ($P = 0.04$) but the decreases for LA and PA infusions were not different from CON.

Glucose treatment decreased plasma NEFA concentration by 242 µEq/L which tended to differ from CON ($P = 0.06$) in which plasma NEFA concentration decreased 56 µEq/L over the same period, whereas, LA and PA did not differ from CON. Propionic acid treatment decreased plasma BHB by 5.8 mg/dL which was greater ($P = 0.02$) than the change for CON of 1.3 mg/dL and both GLU and LA decreased plasma BHB by ~4.4 mg/dL, which tended to be greater than the change for CON ($P \leq 0.09$).

Treatment infusions did not affect plasma concentrations of acetate or lactate compared with CON. However, propionate concentration increased after PA infusion ($P = 0.01$), and tended to increase after GLU infusion ($P = 0.09$) compared with CON. Glucose treatment increased ($P < 0.01$) and LA infusion tended to increase ($P = 0.08$) plasma glucose concentration compared with CON.

Table 3.4. Effect of intra-abomasal infusion of propionic acid (PA), lactic acid (LA) and glucose (GLU) relative to a control (CON) with no infusion on initial, final and change in plasma concentration of insulin, glucagon, metabolites and liver acetyl CoA (AcCoA) content over the 22 h/d infusion for cows in the immediate postpartum period for isoenergetic infusions[1]

						Significance, *P*		
	CON	GLU	LA	PA	SEM	GL vs. CON	LA vs. CON	PA vs. CON
Hepatic AcCoA before, nmol/g	26.3	21.4	21.5	23.1	3.03	0.10	0.10	0.27
Hepatic AcCoA after, nmol/g	19.1	15.5	13.5	18.9	2.46	0.38	0.18	0.97
Change in hepatic AcCoA, nmol/g	5.81	-2.66	-5.3	2.5	3.18	0.10	0.04	0.50
Insulin before, µg/L	0.188	0.208	0.187	0.194	0.04	0.50	0.95	0.83
Insulin after, µg/L	0.181	0.425	0.238	0.254	0.034	<0.0001	0.12	0.05
Change in insulin, µg/L	-0.007	0.222	0.051	0.06	0.019	<0.0001	0.03	0.02
Glucagon before, pg/mL	148.5	149.8	149.6	149.8	8.13	0.84	0.86	0.83
Glucagon after, pg/mL	144.5	134.2	138.5	140.3	10.2	0.09	0.29	0.46
Change in glucagon, pg/mL	2.63	-9.7	-5.45	-5.84	4.53	0.04	0.14	0.13
Insulin to glucagon ratio before	1.29	1.40	1.26	1.37	0.309	0.57	0.88	0.67
Insulin to glucagon ratio after	1.29	3.23	1.73	1.85	0.397	<0.0001	0.18	0.10
Change in insulin to glucagon ratio	0.989	2.46	1.67	1.59	0.181	<0.0001	0.007	0.014
NEFA before, µEq/L	715	613	624	665	103.5	0.13	0.16	0.31
NEFA after, µEq/L	659	398	602	532	92.3	<0.001	0.35	0.05
Change in NEFA, µEq/L	-55.9	-241.7	-22.3	-117.8	5.1	0.06	0.71	0.50
BHB before, mg/dL	11	10.1	10.5	11.1	1.07	0.52	0.73	0.93
BHB after, mg/dL	9.72	5.90	6.12	5.60	0.582	<0.01	<0.01	<0.001
Change in BHB, mg/dL	-1.31	-4.3	-4.42	-5.55	1.18	0.09	0.06	0.02
Triacylglycerides before, mg/dL	4.60	5.27	5.1	5.05	1.18	0.05	0.11	0.15
Triacylglycerides after, mg/dL	4.94	5.06	5.02	4.3	0.923	0.79	0.85	0.15
Change in triacylglycerides, mg/dL	0.338	-0.525	-0.083	-0.754	0.405	0.16	0.48	0.08
Glucose pre infusion, mM	2.81	2.88	2.75	2.86	0.09	0.25	0.36	0.41
Glucose post infusion, mM	2.87	3.21	2.97	2.95	0.06	<0.001	0.19	0.30
Change in glucose, mM	0.059	0.352	0.22	0.085	0.06	0.01	0.08	0.77

Table 3.4. (cont'd)

Lactate before, mM	0.479	0.712	0.427	0.336	0.11	0.13	0.71	0.32
Lactate after, mM	0.518	0.515	0.852	0.574	0.136	0.99	0.12	0.79
Change in lactate, mM	0.039	-0.150	0.425	0.238	0.201	0.49	0.15	0.45
Propionate before, mM	0.230	0.244	0.227	0.222	0.022	0.38	0.79	0.56
Propionate after, mM	0.218	0.204	0.238	0.259	0.02	0.51	0.32	0.05
Change in propionate, mM	-0.012	-0.047	0.012	0.037	0.029	0.09	0.20	0.01

[1]Isoenergetic infusion, 151 kcal/h.

Hepatic gene expression

Infusion treatments increased hepatic expression of *GLUD1 (*glutamate dehydrogenase 1), *GPAM* (glycerol-3-phosphate acyltransferase) and *PCK1* (phosphoenolpyruvate carboxykinase 1) compared with CON [false discovery rate (**FDR**) value < 0.05]. Relative mRNA abundance of *GLUD1* was increased 4.0 fold by GLU (P = 0.01), 3.3 fold by PA (P = 0.02) and 2.7 fold by LA (P = 0.04) compared with CON. Relative mRNA abundance of *GPAM* was increased 3.1 fold by LA (P = 0.01), 2.5 fold by GLU (P = 0.01) and 2.0 fold by PA (P = 0.05) compared with CON. Relative mRNA abundance of *PCK1* was increased 6.0 fold by GLU (P = 0.03) and 5.4 fold by PA (P = 0.03) but was not affected by LA compared with CON. Changes in hepatic expression of *FBP1* (fructose-1,6-bisphosphatase), *GOT2* (aspartate aminotransferase), *PC* (pyruvate carboxylase), *PCCA* (propionyl-CoA carboxylase alpha), and *PPARA* (peroxisome proliferator activated receptor alpha) were considered as potential (FDR < 0.10) hepatic genes affected by infusion of fuels and results are reported in the Appendix.

DISCUSSION

Our results indicate that fuels derived from ruminal fermentation and intestinal digestion of starch result in different effects on DMI and MEI of cows in the PP period compared with a sham control consistent with their expected effects on hepatic oxidation. According to the HOT, anaplerotic fuels extracted by the liver may promote oxidation of AcCoA increasing hepatic energy charge and contributing to satiety (Allen et al., 2009). Of the fuels produced from the fermentation and digestion of starch, propionate is expected to stimulate hepatic oxidation to the greatest extent because it is efficiently extracted from the blood by the liver and is obligatorily anaplerotic, stimulating TCA cycle activity and oxidation of AcCoA (Allen, 2014). Therefore, the greater reduction in DMI and MEI by PA compared with CON among treatments was

expected. Although the change in hepatic AcCoA content over the 22 h infusion period was similar for PA compared with CON, entry and oxidation in the TCA cycle was likely greater for PA compared with CON because PA decreased BHB concentration in plasma but not NEFA concentration compared with CON. Additionally, pulse doses of propionic acid decreased hepatic AcCoA content at 30 and 60 minutes post-dosing and changes in AcCoA content over a day do not reflect changes relative to meals as discussed by Gualdron and Allen (2017)

Intraruminal infusion of PA decreased DMI and MEI of cows in the PP period by decreasing meal size with no effect on meal frequency compared with acetic acid (Stocks and Allen, 2013). Whereas, we expected PA to reduce meal size compared with CON, the effect of PA on meal size was not significant and the reduction in DMI and MEI was from combination of a numerical reduction of 11% in meal size and a tendency for a 14% decrease in meal frequency compared with CON.

The hypophagic effects of propionate compared with acetate have been well established by previous experiments reported in the literature (Allen, 2000). While isomolar amounts of propionate and acetate were generally infused, propionate has greater ME per mole than acetate, and the effects on total MEI accounting for the ME infused were not measured in most studies. However, propionate also decreased total MEI from the diet and treatment infusions in lactating cows compared with acetate when infused intra-ruminally in one study (Oba and Allen, 2003b). Although both propionic acid and acetic acid can be produced by fermentation of starch, we did not include acetic acid as a treatment in the present experiment because increased grain feeding increases ruminal production of propionic acid (Bauman et al., 1971; Sutton et al., 2003) with little effect on acetic acid production (Davis, 1967; Sutton et al., 2003), and because its effects on DMI compared with propionic acid have been established previously (Allen, 2000). The effects

of propionate and acetate on feed intake are consistent with the HOT because little acetate is extracted from the blood by the liver (Knapp et al., 1992) and acetate is not anaplerotic and cannot stimulate hepatic oxidation.

The importance of the anaplerotic effects of fuels was demonstrated by a study in which propionic acid decreased DMI and MEI of cows in the PP period compared with isoenergetic infusion of glycerol (Guadrón-Duarte and Allen, 2017). Whereas glycerol is potentially anaplerotic, its anaplerotic effect is likely greatly diminished when glucose demand is high in the PP period, entering the gluconeogenic pathway in the cytosol instead of stimulating hepatic oxidation and satiety (Guadrón-Duarte and Allen, 2017).

Lactate also is anaplerotic, entering the TCA cycle as oxaloacetate through pyruvate, and can also stimulate oxidation of AcCoA. In support of this, we found that LA infusion decreased hepatic AcCoA content and tended to decrease plasma BHB concentration. However, extraction of lactate from the blood by the liver is much less than propionate (Reynolds et al., 2003) likely because metabolism of lactate to pyruvate is thermodynamically unfavorable when cytolosic NADH to NAD ratio is elevated (Allen et al., 2009). In addition, pyruvate from lactate might be used for alanine conversion through transamination. Therefore, the reduction in DMI by LA compared with control was expected. Whereas LA reduced DMI compared with CON, the effect was not adequate to significantly reduce MEI when the ME of the diet and infusate were combined. The reduction in DMI was from a reduction in meal size indicated that its effect was during the timeframe of meals. The LA treatment tended to increase glucose, consistent with its use as a glucose precursor and increased plasma insulin concentration. Plasma propionate concentration was increased 17% by PA over the 22 h infusion, but plasma lactate concentration

was doubled (numerical only) consistent with their expected efficiency of hepatic extraction from the blood.

Infusion of GLU did not affect DMI or MEI compared with CON, consistent with previous experiments with midlactation cows in which glucose was infused post-ruminally (Frobish and Davis, 1977; Clark et al., 1977) or intravenously (Dowden and Jacobsen, 1960; Al-Trad et al., 2009) in dairy cows. The ruminant liver extracts little glucose from the blood (Stangassinger and Giesecke, 1986) because activities of glucokinase and hexokinase are low (Emmanuel, 1981). Therefore, glucose is unable to stimulate hepatic oxidation directly and its effect on DMI compared with control was as expected. Although a significant effect of glucose on DMI compared with CON was not detected, the numerical reduction in DMI is likely the reason for the lack of effect of GLU on MEI compared with CON because the numerical reduction in MEI of the diet that was similar to the amount of ME infused per day.

Whereas, we did not detect a reduction of DMI by GLU treatment, infusion of graded amounts of glucose (0 to 1,500 g/d) into the duodenum of lactating cows (~60 d postpartum) decreased DMI linearly, but did not affect NE_L intake when the infused glucose was considered (Hurtaud et al., 1998) and infusion of 1,500 g of starch hydrolysate into the rumen or abomasum reduced DMI but increased NE intake of cows in early lactation (24 to 66 d postpartum) when the infused glucose was considered (Knowlton et al., 1998). Variation in effects of glucose infusions on DMI and MEI might be from differences in the dose of glucose, physiological state of cows, differences in diet composition, and length of infusion. Although, the above experiments were not conducted during the PP period, continuous infusion of glucose (1,500 g/d) into the abomasum of cows from 4 to 29 days in milk, decreased DMI ~6 kg/d (Larsen and Kristensen, 2009). We also infused during the PP period but infused a lower dose (~60%) for

only one day. Differences for the effects of glucose infusion between these experiments might be because treatment had a greater effect on increasing plasma insulin concentration for a longer time which might have down-regulated gluconeogenesis, decreasing cataplerosis of the TCA cycle, increasing TCA cycle activity and oxidation of AcCoA, and stimulating satiety sooner, as discussed by Brown and Allen (2013). Effects of glucose infusion on meal size and DMI were dependent upon the diet in an experiment in which glucose was infused at a rate of 1,000 g/d for the first 12 d PP (Brown and Allen, 2013). Therefore, depression of DMI by glucose might be related to its indirect effects on hepatic oxidation, dependent on dose, diet and physiological state of cows.

Increased expression of hepatic *GPAM*, the gene coding for the enzyme responsible for the first and committed step in glycerolipid synthesis, by all infusion treatments was consistent with effects on increasing plasma insulin concentration because *SREBF1* (Mughal et al., 2010), a nuclear factor which up-regulates *GPAM* expression is induced by insulin. Infusion of GLU and PA increased expression of hepatic *PCK1* despite increasing plasma insulin concentration. Insulin has been reported to decrease mRNA abundance of *PCK1* in rat liver cells (Granner et al., 1983) and glucose infusion in cows numerically increased blood insulin concentration 54% and decreased hepatic mRNA abundance of *PCK1* 51% compared to water infusion although the treatments were not directly compared statistically (Zhang et al., 2015). However, in that study hepatic *PCK1* expression was maintained when serum insulin concentration was nearly doubled in cows infused with propionate (Zhang et al., 2015). Differences in effects of propionate and glucose infusion on *PCK1* expression between the current experiment and the experiment of Zhang et al. (2015) might be because we infused propionate and glucose at twice the rate (0.41 and 0.22 mol/h, respectively) compared with (0.21 and 0.11 mol/h, respectively), for longer (22 h

vs. 8 h) to cows in the immediate PP period compared with mid-lactation. Glutamate dehydrogenase 1 (*GLUD1*) converts L-glutamate into alpha-ketoglutarate and plays a key role in anaplerosis of the TCA cycle. However, the mechanism by which all treatments increased its expression is not known.

CONCLUSION

Fuels supplied by the ruminal fermentation and intestinal digestion of starch have different effects on DMI that are consistent with their ability to stimulate hepatic oxidation of AcCoA. Greater ruminal starch fermentation increases the production of propionic acid, which decreased intake of dry matter and total metabolizable energy compared with control. However, the primary fuels from digestion of starch in the small intestine did not decrease metabolizable energy intake of cows compared with control. Depression of feed intake of cows in the PP period by diets containing highly fermentable starch is likely because of the ability of propionic acid to stimulate hepatic oxidation.

ACNOWLEDGEMENTS

We acknowledge the financial support for this project from USDA National Research Initiative Competitive Grant no. 2014-67015-21708 to MSA, and from Fulbright Colombia and Colciencias to LGD. We also thank D. G. Main, R. A. Longuski, J. Liesman, R. West, G. Maldini, K. Kennedy, R. Albornoz, R. Yair and, D. de Sousa (all from Michigan State University) for technical assistance, G.A. Contreras, C. Strider, Norma Manrique-Carpintero and J. Landgraf for assistance on gene expression analysis, R. J. Tempelman and J. P. Steibel for statistical advice, and Kemin, Inc. for supplying the propionic acid treatment.

APPENDICES

APPENDIX A: GENE EXPRESSION

RNA Extraction and Real-Time Quantitative PCR.

