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Influence of Diets Formulated to Increase the Supply of Ketones or Ketone Precursors on the Carbohydrate Status and Performance of Transition Dairy Cows

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INFLUENCE OF DIETS FORMULATED TO INCREASE THE SUPPLY OF KETONES OR KETONE PRECURSORS ON THE CARBOHYDRATE STATUS AND PERFORMANCE OF TRANSITION DAIRY COWS.

BY

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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DEDICATION

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ABSTRACT

INFLUENCE OF DIETS FORMULATED TO INCREASE THE SUPPLY OF KETONES OR KETONE PRECURSORS ON THE CARBOHYDRATE STATUS AND PERFORMANCE OF TRANSITION DAIRY COWS.

Jeffrey M. DeFrain

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Research to date has focused on increasing the supply of glucose precursors in transition dairy cows to reduce ketosis. Ketones and ketone bodies represent energy sources for body tissues; therefore, conserving blood glucose for lactose synthesis in the mammary gland. This led to the hypothesis that transition cows might benefit from diets formulated to stimulate rumen epithelial ketogenesis or dietary ingredients supplying ketone precursors. A series of experiments were conducted to study the impact of diets formulated to increase the supply of ketones (via feeding lactose, glycerol, or an alpha-amylase enzyme preparation) or ketone precursors (feeding Ca soaps of long-chain fatty acids) on the carbohydrate status of transition dairy cows. Feeding lactose to lactating dairy cows increased ruminal butyrate and plasma betahydroxybutyrate (BHBA) without placing cows at risk for developing ketosis. Additional experiments were conducted using transition dairy cows. All comparisons were made against control cows fed a corn-based concentrate mix.

Feeding glycerol decreased precalving feed intake, increased concentrations of rumen butyrate and plasma BHBA while plasma glucose was decreased. Feeding lactose to transition dairy cows resulted in more consistent prepartum DMI, increased proportions of ruminal butyrate, and concentrations of BHBA in plasma without affecting the glycemic status. Transition dairy cows fed an alpha-amylase enzyme preparation had greater concentrations of glucose and BHBA in plasma. Feeding propionate and fat in sufficient quantities decreased fat mobilization as reflected by decreased NEFA concentrations in plasma postpartum while plasma BHBA and glucose were unaffected. With the exception of cows fed glycerol, lactation performance was unaffected by diets formulated to increase the supply of ketones or their precursors in transition dairy cows. It is concluded that formulating diets to increase the supply of ketones via rumen epithelial ketogenesis increases plasma BHBA while minimally affecting the carbohydrate status and performance of transition dairy cows.

Keywords: Transition dairy cow, ketone, betahydroxybutyrate

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
AMA	Alpha-amylase diet
ApoB	Apolipoprotein B
BCFA	Branched chain fatty acids
BCS	Body condition score
BHBA	Betahydroxybutyrate
BW	Body weight
C	Celsius
CON	Control diet
CP	Crude protein
CPM	Cornell Pennsylvania Miner
CV	Coefficient of variation
d	Day
DCAD	Dietary cation-anion difference
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy-corrected milk
h	Hour
HG	High glycerol diet
HILAC	High lactose diet

HPLC	High-performance liquid chromatography
LAC	Lactose diet
LG	Low glycerol diet
LOLAC	Low lactose diet
min	Minute
MUN	Milk urea nitrogen
NDF	Neutral detergent fiber
NEFA	Nonesterified fatty acids
NE_L	Net energy of lactation
NFC	Non-fiber carbohydrate
NH_3-N	Ammonia nitrogen
NRC	National Research Council
OAA	Oxaloacetate
P	Propionate diet
PF1	Propionate + fat level 1 diet
PF2	Propionate + fat level 2 diet
PC	Pyruvate carboxylase
PEP-CK	Phosphoenolpyruvate carboxykinase
RUP	Rumen undegradable protein
SCC	Somatic cell count
SD	Standard deviation
SEM	Standard error of means

SNF	Solids-not-fat
TCA	Tricarboxylic acid cycle
TMR	Total mixed ration
VFA	Volatile fatty acids
VLDL	Very-low density lipoprotein
WHEY	Whey diet

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CHAPTER 1. Review of Literature

Introduction

The goal of dairy producers and industry consultants is to develop management strategies that are effective at maximizing the profitability of the entire dairy enterprise while optimizing herd health. Although an important piece of the management scheme, this reaches far beyond achieving high levels of milk production. Successful enterprises are those that reduce stress by providing the utmost level of cow comfort. This approach ensures high levels of milk production, provides for short calving intervals through sound reproduction, and, most importantly, produces an environment enriched with superior animal health. Any factors that compromise animal health, particularly during the peripartum period, can result in devastating losses to the profitability of the dairy operation. Identifying these factors can increase profitability because there are more diseases and veterinary costs associated with this period than any other time (Shanks et al., 1981).

This period has been coined “the transition period” and defined by both Grummer (1995) and Drackley (1999) as 3 weeks before and 3 weeks after parturition. The transition period continues to be the focal point of research efforts of dairy cattle scientists both nationally and internationally because it is poorly understood relative to our knowledge of the cow during and after peak lactation. Because of the greater level of metabolic disorders associated with the transition period (Jordan and Fourdraine, 1993), these 6 weeks are often

regarded as the most critical phase of the lactation cycle and ultimately determine lactation performance and therefore profitability of the dairy farm.

The transition period is characterized by dramatic changes in nutrient demands, necessitating intricate control of metabolism to meet lactational demands for energy, more specifically, glucose, amino acids, and fatty acids. Metabolic coordination was most clearly acknowledged by Bell (1995). Relative to 1 month before calving, demands for glucose, amino acids, and fatty acids increased 2.7, 2, and 4.5-fold, respectively, by 4 days in milk (DIM). These nutrient demands clearly demonstrate why negative energy balance is inevitable during this time. Energy balance is ultimately controlled by the amount of dry matter intake (DMI) at the time of parturition, which directly influences concentrations of non-esterified fatty acids (NEFA) in plasma. According to data cited by Grummer (1993), as DMI declined by more than 30% during the final week before calving, cows experienced nearly a twofold increase in plasma NEFA between d 17 and 2 before calving and a subsequent two-fold increase during the 48 hours prior to calving. As a result of escalating levels of blood NEFA, fat accumulates within hepatocytes because the rate of NEFA uptake by the liver is in proportion to their supply (Emery et al., 1992). The connection between fatty liver and ketosis is a result of forcing the liver to partition hepatic NEFA between storage as triglyceride or ketogenesis via incomplete oxidation (Herdt, 2000).

A 26-year review of the literature (Kelton et al., 1998) indicated incidences of ketosis ranged from 1.3 to 18.3% (median = 4.8%), typically occurring within the first 6 weeks postpartum. Guard (1994) estimated ketosis to cost $145 per case; a cost which includes the cost of treatment, milk loss, increased days open, culling, and replacement. Therefore, this disease could potentially cost the dairy industry just over $60,000,000 annually. The inability to overcome the unrelenting decrease in feed intake associated with parturition invites the need for dietary manipulations as a means to alleviate postpartum metabolic disorders such as the fatty liver-ketosis complex in dairy cattle. Because of the significant role of propionate in gluconeogenesis, research has been directed toward increasing the delivery of propionate to the liver primarily by feeding more fermentable carbohydrates or feeding/drenching propionate-based supplements. Research attempts to alter other rumen volatile fatty acids (VFA), such as butyrate, or supply ketone precursors to improve the carbohydrate status of transition dairy cows are limited. The purpose of this review is to present a review of the etiology of the fatty liver-ketosis complex and provide a discussion on ruminal ketogenesis, with particular emphasis on the effects of ruminally-derived butyrate and ketones on glucose and ketone body metabolism, and gluconeogenic enzymes.

Fatty liver-ketosis complex

Etiology

Pinpointing all of the causative factors involved in the onset of the fatty liver-ketosis complex, also known as fatty liver syndrome, fat cow syndrome, and hepatic lipidosis, presents one of the most intriguing areas of dairy cattle nutrition. Fatty liver and ketosis are recognized to be interrelated Grummer (1993). In fact, the proximity of the two advocates the use of the adage “Which came first: the chicken or the egg?” and is no doubt applicable to this phenomenon. For the most part, it has been decided that fatty liver precedes ketosis (Veenhuizen et al., 1991; Grummer, 1993).

The universal negative energy balance associated with the first few weeks of lactation prefaces the onset of fatty liver and ketosis (Herdt, 2000). In non-ruminants, carbohydrates provide the primary fuel source and, like their ruminant counterparts, are stored during times of positive energy balance. Body storage of carbohydrates is limited; therefore, when energy needs go beyond amounts supplied, secondary fuels of amino acids, but not fats, are utilized for carbohydrate synthesis, the process of gluconeogenesis. To further derange this carbohydrate balance, ruminal fermentation destroys much of the carbohydrates consumed, limiting the amount of carbohydrates absorbed from the gut. Estimates by Huntington (1997) indicate 28% of total glucose supply is derived from absorbed glucose, 67% from organic acids, and 5% from other sources in a dairy cow yielding 32 kg milk per day.

Baird (1982) declared the cause of ketosis as a carbohydrate insufficiency as a result of high milk yields during a lack of glucose supply. Later, Kronfeld (1982) found it necessary to classify ketosis for management purposes. Classifications are shown in Table 1.

Table 1. Classifications of ketosis.

Ketosis type	Cause
Primary underfeeding	Cow is not offered enough acceptable feed.
Secondary underfeeding	Voluntary intake of the cow is diminished by disease.
Alimentary or ketogenic	Cow is consuming excessive amounts of highly ketogenic feeds, e.g. certain silages.
Spontaneous	Blood ketones are elevated even though the cow is consuming a ration that is "nutritionally adequate in every respect…on the basis of our present knowledge" (Shaw, 1956).

With sound management practices in place, cases of primary and alimentary ketosis are avoidable and generally minimal. Secondary ketosis occurs largely because the activity of the immune system is depressed during the week before and the week after calving (Goff and Horst, 1997). The etiology of spontaneous ketosis is the least understood and has been discussed by Grummer (1993). It is hopeful that this review contributes to this knowledge gap, especially the findings of the experiments reported in the later chapters of this dissertation.