Total RNA was extracted from ~15 mg of hepatic tissue using the RSC simply RNA Tissue Kit (Promega Corp., Madison, WI) and then purified using the RQ1 RNase-Free DNase Kit (Promega Corp., Madison, WI). Purity, concentration, and integrity of mRNA were evaluated using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE) and an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) system. All samples had a RNA integrity number > 6. Conversion to cDNA was performed using the Applied Biosystems High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA).

Quantitative PCR Analysis

Gene expression of predetermined genes was determined using real-time quantitative PCR (qPCR) and primers were designed using Primer Express 3.0 (Life Technologies Corp.) and Lasergene 9 core suite software (DNASTAR) and synthesized commercially (Integrated DNA Technologies, Coralville, IO). Primer sequences and accession numbers are summarized in Supplemental Table 3.5. Complementary DNA was synthesized from 100 ng of total RNA as a template using the High-Capacity cDNA Reverse-Transcription Kit (Life Technologies Corp.), and amplification efficiency was validated for endogenous control and target genes with polled cDNA from samples using a 5-fold dilution series. A non-reverse-transcriptase control sample validated absence of genomic DNA.

For relative gene expression, samples were assayed in triplicate on the Wafergen Smartchip System (WaferGen Biosystems, Fremont, CA) using cDNA at a primer-efficient concentration of 500 pg/µL and 10 uL of Power SYBR Green (Life Technologies Corp.), 10 µL of a forward and reverse primer mix (1 µM each), and 5 µL of sample (0.5 ng of cDNA/µL).

The relative gene expression was determined using *HPRT1*, *PGK1* and *RPS9* as endogenous control genes.

Gene expression was analyzed from raw cycles to threshold ($\mathbf{C_T}$) values obtained using Data Assist software (Applied Biosystems) and then corrected for efficiency using the formula $C_{Tcorrected} = -\log_2 (E^{-C_T})$. The computation of ΔC_T for genes of interest pre and post infusion was calculated using the corrected C_T values for genes of interest and the mean of the corrected C_T for the control genes which included *HPRT1*, *PGK1* and *RPS9I*, according to the formula $\Delta C_T = C_{T\ \text{Target gene pre or post-infusion}} - C_{T\ \text{Mean of control genes}}$, for computing the difference in expression after infusion was used the following formula, $\Delta\Delta C_T = \Delta C_{T\ \text{Target gene post-infusion}} - \Delta C_{T\ \text{Target gene pre-infusion}}$.

ΔC_T and $\Delta\Delta C_T$ values were analyzed with the following model using the Fit Model procedure of JMP (version 12.0, 2015; SAS Institute Inc., Cary, NC):

$$Y_{ijkl} = \mu + S_i + S(C_j)_i + S(P_k)_i + T_l + e_{ijk}$$

where Y_{ijkl} = the ΔC_T of the gene of interest in each observational unit, μ = overall mean, S_i = fixed effect of square (i = 1 to 2), $S(C_j)_i$ = random effect of cow within square (j = 4), $S(P_k)_i$ = random effect of period within square (k = 1 to 4), T_l = fixed effect of treatment (l = 1 to 4), and e_{ijkl} = residual, normally distributed. Treatment effects were declared significant at $P \leq 0.05$ and tendencies for treatment effects at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies for interactions at $P \leq 0.15$. All data are expressed as LSM ± SEM, unless otherwise specified. This model is equivalent to the model described by Steibel et al. (2009).

Results are presented as fold changes relative to the CON samples. These were calculated from difference in least squares mean differences according to the formula 2^{-diff}, where diff = $LSmeans_{(treatment\ )}$ - $LSmeans_{(CON)}$, i.e. it is the difference in the least squares means obtained from the mixed model. After analysis *P* values for treatment effect were subject to Multiple Test correction using a local false discovery rate (FDR) value < 0.05. Results on LSM of the C_T differences after infusion relative to before infusion are reported in Supplemental Table 3.6. Fold changes (FC) on hepatic genes affected by infusion treatments and the confidence intervals for the fold changes are reported in Supplemental Table 3.7 and 3.8 respectively.

Table 3.5. Primer sequences and accession numbers for genes analyzed with real-time quantitative PCR

Gene[1]	Accession number	Forward Sequence (5'-3') Reverse Sequence (5'-3')
ADH1C	NM_001206387.1	CAGGACTACGAGAAACCCATC TCCTACAATGACGCTTACACC
CPT1A	XM_019954456.1	TGACGGCTCTGGCACAAG TTTTAGCTTCAAATACCACTGCCAATC
CS	NM_001044721	ACTAATGCATGTAGTGTGGGTTAGGT AAGAGCCAGATTCCCACTCTGA
FBP1	XM_019966535.1	GGCACGGGCGAGATGA CTTTGACCGCGGTGCAA
G6PC	NM_183364	CCATATCCGAAACCAATCAAGAG GAACAGGCAAGGAGGAGGAGTT
GAPDH	XM_019960295.1	TGCCGCCTGGAGAAACC CGCCTGCTTCACCACCTT
GCGR	XM_019982395.1	GAGCCCACCAGATGCGTTAT GATGAGGGTCAGTGTGGACTTG
GLUD1	NM_182652.2	CGCTCTGCCAGGCAAATC GGTCCAGCCCCAGGTTATACTT
GOT2	XM_019979412.1	GGCTGTGCGCCACTTCAT TTGGCATAGGACTGGCAGAGA
GPAM	NM_001012282	GAAGTCATGAGGGTGACGAGAAA TGCCACAACCTGAGCTTACACT
HMGCS1	XM_019982983.1	AAGCTCCGAGAGGATACTCATCA TCGAAGAGGGAATCTATGGAACTC
HMGCS2	AA112667	CCTGCTGCAATCACTGTCATG TCTGTCCCGCCACCTCTTC
HNF4A	NM_001015557	GGCGTCCCGCCAGACT GCACAGGACCCGATGACATC
HPRT1[2]	NM_001034035	TGGCGTCCCAGTGAAATCA CAGCTGGCCACAGAACAAGA
MCEE	NM_001045995	GGGCTGGCCCTGTATGG GCACTGCGACTGCAACATG
MUT	NM_173939	CTCGCTGCTGGGCACAA AAGGGCGTTGAGTTCCTTGA
PC	NM_177946	AGGCAAGACGCTGCACATC GCCCGCCCGGTTGA
PCCA	NM_001083509	GGTCAGGAAATCTGTGTGATTGAA GTCTTCCCGGCCGTCATA
PCCB	BC109784	CGAATCTGCTGTGACCTGGAT TCCTCCAAGGGCGCTGTAC
PCK1	NM_174737	CAGCCAAGCTGCCCAAGA CCGGCCTTGTCCTTTCG
PCK2	XM_019968310.1	CCCCCACCCCTACTCCAA

Table 3.5 (cont'd)

		TGCTCATAGTTTCTGCAAGCCTAGT
PDK4	NM_001101883	GAGGTGGTGTTCCCCTGAGA
		TTGGTGCAGTGGAGTACGTGTAA
PGK1[2]	NM_001034299.1	CAAGGATGTTTTGTTCTTGAAGGA
		GGGTCAGCACAAGCCTTCTC
PPARA	NM_001034036	CAGCGCCGAGGAGTCATC
		TGTCCCCGCAGATCCTACAC
RPS9[2]	XM_019978604.1	TCCCGCCTCGACCAAGA
		TTTGTTCCGGAGCCCATACT
SLC374A	NM_001193045	TTGTCATGCCGTCGTTGGT
		GGTGATGAGCCCCAAGTCA

[1]*ADH1C* = alcohol dehydrogenase 1C; *CPT1A* = carnitine palmitoyltransferase 1A; *CS* = citrate synthase; *FBP1* = fructose-1,6-bisphosphatase 1; *G6PC* = glucose-6-phosphatase; *GADPH* = glyceraldehyde-3-phosphate dehydrogenase; *GCGR* = glucagon receptor; *GLUD1* = glutamate dehydrogenase 1; *GOT2* = aspartate aminotransferase 2; *GPAM* = glycerol-3-phosphate acyltransferase; *HMGCS1* = HMG-CoA synthase 1; *HMGCS2* = HMG-CoA synthase 2; *HNF4A* = hepatocyte nuclear factor 4 alpha; *HPRT1* = hypoxanthine phosphoribosyltransferase 1; *MCEE* = methylmalonyl CoA epimerase; *MUT* = methylmalonyl CoA mutase; *PC* = pyruvate carboxylase; *PCCA* = propionyl-CoA carboxylase alpha; *PCCB* = propionyl-CoA carboxylase beta; *PCK1* = phosphoenolpyruvate carboxykinase 1; *PCK2* = phosphoenolpyruvate carboxykinase 2; *PDK4* = pyruvate dehydrogenase kinase; *PGK1* = phosphoglycerate kinase 1; *PPARA* = peroxisome proliferator activated receptor alpha; *RPS9* = ribosomal protein S9; *SLC37A4* = solute carrier family 37 A4.
[2]Endogenous control gene.

Table 3.6. Effect of isoenergetic[1] intra-abomasal infusion of glucose (GLU), lactic acid (LA) and propionic acid (PA) and a no infusion control (CON) on least square means of the C_T differences after infusion relative to before infusion[2] on hepatic gene expression for cows in the immediate postpartum period

Gene[3]	CON	GLU	LA	PA	SEM	*P* - value
ADH1C	3.52	0.07	-0.28	0.02	2.285	0.43
CPT1A	0.20	-0.27	0.76	0.53	0.552	0.62
CS	0.08	0.03	-0.39	-0.29	0.480	0.83
FBP1	1.85	-0.35	1.51	0.26	0.670	0.11
G6PC	2.63	0.94	0.79	0.42	1.042	0.40
GAPDH	0.25	0.22	-0.09	0.09	0.307	0.88
GCGR	3.25	0.52	1.30	0.07	1.508	0.29
GLUD1	1.56	-0.44	0.11	-0.14	0.415	0.03
GOT2	1.15	0.22	-0.23	0.10	0.334	0.10
GPAM	1.47	0.12	-0.14	0.45	0.374	0.02
HMGCS1	1.15	-0.45	0.54	0.82	0.667	0.28
HMGCS2	0.36	-0.51	-0.29	-0.08	0.410	0.61
HNF4A	0.98	0.18	-0.20	0.20	0.435	0.51
MCEE	1.39	0.03	0.66	0.47	0.510	0.33
MUT	1.34	0.43	0.39	0.21	0.447	0.36
PC	1.33	0.37	0.55	-0.01	0.369	0.16
PCCA	1.45	0.15	-0.14	0.12	0.473	0.08
PCCB	0.96	-0.12	-0.08	0.24	0.403	0.14
PCK1	2.34	-0.25	1.53	-0.11	0.854	0.06
PCK2	1.12	-0.05	0.41	1.14	0.535	0.43
PDK4	0.69	0.06	0.46	0.26	0.410	0.79
PPARA	1.12	-0.05	0.24	-0.13	0.324	0.10
SLC374A	1.27	0.25	0.04	-0.02	0.551	0.37

[1]Isoenergetic infusion, 151 kcal/h.

[2] $\Delta C_T = C_{T\ \text{Target gene pre or post-infusion}} - C_{T\ \text{Mean of control genes}}$, for computing the difference in expression after infusion was used the formula $\Delta\Delta C_T = \Delta C_{T\ \text{Target gene post-infusion}} - \Delta C_{T\ \text{Target gene pre-infusion}}$.

[3]*ADH1C* = alcohol dehydrogenase 1C; *CPT1A* = carnitine palmitoyltransferase 1A; *CS* = citrate synthase; *FBP1* = fructose-1,6-bisphosphatase 1; *G6PC* = glucose-6-phosphatase; *GADPH* = glyceraldehyde-3-phosphate dehydrogenase; *GCGR* = glucagon receptor; *GLUD1* = glutamate dehydrogenase 1; *GOT2* = aspartate aminotransferase 2; *GPAM* = glycerol-3-phosphate acyltransferase; *HMGCS1* = HMG-CoA synthase 1; *HMGCS2* = HMG-CoA synthase 2; *HNF4A* = hepatocyte nuclear factor 4 alpha; *MCEE* = methylmalonyl CoA epimerase; *MUT* = methylmalonyl CoA mutase; *PC* = pyruvate carboxylase; *PCCA* = propionyl-CoA carboxylase alpha; *PCCB* = propionyl-CoA carboxylase beta; *PCK1* = phosphoenolpyruvate carboxykinase 1; *PCK2* = phosphoenolpyruvate carboxykinase 2; *PDK4* = pyruvate dehydrogenase kinase; *PPARA* = peroxisome proliferator activated receptor alpha; *SLC37A4* = solute carrier family 37 A4.

Table 3.7. Fold changes (FC)[1] on hepatic genes affected by isoenergetic[2] intra-abomasal infusion of glucose (GLU), lactic acid (LA) and propionic acid (PA) relative to a no infusion control (CON) for cows in the immediate postpartum period

			Confidence interval[4]		
Gene[3]	Infusion	FC	Lower	Upper	*P* value
FBP1	GLU	4.60	0.11	2.36	0.04
	LA	1.26	0.40	0.64	0.74
	PA	3.01	0.17	1.55	0.12
GLUD1	GLU	3.99	0.16	2.62	0.01
	LA	2.73	0.24	1.75	0.04
	PA	3.25	0.20	2.11	0.02
GOT2	GLU	1.89	0.37	1.32	0.10
	LA	2.60	0.27	1.80	0.02
	PA	2.07	0.34	1.43	0.06
GPAM	GLU	2.54	0.28	1.83	0.01
	LA	3.05	0.23	2.18	0.00
	PA	2.03	0.35	1.46	0.05
PC	GLU	1.95	0.35	1.32	0.11
	LA	1.72	0.39	1.16	0.19
	PA	2.53	0.27	1.71	0.03
PCCA	GLU	2.04	0.27	1.62	0.05
	LA	3.01	0.22	1.96	0.02
	PA	2.51	0.26	1.64	0.04
PCK1	GLU	6.00	0.08	2.93	0.03
	LA	1.75	0.28	0.85	0.45
	PA	5.44	0.09	2.66	0.03
PPARA	GLU	2.24	0.31	1.58	0.03
	LA	1.84	0.38	1.29	0.11
	PA	2.37	0.30	1.67	0.03

[1]$FC = 2^{-diff}$, where diff= LSM $_{infusion\ treatment}$ − LSM $_{CON}$, i.e. it is the difference in the least squares means obtained from the mixed model.Gene expression fold changes were calculated from LSM differences of the ΔC_T values ($\Delta\Delta C_T$) normalized to the mean of *HPRT1*, *PGK1* and *RPS9* housekeeping genes. The CON treatment was the calibrator for the calculation of $\Delta\Delta C_T$.

[2]Isoenergetic infusion, 151 kcal/h.

[3]Genes listed in the table were subject to Multiple Test correction using a local FDR value <0.10, *FBP1* = fructose-1,6-bisphosphatase 1; *GLUD1* = glutamate dehydrogenase 1; *GOT2* = aspartate aminotransferase 2; *GPAM* = glycerol-3-phosphate acyltransferase; *PC* = pyruvate carboxylase; *PCCA* = propionyl-CoA carboxylase alpha; *PCK1* = phosphoenolpyruvate carboxykinase 1; *PPARA* = peroxisome proliferator activated receptor alpha.

[4] 95% confidence intervals of the $\Delta\Delta C_T$ values were transformed to fold changes and calculations are presented in Supplemental Table 3.8.

Table 3.8. Confidence intervals[1] of hepatic gene expression fold changes (FC) in genes affected by isoenergetic[2] intra-abomasal infusion of glucose (GLU), lactic acid (LA) and propionic acid (PA) relative to a no infusion control (CON) for cows in the immediate postpartum period

Gene[4]	Infusion	Diff	SEM	*P* value	log FC[3]	Upper CI	Lower CI	FC	FC Lower CI	FC Upper CI
FBP1	GLU	-2.20	0.961	0.04	2.20	3.16	-1.24	4.60	0.11	2.36
	LA	-0.34	0.981	0.74	0.34	1.32	0.64	1.26	0.40	0.64
	PA	-1.59	0.963	0.12	1.59	2.56	-0.63	3.01	0.17	1.55
GLUD1	GLU	-2.00	0.609	0.01	2.00	2.61	-1.39	3.99	0.16	2.62
	LA	-1.45	0.639	0.04	1.45	2.09	-0.81	2.73	0.24	1.75
	PA	-1.70	0.619	0.02	1.70	2.32	-1.08	3.25	0.20	2.11
GOT2	GLU	-0.92	0.527	0.10	0.92	1.45	-0.40	1.89	0.37	1.32
	LA	-1.38	0.534	0.02	1.38	1.91	-0.85	2.60	0.27	1.80
	PA	-1.05	0.529	0.06	1.05	1.58	-0.52	2.07	0.34	1.43
GPAM	GLU	-1.35	0.473	0.01	1.35	1.82	-0.87	2.54	0.28	1.83
	LA	-1.61	0.483	0.00	1.61	2.09	-1.13	3.05	0.23	2.18
	PA	-1.02	0.475	0.05	1.02	1.50	-0.54	2.03	0.35	1.46
PC	GLU	-0.97	0.568	0.11	0.97	1.53	-0.40	1.95	0.35	1.32
	LA	-0.79	0.577	0.19	0.79	1.36	-0.21	1.72	0.39	1.16
	PA	-1.34	0.569	0.03	1.34	1.91	-0.77	2.53	0.27	1.71
PCCA	GLU	-1.30	0.611	0.05	1.03	1.91	-0.69	2.04	0.27	1.62
	LA	-1.59	0.617	0.02	1.59	2.21	-0.97	3.01	0.22	1.96
	PA	-1.33	0.615	0.04	1.33	1.94	-0.71	2.51	0.26	1.64
PCK1	GLU	-2.58	1.032	0.03	2.58	3.62	-1.55	6.00	0.08	2.93
	LA	-0.81	1.052	0.45	0.81	1.86	0.24	1.75	0.28	0.85
	PA	-2.44	1.032	0.03	2.44	3.48	-1.41	5.44	0.09	2.66
PPARA	GLU	-1.17	0.502	0.03	1.17	1.67	-0.66	2.24	0.31	1.58
	LA	-0.88	0.513	0.11	0.88	1.40	-0.37	1.84	0.38	1.29
	PA	-1.25	0.504	0.03	1.25	1.75	-0.74	2.37	0.30	1.67

[1] 95% confidence intervals of the $\Delta\Delta C_T$ values were transformed to fold changes

[2]Isoenergetic infusion, 151 kcal/h

[3]Fold changes are relative to gene expression in the CON treatment, normalized to the mean of *HPRT1*, *PGK1* and *RPS9* housekeeping genes.

[4]Genes listed in the table were subject to Multiple Test correction using a local FDR value <0.10, *FBP1* = fructose-1,6-bisphosphatase 1; *GLUD1* = glutamate

Table 3.8. (cont'd)
dehydrogenase 1; *GOT2* = aspartate aminotransferase 2; *GPAM* = glycerol-3-phosphate acyltransferase; *PC* = pyruvate carboxylase; *PCCA* = propionyl-CoA carboxylase alpha; *PCK1* = phosphoenolpyruvate carboxykinase 1; *PPARA* = peroxisome proliferator activated receptor alpha.

APPENDIX B: BLOOD METABOLITES

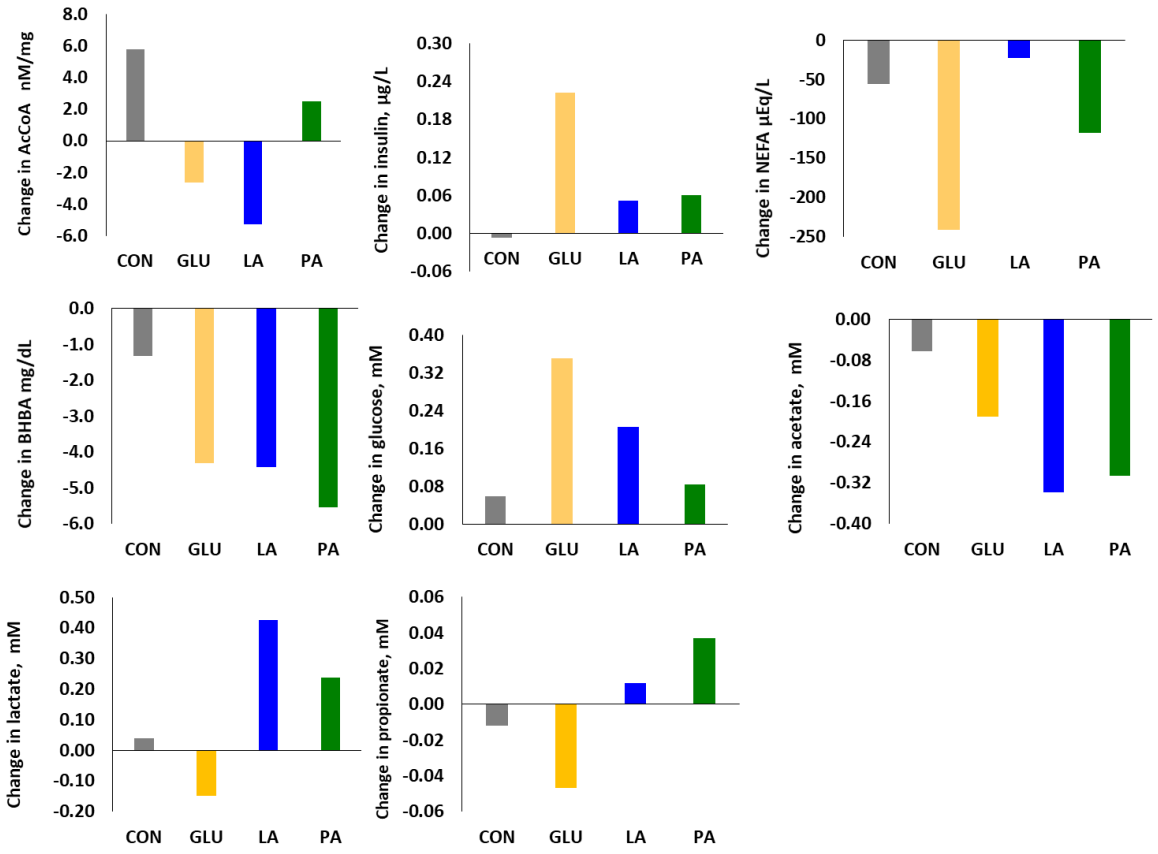

Figure 3.1 Effects of isoenergetic intra-abomasal infusion of glucose (GLU), lactic acid (LA) and propionic acid (PA) relative to a no infusion control (CON) on change in plasma concentration of insulin, metabolites and liver acetyl CoA (AcCoA) content over the 22 h/d infusion for cows in the immediate postpartum period

BIBLIOGRAPHY

BIBLIOGRAPHY

Albornoz, R. I., and M. S. Allen. 2016. Diet starch content and fermentability affects feed intake and milk yield of cows in the postpartum period.. J. Anim. Sci. 94:362-362. doi:10.2527/jam2016-0755.

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. http://dx.doi:10.3168/jds.S0022-0302(00)75030-2.

Allen, M.S. 2014. Drives and limits to feed intake in ruminants. Anim. Prod. 54:1513–1524. http://dx.doi:10.1071/AN14478.

Allen, M.S., B.J. Bradford, and M. Oba. 2009. BOARD-INVITED REVIEW: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. http://dx.doi:10.2527/jas.2009-1779.

Al-Trad, B., K. Reisberg, T. Wittek, G. B. Penner, A. Alkaassem, G. Gabel, M. Fürll, and J. R. Aschenbach. 2009. Increasing intravenous infusions of glucose improve body condition but not lactation performance in midlactation dairy cows. J. Dairy Sci. 92:5645-5658.

AOAC International. 1997. Official Methods of Analysis. 16th ed. A. International, Gaithersburg, MD.

Bauman, D. E., C. L. Davis, and H. F. Bucholtz. 1971. Propionate production in the rumen of cows fed either a control or high- grain, low-fiber diet. J. Dairy Sci. 54:1282−1287.

Bell, A.W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804–2819.

Bradford, B.J., and M.S. Allen. 2005. Phlorizin administration increases hepatic gluconeogenic enzyme mRNA abundance but not feed intake in late-lactation dairy cows. J. Nutr. 135:2206–2211.

Brown, W. E. and M.S. Allen. 2013. Effects of intrajugular glucose infusion on feed intake, milk yield, and metabolic responses of early postpartum cows fed diets varying in protein and starch concentration. J. Dairy Sci. 96:7132-7142.

Clark, J.H., H.R. Spires, R.G. Derrig, and M.R. Bennink. 1977. Milk production, nitrogen utilization and glucose synthesis in lactating cows infused postruminally with sodium caseinate and glucose1. J. Nutr 107:631–644.

Cochran, R.C., D.C. Adams, J.D. Wallace, and M.L. Galyean. 1986. Predicting digestibility of different diets with internal markers: evaluation of four potential markers. J. Anim. Sci. 63:1476–1483.

Dado, R.G., and M.S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J. Dairy Sci. 78:118–133. http://dx.doi:10.3168/jds.S0022-0302(95)76622-X.

Davis, C. L. 1967. Acetate production in the rumen of cows fed either control or low-fiber, high-grain diets. J. Dairy Sci. 50: 1621-1625.

Dowden, D. R., and D. R. Jacobson. 1960. Inhibition of appetite in dairy cattle by certain intermediate metabolites. Nature 188:148–149.

Emery, A.G., and F.G. Benedict. 1911. The heat of combustion of compounds of physiological importance. Am J Physiol 28:301–307.

Emmanuel, B. 1981. Glucokinase, hexokinase, gluconeogenesis, glycogenesis and glycolysis in camel (*Camelus dromedarius*) and sheep (*Ovis aries*). Comp Biochem Physiol B Biochem Mol Biol. 64:547–550.

Frobish, R.A., and C.L. Davis. 1977. Effect of abomasal infusions of glucose and proprionate on milk yield and composition. J. Diry Sci. 60:204–209.

Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analysis (Apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Granner, D., T. Andreone, K. Sasaki, and E. Beale. 1983. Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. Nature 305:549–551

Gualdrón-Duarte, L.B., and M.S. Allen. 2017. Increased anaplerosis of the tricarboxylic acid cycle decreased meal size and energy intake of cows in the postpartum period. J. Dairy Sci. 100:4425–4434. doi:10.3168/jds.2016-12104.

Hach, C.C., B.K. Bowden, A.V. Kopelove, and S.V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. J. Assoc. Off. Anal. Chem. 70:783–787.

Hurtaud, C., H. Rulquin, and R. Verite. 1998. Effects of graded duodenal infusions of glucose on yield and composition of milk from dairy cows. 1. Diets based on corn silage. J. Dairy Sci. 81:3239–3247.

Karkalas, J. 1985. An improved enzymic method for the determination of native and modified starch. J. Sci. Food Agric. 36:1019–1027.

King, T.M., and P.D. Reiss. 1985. Separation and measurement of short-chain coenzyme-A compounds in rat liver by reversed-phase high-performance liquid chromatography. Anal. Biochem. 146:173–179.

Knapp, J.R., H.C. Freetly, B.L. Reis, C.C. Calvert, and R.L. Baldwin. 1992. Effects of somatotropin and substrates on patterns of liver metabolism in lactating dairy cattle. J. Dairy Sci. 75:1025–1035. doi:10.3168/jds.S0022-0302(92)77846-1.

Knowlton, K. F., T. E. Dawson, B. P. Glenn, G. B. Huntington, and R. A. Erdman. 1998. Glucose metabolism and milk yield of cows infused abomasally or ruminally with starch. J. Dairy Sci. 81:3248–3258.

Kotarski, S.F., R.D. Waniska, and K.K. Thurn. 1992. Starch hydrolysis by the ruminal microflora. J. Nutr. 122:178–190.

Labedeva, N.D. 1964. Heats of combustion of monocarboxilic acids. Russ. J. Phys. Chem (Engl.Transl). 38:1435-1437.

Larsen, M., and N.B. Kristensen. 2009. Effect of abomasal glucose infusion on splanchnic amino acid metabolism in periparturient dairy cows. J. Dairy Sci. 92:3306–3318. doi:10.3168/jds.2008-1889.

Larson, J., and P.C. Hoffman. 2008. Technical Note: A method to quantify prolamin proteins in corn that are negatively related to starch digestibility in ruminants. J. Dairy Sci. 91:4834–4839. doi:10.3168/jds.2008-1378.

Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217–1240.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle . 7th rev. ed. Natl. Acad. Press, Washington, DC.

Oba, M., and M.S. Allen. 2003a. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174–183. doi:10.3168/jds.S0022-0302(03)73598-X.

Oba, M., and M.S. Allen. 2003b. Intraruminal infusion of propionate alters feeding behavior and decreases energy intake of lactating dairy cows. J. Nutr. 133:1094–1099.

Overton, T.R., and M.R. Waldron. 2004. Nutritional management of transition dairy cows:strategies to optimize metabolic health. J. Dairy Sci. 87:E105–E119. doi:10.3168/jds.S0022-0302(04)70066-1.

Reynolds, C.K., P.C. Aikman, B. Lupoli, D.J. Humphries, and D.E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86:1201–1217. doi:10.3168/jds.S0022-0302(03)73704-7.

Saville, G., and H.A. Gundry. 1959. The heats of combustion solution and ionization of lactic acid. Transactions of the Faraday Society. 2036–2038.

Stangassinger, M., and D. Giesecke. 1986. Splanchnic metabolism of glucose and related energy substrates. Pages 347–366 in Control of Digestion and Metabolism in Ruminants. L. P. Milligan, W. L. Grovum, and A. Dobson, ed. Prentice Hall,Englewood Cliffs, NJ.

Steibel, J.P., R. Poletto, P.M. Coussens, and G.J.M. Rosa. 2009. A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. Genomics. 94:146-152.

Stocks, S.E., and M.S. Allen. 2012. Hypophagic effects of propionate increase with elevated hepatic acetyl coenzyme A concentration for cows in the early postpartum period. J. Dairy Sci. 95:3259–3268. http://dx.doi:10.3168/jds.2011-4991.

Stocks, S.E., and M.S. Allen. 2013. Hypophagic effects of propionic acid are not attenuated during a 3-day infusion in the early postpartum period in Holstein cows. J. Dairy Sci. 96:4615–4623. http://dx.doi:10.3168/jds.2013-6653.

Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J. Dairy Sci. 86:3620-3633.

Yang, J., Y. Chi, B.Burkhardt, Y. Guan, and B. Wolf. 2010. Leucine metabolism in regulation of insulin secretion from pancretic beta cells. Nutr. Rev. 68:270-279. doi:10.1111/j.1753-4887.2010.00282.x

Zhang, Q., S. Koser, B. Bequette, and S. Donkin. 2015. Effect of propionate on mRNA expression of key genes for gluconeogenesis in liver of dairy cattle. J. Dairy Sci. 98:8698-8709.