The events associated with lactogenesis impose tremendous physiological challenges to the homeostatic mechanisms of the cow. Following parturition, hypoglycemic and therefore hypoinsulinemic conditions are nearly

imminent because of an estimated drain of 2 kg/d of glucose for lactose synthesis and excretion in milk during the first 3 weeks of lactation (Drackley et al., 2001). Clearly, the single most important nutrient required for milk synthesis is glucose, yet nearly all glucose consumed by the dairy cow is degraded in the rumen to VFA which are absorbed and transported to the liver. The liver of the ruminant plays a pivotal role in converting propionate into glucose via gluconeogenesis. The glucose released from the liver is exported and used primarily for lactose synthesis in the mammary gland. However, when feed consumption, and therefore hepatic propionate supply, is insufficient to satisfy glucose demands for milk synthesis and the mandatory glucose needs of other tissues, the dairy cow mobilizes large amounts of body fat setting up fatty liver and ketosis which can trigger a myriad of other metabolic disorders (Correa et al., 1993).

Biochemical Aspects

During early lactation, energy requirements for milk and maintenance exceed amounts supplied by the diet and available stores of endogenous glucose precursors are insufficient and rapidly depleted. The result is the release of body fat in the form of NEFA to fulfill body energy needs, a process which, according to Baird (1982), is exacerbated by the hypoinsulinemic conditions. The elevation of NEFA in blood initiates the cascade of metabolic events leading up to the point at which cows succumb to the fatty liver-ketosis complex.

The NEFA released from adipocytes circulate the peripheral blood and is delivered to the liver. The fate of NEFA within the hepatocyte is esterification into triglyceride and export as very low-density lipoprotein (VLDL), complete oxidation to ATP and CO_2, or incomplete oxidation to acetate and ketone bodies (Gruffat et al., 1996). The tricarboxylic acid cycle (TCA) cycle has a limited capacity to completely oxidize fatty acids as a source of body fuels. When this limit is reached, the acetyl CoA not accepted into the TCA is diverted toward acetoacetate and betahydroxybutyrate (BHBA) synthesis. Baird (1982) indicated the limited capacity to oxidize fatty acids largely results from a decreased concentration of liver oxaloacetate, the point of entry of acetyl CoA into the TCA. Diagnosis of ketosis is reliant upon the appearance of these ketone bodies in blood, milk, and urine.

Grummer (1993) concluded the fatty liver component of the fatty liver-ketosis complex is a result of rapid rates of fatty acid esterification and the concomitant storage of these triglycerides due to the low rate of VLDL triglyceride export. The rate at which hepatic triglycerides are exported within the VLDL vesicle is likely to be the most significant determinant in the development of fatty liver. Pullen et al. (1990) found the ability of the liver to export triacylglycerol in VLDL was proportional to the lipogenic capacity of the liver. In the case of sheep, cattle, pigs, and guinea pigs, where the liver is a minor contributor to lipogenesis, very little liver triacylglycerol was secreted from NEFA. Paradoxically, liver slices from species in which lipogenesis occurs

predominately in the liver (chicken and fish) secreted triacylglycerol in VLDL in greater capacity. An important component of the VLDL secretion vessel is apolipoprotein B (ApoB). Relative to pregnant, non-lactating cows, Gruffat et al. (1997) found hepatic concentrations of ApoB to be lower in lactating cows at 1, 2, and 4 weeks postpartum. They (Gruffat et al., 1997) concluded ApoB synthesis is regulated post-transcriptionally via a decrease in translation or increased rate of ApoB degradation, a conclusion later supported by Rustaeus et al. (1999). Most recently, Chen and Grummer (2001) outlined the importance of ApoB but also apolipoprotein E, microsomal triglyceride transfer protein, and soluble low-density lipoprotein receptor as regulatory factors involved in hepatic VLDL synthesis and secretion.

Nutritional intervention

Many feed additives have been developed to aid in the prevention of the fatty liver-ketosis complex; however, the “silver bullet” has yet to surface. Some, but not all, of these feed additives have merit and are backed by sound nutritional biochemistry research. Generally speaking, most of the additives are targeting one of two scenarios in the field: 1) Can I improve the glycemic status of my transition cows to avoid ketosis? or 2) My cows are over-conditioned, what should I do? Therefore, the following intervention strategies have been discussed accordingly.

Scenario 1. Because of the importance of propionate during gluconeogenesis, most feed additives designed to increase the glucose status of

transition cows involve the use of propylene glycol, propionate, are a combination of the two. Propylene glycol and salts of propionate have elicited an insulin response and reduced the mobilization of body fat. These effects have been clearly documented. When administered as a drench, Studer et al. (1993) and Grummer et al. (1994) found propylene glycol to increase blood glucose and insulin and decrease NEFA and BHBA in blood. Similarly, Schultz (1958) and Schmidt and Schultz (1958) have observed increases in blood glucose and decreases in concentrations of BHBA in blood of cows fed propionate. Obviously feeding glucose precursors is less laborious compared to drenching. To determine the most effective delivery method, Christensen et al. (1997) administered propylene glycol as an oral drench, mixed with a concentrate fed separately, or mixed in the total mixed ration. Because of differences in concentrations of NEFA in plasma, Christensen et al. (1997) recommended drenching or mixing propylene glycol with a concentrate fed separately instead of incorporating it into a total mixed ration.

Scenario 2. Feed additives for over-conditioned transition cows have been designed to improve the efficiency of lipid export from hepatic tissue. Feeding rumen-protected choline has generated favorable results. Piepenbrink and Overton (2003) hypothesized that choline, a methyl donor, may be beneficial in periparturient dairy cows because of its role in cell membrane structure and lipoprotein metabolism. Feeding rumen-protected choline decreased hepatic capacity for storage of radiolabeled palmitate as esterified products within liver,

suggesting a greater capacity for export as VLDL. Methionine also serves as a methyl donor, contributing to the synthesis of phophatidylcholine and subsequently VLDL (Grummer, 1993). Using cows with hepatic steatosis, Pechova et al. (2000) concluded a shortage of methionine could potentially serve as a predisposing factor in the onset of fatty liver. However, recent attempts to alleviate fatty liver by feeding methionine hydroxy analog have been unsuccessful (Bertics and Grummer, 1999). Lastly, the role of niacin in alleviation of fatty liver has received considerable attention. Niacin exerts indirect effects on hepatic fatty acid metabolism because it does not directly influence the export of hepatic fatty acids per se. Niacin appears to act by decreasing the amount of NEFA released from adipose tissue, more specifically, via inhibition of adenylate cyclase (Atkins et al., 1988). Minor et al. (1998) fed 12 grams of niacin/day from 19 days prior to parturition through 40 weeks postpartum and observed no effects on blood and liver metabolites. NRC (2001) recognizes the nutritional role of niacin, but does not support its routine use to minimize the risk of fatty liver in dairy cattle.

Based upon these scenarios, the importance of propionate cannot be disregarded, nor can the potential of using the aforementioned feed additives to fortify lipid export mechanisms from the liver. Merely because of a lack of available data, it seems necessary to engage in learning more about the effects of other ruminal VFA, butyrate in this case, particularly as butyrate affects

carbohydrate metabolism in transition cows and therefore, the fatty liver-ketosis complex.

Ruminal butyrate

The fermentation of organic matter by the ruminal microorganisms leads to the production of VFA, largely acetate, propionate, and butyrate. Acetate and propionate are the first and second (respectively) most abundant VFA produced in the rumen, with butyrate noted as the third most abundant (Trenkle, 1970). In general, diet composition is one of the most influential factors affecting molar proportions of ruminal VFA. Altering the molar proportion of ruminal VFA can lead to significant changes in animal production and performance.

Typical concentrations of ruminal butyrate, a 4-carbon fatty acid, are usually less than 15% of the total VFA on a molar basis. Even though butyrate concentrations remain low compared to the levels of acetate and propionate in the rumen, it is the most extensively metabolized fatty acid (70 to 90%) during its absorption into portal circulation by the ruminal epithelium (Stevens and Stettler, 1966). In vivo research by Weigand et al. (1975) demonstrated that 90% of absorbed butyrate carbon was converted to ketone bodies (mainly BHBA and acetoacetate) prior to its release into portal circulation. The extensive metabolism by the rumen epithelial tissue has led to the conclusion that ruminal butyrate (as well as propionate) plays a significant role in epithelial growth in the young calf (Sakata and Tamate, 1976, 1978; Sander et al., 1959). It is equally important to note that the oxidation of ketone bodies synthesized from ruminally-

absorbed butyrate is not wasteful by any means as they (ketone bodies) provide necessary energetic substrate for cardiac and skeletal muscle and contribute to the synthesis of fatty acids in the mammary gland.

Interactions Between Ruminal Butyrate and Propionate

Propionate is the sole glucogenic VFA produced and absorbed from ruminal fermentation processes. This makes it a major contributor to the glucose pool of ruminants and is likely the reason research efforts have focused on increasing its supply to the liver in the hypoglycemic early postpartum dairy cow. Propionate increases blood glucose (Baird et al., 1980), decreases blood BHBA (Goff et al., 1996), decreases liver oxidation of NEFA (Armentano et al., 1991), and alters the activity of key enzymes such as propionyl CoA (Ash and Baird, 1973). However, research on the effect of other ruminal VFA on these same blood metabolites is limiting.

Increases in the concentration of ruminal propionate results in significant effects on the absorption and metabolism of ruminal butyrate. Dijkstra et al. (1993) used two multiparous lactating Holstein cows to study the effect of VFA concentration on VFA absorption. They found the fractional absorption rate of propionate to decrease as the level of concentration increased from 20 to 100 m*M*. Paradoxically, the fractional absorption rate of butyrate from the rumen was not altered by increases in its concentrations (from 20 to 100 m*M*). Differences were attributed to the fact that the absorption of VFA is a diffusion process and the rumen epithelial tissue extensively metabolizes both propionate and butyrate.

The effect of individual VFA on epithelial metabolism is of particular interest in the hypoglycemic transition dairy cow because of the contribution of ruminal butyrate to epithelial ketogenesis.

Increasing ruminal propionate as a means of supplying more hepatic glucogenic substrate appears to be somewhat counteracted by the epithelial metabolism of ruminal butyrate. Baldwin and Jesse (1996) found propionate to stimulate the conversion of butyrate to BHBA in isolated sheep ruminal epithelial cells. This is contradictory to in vivo results reported by Harmon et al. (1988) who found epithelial output of ketone bodies was not altered by increasing concentrations of ruminal propionate. Others (Bush et al., 1970) have indicated propionate alters only the ratio of BHBA to acetoacetate and not total ketone body output in vitro. All of these results demonstrate the potential effects of propionate on epithelial metabolism of butyrate. Perhaps the response of propionate-stimulating feed supplements in diets for transition dairy cows is masked by the effect of propionate concentrations on epithelial ketogenesis.