CHAPTER 4. EFFECTS OF ACETIC ACID OR SODIUM ACETATE INFUSED INTO THE RUMEN OR ABOMASUM ON FEEDING BEHAVIOR AND METABOLIC RESPONSE OF COWS IN THE POSTPARTUM PERIOD

ABSTRACT

Effects of continuous isomolar infusions of acetic acid (AA) or sodium acetate (NA) infused into the rumen (RU) or into the abomasum (AB) on feeding behavior, dry matter intake (DMI), and metabolic response of cows in the early postpartum period were evaluated. Six rumen-cannulated multiparous Holstein cows (11.8 ± 3.9 days in milk) were utilized in a 6 x 6 Latin square design experiment balanced for carryover effects with a 2 x 3 factorial arrangement of treatments. Treatments were AA and NA, with sodium chloride (CON) as a control, infused at a rate of ~0.75 mol/h (0.5 L/h) into the RU or AB for the first 8 h following feeding with a rest day between infusion days. Treatment sequences were assigned randomly to cows. Feeding behavior was recorded by a computerized data acquisition system and blood was sampled at 0, 4, and 8 h relative to the start of infusion. We hypothesized that AA is more hypophagic than NA, and that infusion into the AB is more hypophagic than into the RU. Treatments decreased dry matter intake (DMI) 30% (4.3 kg/8h vs. 6.1 kg/8h) compared with CON by decreasing meal frequency but their effects on DMI differed; NA-RU did not reduce DMI (7.0 kg/8 h), whereas AA-RU (2.6 kg/8 h), AA-AB (3.7 kg/8 h) and NA-AB (4.0 kg/8 h) decreased DMI compared with CON. There was a residual effect following infusions of AA compared with NA on DMI for the remainder of the day but treatments did not affect DMI during the rest day. Treatments increased plasma acetate and ß-hydroxybutyrate concentrations over time (interaction, $P < 0.01$) and decreased plasma insulin concentration compared with CON. Plasma glucose concentration decreased over time after AA-AB infusion compared with other treatments and CON. Plasma

nonesterified fatty acid concentration increased over time for AA compared with NA and CON suggesting the increase in lipolysis to compensate the decrease in DMI. In contrast to the other treatments, NA-RU did not decrease DMI compared with control but the reason cannot be determined from the data available from the current study.

INTRODUCTION

Acetic acid is the primary end-product of ruminal fermentation, typically comprising over 50% of the fermentation acids produced in the rumen on molar basis. It is used for fatty acid synthesis in adipose and mammary tissue and as an energy source by various tissues. Intraruminal infusions of acetic acid (**AA**) or sodium acetate (**NA**) have been used to test their effects on yields of milk and milk components and feed intake of lactating cows (Rook and Balch, 1961; Reynolds et al., 1979; Sheperd and Combs, 1998; Urrutia and Harvatine, 2017). Whereas some studies have infused AA intraruminally (**RU**) without detrimental effects on DMI (Rook and Balch, 1961; Choi and Allen, 1999), others have reported a reduction in DMI (Montgomery et al., 1963; Wilson et al., 1967) or adverse physiological effects on the animals (*e.g.* cessation of rumination, decrease in ruminal pH and symptoms of metabolic acidosis (Montgomery et al., 1963; Sheperd and Combs, 1998).

Our laboratory has used AA alone or partially neutralized with sodium hydroxide intraruminally as control treatments to study the effects of propionate on feeding behavior of lactating cows. Infusions up to 28 moles of NA (1.56 mol/h) or ~20 moles of AA (0.75 mol/h) had no detrimental effect on the animals or reduction on feed intake (Choi and Allen, 1999; Oba and Allen, 2003a, b, c; Stocks and Allen, 2013, 2014). We considered that propionate is hypophagic compared with acetate because propionate is anaplerotic and stimulates hepatic oxidation of acetyl CoA (**AcCoA**) in the tricarboxylic acid (**TCA**) cycle whereas acetate does not

(Allen et al., 2009). Feeding behavior is affected by osmotic changes in the rumen so we rationalized that isosmotic infusions of acetate were appropriate controls for the propionate treatment. All previous experiments to our knowledge have infused AA alone or partially neutralized with sodium hydroxide into the rumen. However, we recently observed adverse effects of abomasal (**AB**) infusion of 20.3 mol/d of AA (4.2 Mcal) at a rate of 0.5 L/h (0.92 mol/h) to cows in the postpartum (**PP**) period (Gualdrón-Duarte and Allen, unpublished) in which AA decreased DMI 64% compared with control (no infusion). This was not expected because intraruminal infusions of AA at this rate did not decrease DMI in previous experiments.

The objective of this experiment was to evaluate the effects of continuous isomolar infusions of AA or NA infused into the RU or into the AB on feeding behavior, DMI and metabolic responses of cows in the PP period. We hypothesized that AA would be more hypophagic than NA, and that infusion into the AB would be more hypophagic than into the RU.

MATERIALS AND METHODS

Animals, and Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Cows were ruminally cannulated at least 45 d before parturition and housed in individual tie-stalls for the 12-d duration of the experiment. All cows were fitted with a single jugular catheter 2 d before the beginning of the experiment, according to Bradford et al. (2006). Catheter patency was checked daily until removed at the end of the experiment. Cows were withheld from feed at 0800 h and were not allowed access to feed until all blood samples were collected. Cows were fed once daily (900 h) at 125% of expected *ad libitum* intake, and milked twice daily (0530 and 1730 h). The amounts of feed offered and orts

were weighed for each cow daily.

Experimental Design and Treatment Diets

Six ruminally cannulated multiparous, lactating Holstein cows (11.8 ± 3.9 DIM; mean ± SD) at the Michigan State University Dairy Teaching and Research Center were used in a 6 x 6 Latin square design experiment with a 2 x 3 factorial arrangement of treatments. Cows were assigned randomly to stalls and a treatment sequence balanced for carry-over effects. Infusion periods were 8-h long with continuous isomolar infusions (0.75 mol/h) of AA (pH 2.3, > 99.7%, Sigma Aldrich, Chemical Co., St. Louis, MO), NA (pH 5.9, AA + NaOH,) or NaCl (CON, > 99.5%, Sigma Aldrich, Chemical Co., St. Louis, MO) as a control; infused into the AB or RU at a rate of 0.5 L/h. Concentration of solutions and duration of infusions were 0.75 mol/h and 8 h respectively to reduce adverse the physiological effects observed in a previous experiment (Gualdrón-Duarte and Allen, unpublished) when solutions were infused at 0.91 mol/h for 22 h/d into the AB. Solutions were infused into the RU or AB using Baxter Flo-Gard 6201 infusion pumps (Baxter Medical Products, Deerfield, IL) through two vinyl tubes (0.95 cm OD, 0.71 cm ID), the first connected to a Nalgene bottle (3.8 cm diameter, 8.5 cm long) and placed into the AB and held by a semihard flexible rubber flange as described in Gualdrón-Duarte and Allen (2017), and the second tube placed into the RU. A recovery period of 40 h was included between infusions.

All cows received a common experimental diet from parturition through the end of the experiment. The experimental diet (Table 4.1) was composed of corn silage, ground corn, soybean meal, alfalfa silage, alfalfa hay, soy hulls, and a vitamin and mineral mix and formulated to meet requirements for absorbed protein, minerals, and vitamins (NRC, 2001).

Sample and Data Collection

Feed offered and refused was measured daily. Cows were blocked from feed from 0800 to 0900 h daily. Samples (0.5 kg) of all dietary ingredients and the TMR were collected daily throughout the experiment, and orts for each cow were taken at the end of each infusion period and stored in plastic bags at -20°C until processed. Feeding behavior data (feed disappearance and water intake) were recorded continuously for 22 h daily for each cow during infusion days via computer every 5 s, and size, length, and frequency of meals, intermeal interval, total eating time, and water intake were calculated (Dado and Allen, 1995).

Table 4.1. Ingredients and nutrient composition of the experimental diet

Item	Amount
Ingredient, % of DM	
Corn silage	29.9
Ground corn	19.5
Soybean meal	16.4
Alfalfa silage	15.1
Alfalfa Hay	13.6
Soybean hulls	1.4
Vitamin and mineral mix[1]	2.0
Sodium bicarbonate	1.1
Limestone	1.1
Nutrient composition	
DM, %	52.4
NDF, % of DM	28.7
Forage NDF, % of DM	24.3
Starch, % of DM	22.0
CP, % of DM	17.4
Ash, % of DM	8.9

[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.

Milk yield was recorded and milk samples were collected daily at each milking and stored with preservative (Bronopol tablet, D&F Control Systems, San Ramon, CA) at 4ºC for component analysis (Universal Lab Services, East Lansing, MI). Milk samples were analyzed for fat, true protein, lactose, and milk urea nitrogen (**MUN**) by Michigan DHIA (AOAC, 1997).

Blood samples were collected immediately before and at 4 and 8 h following the start of infusions. Blood was sampled from a jugular catheter and collected into 3 evacuated tubes, 2 containing K_2EDTA and 1 containing potassium oxalate with NaF as a glycolytic inhibitor, and placed on ice until centrifugation. Tubes were centrifuged at 3,000 x *g* for 15 min at 5°C immediately after sample collection. Plasma was harvested and frozen at -20°C until analysis. Before and 4 h after the start of infusions, blood samples were taken in a heparinized syringe, transported to the laboratory and analyzed with a blood gas analyzer.

Analysis of Samples

Diet ingredients and orts were dried in a 55°C forced-air oven for 72 h and were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for CP, starch, NDF, and ash. Crude protein was determined according to Hach et al. (1987). Starch was analyzed using an enzymatic method (Karkalas, 1985) after samples were gelatinized with NaOH; glucose concentration was measured by a glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Content of NDF was analyzed according to Mertens (2002) with the inclusion of amylase and Na sulfite. Ash content was determined after 6 h of oxidation at 500°C in a muffle furnace. All nutrients are expressed as percentages of DM that was determined by drying at 105°C in a forced-air oven for more than 8 h.

Plasma samples were analyzed colorimetrically with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp.) to determine concentrations of metabolites. Commercial kits were used to determine concentrations of nonesterified fatty acids (**NEFA**; NEFA HR kit, Wako Chemicals USA, Richmond,VA) and β hydroxybutyrate (**BHB**; kit no. 2240, Stanbio Laboratory, Boerne, TX), and glucose was determined using a glucose oxidase method (Sigma Chemical. Co.). Insulin was determined using an ELISA kit (kit no. 10-1201-01, Mercodia,

Uppsala, Sweden). Plasma acetate, propionate and lactate concentrations were analyzed by HPLC (Waters Corp., Milford, MA). Plasma (600 μL) was mixed with 600 μL of 3.6 M $Ca(OH)_2$ and 300 μL of 0.5 M $CuSO_4$ containing crotonic acid as an internal marker in 1.7 mL microcentrifuge tubes. Samples were frozen, thawed and centrifuged at 12,000 × *g* for 10 min, and supernatant (1,000 μL) was mixed with 42 μL of 98% H_2SO_4 in 1.5-mL microcentrifuge tubes. Samples were frozen and thawed twice, and centrifuged at 12,000 × *g* for 10 min to precipitate and remove protein. Supernatant was transferred to HPLC vials. The column used was Aminex HPX-87H (catalog number 125-0140; 300 x 7.8 mm; Bio-Rad Laboratories, Richmond, CA). Column temperature was 50°C, and the solvent was 0.015N H_2S0_4. Detection was by refractive index (Waters 410; Millipore Corp., Milford, MA). Blood chemistry and gas analysis was performed in a Stat Profile pHOX Ultra Analyzer (Nova Biomedical Corp., Waltham, MA) by Michigan State University Diagnostic Center for Population and Animal Health (East Lansing) for direct measurement of pH (hydrogen ion selective glass membrane), partial pressure of CO_2 (modified hydrogen ion selective glass membrane), partial pressure of O_2 (gas permeable membrane), saturation percentage of O_2 (reflectance photometry system), Na^+, K^+, Cl^-, Ca^{++}, Mg^{++} (ion selective membrane), glucose, lactate and creatinine (platinum electrode), and blood urea nitrogen (ammonium ion selective membrane). Values for HCO_3, total CO_2, base excess blood, anion gap, osmolality and standard bicarbonate were determined indirectly by calculation.

Statistical Analysis

Feeding behavior, intake, yields of milk and milk components, concentration of milk components, and plasma composition were analyzed using the Fit Model procedure of JMP (version 12.0, 2015; SAS Institute Inc., Cary, NC) using the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk}$$

where Y_{ijk} = the dependent response variable of interest, μ = overall mean, T_i = fixed effect of treatment (i = 1 to 6), P_j = fixed effect of period (j = 1 to 6), C_k = random effect of cow (k = 6), and e_{ijk} = residual, normally distributed.

Plasma concentrations of glucose, NEFA, BHB, acetate, propionate, lactate and insulin were analyzed using the MIXED procedure of SAS (version 9.4, 2013; SAS Institute Inc., Cary, NC) with repeated measures, using the following model:

$$Y_{ijkl} = \mu + T_i + P_j + H_k + T_i H_k + C_l + e_{ijkl}$$

where Y_{ijkl} = the response variable; μ = overall mean; T_i = fixed effect of treatment (i = 1 to 6); P_j = fixed effect of period (j = 1 to 6); H_k = fixed effect of time (k = 1 to 3); T_iH_k = interaction of treatment and time; C_l = random effect of cow (l = 6); and e_{ijkl} = residual. Time was included in the repeated statement. Covariance structure was first-order autoregressive. Treatment differences at each time point were analyzed using the SLICE option. Cow by treatment interaction was implicitly used as the error term for treatment. Denominator degrees of freedom were estimated by using the Between-Within option.

For all statistical models used, residuals were normally distributed as determined by the Shapiro-Wilk test.

Blood chemistry results at 4 h following the start of infusion were analyzed using the Fit Model procedure of JMP (version 12.0, 2015; SAS Institute Inc., Cary, NC) with the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + Cov + e_{ijk}$$

where Y_{ijk} = the dependent response variable of interest, μ = overall mean, T_i = fixed effect of treatment (i = 1 to 6), P_j = fixed effect of period (j = 1 to 6), C_k = random effect of cow (k = 6), Cov = effect of covariate, and e_{ijk} = residual, normally distributed. Data for samples preceding each infusion were included as covariates to account for differences in baseline measurements.

Treatment effects were declared at $P \leq 0.05$ and tendencies for treatment effects at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies for interactions at $P \leq 0.15$. All data are expressed as least squares means and standard error of the means, unless otherwise specified. Means were compared with preplanned contrasts.

RESULTS

Feed Intake and Feeding Behavior

Treatments decreased DMI during the infusion period 30% (4.3 kg/8h vs. 6.1 kg/8 h, $P < 0.01$) compared with CON by decreasing meal frequency ($P < 0.05$) but their effects on DMI differed (interaction between type and site of infusion, $P < 0.01$). The treatment NA-RU did not reduce DMI during the 8h of infusion (7.0 kg/8h), whereas AA-RU (2.6 kg/8 h), AA-AB (3.7 kg/8 h) and NA-AB (4.0 kg/8 h) decreased DMI compared with CON. . Acetic acid decreased DMI 32% compared with NA infusions during the 15 h following infusions and the effects of treatment on DMI for the day of infusion was similar to those observed during the infusion period. Treatments did not affect DMI ($P = 0.22$) during the rest day. Treatments decreased water intake 33% (31.4 L/8 h vs. 47.1 L/8 h, $P < 0.01$) compared with CON and AA decreased water intake during the infusion period by 56% (19.1 L/8h vs. 43.7 L/8h, $P < 0.01$) compared

with NA. However, treatments did not affect water intake during the 15h following infusions (Table 4.2).