Rumen Epithelial Metabolism and Absorption

Because of the rapid conversion of ruminal butyrate to BHBA as noted by Weigand et al. (1975), it might seem unlikely that butyrate absorption from the rumen could trigger an insulin release as a means to minimizing the onset of fatty liver. One possible way to overcome the epithelial metabolism of butyrate would be to challenge (saturate) the enzyme system responsible for the conversion of butyrate to BHBA. This approach could be used to increase the

absorption of butyrate. In vitro work by Stevens and Stettler (1966) clearly demonstrate the butyrate-conversion enzyme system is saturable. Increasing butyrate from 30 to 90 m*M* resulted in a 1.5-fold increase in butyrate absorption and a 3 to 4-fold increase in butyrate transport without affecting the production of ketone bodies. These in vitro findings were later confirmed by Weigand et al. (1972) who conducted a series of experiments and concluded the extent of butyrate conversion was affected by ruminal pH and VFA concentrations and was negatively correlated to the rate of butyrate absorption.

Relative to propionate, limited amounts of ruminal butyrate reach the systemic circulation. However, butyrate is reportedly more insulinotropic than propionate (Stern et al., 1970). Stern et al. (1970) found jugular injections of butyrate (1 mmole/min for 20 min) to increase plasma immunoreactive insulin in goats greater than a similar injection of propionate. A similar response was observed in sheep by the earlier work of Horino et al. (1968). DeJong (1982) concluded increases in butyrate absorption served as a postprandial regulator of insulin and glucagon. Effects of BHBA on insulin have also been described. A review on ketone body utilization in ruminants by Heitmann et al. (1987) reported BHBA infusions in sheep stimulated insulin secretion and production by the pancreas, decreased NEFA, decreased hepatic uptake of NEFA and subsequent hepatic ketogenesis. Sano et al. (1995) recently refuted the role of butyrate in glucagon regulation as described by DeJong (1982). Sano et al. (1995) found intravenous infusion of butyrate in sheep to affect insulin secretion, but not

glucagon. It is important to recognize the need of ketone bodies as an energy substrate within the body. It is likely that the total amount of ketones synthesized within the body would remain unchanged, as rumen epithelial ketogenesis would likely become greater than hepatic ketogenesis in cows fed butyrate-inducing diets.

Effects of Butyrate and BHBA on Glucose Metabolism

As previously noted, the onset of lactation necessitates a rapidly available source of energy which far exceeds the energy supplied by the amount of feed consumed. This additional energy is supplied from the mobilization of body tissue. Insulin plays a significant role in the degree of lipolysis which ultimately dictates the amount of fat infiltrating the liver and the subsequent chance for developing the fatty liver-ketosis complex. In an elegant review of the role of insulin, Zammit (1996) describes how increases in the portal circulation of insulin are accompanied by increased hepatic activity of key lipogenic enzymes, acetyl-CoA and malonyl-CoA carboxylases. Decreases in insulin results in an increase in mitochondrial ß-oxidation of fatty acids. Because propionate is glucogenic and butyrate is ketogenic, one would hypothesize propionate supply would be the key to decreasing the incidence and degree of hypoglycemia in the immediate postpartum period. However, the role of butyrate and BHBA on glucose metabolism is not well characterized.

Although the extent of butyrate metabolism may be saturable, one would expect concentrations of BHBA to increase to some degree in cows consuming

feeds which ferment to butyrate. Increasing circulating butyrate and BHBA in transition dairy cows would be contradictory to current recommendations. However, it seems as if the effects of butyrate and BHBA on glucose metabolism may actually be misconceived. Transfer quotients of ^{14}C-Na bicarbonate to expired CO_2 and ^{14}C-glucose utilized by Anand and Black (1970) found intravenous butyrate injections in goats stimulated glycogenolysis, whereas injections in cattle stimulated gluconeogenesis. Anand and Black (1970) referred to earlier work conducted with lambs that responded similarly to the goats. In their explanation for the observed differences between species, they suggested in the goats, the infused butyrate acted on the glucagon-secreting cells, causing an increase in glucagon release, activating phosphorylase and the subsequent breakdown of glycogen. However, because more of the infused ^{14}C-Na bicarbonate was incorporated into glucose in cattle, the researchers suggested that cattle responded by increasing gluconeogenesis. The metabolism of butyrate increases acetyl-CoA entry into the TCA cycle and possibly reduces the oxidation of pyruvate. Because elevated concentrations of acetyl CoA stimulate pyruvate carboxylase (PC) activity (Utter and Keech, 1963), pyruvate is diverted to oxaloacetate (OAA), stimulating gluconeogenesis.

Effects on Gluconeogenic Enzymes

Both PC and phosphoenolpyruvate carboxykinase (PEP-CK) are required for hepatic gluconeogenesis. Pyruvate carboxylase catalyzes the formation of OAA from pyruvate. Phosphoenolpyruvate carboxykinase coverts the OAA

formed by PC to phosphoenol pyruvate which can proceed through the gluconeogenic pathway. Because acetyl-CoA is a direct allosteric activator of PC and inhibits pyruvate dehydrogenase (Voet and Voet, 1990), ruminally absorbed butyrate, and therefore increased acetyl-CoA, could potentially serve to stimulate gluconeogenesis in the hypoglycemic transition dairy cow. Black et al. (1966) concluded butyrate metabolism acts to spare the oxidation of pyruvate, a glucogenic precursor, and enhance the conversion of pyruvate to oxaloacetate. In support of conclusions from Black et al. (1966), tracer work by Anand and Black (1970) found butyrate injections to stimulate gluconeogenesis in cattle. Attempts to increase ruminal butyrate in transition dairy cows are unavailable; perhaps due to the potential these conditions might encourage the onset of ketosis.

Changes in gluconeogenic enzymes, PC and PEP-CK, have been characterized in bovine hepatic tissue. In liver biopsies collected at 2-wk intervals from calves from 14 to 84 d of age, Donkin et al. (1998) found PC expression to decrease by 38%. Agca and Donkin (2000) quantified PC and PEP-CK in liver biopsies from 7 cows at 28 d prepartum and again at 1 d postpartum. The ratio of PEP-CK to PC was 35 and 9 at 28 d prepartum and 1 d postpartum, respectively. Agca and Donkin (2000) concluded the increase in PC could increase gluconeogenesis from pyruvate even though PEP-CK remained constant. This was supported by the work of Greenfield et al. (2000) who concluded PC and PEP-CK mRNA expression was responsive to the onset of

lactation. Interestingly, peak PEP-CK activity lagged behind PC activity by approximately 28 d. One explanation for this response could likely be attributed to the fact that the role of PEP-CK occurs metabolically “downstream” from that of PC. An alternative explanation would be that the mobilization of body tissue increased acetyl-CoA, allosterically affecting PC, resulting in a dramatic increase in intracellular OAA, which overwhelmed PEP-CK.

As emerging reports continue to describe the dynamics of these key hepatic enzymes during the transition period, others are focusing on metabolic substrates that may influence these changes. Overton et al. (1999) reported on the gluconeogenicity of alanine relative to propionate in hepatocytes. To induce acute hypoglycemia, Dorset wethers were injected with phlorizin to cause urinary excretion of glucose. Hepatic tissue was harvested and gluconeogenesis from propionate and alanine were determined in vitro. The conversion of propionate to glucose was increased to 166% of controls (23.2 vs. 14.0 µmol/h[h·g cell dry matter {DM}]) whereas that of alanine was more than doubled (285% of controls; 6.9 vs 2.4 µmol/h[h·g cell DM]). Although Overton et al. (1999) did not report on PC activity, one could speculate that the increased conversion of alanine to glucose upregulated PC expression. It is hypothesized that the role of butyrate in the transition dairy cow may be an upregulation of PC expression by increasing acetyl-CoA. Increasing ruminal butyrate could ultimately increase gluconeogenesis and prepare the ketone-utilizing machinery for the overwhelming supply of ketones delivered during lactogenesis.

Feedstuffs supplying ketones or ketone precursors

Lactose

Lactose, a disaccharide composed of glucose and galactose, is the major carbohydrate (~70% of DM) found in whey, a liquid byproduct of cheese manufacturing. Whey also contains high quality protein as well as major minerals such as calcium, phosphorus, and potassium. In general, liquid whey is inexpensive for dairies located in close proximity to cheese plants and possesses a desirable chemical composition and physical properties, and has become an attractive ingredient in diets for lactating dairy cattle.

Relative to starches, sugars generally ferment rapidly in the rumen and lead to significant changes in ruminal fermentation profiles. Although classified as a sugar, lactose has not necessarily followed this trend. Using a gas production technique, Hussain and Miller (1998c) found lactose and whey permeate to ferment slowly compared to glucose, sucrose, and starch. This is supported by the earlier work of Chamberlain et al. (1993) who found supplementing grass silage diets with lactose resulted in the least postprandial depression in rumen pH compared to xylose, starch, fructose, and sucrose. Lactose has minimally affected ruminal pH in dairy cattle fed large amounts of lactose (5 to 38% of diet DM, Huber et al., 1967; King and Schingoethe, 1983). Paradoxically, others (Poncet and Rayssiguier, 1980; Pinchasov et al., 1982; Susmel et al., 1995) found lactose to decrease rumen pH; however, lactose was either consumed rapidly, fed as liquid whey, or force-fed through a ruminal

cannula, respectively, which would result in a more rapid fermentation than when lactose was voluntarily consumed.

Data available on the ruminal fermentation of whey has consistently supported an increase in ruminal butyrate concentrations (Maiga et al., 1995; Schingoethe, 1976). Concentrations of ruminal butyrate were 23 and 17 for steers fed up to 60% of diet DM as dried whole whey or 40% as lactose, respectively; nearly two-fold greater than steers fed the control diet (Schingoethe et al., 1980). Grummer et al. (1983) fed steers dried whole whey at 45% of diet DM and reported butyrate concentrations as high as 25% of the total VFA concentration.