Table 4.2. Effects of isomolar[1] intra-ruminal (RU) or intra-abomasal (AB) infusion (8 h/d) of sodium chloride (CON), acetic acid (AA) and sodium acetate (NA) on feeding behavior and water intake for cows in the immediate postpartum period

	Infusion											
	CON-RU	CON-AB	AA-RU	AA-AB	NA-RU	NA-AB	SEM	Trt[2]	CON vs. AA+NA	NA vs. AA	AB vs. RU	Int[3]
During infusion, /8h												
DMI, kg	6.23	6.05	2.57	3.72	7.02	4.01	0.99	< 0.001	< 0.01	< 0.01	0.19	0.01
Meal frequency, meals	4.67	4.67	2.83	2.46	5.12	4.08	0.772	0.15	0.14	0.03	0.4	0.69
Meal size, kg	1.37	1.34	1.45	2.04	1.39	1.22	0.315	0.41	0.46	0.13	0.48	0.19
Water intake, L	49.6	44.6	17.7	20.4	46.2	41.2	7.88	< 0.01	< 0.01	< 0.01	0.86	0.56
Drinking frequency,	7.17	5.5	2.93	3.5	7.35	5.6	1.47	< 0.01	0.05	< 0.01	0.5	0.19
Following infusion, /15 h												
DMI, kg	6.70	7.58	4.08	5.92	7.30	7.43	1.08	0.06	0.22	0.02	0.3	0.37
Water intake, L	31.5	30.4	26.9	27.0	32.4	28.6	6.04	0.8	0.43	0.32	0.6	0.59
Total for infusion day, /23 h												
DMI, kg/d	13.0	13.6	6.67	9.62	14.2	11.4	1.76	< 0.001	0.01	<	0.97	0.02
Rest day, /23 h												
DMI, kg	13.6	14.4	10.7	13.1	14.2	11.5	2.67	0.33	0.21	0.54	0.9	0.11
Water, L	64.5	78.4	66.8	69.5	62.9	72.7	11.8	0.92	0.62	0.96	0.46	0.67

[1]Isomolar infusion, 0.75 mol/h

[2]Trt = treatment

[3]Int=interaction of acetate type (AA vs. NA) and site of infusion (AB vs. RU).

Table 4.3. Effects of isomolar[1] intra-ruminal (RU) or intra-abomasal (AB) infusion (8h/d) of sodium chloride (CON), acetic acid (AA) and sodium acetate (NA) on milk yield and milk components for cows in the immediate postpartum period

	Infusion							Significance, *P*				
	CON-RU	CON-AB	AA-RU	AA-AB	NA-RU	NA-AB	SEM	Trt[2]	CON vs. AA+NA	NA vs. AA	AB vs. RU	Int[3]
PM milking (at the end of infusions)												
Yield, kg												
Milk	16.5	18.0	17.0	17.5	16.9	14.9	1.74	0.30	0.37	0.14	0.42	0.19
Fat	0.90	0.86	0.69	0.86	0.88	0.74	0.11	0.16	0.17	0.60	0.89	0.04
Protein	0.46	0.48	0.48	0.46	0.45	0.41	0.04	0.33	0.41	0.10	0.26	0.62
Lactose	0.79	0.87	0.81	0.82	0.83	0.73	0.08	0.53	0.42	0.44	0.38	0.23
Composition												
Fat, %	5.42	4.75	4.16	4.78	5.24	4.87	0.42	0.15	0.24	0.09	0.71	0.15
Protein, %	2.84	2.65	2.83	2.65	2.69	2.78	0.08	0.42	0.85	0.95	0.56	0.08
Lactose, %	4.78	4.85	4.76	4.73	4.92	4.92	0.06	< 0.001	0.44	< 0.01	0.67	0.77
MUN, mg/dL	12.6	11.7	13.6	13.9	12.3	13.0	0.88	0.14	0.06	0.11	0.43	0.79
AM milking (12 h after infusions)												
Yield, kg												
Milk	15.4	15.6	14.0	11.1	14.5	13.2	1.85	0.01	< 0.01	0.14	0.03	0.38
Fat	0.65	0.62	0.77	0.60	0.58	0.56	0.09	0.02	0.88	0.02	0.04	0.13
Protein	0.42	0.41	0.38	0.31	0.39	0.35	0.05	0.02	0.01	0.33	0.03	0.59
Lactose	0.73	0.75	0.64	0.51	0.70	0.63	0.09	< 0.01	< 0.01	0.05	0.03	0.50
Composition												
Fat, %	4.15	3.98	5.60	5.54	4.09	4.37	0.36	< 0.001	< 0.001	< 0.001	0.68	0.54
Protein, %	2.80	2.59	2.74	2.72	2.71	2.64	0.08	0.90	0.92	0.49	0.52	0.75
Lactose, %	4.72	4.77	4.60	4.62	4.81	4.75	0.08	0.08	0.35	0.01	0.72	0.52
MUN, mg/dL	11.7	12.0	15.4	14.8	11.6	13.7	0.97	0.01	0.01	0.01	0.41	0.15

[1]Isomolar infusion, 0.75 mol/h

[2]Trt = treatment

[3]Int=interaction of acetate type (AA vs. NA) and site of infusion (AB vs. RU).

Milk Production and Components

At the end of the infusion period (PM milking), there was no effect of treatment on milk yield. Treatments interacted ($P = 0.04$) to affect fat yield and tended to interact ($P = 0.15$) to affect fat concentration with NA increasing, and AA decreasing fat yield when infused in the RU compared with the AB (Table 4.3). Protein yield tended to be increased by AA compared with NA (0.47 vs. 0.43 kg, $P = 0.10$) but protein concentration was not affected by treatment. Lactose concentration was increased by NA compared with AA (4.92% vs. 4.75%, $P < 0.01$) but lactose yield was not affected by treatment. Concentration of MUN tended to be increased by AA and NA treatments compared with control (12.2 vs. 10.0 mg/dl, $P = 0.06$).

Effects of treatment on milk yield and composition for the AM milking (12 h after infusion) are reported in Table 4.3. The AA and NA treatments decreased milk yield 15% compared with CON (13.2 kg vs. 15.5 kg, $P < 0.01$) and infusion into the AB decreased milk yield 15% compared with infusion into the RU (12.2 kg vs. 14.3 kg, $P = 0.03$). Fat yield was affected by treatment ($P = 0.02$) with AA-RU tending to increase fat yield compared with AA-AB, NA-AB, and NA-RU (interaction, $P = 0.13$). Treatments increased fat concentration 16% compared with CON (4.9 vs. 4.1%, $P < 0.001$) and AA increased fat concentration compared with NA (5.6 vs 4.2%, $P < 0.001$). Treatments decreased protein yield 14% compared with CON (0.36 vs. 0.42 kg, $P = 0.01$) and infusion into the AB decreased protein yield 14% compared with RU (0.33 vs. 0.39, $P = 0.03$). No effect of treatment on protein concentration was observed. Treatments decreased lactose yield 16% compared with CON (0.62 vs 0.74 kg, $P < 0.01$), with a decrease of 13% for AA compared with NA (0.58 vs. 0.67 kg, $P = 0.05$) and a decrease of 15% for infusion into the AB compared with RU (0.57 vs. 0.67, $P = 0.03$). The AA treatment decreased lactose concentration 3.6% compared with NA (4.61 vs. 4.78%, $P = 0.01$). Treatments

increased MUN concentration 14% compared with CON (13.9 vs. 11.9 mg/dL, $P = 0.01$), but their effects tended to differ with AA increasing, and NA decreasing, MUN concentration when infused into the RU compared with the AB (interaction $P = 0.15$).

Blood Metabolites and Insulin Concentration

Treatments interacted with time to affect plasma concentrations of glucose ($P = 0.01$), NEFA ($P = 0.02$), BHB ($P < 0.01$), acetate ($P < 0.0001$) and propionate ($P = 0.03$; Table 4.4, Fig. 4.1). Compared with CON, treatments decreased plasma lactate concentration 8% ($P = 0.01$), decreased plasma insulin concentration 38% ($P = 0.01$), increased plasma acetate concentration 122% ($P < 0.001$) and tended ($P = 0.07$) to increase propionate concentration 21%. However, no differences were detected between infusion treatments (AA vs. NA), site of infusion (RU and AB), or their interaction for any plasma metabolite or hormone.

Blood Chemistry

Acetic acid decreased pH (7.42 vs. 7.44, $P = 0.04$), HCO_3^- (22.0 vs. 25.3 mmol/L, $P < 0.01$), total CO_2 (23.0 vs. 26.4 mm/L, $P < 0.01$), base excess (-1.10 vs. 1.89 mmol/L, $P < 0.01$) and osmolality (273 vs. 278 mOsm/L, $P < 0.01$) compared with NA but interacted with site of

Table 4.4. Effects of isomolar[1] intra-ruminal (RU) or intra-abomasal (AB) infusion (8h/d) of sodium chloride (CON), acetic acid (AA) and sodium acetate (NA) on plasma metabolites and insulin for cows in the immediate postpartum period

	Infusion							Significance, *P*						
	CON-AB	CON-RU	AA-AB	AA-RU	NA-AB	NA-RU	SEM	Trt[2]	Time	Trt*time	CON vs. AA+NA	NA vs. AA	AB vs. RU	Int[3]
Glucose, mg/dL	42.0	45.3	41.3	39.0	41.2	43.0	4.2	0.56	< 0.01	0.01	0.24	0.43	0.67	0.41
NEFA, µEq/L	601	527	676	773	604	653	137	0.34	< 0.001	0.02	0.11	0.25	0.73	0.77
BHB, mg/dL.	23.2	22.0	25.5	30.2	27.8	29.0	6.7	0.59	<0.0001	< 0.01	0.10	0.89	0.63	0.65
Lactate, mmol	0.333	0.368	0.337	0.309	0.336	0.309	0.01	0.01	0.06	0.71	0.01	0.98	0.51	0.97
Acetate, mmol	1.46	1.26	2.33	2.98	2.82	3.95	0.54	< 0.01	<0.0001	<0.0001	< 0.001	0.12	0.18	0.61
Propionate, mmol	0.117	0.108	0.145	0.155	0.125	0.142	0.03	0.40	0.03	0.03	0.07	0.39	0.71	0.87
Insulin, µg/L	0.180	0.182	0.105	0.093	0.131	0.119	0.04	0.17	0.94	0.43	0.01	0.40	0.77	1.00

[1]Isomolar infusion, 0.75 mol /h

[2]Trt = treatment

[3]Int=interaction of acetate type (AA vs. NA) and site of infusion (AB vs. RU).

infusion with decreases for AA and increases for NA when infused into the AB compared with the RU (interaction $P \leq 0.10$). Acetic acid increased anion gap (16.3 vs. 15.1 mmol/L, $P = 0.04$), and ionized magnesium (1.33 vs. 1.20 mg/dl, $P < 0.01$) and decreased sodium (139 vs. 142 mmol/L, $P < 0.01$) and tended to decreased creatinine (0.946 vs. 0.999, $P = 0.08$) and ratio of calcium to magnesium (2.32 vs. 2.43, $P = 0.08$) compared with NA. Acetic acid decreased blood urea nitrogen (BUN, 12.3 vs. 13.0 mg/dL) and the ratio of BUN to creatinine (12.7 vs. 13.1) when infused into the RU, but increased BUN (13.1 vs. 12.5 mg/dL) and BUN/creatinine (14.0 vs. 13.2) when infused into the AB (interaction $P = 0.02$). Acetic acid and NA treatments decreased chloride (1.05 vs. 1.08 mmol/L, $P < 0.001$) and tended to decrease normalized calcium (4.91 vs. 5.10 mg/dL. $P = 0.06$) compared with CON. Plasma potassium concentration was not affected by treatment ($P > 0.15$).

DISCUSSION

This study was conducted to investigate the mechanism by which abomasal infusion of AA decreased DMI by cows in the immediate PP period in a previous experiment (Gualdrón-Duarte and Allen, unpublished). Our objective was to evaluate the effects of continuous isomolar infusions of AA or NA infused into the RU or into the AB on feeding behavior, DMI and metabolic response of cows in the PP period.

In agreement with our hypothesis, AA was more hypophagic than NA but NA-AB decreased DMI whereas NA-RU did not compared with CON. The decrease in DMI by AA was because AA decreased meal frequency and not meal size compared with NA, indicating that AA did not affect satiety within the timeframe of meals but delayed hunger. Choi and Allen (1999).

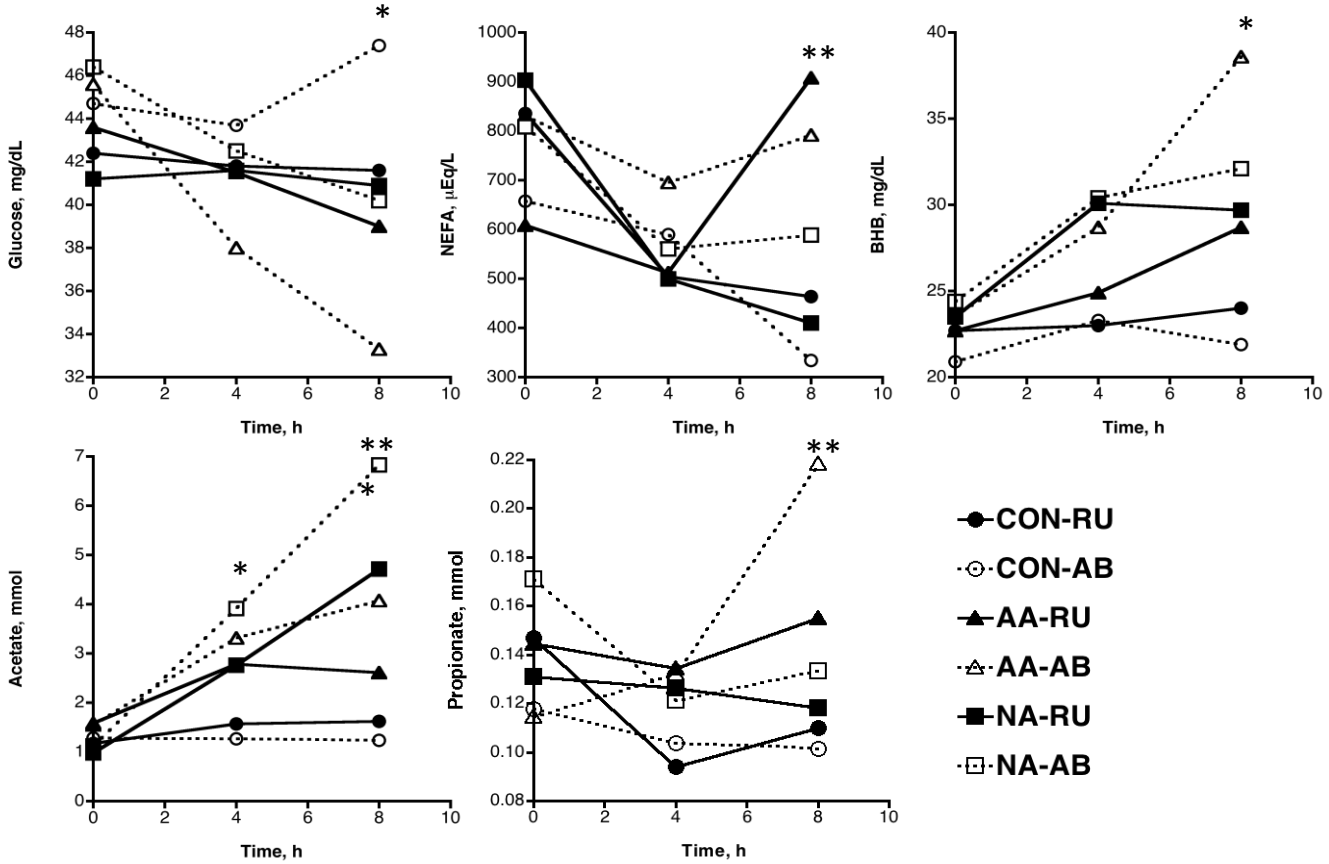

Figure 4.1. Effects of isomolar intra-ruminal (RU) or intra-abomasal (AB) infusion (8h/d) of sodium chloride (CON), acetic acid (AA) and sodium acetate (NA) on plasma metabolites for cows in the immediate postpartum period. Treatments were: continuous isomolar infusions (0.75 mol/h) of acetic acid (AA, black solid circle), sodium acetate (NA, solid triangle,) or sodium chloride (CON, solid square) as a control; infused into the abomasum (AB, solid line) or rumen (RU, broken line) at a rate of 0.5 L/h