Two theories have surfaced describing reasons for the increase in butyrate observed during the ruminal fermentation of lactose: 1) a reduction in ruminal protozoa and 2) a disturbance in the oxidation-reduction balance of the rumen. Grummer et al. (1983) fed steers a diet containing 32% lactose and observed the typical elevated butyrate concentrations. The steers were then defaunated and fed the same diet and an increase in ruminal propionate was observed. More recently, Hussain and Miller (1998a; 1998b) observed a decrease in protozoa populations when lactose was used as a substrate both in vitro and in vivo; however, in vitro results were confounded by a subsequent decrease in pH. A follow-up in vitro study by Hussain and Miller (1999) reported a similar observation when pH was maintained with sodium bicarbonate. Secondly, the synthesis of butyrate is apparently favored as a means of

maintaining the ruminal oxidation-reduction balance (Satter and Esdale, 1968). This is most evident considering the oxidation of lactate to pyruvate generates two hydrogens and necessitates the formation of butyrate from extracellular acetate as an electron sink for these two hydrogens. However, this theory is somewhat ambiguous if lactic acid production is favored at lower pH (Strobel and Russell, 1996), a condition typically not observed in cows fed lactose. Certainly both theories can be used to explain the increase in ruminal butyrate but can obviously dependent upon the ruminal environment resulting from diet characteristics.

Feeding lactose has also improved ruminal nitrogen efficiency as measured by changes in ammonia nitrogen. Relative to unsupplemented or corn and soybean meal-based controls, lactose has decreased concentrations of ruminal ammonia nitrogen when fed in both high forage (Poncet and Rayssiguier, 1980) and high concentrate (King and Schingoethe, 1983) diets. In vitro work by Hussain and Miller (1998a; 1999) obtained similar results. Paradoxically, results from Pinchasov et al. (1982) found ruminal ammonia nitrogen was greater in cows fed liquid acid whey relative to unsupplemented controls. The time at which ruminal sampling occurred relative to feeding was not clearly stated by Pinchasov et al. (1982) and may have contributed to the contradictory results. Because feeding lactose resulted in ruminal defaunation (previously discussed) and therefore predation of bacteria by protozoa, Hussain and Miller (1998a) speculated lactose might increase microbial protein supply.

Using urinary allantoin excretion, Chamberlain et al. (1993) and Susmel et al. (1995) have shown tendencies for greater microbial nitrogen yields from feeding lactose compared to starch.

Glycerol

Glycerol is an energy dense (2.3 Mcal/kg, Schröder and Südekum, 1999), palatable (Fisher et al., 1971), trihydric alcohol. The recent turn toward renewable energy resources, in this case soydiesel, has resulted in a surplus of glycerol stores. Even with the more than 1,500 current uses of glycerol, Crandall (2004) indicated the forecasted abundant glycerol stores could only lead to a cheaper supply. Because glycerol is odorless, becoming more available, and energy dense, it will likely be a valuable ingredient in ruminant diets in the future.

Hobson and Mann (1961) provided some of the earliest data characterizing in vitro glycerol fermentation. They (Hobson and Mann, 1961) observed no noticeable change in the number glycerol-fermenting bacteria in sheep fed glycerol at 0 or 20 g/d. However, it was concluded that the predominant glycerol-fermenting organisms were strict anaerobes of the group *Selenomonas ruminantium* and that facultative anaerobes have little to do with the fermentation of glycerol. Recognizing that this group is a basic part of the rumen flora, it is likely that the selenomonads form a major component of the glycerol-utilizers present. These gram-negative rods produced predominantly propionic acid, although other acids were present such as lactate and succinate.

Glycerol merely acts as an intermediate during the hydrolysis of triglycerides within the rumen. Garton et al. (1961) was unable to detect glycerol following the nearly complete hydrolysis of linseed oil. Furthermore, anaerobic incubations of 0.5 g of glycerol at 37°C for 24 h with 100 mL of rumen contents also led to undetectable glycerol concentrations. Because they thought their sampling regimen was inadequate, they repeated the experiment and obtained the first sample 2 hours after glycerol addition. Following 2 h of incubation, nearly 25% of the glycerol had disappeared and 8 h following glycerol addition, nearly 90% of the glycerol was undetectable. This would then explain why previous attempts at determining glycerol concentrations after a 24-hour incubation were unsuccessful.

Other studies designed to evaluate the effect of glycerol on rumen microbial populations were unavailable. Although not an extremely intensive experiment, Schröder and Südekum (1999) investigated the effect of glycerol on ruminal microbial biomass using four ruminally cannulated steers fed 1.1 kg of glycerol. Microbial biomass was estimated from bacterial DM concentration using optical density measured at 600 nm. They observed a slight decrease in microbial biomass when steers were fed glycerol relative to those fed a glycerol-free diet. These data certainly warrant further investigations with regards to the effect of glycerol on rumen microorganisms using a more intensive experimental design.

The fraction of glycerol disappearing from the rumen via absorption as glycerol or converted into VFA appears to be determined by the amount of glycerol fed. Similar to Garton et al. (1961), Rémond et al. (1993) indicated glycerol disappears rapidly (< 4 h) when fed at 240 or 480 g/d. Countless other reports (Rémond et al., 1993; Khalili et al., 1997; Schröder and Südekum, 1999; Fisher et al., 1971) found glycerol to increase concentrations of ruminal butyrate. Fisher et al. (1971) was one of the first studies to report an increase in butyrate in lactating Holstein cows consuming glycerol at 472 g/d. Rémond et al., (1993) also found the portion of glycerol fermented in the rumen yields a higher proportion of butyrate in the VFA than conventional feeds. Rémond et al., (1993) also determined the fate of glycerol using liquid flux and volume data (determined via polyethylene glycol), ruminal glycerol concentrations, and in vitro glycerol fermentation data. It was concluded that of the 240 g administered via ruminal cannula, 13, 43, and 44% of the glycerol was delivered to the omasum, fermented in the rumen, and absorbed from the rumen, respectively. Therefore, nearly half of the glycerol consumed is potentially fermented to VFA within the rumen, leading to the changes in VFA profiles as reported by Fisher et al. (1971) and Rémond et al. (1993). More recently, Schroder and Sudekum (1999) observed a similar response in concentrations of ruminal butyrate in steers fed 1.1 kg glycerol. Ruminal butyrate concentrations were increased 3 h after feeding relative to steers fed 2.1 kg starch.

Conversely, drenching cows with extremely high doses of glycerol appears to have minimal effects on ruminal metabolism. Data from Goff and Horst (2001) suggest that higher levels of glycerol actually decrease its rate of ruminal degradation. In the first experiment, cows were administered 1, 2, or 3 L of glycerol via esophageal pump. Thirty minutes after dosing concentrations of blood glucose increased by 16, 20, and 25% for cows treated with 1, 2, or 3 L, respectively. In the second experiment, two cows diagnosed with clinical ketosis were treated with 1 L of a glycerol drench. Both cows responded with higher levels of blood glucose, reduced urinary ketone body excretion, and increased milk production while plasma BHBA concentrations were not reported. Goff and Horst (2001) observed no effect of drenching glycerol on rumen pH, which is similar to observations by Schröder and Südekum (1999) who fed glycerol. Rumen acid production and disappearance are indirectly related to rumen pH (Van Soest, 1994); therefore, the fermentation of glycerol should induce changes in rumen pH. Perhaps drenching large quantities of glycerol disrupted the osmotic pressure of the rumen and subsequently altered VFA absorption. More intensive rumen sampling regimens are necessary to delineate effects of glycerol on rumen pH.

Because of the rumen epithelial metabolism of butyrate, increases in plasma BHBA could indirectly indicate the amount of glycerol undergoing fermentation to butyrate in the rumen. Fisher et al. (1971) reported increased plasma BHBA in cows fed glycerol at 472 g/d. Paradoxically, Johnson (1955)

observed dramatic decreases in plasma ketones in cows provided 2000 g of glycerol delivered via esophageal drench. This suggests that the delivery method and quantity are equally important in determining the fate of glycerol because glycerol was fed in all of the other studies.

Exogenous fatty acids

Providing acetyl-CoA to energize gluconeogenic processes could also be achieved by exogenous fatty acids supply. Ferre et al. (1978) speculated the hepatic oxidation of fatty acids could provide the ATP, acetyl CoA, and NADH cofactors necessary for maximal gluconeogenic capacity. These speculations were made using neonatal rats, which were fasted for 16 h following birth. A mixture of glucogenic substrates containing lactate (130), pyruvate (10), glycerol (30), alanine (30), glutamine (30), and serine (30 mg/ml) were then interperitoneally injected (50 µl) and induced a two-fold increase blood glucose. However, a similar injection of glucogenic precursors in rats previously fed triglycerides resulted in a 4-fold increase in blood glucose. Fatty acid supply and their subsequent oxidation to supply acetyl-CoA in hepatocytes appear to be essential in sustaining of high rates of gluconeogenesis.

The impact of fatty acids on glucogenic pathways appear to exert effects on propionate metabolism as well. Lomax et al. (1986) proposed the reducing equivalents from fatty acid metabolism affect the transport of carbon atoms across the mitochondrial membrane. Liver cells were isolated from starved sheep and incubated using palmitate and octanoate as the source of fatty acids.

Because the mitochondrial ratio of BHBA:AcAc increased with the addition of fatty acids (both palmitate and octanoate), they (Lomax et al., 1986) suggested the most pronounced effect of fatty acids is on the redox state of the cell. As the cell becomes more reduced, it is likely that malate efflux would increase, subsequently directing pyruvate and propionate carbon towards gluconeogenesis. Therefore, the impact of ruminally inert fatty acids in peripartum dairy cattle nutrition may be two-fold: 1) to increase the supply of acetyl-CoA to stimulate hepatic PC activity and 2) to orchestrate propionate carbon towards gluconeogenesis through changes in cellular redox state.

Conclusions

There are numerous interrelated changes forced upon the transition dairy cow including (but not limited to): non-lactating to lactating, feed intake, dietary, facilities, disturbances such as milking, pen changes and re-establishment of social hierarchy, immunological challenges, etc. More importantly, we have engineered a cow that can produce milk at level that far exceeds her ability to consume enough feed to support this level of production. All of these dynamics place these animals under an enormous amount of stress, which increases the likelihood of incidences of fatty liver and ketosis. Because ruminal propionate is the sole glucogenic VFA, research efforts to date, accompanied by some degree of success, have reported on the efficacy of costly propionate-based feed additives and drenches as means of supplying more gluconeogenic substrate to

the liver. These reports have provided a wealth of information towards hepatic metabolism rather than a solution to ketosis in early lactation.