Table 4.5. Effect of isomolar[1] intra-ruminal (RU) or intra-abomasal (AB) infusion (8h/d) of sodium chloride (CON), acetic acid (AA) and sodium acetate (NA) on blood chemistry for cows in the immediate postpartum period (samples taken 4 h after starting of infusions)

	Infusion							Significance, *P*				
	CON-RU	CON-AB	AA-RU	AA-AB	NA-RU	NA-AB	SEM	Trt[2]	CON vs. AA+NA	NA vs. AA	AB vs. RU	Int[3]
pH	7.43	7.42	7.43	7.41	7.43	7.45	0.009	0.06	0.29	0.04	0.96	0.05
pCO_2, mm Hg[4]	35.1	34.1	33.7	33.2	36.04	37.4	1.26	0.05	0.76	0.92	0.94	0.94
pO_2, mm Hg[5]	43.2	46.2	48.1	46.8	44.1	46.4	2.16	0.48	0.29	0.23	0.79	0.33
p50, mm Hg	27.1	27	26.7	27	27.1	26.9	0.23	0.62	0.32	0.37	0.9	0.23
Total CO_2, mm/L	24.6	23.2	23.6	22.3	25.5	27.3	1.07	0.02	0.27	< 0.01	0.77	0.09
Saturation O_2, %	77.6	81.2	83.5	81.2	78.4	83.2	2.54	0.30	0.22	0.46	0.56	0.1
HCO_3, mmol/L	23.5	22.2	22.6	21.3	24.4	26.1	1.03	0.02	0.28	< 0.01	0.8	0.1
$sHCO_3$, mmol/L[6]	24.2	23.2	23.8	22.4	24.9	26.5	0.79	0.03	0.22	< 0.01	0.87	0.06
BEb, mmol/L[7]	0.27	-1.08	-0.31	-1.88	1.09	2.69	0.93	0.03	0.6	< 0.01	0.99	0.08
BEecf, mmol/L[8]	-3.5	-1.81	-2.55	-1.04	1.86	-0.004	1.1	0.02	0.24	< 0.01	0.91	0.01
Anion gap, mmol/L	14.5	14.5	16.3	16.3	15.3	14.9	0.53	0.07	0.02	0.04	0.86	0.66
Osmolality, mOsm/kg	278	279	274	271	277	279	1.42	0.01	0.05	< 0.01	0.83	0.1
BUN, mg/dL	13.3	12.7	12.3	13.1	13.0	12.5	0.333	0.11	0.24	0.82	0.61	0.02
Creatinine, mg/dL	0.982	0.955	0.959	0.933	1.003	0.995	0.03	0.55	0.87	0.08	0.58	0.75
BUN/Creatinine	13.6	13.7	12.7	14	13.1	13.2	0.427	0.02	0.09	0.51	0.01	0.02
Glucose, mg/dL	44.8	48.1	42.2	43.8	46.3	44.5	2.26	0.56	0.29	0.33	0.97	0.49
Sodium, mmol/L	140	141	139	138	141	142	0.782	0.02	0.14	< 0.01	0.9	0.12
Potassium, mmol/L	3.97	3.86	3.86	3.85	3.86	3.51	0.129	0.18	0.19	0.18	0.16	0.18
Chloride, mmol/L	107	108	105	105	105	103	0.778	0.03	< 0.001	0.27	0.36	0.43
Lactate, mmol/L	0.471	0.458	0.447	0.421	0.44	0.471	0.021	0.43	0.26	0.27	0.9	0.17
iCa, mg/dL[9]	5.21	5.06	5.03	5	5.14	4.84	0.115	0.18	0.12	0.81	0.11	0.2
nCa, mg/dL[10]	5.13	5.06	4.94	4.94	5.05	4.71	0.122	0.12	0.06	0.58	0.13	0.13
iMg, mg/dL	1.25	1.37	1.28	1.38	1.2	1.19	0.045	0.02	0.28	0.01	0.4	0.3
nMg, mg/dL	1.29	1.34	1.33	1.33	1.28	1.27	0.037	0.66	< 0.001	0.02	<0.0001	<0.0001
Ca/Mg	2.47	2.33	2.33	2.3	2.46	2.4	0.064	0.33	0.6	0.08	0.5	0.86

[1]Isomolar infusion, 0.75 mol /h

Table 4.5. (cont'd)

[2]Trt = treatment

3Int=interaction of acetate type (AA vs. NA) and site of infusion (AB vs. RU).

[4] pCO_2 = Carbon dioxide partial pressure

[5]pO_2 = Oxygen partial pressure

[6]HCO_3 = standard bicarbonate ion concentration after the blood has been equilibrated to a pCO2 of 40 mmHg

[7]Base excess blood

[8]Base excess extracellular fluid

[9]Ionized

[10]Normalized to pH 7.4

reported no effect of AA, NA or sodium chloride on meal size of lactating cows when infused into the rumen at spontaneous meals consistent with the results of the current experiment However, unlike the present experiment, effects of AA did not affect hunger as indicated by intermeal interval or DMI compared with NA (Choi and Allen, 1999). Decreased water intake for AA compared with CON and NA is consistent with results reported by Choi and Allen (1999) and is likely because of differences in intakes of Na and DM Murphy et al. (1983) reported that DMI explains most of the variation in water intake which is also correlated with milk production; and that one gram of additional sodium fed increases 50 ± 23 mL of water.

Studies that have evaluated effects of intraruminal infusions of acetate salts or acetic acid on DMI have used different treatment conditions, including rate of infusion, solution concentration, and duration of treatment, and cows varying in physiological state. In general, infusion of AA or NA do not alter DMI of cattle (lactating or non-lactating) when the dose is 1.2 mol/h or less for up to 6 weeks (Huntington et al., 1983; Maxin et al., 2011; Orskov, 1969; Reynolds et al., 1979; Rook and Balch, 1961; Storry and Rook, 1965; Wilson et al., 1967; Urrutia and Harvatine, 2017). However, AA decreases DMI when infused into the rumen at rates greater than 1.2 moles per h. Montgomery et al. (1963) infused AA at 3.5 mol/h for 4 h into rumens of lactating and non-lactating cows and found that it greatly decreased DMI; however, after partial neutralization to pH 5.0, with NaOH, acetate did not alter DMI. Similarly, infusion of AA at 1.5 mol/h into rumens of mid-lactation cows stopped rumination and induced metabolic alkalosis within 8 h but had no adverse effects when partially neutralized (Sheperd and Combs, 1998).

Response in feed intake to ruminal infusions of AA might be affected by stage of lactation or site of infusion. Wilson et al. (1967) reported decreased DMI when AA was infused

at rates greater than 0.44 mol/h to cows in the PP period, consistent with results of the present experiment. Similarly, infusion of AA into the AB of cows in the PP period at 0.91 mol/h decreased DMI 64% (Gualdrón-Duarte and Allen, unpublished). Feed intake response to infusions of acetate salts or acetic acid might be affected by site of infusion; AA and NA caused diarrhea and cessation of eating within 24 h and 48 h, respectively, when infused into the duodenum of non-pregnant, non-lactating cows (Tyrrell et al., 1979).

Infusion of AA and NA increased plasma acetate over time more than CON, as expected, but the reason for the wide range response to AA and NA treatments at 8 h post-infusion is not apparent. Interaction of treatment and time for plasma propionate concentration appears to be from greater propionate concentration for AA-AB after 8 h of infusion compared to other treatments but the reasons are not apparent. Effects of treatment on plasma insulin concentration is consistent with their effects on DMI except that an interaction of AA and NA and site of infusion was not observed; whereas NA-RU did not decrease DMI, it did decrease plasma insulin concentration. Effects of treatment on plasma concentrations of NEFA and BHB increased over time and were generally opposite of their effects on DMI and plasma insulin concentration, as expected.

The effect of treatments on blood gasses and chemistry showed some differences among treatments in some of the measurements, but most of them were within the normal range for dairy cows according to Michigan State University Diagnostic Center for Population and Animal Health (East Lansing). However all infusions had abnormally high values for anion gap and abnormally low osmolality and sodium concentration for lactating cows. Differences among the parameters measured could not explain the effects of treatment on DMI because all values for NA-RU, which did not reduce DMI, were intermediate among treatments.

CONCLUSION

Our results indicate that infusion of Na acetate into the rumen at up to 6 mol/d does not reduce DMI of cows in the early postpartum period compared with sodium NaCl as an osmotic control. However, infusion of acetic acid into the rumen or either acetic acid or Na acetate into the abomasum at similar rates greatly decreased DMI compared with control. Evaluation of changes in plasma concentrations of metabolites, insulin, blood gasses, and blood chemistry did not reveal clues for potential mechanisms responsible for different effects of treatments, which requires further investigation.

ACKNOWLEDGEMENTS

We acknowledge the financial support for this project by USDA National Research Initiative Competitive Grant no. 2014-67015-21708 to MSA, and from Fulbright Colombia and Colciencias to L. Gualdrón-Duarte. We also thank D. G. Main, J. Liesman, R. West, G. Maldini, K. Kennedy, R. Albornoz, R. Yair, D. de Sousa and R. West (all from Michigan State University) for technical assistance.

APPENDIX

APPENDIX

EFFECTS OF FUELS DERIVED FROM STARCH DIGESTION ON FEED INTAKE, FEEDING BEHAVIOR AND METABOLIC RESPONSE OF COWS IN THE POSTPARTUM PERIOD

ABSTRACT

The objective of this study was to determine the effects of fuels derived of starch digestion on feeding behavior of cows in the postpartum (PP) period. Continuous isoenergetic infusions (194 kcal/h) of glucose (GLU, 48.1 g/h), lactic acid (LA, 55.1 g/h), acetic acid (AA, 57.5 g/h), propionic acid (PL, 40.7g/h; and propionic acid (PH, 54 g/h) infused at the mean of the amount infused for GLU, AA and LA, were administered abomasally to six ruminally cannulated multiparous Holstein cows (13.7 ± 3.9 days in milk) in a 6 x 6 Latin Square design experiment balanced for carryover effects. Treatment sequences were assigned to cows randomly, and treatments included control (CON, no infusion), PL (99.5%, 0.51 mol/h), PH (99.5%, 0.70 mol/h), LA (88.0%, 0.63 mol/h) and GLU (99.9%, 0.29 mol/h). Solutions containing treatments were infused at 485 mL/h for 22h/d. Feeding behavior was recorded by a computerized data acquisition system and blood was sampled at 0 and 24 h relative to the start of infusion. Data were analyzed statistically with a mixed model including the fixed effects of treatment and period and the random effect of cow; treatments were compared with preplanned contrasts. We hypothesized that fuel from starch digestion affect feed intake differently according with their capacity to increase oxidation in the liver and that PH is more hypophagic than PL. Compared with CON, PH decreased dry matter intake (DMI) by 44% (9.93 vs. 17.6 kg/d) by decreasing meal size 43% (0.83 vs. 1.46 kg/meal). Glucose, LA and PL infusions did not affect DMI. Abomasal infusions of AA unexpectedly showed adverse physiological effects with signs for

nervous ketosis in some cows and decreased DMI by 64% compared with CON (6.28 vs. 17.6 kg/d). The reduction in DMI was explained by a 32% decrease of meal size (1.00 vs. 1.46 kg/meal) and by a 104% increase of the eating interval (198.8 vs. 97.1 min), but the mechanism causing this response is not known. Among fuels metabolized in the liver, only PH decreased intake by decreasing meal size 43% (0.83 vs. 1.46 kg/d), and increased satiety ratio almost twice (0.034 vs. 0.018) compared with CON. In conclusion increasing starch content and ruminal fermentability in diets may decrease DMI of cows in the PP period likely by increasing oxidation in the liver. Further research is required to determine the effect of abomasal infusions of buffered and unbuffered solutions of AA on feed intake and feeding behavior during the PP period.

INTRODUCTION

The transition period from gestation to lactation in dairy cows causes vast homeostatic and homeorhetic changes highly related with a decrease in DMI with the resulting negative energy balance. The decrease in plasma insulin concentration and insulin sensitivity in adipose tissue close to parturition promotes fatty acid mobilization leading the cows to a lipolytic state (Drackley, 1999). Hepatic uptake and increased oxidation of nonesterified fatty acids (**NEFA**) likely suppress feed intake by increasing the hepatic energy charge signaling for satiety at the brain feeding centers. According to the hepatic oxidation theory (**HOT**), the continuous supply of acetyl CoA (**AcCoA**) from ß-oxidation of NEFA in the hepatocytes increases the activity of the tricarboxylic acid (**TCA**) cycle, increasing ATP production and liver energy charge (Allen et al., 2009).

Starch is the principal energy source used in diets for dairy cows to increase energy intake and meet the high demand for glucose precursors. However, it has been shown that increasing ruminal starch fermentability decreases feed intake (Dann and Nelson, 2011;

Albornoz and Allen, 2016)

The hypophagic effect of most fermentable starch source is related to the effect of propionic acid on promoting oxidation of the hepatic pool of AcCoA (Stocks and Allen, 2012; 2013). The fermentability of starch can vary according to many factors as variety, processing, and growing and harvesting conditions among others (Larson and Hoffman, 2008). Changing starch fermentability alters the type and patterns of fuels obtained through digestion. The principal end products of starch digestion are acetic, propionic and butyric acid from ruminal fermentation, and glucose from pancreatic amylase action in the small intestine. Lactic acid can be produced both from ruminal fermentation and from partial metabolism of glucose by enterocytes. After absorption, all this fuels reach the liver through portal circulation. Fuels that are taken up by the liver and are metabolized increasing energy charge through oxidation decrease firing rate of hepatic vagus nerve sensing for satiety, thus decreasing intake (Allen et al., 2009).

Despite the evidence that propionic acid is more hypophagic when cows are in lipolytic state (Stocks and Allen, 2012; 2013) and during the immediate postpartum (**PP**) period (Oba and Allen, 2003b; Stocks and Allen, 2012; 2013), abomasal infusion of end products from starch digestion on cows in the PP period allow to test for effects of these fuels on feeding behavior and metabolic response. The objective of this experiment was to evaluate the effect of fuels derived from starch digestion on feeding behavior and metabolic response of cows in the PP period. We hypothesized that the fuels from starch digestion metabolized in the liver (propionic acid and lactic acid) are hypophagic and that propionic acid is more hypophagic than lactic acid.