In summary, the fatty liver-ketosis complex is a result of a carbohydrate insufficiency coupled with an inability to keep pace with excessive body fat mobilization. Diets can be formulated to increase the supply of ketones or ketone precursors to influence hepatic gluconeogenic processes. Lactose has been shown to be ketogenic via its conversion to BHBA in rumen epithelium. Glycerol leads to fermentations high in butyrate both in vitro and in vivo whereas the metabolism of exogenous fatty acids increases hepatic acetyl CoA supply.

INTRODUCTION TO EXPERIMENTS

The impact of formulating diets to increase the supply of ketones or their precursors in transition dairy cows is largely unexplored. It was hypothesized that increasing the supply of ketones or their precursors would decrease the degree of hypoglycemia in transition dairy cows and improve performance ultimately via increased hepatic acetyl-CoA supply and subsequent activation of PC. This research should be useful in formulating diets for transition dairy cows to decrease the severity of hypoglycemia and improve animal productivity.

CHAPTER 2. Experiment 1. Feeding Lactose Increases Ruminal Butyrate and Plasma Betahydroxybutyrate in Lactating Dairy Cows

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Abstract

Ruminal fermentation of lactose increases molar proportions of butyrate which is metabolized by the ruminal epithelium to BHBA. To determine the effects of feeding whey, and specifically lactose, on concentrations of ruminal and blood VFA and blood BHBA, eight Holstein and four Brown Swiss multiparous cows (210 ± 33 days in milk) were blocked by breed and randomly assigned to one of three 4x4 Latin squares. Treatments were control (CON, 7.1% of diet DM as corn starch), liquid whey (WHEY, 9.4% of diet DM) containing 70% lactose on a DM basis, low lactose (LOLAC, 7.1% lactose), or high lactose (HILAC, 14.3% lactose). Diets contained 53% forage as corn silage, alfalfa hay, and grass hay (DM basis) and a corn and soybean meal-based concentrate. Average diet percent crude protein and energy density (Mcal/kg NE_L) were 16.8 and 1.47, respectively. Feeding lactose increased DM intake. Milk production and composition were not affected by diet, with the exception of decreased urea nitrogen in milk from cows fed lactose. Greater proportions of ruminal propionate were observed in cows fed CON relative to those fed WHEY and LOLAC. Increasing dietary lactose increased proportions of ruminal butyrate and decreased acetate and branched chain VFA. Concurrent with the increase in ruminal butyrate concentrations, there was an increase in

plasma BHBA as lactose in the diet increased. Concentrations of VFA in plasma were not affected by diet with the exception of the branched chain VFA, which were increased in cows fed LOLAC compared with WHEY. These data indicate lactose fermentation increases proportions of ruminal butyrate and plasma BHBA in lactating dairy cows; however, the observed increase in plasma BHBA is not sufficient to subject cows to ketosis.

Key Words: butyrate, ketones, rumen

Introduction

Lactose is the major carbohydrate (approximately 70% of DM) found in whey, a liquid by-product of cheese manufacturing. Whey also contains high-quality protein as well as major minerals such as calcium, phosphorus, and potassium. Because liquid whey is inexpensive and possesses a desirable chemical composition and physical properties, it has become an attractive ingredient in diets for lactating dairy cattle for producers relatively close to cheese plants.

The ruminal fermentation of lactose and whey has consistently increased ruminal butyrate concentrations (Schingoethe, 1976; Maiga et al., 1995;). Grummer et al. (1983) fed steers dried whole whey at 45% of diet DM and reported butyrate concentrations as high as 25% of the total VFA concentration. Typically, concentrations of ruminal butyrate remain less than 15% of the total VFA on a molar basis. Even though butyrate concentrations remain low

compared with concentrations of acetate and propionate in the rumen, it is the most extensively metabolized fatty acid (70 to 90%) during its absorption into portal circulation by the ruminal epithelium (Stevens and Stettler, 1966). Weigand et al. (1975) demonstrated that 90% of absorbed butyrate carbon is converted to ketone bodies (mainly BHBA and acetoacetate) prior to release into portal circulation. This coincides with data from Krehbiel et al. (1992) who infused butyrate into the rumen of steers and reported an increase in blood ketones and a decrease in blood glucose. As a result, the overproduction or under-utilization of ketone bodies in combination with low blood glucose would promote the onset of lactational ketosis in high producing dairy cows, especially within the first 3 to 4 wk after calving when feed intake is insufficient to meet the energy demands of lactation.

There appear to be differences in the observed responses between the ruminal infusion of butyrate and the heightened production of butyrate by the rumen microorganisms from the fermentation of feedstuffs such as whey or lactose. Krehbiel et al. (1992) infused butyrate ruminally and observed an increase in plasma BHBA and a decrease in glucose concentrations. Alternatively, Doreau et al. (1987) fed lactose and observed an increase in ruminal butyrate and plasma BHBA without affecting plasma glucose concentrations. These studies suggest feeding whey during early lactation may result in ruminal fermentation high in butyrate, increase blood ketones, and subsequently increase the likelihood of cows developing ketosis.

To our knowledge, the relationship between ketone body production and the feeding of whey or lactose has not been investigated. The objectives of this experiment were to determine the effect of feeding whey, and specifically lactose, on concentrations of ruminal and blood VFA and blood BHBA. Our hypothesis was that feeding these products would increase ruminal butyrate concentrations without significantly affecting circulating concentrations of ketone bodies in plasma. In addition, these findings are complementary to the larger scope of our laboratory group, which encompasses the characterization of the effects of ruminal butyrate on key metabolic indicators.

Materials and Methods

Animals, Diets, and Sampling

Eight multiparous Holstein cows (220 ± 45 DIM) and 4 multiparous Brown Swiss cows (200 ± 21 DIM) were blocked by breed and randomly assigned to one of three 4x4 Latin squares with 21-d periods to evaluate the effect of feeding whey or lactose on blood metabolites and ruminal VFA. Cows were housed at the South Dakota State University Dairy Teaching and Research Center (Brookings, SD). The first 2 wk of each period were used to adapt cows to diets. Data were collected during the third week of each period. Animal care and use was according to a protocol approved by the South Dakota State University Institutional Animal Care and Use Committee.

Dietary treatments (Table 2) were corn starch (Cargill, Inc., Minneapolis, MN) at 7.1 % of diet DM (CON); fresh liquid whey containing 70% lactose (DM

basis; First District Ag Service, Litchfield, MN) at 9.4% of diet DM (WHEY); lactose (First District Ag Service) at 7.1% of diet DM (LOLAC); and lactose at 14.3% of diet DM (HILAC). The CON and LOLAC were formulated to contain corn starch and lactose, respectively, similar to the level of lactose found in WHEY. The HILAC was formulated to contain twice the concentration of lactose found in LOLAC and WHEY diets. Diets were formulated to be isocaloric (1.54 Mcal NE_L/kg) and isonitrogenous (17% crude protein [CP]) and meet or exceed NRC (2001) guidelines for 35 kg/d milk production. Because of the high concentrations of minerals in liquid whey, diet Ca, P, Na, K, Mg, and Cl were equalized across treatments. With the exception of WHEY, water was added to all diets in order to balance DM content. The liquid whey contained Myco Curb (Kemin Industries, Inc., Des Moines, IA), and therefore 100 ppm was added to all other diets on an as-fed basis.

Table 2. Ingredient and nutrient composition of control (CON), liquid whey (WHEY), low lactose (LOLAC), and high lactose (HILAC) diets.

	Diet			
Item	CON	WHEY	LOLAC	HILAC
	----------(% of DM)----------			
Ingredient				
Alfalfa hay	16.2	16.1	16.2	16.2
Brome grass hay	3.7	3.7	3.7	3.7
Corn silage	32.5	32.3	32.5	32.7
Whole cottonseed	7.0	6.9	7.0	7.0
Corn grain, ground	12.1	12.1	12.1	2.4
Corn starch	7.1	-	-	-
Liquid whey	-	9.4	-	-
Pure lactose	-	-	7.1	14.2
Dried distillers grain	7.8	7.7	7.8	9.1
Soybean meal, 44%	10.5	10.7	10.5	11.7
Urea	0.26	-	0.26	0.26
Limestone	0.66	0.92	0.65	0.55

Potassium carbonate	0.65	-	0.66	0.66
Salt	0.91	-	0.91	0.91
Dicalcium phosphate	0.39	-	0.39	0.39
Magnesium oxide	0.032	-	0.032	0.032
Vitamin A, D, & E premix[1]	0.194	0.193	0.194	0.194
Nutrient				
DM, % as fed	48.0	48.9	48.8	48.9
CP	16.8	16.2	16.6	16.8
RUP, % of CP[2]	35.6	35.5	35.6	35.5
NE_L[2], Mcal/kg	1.47	1.47	1.45	1.47
ADF	23.0	23.5	23.2	23.6
NDF	33.5	33.8	32.9	32.1
NFC[3]	36.6	36.7	37.2	38.4
Starch	15.0	13.8	7.9	13.7
Lactose	-	5.3	6.1	13.0
Ether extract	3.63	3.34	3.14	3.68
Ash	9.25	9.36	9.92	9.47
Ca	0.94	0.81	0.77	0.81
P	0.43	0.43	0.41	0.44
Mg	0.31	0.31	0.30	0.29
K	1.67	1.72	1.67	1.78
Na	0.45	0.41	0.46	0.28
Cl	0.73	0.68	0.82	0.63
S	0.45	0.24	0.26	0.25
DCAD[4], meq/kg	136	277	234	243

[1]Contains 4,400,000 IU of vitamin A, 880,000 IU vitamin D, and 440 IU of vitamin E per kg.
[2]Calculated using NRC (2001).
[3]Non-fiber carbohydrate = 100 – (% NDF + % CP + % Ether extract + % Ash).
[4]Dietary cation-anion difference as [(Na + K) – (Cl + S)] in milliequivalents per kg of DM.

Cows were housed in a free-stall barn. Diets were mixed and fed as a total mixed ration daily at 0800 h for ad libitum consumption using the Calan Broadbent feeding system (American Calan, Inc., Northwood, NH). Orts were removed and weighed once daily and amount of diet offered was adjusted to ensure 10% feed refusal. Samples of diets were collected on d 16, 19, and 21 of each period. Cows were milked at 0600, 1400, and 2100 h, and milk yield was

recorded. Milk samples were collected on d 19, 20, and 21 of each period from all three milkings on each day. Samples were made into composites by day and analyzed for protein, fat, lactose, milk urea nitrogen (MUN), and somatic cell count (SCC) and solids-not-fat (SNF) was calculated.