MATERIALS AND METHODS

Animal Housing and Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Cows were ruminally cannulated at least 45 d before calving and housed in individual tie-stalls for the 12-d duration of the experiment. Cows were fed once daily (1200 h) at 125% of expected intake, and milked twice daily (0530 and 1730 h). The amounts of feed offered and orts were weighed for each cow daily.

Design and Treatment Diets

Six ruminally cannulated multiparous, lactating Holstein cows at the Michigan State University Dairy Field Laboratory (13.7 ± 3.9 DIM; mean ± SD) were used in a 6 x 6 Latin square experiment, with 5 infusion treatments and a sham control (**CON**, no infusion). Cows were randomly assigned to stalls and a treatment sequence balanced for carry over effects. Infusion periods will be 22-h long with 24-h recovery periods between infusions. Infusion treatments were continuous isoenergetic infusions (194.2 kcal ME/h) of acetic acid (**AA**, >99.7%, Sigma Aldrich, Chemical Co., St. Louis, MO), propionic acid (**PL**; food grade, 99.5%, Kemin Industries, Inc., Des Moines, IA), lactic acid (**LA;** food grade 88.0%, Sena International Inc., Polo, IL) and glucose (**GLU**; ≥ 99.5%, Sigma Aldrich, Chemical Co., St. Louis, MO); and an isoamount (~ 55 g/h) infusion of propionic acid (**PH**; food grade, 99.5%, Kemin Industries, Inc., Des Moines, IA) compared with the other infusion treatments; infused into the abomasum at a rate of 0.5 L/h. The solutions were infused into the abomasum using Baxter Flo-Gard 6201 volumetric infusion pumps (Baxter Medical Products, Deerfield, IL) through vinyl tubing (0.95 cm OD, 0.71 cm ID, 1.5 m L) connected to a Nalgene bottle (3.8 cm diameter, 8.5 cm long) as described in Gualdrón-Duarte and Allen ((Gualdrón-Duarte and Allen, 2017).

The experimental diet (Table 4.6) was composed of corn silage, alfalfa silage, alfalfa hay, ground corn, soybean meal, soy hulls, and a vitamin and mineral mix and formulated to meet requirements for absorbed protein, minerals, and vitamins (NRC, 2001).

Table 4.6. Ingredients and nutrient composition of the experimental diet

Item	Amount
Ingredient, % of DM	
Corn Silage	39.6
Soybean meal	16.1
Alfalfa silage	14.2
Ground corn	10.4
Alfalfa Hay	9.9
Soybean hulls	5.7
Vitamin and mineral mix[1]	2.0
Sodium bicarbonate	1.1
Limestone	1.1
Nutrient composition	
DM, %	53.0
Starch, % of DM	19.8
NDF, % of DM	35.1
Forage NDF, % of DM	29.5
CP, % of DM	16.6

[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, 2,220 ppm Fe, 2,083 ppm Mn, 15 ppm Se, 2,025 ppm Zn, 300 KUI/kg vitamin A, 50 KIU/kg vitamin D, 1,500 KIU/Kg vitamin E.

Data and Sample Collection

Cows were blocked from feed from 1000 to 1200 h daily, and the amount of feed offered and orts were weighed for each cow daily. Samples (0.5 kg) of all dietary ingredients and the TMR were collected daily throughout the experiment, and orts for each cow were taken at the end of each infusion period and stored in plastic bags at -20°C until processed. Feeding behavior data (feed disappearance and water intake) were recorded continuously for 22 h daily for each cow during infusions via computer every 5 s (Dado and Allen, 1995), and size, length, and frequency of meals, intermeal interval , and water intake were calculated. 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).

Milk yield was recorded daily 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 4ºC. Milk samples were analyzed for fat, true protein, lactose, and milk urea nitrogen by Michigan DHIA (AOAC, 1997).

Blood was sampled every day at the beginning and end of each infusion period 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 placed on ice until centrifugation. Tubes were centrifuged at 3,000 x *g* for 15 min at 5°C immediately after sample collection. Plasma was harvested and frozen at -20°C until analysis. Liver samples were collected by needle biopsy (Bradford and Allen, 2005) between 1030 h and 1200 h (before feeding) before each infusion and at the end of infusions for each period for analysis of hepatic AcCoA content. Sample were flash frozen in liquid nitrogen and stored on dry ice for transportation. Samples were stored at -80°C until further analysis.

Analysis of Samples

Feed ingredients and orts samples were dried in a 55°C forced-air oven for 72 h to determine DM content. All samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for ash, NDF, crude protein and starch. All nutrients are expressed as percentages of DM, determined by drying at 105ºC in a forced-air oven for more than 8 h. Ash content was determined after 6 h of oxidation at 500°C in a muffle furnace. 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). Content of NDF was analyzed according to (Mertens, 2002) with the inclusion of amylase and Na sulfite.

Plasma samples were analyzed using commercial kits for concentration of NEFA (NEFA HR kit, Wako Chemicals USA, Richmond, VA), **BHB** (kit no. 2240, Stanbio Laboratory, Boerne, TX). Plasma concentration of metabolites were analyzed colorimetrically with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp.) Liver AcCoA was determined by HPLC following the King and Reiss (1985) procedure with modifications (Stocks and Allen, 2012). Liver AcCoA content was expressed per g of wet liver.

Statistical Analysis

Feeding behavior, intake, blood metabolites, yield of milk, milk components, concentration of milk components, and hepatic AcCoA content, were analyzed using the Fit Model procedure of JMP (version 12.0, 2015; SAS Institute Inc., Cary, NC) with the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk}$$

where Y_{ijk} = the dependent response variable of interest, μ = overall mean, T_i = fixed effect of treatment (i = 1 to 6), P_j = fixed effect of period (j = 1 to 6), C_k = random effect of cow (k = 6), and e_{ijk} = residual, normally distributed. Treatment effects were declared significant at $P \leq 0.05$ and tendencies for treatment effects at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies for interactions at $P \leq 0.15$. All data are expressed as least squares means and standard error of the means, unless otherwise specified. Contrasts were conducted between each infusion treatment and the CON. Six cows started the experiment; however, one cow was removed from the study due to displaced abomasum after the second period.

RESULTS

Feed intake and Feeding Behavior

Infusion of unbuffered diluted AA solutions into the abomasum resulted in an unexpected decrease in intake causing severe ketosis and signs of nervous ketosis and erratic behavior in some cows. One cow was completely removed from the experiment after AA infusion once the decrease in intake was still more than 40% of expected normal intake during the day after infusion. Acetic acid and PH infusions, relative to CON decreased DMI 64% ($P < 0.0001$) and 44% ($P < 0.01$) respectively (Table 4.7). The reduction in DMI was because AA decreased meal frequency 59% ($P <0.01$), and tended to reduce meal size (1.0 kg/meal vs. 1.46 kg/meal, $P = 0.06$) compared with CON. The decrease in DMI for PH infusion was explained by the decrease in meal size 43% ($P = 0.01$) compared with CON. Only AA increased eating interval in 101.7 min ($P = 0.04$) compared with CON. Infusions with AA and PH decreased hunger ratio by 75% ($P = 0.01$) and 50% ($P = 0.05$), and satiety ratio by 70% ($P = 0.02$) and 47% ($P = 0.09$) respectively compared with CON. Infusion with PL tended ($P = 0.09$) to decrease satiety ratio by 47% compared with CON. Reduction in water intake compared with CON follow the same trend that DMI where AA and PH decreased 61% ($P < 0.001$) and 48% ($P < 0.01$) by decreasing drink frequency 46% ($P = 0.01$) and 31% ($P = 0.08$) respectively compared with CON.

Table 4.7. Effects of isoenergetic[1] intra-abomasal infusions (22h/d) of glucose (GLU), acetic acid (AA), lactic acid (LA), propionic acid (PL), and isoamount[2] intra-abomasal infusion of propionic acid (PH), relative to a control (CON) with no infusion on feeding behavior and water intake for cows in the immediate postpartum period

	Infusion							Significance, *P*					
	CON	GL	AA	LA	PL	PH	SEM	Trt[3]	AA vs. CON	GL vs. CON	LA vs. CON	PL vs. CON	PH vs. CON
DMI, kg/d	17.6	16.0	6.28	16.6	15.2	9.93	1.53	< 0.001	< 0.0001	0.45	0.64	0.25	< 0.01
Feeding behavior (22h/d)													
Meal frequency,	10.6	11.2	4.34	10.1	10.3	7.94	1.25	0.01	< 0.01	0.74	0.78	0.89	0.12
Meal length, min/meal	24.5	21.1	24.6	22.7	21.8	20.4	2.22	0.56	0.97	0.24	0.54	0.35	0.13
Meal size, kg/meal	1.46	1.25	1.00	1.33	1.07	0.83	0.153	0.09	0.06	0.36	0.57	0.10	0.01
Eating interval, min	97.1	97.5	198.8	108.2	108.6	111.4	33.7	0.29	0.04	0.99	0.82	0.81	0.75
Hunger ratio	0.028	0.026	0.007	0.026	0.02	0.014	0.005	0.05	0.01	0.72	0.73	0.28	0.05
Satiety ratio	0.034	0.031	0.01	0.032	0.017	0.018	0.006	0.09	0.02	0.76	0.81	0.09	0.09
Water intake, L/d	77.7	74.6	30.2	70.4	59.4	40.6	8.75	< 0.01	< 0.001	0.75	0.46	0.08	< 0.01
Drink frequency,	10.5	9.95	5.66	11.9	7.83	7.28	1.52	0.05	0.01	0.75	0.43	0.15	0.08
Liters infused, L/d	0	10.7	10.6	11.1	10.3	10.6	0.19						
Moles infused, mol/d	0	6.46	20.3	13.8	11.3	15.3	0.27						
Calories infused, kcal/d	0	4300	4233	4430	4124	5601	89.5						

[1]Isoenergetic infusion, 194.2 kcal/h
[2]Isoamount infusion, ~ 50 g/h
[3]Trt = treatment

Milk Production and Components

Only infusion with AA compared with CON had an effect on milk yield and milk components (Table 4.8). Milk yield was decreased by 7.2 kg/d ($P < 0.01$), with an increase in 24% on the percentage of fat ($P < 0.01$) and a numerical increase of 9.0% on the percentage of true protein ($P = 0.06$). Lactose and total solids yield was reduced by 19% ($P = 0.02$).

Plasma Metabolites and Liver AcCoA Content

Hepatic content of AcCoA and plasma BHB concentration increased 11.6 nmol/g ($P = 0.02$) and 28.6 mg/dL ($P < 0.01$) respectively, after infusion with AA compared with CON. Infusion with GLU compared with CON decreased NEFA 363 µEq/mL ($P < 0.01$). Plasma glucose concentration was affected by infusion treatments. Compared with CON, GLU infusion increased plasma glucose concentration (11.4 mg/dL, $P = 0.01$) and LA had a numerical increase of glucose in plasma (6.93 mg/dL, $P = 0.08$). Infusion with AA decreased plasma glucose concentration by17.8 mg/dL ($P < 0.001$) compared with CON.

DISCUSSION

According to the HOT, fuels derived from starch digestion that can be extracted from blood circulation by the liver (propionate, butyrate and lactate) and be metabolized, have the potential to increase hepatic energy charge and decrease intake (Allen et al., 2009). The present study shows the effect of isoenergetic abomasal infusions (except for PH with isoamount ~50 g/h in relation to other treatments) of fuels derived from starch digestion on feed intake. In the current research, as expected, abomasal GLU infusion does not affect feed intake or any of the feeding behavior parameters measured compared with CON. Glucokinase activity in ruminant

Table 4.8. Effects of isoenergetic[1] intra-abomasal infusions (22h/d) of glucose (GL), acetic acid (AA), lactic acid (LA), propionic acid (PL), and isoamount[2] intra-abomasal infusion of propionic acid (PH), relative to a control (CON) with no infusion on milk yield and milk components for cows in the immediate postpartum period

	Infusion							Significance, *P*					
	CON	GL	AA	LA	PL	PH	SEM	Trt[3]	AA vs. CON	GL vs. CON	LA vs. CON	PL vs. CON	PH vs. CON
Milk yield, kg/d	33.3	33.9	26.1	32.0	34.4	32.5	2.48	0.01	< 0.01	0.78	0.58	0.60	0.68
Fat, %	4.73	4.68	5.88	5.01	4.73	4.98	0.3	0.02	< 0.01	0.89	0.46	1.00	0.45
Fat yield, kg/d	1.63	1.54	1.73	1.57	1.6	1.63	0.17	0.90	0.56	0.59	0.73	0.84	0.98
True protein, %	3.01	2.86	3.27	3.05	3.00	2.90	1.93	0.05	0.06	0.26	0.81	0.93	0.37
True protein yield, kg/d	1.08	0.964	0.968	1.02	1.05	0.94	0.09	0.80	0.33	0.33	0.62	0.78	0.24
Lactose, %	4.63	4.65	4.46	4.59	4.78	4.69	0.12	0.22	0.17	0.91	0.76	0.28	0.69
Lactose yield, kg/d	1.62	1.52	1.31	1.51	1.63	1.53	0.13	0.13	0.02	0.43	0.42	0.92	0.46
Solids, %	5.51	5.52	5.31	5.46	5.68	5.57	0.14	0.25	0.17	0.94	0.75	0.30	0.70
Total solids, kg/d	1.92	1.81	1.56	1.79	1.93	1.82	0.16	0.14	0.02	0.43	0.42	0.94	0.46
MUN, mg/dL	11.2	10.7	11.3	11.8	11.4	12.2	1.54	0.85	0.95	0.70	0.67	0.90	0.41

[1]Isoenergetic infusion, 194.2 kcal/h
[2]Isoamount infusion, ~50 g/h
[3]Trt = treatment

Table 4.9. Effects of isoenergetic[1] intra-abomasal infusions (22h/d) of glucose (GL), acetic acid (AA), lactic acid (LA), propionic acid (PL), and isoamount[2] intra-abomasal infusion of propionic acid (PH), relative to a control (CON) with no infusion on change in concentrations of insulin and metabolites in plasma, and liver acetyl CoA (AcCoA) content over the infusion day for cows in the immediate postpartum period

	Infusion							Significance, *P*					
	CON	GL	AA	LA	PL	PH	SEM	Trt[3]	AA vs. CON	GL vs. CON	LA vs. CON	PL vs. CON	PH vs. CON
AcCoA start, nmol/g	20.8	21.7	29.68	19.8	19.2	20.6	2.7	0.15	0.04	0.82	0.82	0.70	0.96
AcCoA end, nmol/g	18.2	17.2	38.7	18.5	16.1	21.4	4.64	0.01	< 0.01	0.86	0.95	0.70	0.56
AcCoA change,	-2.55	-4.46	9.06	-1.83	-3.24	0.9	3.79	0.08	0.02	0.67	0.88	0.88	0.45
Glucose start, mg/dL	49.2	45.9	44.3	46.8	45.7	45	3.88	0.73	0.15	0.32	0.48	0.30	0.22
Glucose end, mg/dL	49.5	57.6	28.2	53.5	52.2	52.3	2.04	<0.0001	<0.0001	< 0.01	0.08	0.21	0.20
Glucose change,	-2.03	9.35	-19.8	4.25	4.15	4.90	2.55	<0.0001	< 0.001	0.01	0.11	0.12	0.08
NEFA start, µEq/mL	436.8	637.3	554.1	660.6	543.3	663.2	88.32	0.09	0.17	0.03	0.02	0.21	0.01
NEFA end, µEq/mL	555.4	393.1	696.1	459	553.8	550.4	140.7	0.61	0.42	0.35	0.58	0.99	0.98
NEFA change,	121.8	-240.9	148	-198.4	13.8	-109.6	111.6	0.11	0.87	0.03	0.06	0.50	0.16
BHB start, mg/dL	16.3	16.6	17	9.61	15.2	16.9	6.07	0.86	0.91	0.96	0.34	0.88	0.93
BHB end, mg/dL	12.9	4.76	38.4	4.94	3.52	3.37	4.07	<0.0001	< 0.001	0.15	0.16	0.10	0.10
BHB change, mg/dL	-1.21	-9.75	27.4	-2.54	-9.61	-11.4	5.04	< 0.01	< 0.01	0.28	0.86	0.28	0.20

[1]Isoenergetic infusion, 194.2 kcal/h
[2]Isoamount infusion, ~50 g/h
[3]Trt = treatment

liver is very low (Emmanuel, 1981) and glucose is spared for other tissues mostly for biosynthetic purposes. Glucose infusion increased concentration of plasma glucose and decreased concentration of plasma NEFA likely because lipolysis was reduced. The infusion of PH (0.7 mol/h) decreases intake 44% compared with CON by reduction in meal size, consistent with the hypophagic effect of propionic acid reported by previous research (Oba and Allen, 2003a; Stocks and Allen, 2012; Gualdrón-Duarte and Allen, 2017) and by an increase of 104% in the interval between meals. None of the metabolites measured in plasma or the hepatic AcCoA content was affected with PH infusion. In the current study PL treatment did not affect intake, even with the numerical decrease in DMI of 2.4 kg/d, and it was likely because of the higher variability in intake among cows caused by carry over effect of AA infusion. The lack of effect on intake could be also related to the 7% less calories infused compared with the expected amount because of the higher variability in the infusion rate. None of the feeding behavior parameters, blood metabolites or hepatic AcCoA content were affected, in contrast to Gualdrón-Duarte and Allen (Gualdrón-Duarte and Allen, 2017) which report decrease in DMI and differences in feeding behavior and metabolic response infusing the same moles of propionic acid per day on cows in the postpartum period.