Cows were weighed and body condition was scored according to Wildman et al. (1982) immediately after the 1400 h milking on two consecutive day at the beginning of the study and upon completion of the experiment. Approximately 4 h after feeding on d 19, 20, and 21, blood samples (~ 15 mL) were collected from a coccygeal vessel into evacuated tubes (Becton Dickinson and Co., Franklin Lakes, NJ) containing K-EDTA (glucose, NEFA, and BHBA analysis) and sodium heparin (VFA analysis). Blood was immediately placed on ice for transport to the laboratory. Blood was centrifuged and plasma was harvested and stored at -20°C until further analysis. Ruminal fluid was collected on d 20 and 21 of each period approximately 4 h after feeding by applying vacuum pressure to an esophageal tube fitted with a suction strainer. Collected fluid was immediately analyzed for pH using a portable pH meter equipped with a combination electrode. Following pH determination, a 10-mL sample was mixed with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen at -20ºC until analyzed for concentrations of VFA and NH_3.

Laboratory Analysis

Samples of diets were dried at 55ºC in a forced-air oven and allowed to air-equilibrate before being ground to pass a 1-mm screen (Brinkman

ultracentrifuge mill, Brinkman Instruments Co., Westbury, NY). Samples were composited by period and analyzed for DM, Kjeldahl N, ether extract, and ash according to AOAC methods (1997). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured using the ANKOM A200 (ANKOM Technology Corp., Fairport, NY) filter bag technique. Determinations of ADF was according to AOAC (973.18 C, 1997) whereas NDF was according to Van Soest et al. (1991) with the addition of 4 mL of alpha amylase and 20 g of sodium sulfite. Diet samples were composited across period and analyzed by Dairyland Laboratories, Inc. (Arcadia, WI) for concentrations of starch, Ca, P, K, Mg, Cl, Na, and S. Starch was measured as dextrose after treating samples with glucoamylase using a YSI 2700 SELECT Biochemistry Analyzer (Yellow Springs, OH) based on methods from Holm et al. (1986). Minerals were quantified according to AOAC methods (985.01, 1997) using inductively coupled plasma spectrometer (Thermo Jarrell Ash, Franklin, MA). Samples were also analyzed for lactose according to AOAC (974.06, 1990) using an HPLC (Waters Corporation, Milford, MA) equipped with a refractive index detector and a 300 mm $\times$ 7.8 mm column (HPX-87H, Bio-Rad Laboratories, Hercules, CA) using a flow rate of 0.6 mL/min of 0.01 N H_2SO_4.

Milk compositional analysis was conducted by Heart of America DHI Laboratory (Manhattan, KS) according to approved procedures of AOAC (1990). Milk true protein, fat, and lactose were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska,

MN). Concentration of MUN was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments).

Coccygeal plasma samples were thawed and concentrations of glucose were determined using glucose oxidase (Sigma Kit #315, Sigma Diagnostics, St. Louis, MO) according to the procedures of Trinder (1969). Concentration of BHBA in plasma was determined (Sigma Kit 310-A, Sigma Diagnostics, St. Louis, MO) following the methods of Williamson et al. (1962) and plasma NEFA concentrations were determined using a colorimetric assay (NEFA-C Kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993). Insulin was quantified by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) with an intraassay coefficient of variation (CV) of 3.5%. Plasma VFA samples were extracted using ion exchange procedures of Reynolds et al. (1986) and analyzed by gas chromatography (Model 6890, Hewlett-Packard, Avondale, PA). Volatile fatty acids were separated on a 15 m $\times$ 0.25 mm i.d. column (Nukol, 17926-01C, Supelco, Inc., Bellefonte, PA) with a flow of 1.0 mL/min He. A splitless injection port (280ºC) was configured with a purge time and flow of 0.10 min and 10 min/mL of He, respectively, while the flame ionization detector was maintained at 300ºC. Initial oven temperature was 100ºC for 6 min, then increased (5ºC/min) to 120ºC, and held for 5 min.

Ruminal samples collected for NH_3-N and VFA were thawed and centrifuged at 30,000 × g for 20 min at 4ºC. Ammonia concentrations were determined following the general protocol of Broderick and Kang (1980). Concentrations of VFA were measured by gas chromatography (Model 6890, Hewlett-Packard) using a flame ionization detector. The split ratio in the injector port (250ºC) was 100:1 with the column described and a flow of 1.3 mL/min of He. Column and detector temperature were maintained at 130 and 225ºC, respectively.

Statistical Analysis

Weekly means of DMI and milk yield during the final week of each period were used for statistical analysis. Means were also calculated for data collected on d 19, 20, and 21 (milk composition and blood metabolites) and d 20 and 21 (ruminal fluid) and used for statistical analysis. Analysis of variance was conducted using the MIXED procedure (Littell et al., 1996) of SAS (2001). Cow served as the experimental unit. The model was Y = treatment + breed + treatment × breed + period, using cow(breed) as a random variable. Preplanned contrasts were designed to test for the linear effect of dietary lactose inclusion level (CON vs. LOLAC vs. HILAC), source of lactose (WHEY vs. LOLAC), and source of carbohydrate (CON vs. WHEY or LOLAC). Significance was declared at $P < 0.05$, unless otherwise noted.

Results

General Observations

The experiment was conducted during May, June, and July of 2002 and average daily temperature was 33ºC. Ingredient and nutrient composition of diets are shown in Table 2. The low CP content of WHEY was attributed to the lower than expected CP content of the fresh, liquid whey. Data from one cow during the first period were deleted and analyzed as missing data for reasons unrelated to treatment. Average body weight (BW) and body condition score (BCS) during the experiment were 707 ± 72 kg and 3.33 ± 0.47 and 656 ± 65 kg and 3.44 ± 0.30 for Holsteins and Brown Swiss, respectively.

Dry Matter Intake, Milk Yield, and Milk Composition

Dry matter intake, milk yield, and milk compositional data are shown in Table 3. Increasing the level of dietary lactose tended (P = 0.09) to increase DMI. Dry matter intake of cows fed WHEY and LOLAC were similar (22.6 and 22.3 kg/d, respectively) while the greatest DMI (23.3 kg/d) was observed in cows fed HILAC. The increase in DMI with lactose addition did not translate into an

Table 3. Dry matter intake, milk yield, milk composition, milk SCC, BW, and BCS of cows fed control (CON), liquid whey (WHEY), low lactose (LOLAC), and high lactose (HILAC) diets.

	Diet					Contrast[1]		
Item	CON	WHEY	LOLAC	HILAC	SEM[2]	1	2	3
						----------*P*----------		
DMI, kg/d	21.7	22.6	22.3	23.3	1.16	0.09	0.59	0.52
Milk, kg/d	25.7	24.9	25.8	25.5	2.40	0.85	0.58	0.81
ECM[3], kg/d	26.7	25.4	27.3	27.2	2.40	0.43	0.29	0.81
ECM/DMI	1.29	1.20	1.29	1.18	0.111	0.63	0.50	0.69
Milk fat								
%	3.37	3.38	3.48	3.35	0.086	0.90	0.31	0.47
kg/d	0.89	0.86	0.93	0.92	0.085	0.41	0.27	0.91
Milk protein								
%	3.38	3.32	3.34	3.31	0.080	0.49	0.80	0.36
kg/d	0.89	0.82	0.89	0.89	0.073	0.46	0.25	0.50
Milk SNF								
%	9.81	9.72	9.75	9.65	0.137	0.49	0.85	0.66
kg/d	2.62	2.47	2.64	2.65	0.228	0.42	0.31	0.62
Milk lactose								
%	5.16	5.14	5.15	5.09	0.119	0.62	0.93	0.92
kg/d	1.39	1.32	1.40	1.41	0.129	0.46	0.40	0.72
SCC[4]	110	168	98	103	52	0.31	0.12	0.56
MUN, mg/dL	15.1	13.2	14.8	13.8	0.45	0.49	<0.01	<0.01

[1]1 = Linear lactose (CON vs. LOLAC vs. HILAC), 2 = wet vs. dry lactose (WHEY vs. LOLAC), 3 = carbohydrate source (CON vs. WHEY and LOLAC).
[2]Standard error of means.
[3]ECM = [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)] Orth, 1992.
[4]SCC x 10^3/mL.

increase in yields of milk or energy-corrected milk (ECM). Average milk and ECM yields were 25.5 and 26.7 kg/d, respectively. Production efficiencies (ECM/DMI) were similar among dietary treatments. Percentages and yields of fat, true protein, SNF, and lactose in milk and milk SCC were not affected by dietary treatments; however, a breed effect ($P = 0.01$) was observed for SNF as Brown Swiss produced milk with greater SNF relative to Holsteins (9.97 vs. 9.51%, respectively). Feeding WHEY decreased ($P < 0.05$) MUN relative to cows fed LOLAC (13.2 and 14.8 mg/dL, respectively). Additionally, a carbohydrate effect ($P < 0.01$) was observed as MUN concentrations were greater in milk from cows fed CON than from those fed WHEY and LOLAC (15.1 vs. 13.2 and 14.8 mg/dL, respectively), and Brown Swiss cows yielded milk with greater ($P < 0.01$) concentrations of MUN relative to the Holsteins (15.4 vs. 13.0 mg/dL).

Rumen Fluid and Plasma Metabolites

Ruminal pH, NH_3-N concentrations, and VFA proportions are shown in Table 4. Dietary treatments did not affect ruminal pH. It is important to note that pH values represent samples collected using an esophageal tube and may have been contaminated with saliva. Concentrations of NH_3-N, total VFA, and acetate:propionate ratio were not different among dietary treatments; however, a breed effect was observed for NH_3-N and total VFA. Ruminal NH_3-N in Brown Swiss cows was nearly two-fold greater than in the Holsteins ($P < 0.01$; 6.3 vs. 3.3 mg/dL, respectively) and total VFA concentrations were 20% greater for

Table 4. Ruminal pH, NH_3-N (mg/dL), VFA proportions (% of total VFA), and total VFA (mM) of cows fed control (CON), liquid whey (WHEY), low lactose (LOLAC), and high lactose (HILAC) diets.

	Diet					Contrast[1]		
Item	CON	WHEY	LOLAC	HILAC	SEM	1	2	3
						----------*P*----------		
pH	6.69	6.68	6.78	6.68	0.074	0.92	0.31	0.59
NH_3-N	5.55	4.11	4.57	5.02	0.650	0.86	0.62	0.13
Acetate	60.6	59.9	59.5	57.3	0.64	<0.01	0.66	0.25
Propionate	22.3	20.8	20.9	21.6	0.54	0.85	0.96	0.02
Isobutyrate	0.81	0.67	0.71	0.53	0.068	0.01	0.68	0.14
Butyrate	13.9	16.1	16.3	18.0	0.38	<0.01	0.63	<0.01
Isovalerate	1.02	0.94	1.02	0.74	0.104	0.04	0.54	0.71
Valerate	1.19	1.40	1.42	1.66	0.057	<0.01	0.87	<0.01
BCFA[2]	1.83	1.62	1.73	1.27	0.162	0.02	0.57	0.37
Total VFA	94.5	98.4	97.6	104.3	4.42	0.17	0.90	0.51
A:P[3]	2.75	2.91	2.88	2.67	0.096	0.20	0.77	0.18

[1]1 = Linear lactose (CON vs. LOLAC vs. HILAC), 2 = wet vs. dry lactose (WHEY vs. LOLAC), 3 = carbohydrate source (CON vs. WHEY and LOLAC).
[2]BCFA = Branched chain volatile fatty acids.
[3]Acetate to propionate ratio.

Brown Swiss relative to the Holsteins ($P < 0.01$; 107.6 vs. 90.0 m*M*, respectively). Increasing dietary lactose resulted in a decrease ($P < 0.05$) in molar proportions of acetate, isobutyrate, isovalerate, and branched chain VFA, being least for cows fed HILAC. Ruminal fluid from cows fed CON contained a greater proportion ($P = 0.02$) of propionate relative to those fed WHEY or LOLAC (22.3 vs. 20.8 and 20.9%, respectively). Increasing dietary lactose resulted in an increase ($P < 0.01$) in ruminal butyrate proportions. Ruminal butyrate was 13.9% of total VFA for cows fed CON and 18% for those fed HILAC. Additionally, a ruminal fermentation difference was observed for starch and lactose, as feeding WHEY or LOLAC resulted in an increase in proportions of butyrate ($P < 0.01$) and valerate ($P < 0.01$) and a decrease in proportions of propionate ($P = 0.02$) relative to those fed CON.

Effects of experimental diets on plasma constituents are shown in Table 5. Increases in dietary lactose resulted in a linear decrease ($P = 0.04$) in concentrations of plasma glucose. This decreased plasma glucose did not affect concentrations of insulin in plasma, which averaged 93.3 μIU/mL. There was a tendency for feeding LOLAC to result in an increase in concentrations of NEFA in plasma compared with cows fed WHEY. Plasma BHBA concentrations averaged 2.7 mg/dL and increased ($P < 0.01$) in response to increases in dietary lactose inclusions. Furthermore, cows fed CON tended ($P = 0.09$) to have lower BHBA concentrations than those fed diets containing lactose. Although concentrations of propionate in plasma were not affected by level of dietary

Table 5. Plasma glucose (mg/dL), insulin (pg/mL), NEFA (µEq/L), BHBA (mg/dL), and VFA (µmol/L) in cows fed control (CON), liquid whey (WHEY), low lactose (LOLAC), and high lactose (HILAC) diets.

	Diet					Contrast[1]		
Item	CON	WHEY	LOLAC	HILAC	SEM	1	2	3
						----------*P*----------		
Glucose	68.0	67.7	66.5	65.5	1.5	0.04	0.38	0.45
Insulin	150.8	162.0	122.4	165.0	30.7	0.92	0.78	0.28
NEFA	82	77	94	85	6	0.32	0.06	0.63
BHBA	2.01	2.70	2.55	3.61	0.30	<0.01	0.72	0.09
Acetate	861	873	967	782	87	0.51	0.42	0.56
Propionate	74.2	63.0	101.1	63.9	14.9	0.97	0.08	0.66
Isobutyrate	0.80	0.62	1.92	0.68	0.43	0.76	0.04	0.38
Butyrate	13.2	11.0	22.0	11.6	4.4	0.84	0.08	0.53
Isovalerate	19.4	14.9	43.2	16.0	9.5	0.80	0.04	0.40
Valerate	6.0	3.8	13.7	4.3	3.5	0.85	0.05	0.52
BCFA[2]	1.77	1.59	2.24	1.76	0.22	0.57	0.04	0.60
Total VFA	975	967	1148	878	116	0.65	0.25	0.54
A:P[3]	13.4	15.1	14.2	13.9	0.74	0.69	0.37	0.17

[1]1 = Linear lactose (CON vs. LOLAC vs. HILAC), 2 = wet vs. dry lactose (WHEY vs. LOLAC), 3 = carbohydrate source (CON vs. WHEY and LOLAC).
[2]BCFA = Branched chain volatile fatty acids
[3]Acetate to propionate ratio.

lactose inclusion, propionate tended (P = 0.08) to be greater for cows fed LOLAC relative to cows fed WHEY. Concentrations of isobutyrate, isovalerate, valerate, and branched chain VFA in plasma were greater (P < 0.05) for cows fed LOLAC compared with cows fed WHEY.

Discussion

Increasing dietary lactose from 0 to 13% (DM basis) tended to increase DMI. In other studies, feeding lactose as either liquid acid whey (Pinchasov et al., 1982) or dried whey (Schingoethe and Rook, 1976) to lactating dairy cattle also resulted in greater DMI. Similar dietary lactose levels (up to 13% lactose DM) have been fed to lactating dairy cattle (Schingoethe and Rook, 1976; Doreau et al., 1987; Maiga et al., 1995) without affecting feed intake. In addition, dietary lactose inclusions as high as 60% (DM basis) have been fed to beef cattle (King and Schingoethe, 1983; Susmel et al., 1995) without affecting feed intake or resulting in digestive upsets; however, the number of animals used and the length of these experiments were limited and these studies should be interpreted with caution. The greatest effect of feeding lactose appears to be its ability to alter ruminal fermentation and therefore blood metabolites and milk composition, not necessarily to increase DMI as observed in the present study.

The absence of any treatment effect on milk fat contradicts results of others who substituted lactose (Bowman and Huber, 1967) and dried whey (Schingoethe et al., 1976) for shelled corn in component fed diets to cows in early lactation (<100 DIM). The ability of lactose to maintain milk fat has been

described by Schingoethe (1976) but is likely to be influenced by stage of lactation. Schingoethe et al. (1976) fed diets containing 3.5% lactose on a DM basis to cows in late lactation (180 DIM), similar to the stage of lactation of cows in the present experiment (220 DIM), and did not affect milk fat.

Feeding lactose from fresh liquid whey or pure lactose did not affect milk true protein, which agreed with results from Bowman and Huber (1967) and Pinchasov et al. (1982) but not Schingoethe et al. (1976). In the latter study, feeding dried whole whey (5% diet DM) to replace shelled corn during late lactation (180 DIM) led to an increase in milk protein percent over control-fed cows (3.95 and 3.80%, respectively). Differences in response to feeding lactose and whey products could possibly be attributed to the lower dietary CP (15.8%) and milk yields (16.5 kg/d) in the Schingoethe et al. (1976) experiment relative to the present study (dietary CP = 16.6%; milk yield = 25 kg/d).

Regardless of amount or source, feeding lactose decreased MUN relative to CON. WHEY was the most effective at decreasing MUN. Furthermore, feeding fresh, liquid whey decreased MUN relative to cows fed similar levels of pure, granular lactose (5.3 and 6.1% lactose, respectively). Perhaps some of the differences observed in MUN response could be attributed to the lower diet CP content of WHEY (16.2%) relative to the other diets which averaged 16.7%. Lactose was more effective at decreasing MUN than corn starch when substituted 1:1 (DM basis). Effects of lactose on MUN were not a primary objective of the current study; however, these data indicate the need for further

investigations to study the impact of feeding lactose on MUN as this area remains largely unexplored.

An elegant review by Schingoethe (1976) summarized the consistent increase in concentrations of ruminal butyrate typically observed in cows fed whey. The butyrate-stimulating effect of lactose was again observed in the present study as ruminal butyrate proportions increased with an increase in dietary lactose DM (Table 4). The greatest ruminal butyrate proportions (18% of total VFA) were recorded for cows fed HILAC. This increase in ruminal butyrate was primarily at the expense of acetate, and to a lesser extent, branched chain VFA. Other researchers have fed similar levels of dietary lactose. Chamberlain et al. (1993) and Susmel et al. (1995) fed 5 and 15% of dietary DM as lactose and found ruminal butyrate proportions of 14.5 and 13.4%, respectively, which was nearly 1.5 times greater than proportions found in controls (7.8 and 9.8 %, respectively). From 1967 to 1995, there were ten published studies reporting the effects of feeding lactose on proportions of ruminal butyrate. Ranges and (standard deviations) of the amounts of lactose fed (% of diet DM) and ruminal butyrate proportions were 2.2 to 42.6% (12.5) and 6.2 to 30.3% (6.7), respectively. Studies with the greatest proportions of ruminal butyrate (> 10 percentage points over controls) were conducted using a limited number of animals (< 5 animals per treatment). The changes in ruminal butyrate reported here are consistent with previous results.

Increasing the level of dietary lactose increased ruminal butyrate and plasma BHBA and decreased concentrations of glucose in plasma. Although our changes in plasma glucose and BHBA were not as great, these results are in general agreement with Krehbiel et al. (1992) who infused butyrate ruminally. They recorded ruminal butyrate proportions 1.5-fold greater (28% of total VFA) than those observed in the present study which resulted in an increase in plasma BHBA from 3.5 to 7.3 mg/dL and a decrease in concentrations of plasma glucose from 69.4 to 63.4 mg/dL for control and ruminally infused animals, respectively. They hypothesized butyrate stimulated glucose utilization by peripheral tissues that resulted in the decrease in blood glucose. Perhaps the ruminal butyrate proportions in our study were not great enough to affect glucose utilization in tissues as suggested by Krehbiel et al. (1992).

These data are the first to report effects of diet-induced elevated ruminal butyrate proportions on plasma insulin concentrations. These results are similar to those of Krehbiel et al. (1992), although they infused butyrate into the rumen. A review on ketone body utilization by Heitmann et al. (1987) reported BHBA infusions at rates simulating maximum utilization in sheep (0.4 g/kg$^{3/4}$/h) stimulated insulin secretion and production by the pancreas. Furthermore, intravenous infusions of butyrate in sheep resulted in a dose-dependent increase in plasma insulin (Sano et al., 1995). Based on conclusions by Sano et al. (1995), because concentrations of butyrate in plasma (Table 5) were not affected

by feeding lactose, it is likely that plasma insulin concentrations would remain unchanged by the amounts of lactose fed in the present study.