Abomasal infusion of AA unexpectedly resulted on clinical signs of ketosis and decreased intake. However the reduction in intake is likely through a mechanism that is not related to hepatic oxidation. Ruminant liver has a very low activity of AcCoA synthetase, the enzyme necessary to extract acetate from blood for further metabolism in tissues (Allen, 2000). Some studies have reported harmful effects when buffered and unbuffered solutions of acetic acid were infused into the rumen (Sheperd and Holcomb, 1998; Wilson et al., 1967; Montgomery et al., 1963) but others do not report any detrimental consequence (Stocks and

Allen, 2012; 2013; Choi and Allen, 1999; Rook and Balch, 1961). The dramatic decrease in intake (64%) compare to CON was explained by the reduction in number of meals and a tendency to decrease meal size. There was no effect on the duration of the meals. The decrease in glucose concentration to hypoglycemic values (28.2 mg/dL) was likely by the decrease in intake of gluconeogenic precursors and the high glucose demand after calving. Plasmatic NEFA levels were not affected compared with CON likely because peripheral tissues, including the mammary gland, extracted the acetic acid infused. However, content of hepatic AcCoA was highly increased and likely exported as BHB, which could explain the increased plasmatic concentration of BHB over the physiological normal levels (38.4 mg/dL). The effect in intake decrease milk yield 7.2 L/d. Total solids yield decrease but protein and fat concentration increased (9% and 24%, respectively) compared with CON. In previous experiments with long-term intraruminal infusions of sodium acetate has been reported similar increase (20%) in fat concentration (Sheperd and Holcomb, 1998) or linear increase (6% to 11%) with increased doses of sodium acetate infused (Urrutia and Harvatine, 2017) but they also report an increase in fat yield (Sheperd and Holcomb, 1998; Urrutia and Harvatine, 2017).

CONCLUSIONS

It is difficult to make conclusions from the results of this experiment because of the unexpected dramatic decrease in DMI when acetic acid was infused into the abomasum. This increased the standard error and likely resulted in a carryover effect of treatment. The experiment described in Chapter 3 was conducted to compare isoenergetic infusions of glucose, lactic acid and propionic acid into the abomasum of cows in the postpartum period without the acetic acid treatment. In addition, the experiment described in Chapter 4 was conducted to better understand the hypophagic effects of abomasal infusion of acetic acid. This experiment was

included in the appendix of this dissertation to document the effects of treatment and because the numerical differences in DMI among treatments (except acetic acid) were as we hypothesized.

CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

This research shows that fuels absorbed from the gastrointestinal tract can have different effects on feed intake, likely related to their ability to stimulate hepatic oxidation. Results from abomasal infusions of isoenergetic and isomolar solutions of propionic acid (**PA**) and glycerol (**GL**); and isoenergetic solutions of fuels derived from starch digestion showed that gluconeogenic substrates infused into the abomasum have different effects on feeding behavior and DMI and that the effects are independent of their energy content. Glycerol did not decrease intake either in isoenergetic or isomolar infusions compared with PA, and PA decreased hepatic acetyl CoA (**AcCoA**) content compared with GL when pulse-dosed into the abomasum likely by increasing oxidation in the tricarboxylic acid (**TCA**) cycle.

When fuels resulting from starch digestion were infused directly into the abomasum in isoenergetic solutions they resulted in different effects on DMI and energy intake. Infusion of glucose (**GLU**), which is not taken up from the blood by the liver in mature ruminants, did not reduce intake, whereas lactic acid (**LA**) and PA, both extracted by the liver and anaplerotic, decreased DMI while only PA decreased metabolizable energy intake. Hepatic extraction of propionate is greater than lactate. Absorption of propionate is high because of the high activity of propionyl-CoA synthase whereas conversion of lactate to pyruvate is dependent of the redox state of the cell and likely that explains the differences found between both fuels. The hypophagic effect of PA is likely because it is an obligatory anaplerotic compound and can stimulate oxidation of hepatic AcCoA content whereas GL and LA are less so. The reduction of AcCoA content during the timeframe of a meal possibly explained the reduction in meal size when PA was infused.

Infusion 20 mol/d of acetic acid into the abomasum as a control treatment for isoenergetic infusions of fuels dramatically decreased in DMI unexpectedly. Acetate is the principal end-product from ruminal fermentation but little acetate is extracted from the blood by the liver, so the observed decrease in intake is from a mechanism unrelated to hepatic oxidation. We evaluated the effect of isosmotic solutions of acetic acid infused as an acid or as a salt into the abomasum or into the rumen and we conclude that up to 6 mol/8h of sodium acetate infused into the rumen did not decrease DMI of cows in the early PP period, and this treatment could be used as the isosmotic control in studies to evaluate the effect of VFA or other fuels on feed intake. However the reasons explaining why other treatments decreased DMI are not understood and require further investigation.

In addition to the measurement of hepatic AcCoA content, still more work is required to evaluate the effects of PA and other fuels on TCA cycle flux intermediates and flux-controlling enzymes, and energy charge in the liver. Pulse-dose infusion experiments of fuels, and sampling at different time points within the timeframe of a meal could give insight into the more active metabolic pathways or possible metabolic bottlenecks; and changes in ATP to ADP ratio and phosphorylation potential to explain the changes observed on feeding behavior, DMI and metabolic responses along the experiments presented. Research to evaluate the effects of feeding more fermentable starch sources in diets for cows in the PP period is needed. It is important to conduct studies 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, and results from those experiments will provide practical information about feeding strategies to increase DMI after calving.

The experiments conducted for this dissertation provide strong support for the control of feed intake through hepatic oxidation. Changing the amount and source of starch for diets alters the ruminal fermentability and the availability of substrate for intestinal digestion and thus modifies the type and pattern of fuels from starch digestion. Fuels have different effects on DMI and feeding behavior consistent with their ability to promote AcCoA oxidation in the liver. More fermentable starch sources in diets for cows in the immediate PP period increases PA production and availability for the liver and might reduce energy intake of cows by stimulating hepatic oxidation.

BIBLIOGRAPHY

BIBLIOGRAPHY

Albornoz, R. I., and M. S. Allen. 2016. Diet starch content and fermentability affects feed intake and milk yield of cows in the postpartum period. J. Anim. Sci. 94:362-362. doi:10.2527/jam2016-0755

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. http://dx.doi:10.3168/jds.S0022-0302(00)75030-2.

Allen, M.S., B.J. Bradford, and M. Oba. 2009. BOARD-INVITED REVIEW: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. http://dx.doi:10.2527/jas.2009-1779.

AOAC International. 1997. Official Methods of Analysis. 16th ed. A. International, Gaithersburg, MD.

Bradford, B.J., and M.S. Allen. 2005. Phlorizin administration increases hepatic gluconeogenic enzyme mRNA abundance but not feed intake in late-lactation dairy cows. J. Nutr. 135:2206–2211.

Bradford, B.J., A.D. Gour, A.S. Nash, and M.S. Allen. 2006. Propionate challenge tests have limited value for investigating bovine metabolism. J. Nutr.136:1920–2006.

Choi, B.R., and M.S. Allen. 1999. Intake regulation by volatile fatty acids and physical fill. S. Afr. J. Anim. Sci. 29:40–41(abstr.).

Dado, R.G., and M.S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J. Dairy Sci. 78:118–133. http://dx.doi:10.3168/jds.S0022-0302(95)76622-X.

Dann, H. M., and B. H. Nelson. 2011. Early lactation diets for dairy cattle focus on starch. Proc. Cornell Nutrition Conference for Feed Manufacturers. Syracuse, NY.

Drackley, J.K. 1999. ADSA FOUNDATION SCHOLAR AWARD Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259–2273. doi:10.3168/jds.S0022-0302(99)75474-3.

Engku Azahan, E.A., and J.M. Forbes. 1992. Effects of intraruminal infusion of sodium salts on Forbes, J.M. 2007. A personal view of how ruminant animals control their intake and choice of food: minimal total discomfort. Nut. Res. Rev. 20:132–146. doi:10.1017/S0954422407797834.

selection of hay and concentrate foods by sheep. Appetite. 18:143–154.

Gualdrón-Duarte, L.B., and M.S. Allen. 2017. Increased anaplerosis of the tricarboxylic acid cycle decreased meal size and energy intake of cows in the postpartum period. J. Dairy

Sci.1–10. doi:10.3168/jds.2016-12104.

Hach, C.C., B.K. Bowden, A.V. Kopelove, and S.V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. J. Assoc. Off. Anal. Chem. 70:783–787.

Huntington, G.B., P.J. Reynolds, and H.F. Tyrrell. 1983. Net absorption and ruminal concentrations of metabolites in nonpregnant dry holstein cows before and after intraruminal acetic acid infusion. J. Dairy Sci. 66:1901–1908. doi:10.3168/jds.S0022-0302(83)82028-1.

Karkalas, J. 1985. An improved enzymic method for the determination of native and modified starch. J. Sci. Food Agric. 36:1019–1027.

King, T.M., and P.D. Reiss. 1985. Separation and measurement of short-chain coenzyme-A compounds in rat liver by reversed-phase high-performance liquid chromatography. Anal. Biochem. 146:173–179.

Larson, J., and P.C. Hoffman. 2008. Technical Note:. A method to quantify prolamin proteins in corn that are negatively related to starch digestibility in ruminants. J. Dairy Sci. 91:4834–4839. doi:10.3168/jds.2008-1378.

Maxin, G., F. Glasser, C. Hurtaud, J.L. Peyraud, and H. Rulquin. 2011. Combined effects of *trans*-10,*cis*-12 conjugated linoleic acid, propionate, and acetate on milk fat yield and composition in dairy cows. J. Dairy Sci.94:2051–2059. doi:10.3168/jds.2010-3844.

Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217–1240.

Montgomery, M.J., L.H. Schultz, and B.R. Baumgardt. 1963. Effect of intraruminal infusion of volatile fatty acids and lactic acid on voluntary hay intake1. J. Dairy Sci. 46:1380–1384. doi:10.3168/jds.S0022-0302(63)89285-1.

Murphy, M.R., C.L.Davis, and G.C.McCoy.1983. Factors affecting water consumption by Holstein cows in early lactation. J. Dairy Sci. 66:35– 38.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle . 7th rev. ed. Natl. Acad. Press, Washington, DC.

Oba, M., and M.S. Allen. 2003a. Dose-response effects of intrauminal infusion of propionateon feeding behavior of lactating cows in early or midlactation. J. Dairy Sci. 86:2922–2931. doi:10.3168/jds.S0022-0302(03)73889-2.

Oba, M., and M.S. Allen. 2003b. Extent of hypophagia caused by propionate infusion is related to plasma glucose concentration in lactating dairy cows. J. Nutr. 133:1105–1112.

Oba, M., and M.S. Allen. 2003c. Effects of intraruminal infusion of sodium, potassium, and ammoniumon hypophagia from propionate in lactating dairy cows. J. Dairy Sci. 86:1398–

1404. doi:10.3168/jds.S0022-0302(03)73723-0.

Oba, M., and M.S. Allen. 2003d. Hypophagic effects of ammonium are greater when infused with propionate compared with acetate in lactating dairy cows. J. Nutr. 133:1100–1104.

Oba, M., and M.S. Allen. 2003e. Intraruminal infusion of propionate alters feeding behavior and decreases energy intake of lactating dairy cows. J. Nutr. 133:1094–1099.

Orskov, E.R., W.P. Flatt, P.W. Moe, A.W. Munson, R.W. Hemken, and I. Katz. 1969. The influence of ruminal infusion of volatile fatty acids on milk yield and composition and on energy utilization by lactating cows. Br. J. Nutr. 23:443–453.

Reynolds, P.J., H.F. Tyrrell, and P.W. Moe. 1979. Effects of ruminal infusion of acetic acid onto three hay to concentrate ratios on apparent digestibilities and rumen parameters in cattle. J. Anim. Sci. 48:1491–1500.

Rook, J.A.F., and C.C. Balch. 1961. The effects of intraruminal infusions of acetic, propionic and butyric acids on the yield and composition of the milk of the cows. Br. J. Nutr. 15:361–369.

Sheperd, A.C., and C.S. Holcomb. 1998. Long-term effects of acetate and propionate on voluntary feed intake by midlactation cows. J. Dairy Sci. 81:2240–2250.

Stocks, S.E., and M.S. Allen. 2012. Hypophagic effects of propionate increase with elevated hepatic acetyl coenzyme A concentration for cows in the early postpartum period. J. Dairy Sci. 95:3259–3268. http://dx.doi:10.3168/jds.2011-4991.

Stocks, S.E., and M.S. Allen. 2013. Hypophagic effects of propionic acid are not attenuated during a 3-day infusion in the early postpartum period in Holstein cows. J. Dairy Sci. 96:4615–4623. http://dx.doi:10.3168/jds.2013-6653.

Storry, J.E., and J.A.F. Rook. 1965. Effect in the cow of intraruminal infusions of volatile fatty acids and of lactic acid on the secretion of the component fatty acids of the milk fat and on the composition of blood. Biochem. J. 96:210–217.

Tyrrell, H.F., P.J. Reynolds, and P.W. Moe. 1979. Effect of diet on partial efficiency of acetate use for body tissue synthesis by mature cattle. J. Anim. Sci. 48:598–606.

Urrutia, N.L., and K.J. Harvatine. 2017. Acetate dose-dependently stimulates milk fat synthesis in lactating dairy cows. J. Nutr. jn245001. doi:10.3945/jn.116.245001.

Wilson, G.F., A.W.F. Davey, and R.M. Dolby. 1967. Milk composition as affected by intra-ruminal infusion of volatile fatty acids to cows on a restricted ration. N. Z. J. Agric. Res. 10:215–225.