Feeding lactose resulted in increases in concentrations of plasma BHBA. According to data from Nielen et al. (1994), plasma BHBA concentrations greater than 12.5 mg/dL indicate cows with subclinical ketosis. The greatest concentration of plasma BHBA was recorded for cows fed HILAC (3.6 mg/dL), indicating that cows were not at risk for developing ketosis. To our knowledge, only one other study has reported plasma BHBA concentrations in cows fed lactose (Doreau et al., 1987). Doreau et al. (1987) observed a 2.5-fold increase (5.4 vs. 13.5 mg/dL) in plasma BHBA in late-lactating cows fed 13% lactose (DM basis) from milk. Differences in results between our study and those of Doreau et al. (1987) were likely attributable to differences in experimental diets, as the later study fed a diet containing 60% hay and 10% fat (DM basis). Poncet and Rayssiguier (1980) suggested changes in ruminal VFA molar proportions with lactose-supplemented diets depend on such factors as the nature of the diet, rate of intake, and rumen pH. Although plasma BHBA concentrations were not reported by Poncet and Rayssiguier (1980), they observed an increase in ruminal propionate proportions in sheep fed 700 g/d lucerne hay with 400 g/d lactose compared to unsupplemented controls.

Weigand et al. (1972) suggested the enzyme system involved in rumen epithelial ketogenesis may become saturated. Our intentions were to saturate this enzyme system by feeding diets fermenting to high ruminal butyrate

concentrations and increase concentrations of butyrate in plasma. Although increases in ruminal butyrate were observed, we were unable to affect concentrations of butyrate in plasma (Table 5). In addition, differences were observed for molar percentages of acetate and propionate in the rumen; however, concentrations of these acids in plasma were similar among treatments. In vivo data presented by Bergman (1990) found 30, 50, and 90% of ruminal acetate, propionate, and butyrate, respectively, did not appear in the portal blood. These data were in agreement with in vitro data using isolated sheets of rumen epithelium where 45, 65, and 85% of acetate, propionate, and butyrate, respectively, disappeared from the lumen side. This review (Bergman, 1990) emphasizes the extensive metabolic activity occurring within the ruminal epithelium and partially explains the differences in VFA profiles in rumen liquor relative to those observed in plasma in the present study.

Earlier findings by Weigand et al. (1972) and Krehbiel et al. (1992) suggesting rumen epithelial ketogenic capacity reaches a plateau with low butyrate loads (15 to 28% of total VFA) was somewhat disputed by Kristensen et al. (2000). Kristensen et al. (2000) found the extent of epithelial butyrate oxidation to be overestimated and the portal recovery of butyrate carbon to be underestimated if only portal net appearance rates of butyrate and BHBA are considered because of the metabolism of BHBA by the portal-drained viscera. Diet and consequently concentrations of ruminal VFA, pH, and liquid volume are known to affect rates of VFA absorption (Dijkstra et al., 1993). Conclusions from Kristensen et al.

(2000) were based on Leicester ewes fed a 100% forage diet and an average ruminal pH of 6.85 whereas Weigand et al. (1972) and Krehbiel et al. (1992) utilized Holstein steers fed diets containing 60% concentrate and average ruminal pH of 6.00 and 6.54, respectively. Consequently, the ability to alter plasma butyrate concentrations can indeed be largely influenced by diet and therefore conditions within the ruminal environment.

Conclusions

Cows fed diets high in butyric acid, such as poorly fermented forages (Andersson and Lundström, 1985) or cows ruminally infused with butyrate are more susceptible to ketosis (Krehbiel et al., 1992). Although the metabolic events occurring within the ruminal epithelial tissue lead to the production of ketone bodies, largely BHBA, the connection between feeding whey, a feedstuff fermenting to butyrate, and the onset of ketosis has not been established. We hypothesized that significant increases in ruminal butyrate may induce ketosis because of the conversion of butyrate to BHBA within the rumen epithelium. Substituting dietary lactose for corn starch in an alfalfa hay and corn silage-based diets increased proportions of ruminal butyrate and concentrations of BHBA in plasma; however, increases in plasma BHBA observed in this experiment were not great enough to place cows at risk for developing ketosis. It is concluded that lactose from fresh, liquid whey or in pure, granular form may be substituted for corn starch in diets of late-lactation dairy cows without leading to

the onset of physiological conditions favoring ketosis and may improve nutrient utilization as evidenced by the observed decreases in MUN.

CHAPTER 3. Experiment 2. Feeding Glycerol to Transition Dairy Cows: Effects on Blood Metabolites and Lactation Performance

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Abstract

Glycerol can alleviate the symptoms of ketosis when delivered as an oral drench. The addition of glycerol to the diet would eliminate the need for restraining cows for drenching yet deliver a glucogenic substrate, potentially alleviating the fatty liver-ketosis complex, and improving lactational performance. Twenty-one multiparous and nine primiparous Holstein cows blocked by parity and expected calving date were used in a randomized block design to evaluate the effects of feeding glycerol from 14 d prepartum to 21 DIM. Treatments (kg/d dry matter basis) were: 0.86 of corn starch (CON), 0.43 corn starch + 0.43 glycerol (LG), or 0.86 glycerol (HG), topdressed, and hand-mixed into the upper 1/3 of the daily ration. All cows were fed a common diet from 22 to 70 DIM. Prepartum DMI was greater for cows fed CON compared with LG or HG (13.3, 10.8, and 11.3 ± 0.50 kg/d, respectively). Prepartum plasma glucose, insulin, ß-hydroxybutyrate, non-esterified fatty acids, and ruminal profiles were not affected by treatment. Rumen fluid collected postpartum from cows fed LG and HG had greater total VFA, greater molar proportions of propionate, and a decreased ratio of acetate to propionate. Furthermore, concentrations of butyrate tended to be greater in rumens of cows fed LG and HG. Postpartum concentrations of

glucose in plasma were greatest for cows fed CON relative to LG and HG (66.0 vs. 63.1 and 58.4 mg/dL, respectively) and decreased sharply at 21 DIM, after treatments ended, for cows fed HG (diet × day interaction). Body weight and condition loss, plasma non-esterified fatty acids, and liver lipid during the first 21 DIM were similar among treatments. Postpartum DMI was not affected by treatments; however, a tendency was observed for a diet × day interaction for body weight, as cows fed LG gained more body weight from 21 to 70 DIM relative to cows fed HG. Yield of energy-corrected milk during the first 70 DIM tended to be greatest for cows fed CON. The LG and HG diets decreased urea nitrogen concentrations in milk relative to CON. Based upon prepartum DMI and concentrations of glucose and BHBA in blood postpartum, feeding glycerol to dairy cows at levels used in this experiment increased indicators used to gauge the degree of ketosis in dairy cattle.

Key words: periparturient, glycerol, metabolites

Introduction

Satisfying the nutritional requirements of the high producing dairy cow is a challenge, particularly near parturition. Feed consumption during the week prior to calving declines 30% (Bertics et al., 1992). Typically, feed intake during the first 5 weeks of lactation continues to be insufficient to match the increasing energy demands of lactation. During this time, the cow is in a negative energy balance; energy output in the form of milk exceeds energy input in the form of feed.

Because of our inability to overcome the intake depression observed around calving, producers use oral drenches and pastes as a means to deliver glucose precursors, such as calcium-propionate and propylene glycol, to decrease ketosis and other fresh-cow metabolic disorders. Glycerol is an effective treatment against lactation ketosis in dairy cattle when administered in this manner and enters the metabolic pathway to glucose in a different location than other glucogenic precursors (Leng, 1970). Johnson (1955) reported the oral administration of 2 kg of glycerol was more effective in alleviating ketosis when compared with propylene glycol. Fisher et al. (1971) concluded the mode of action behind feeding glycerol could be attributed to an increase in feed intake and subsequent supply of more glucogenic substrate. Additional work by Fisher et al. (1973) found cows fed glycerol at 374 g/d lost less body weight and remained in a more positive energy balance than those fed glycerol or propylene glycol at 174 g/d. From these data, one could conclude that feeding glycerol as

a topdress (vs. drenching) could potentially improve the health and lactational performance of peripartum dairy cows.

Recent interest in biodiesel as a renewable energy resource will create a surplus of glycerol stores (Crandall, 2004), a byproduct of biodiesel production. As glycerol becomes more available it will likely become less cost prohibitive as an aid in the treatment of ketosis. Australian workers (Schröder and Südekum, 1999) fed sheep 48, 78, 131, or 185 g/d of glycerol (DM basis) in a low-starch, concentrate diet and found either no effect or positive effects on digestibility of organic matter, starch, and cell-wall components. However, feeding the same levels of glycerol in high-starch concentrate diets resulted in a decrease in cell-wall digestibility but no effect on the digestion of organic matter or starch. Furthermore, Schröder and Südekum (1999) found feeding glycerol decreased the acetate:propionate ratio and stimulated water intake, both of which would benefit transition dairy cows.

Incorporation of glycerol into a complete diet has not been evaluated in peripartum dairy cows. The objectives of this experiment were to determine the effects of feeding glycerol 2 wk before and 3 wk after parturition on plasma metabolites, health, and lactation performance. We hypothesized that feeding glycerol would deliver a glucogenic substrate and presumably alleviate the fatty liver-ketosis complex and improve lactation performance.

Materials and Methods

Cows and Sampling

Animal care and use was according to a protocol approved by the South Dakota State University Institutional Animal Care and Use Committee. The experiment was conducted from July through November of 2002 at the South Dakota State University Dairy Teaching and Research Facility (Brookings, SD). Twenty-one multiparous and nine primiparous Holstein cows, blocked by parity and expected calving date, were arranged into a randomized block design. The treatment groups consisted of three topdresses which were hand-mixed into the upper one-third of the close-up (starting 21 d prior to expected calving) and fresh-cow (calving through 21 DIM) diets. The topdresses were (DM basis): 0.86 kg/d corn starch (CON), 0.43 kg/d corn starch + 0.43 kg/d glycerol (LG), and 0.86 kg/d glycerol (HG). All cows received a common diet from 22 to 70 DIM.

The ingredient and nutrient composition of the close-up, fresh, and high group diets are shown in Table 6. The glycerol was produced by a soy-diesel facility (West Central Soy, Ralston, IA) and contained 80.2% glycerol, 11.5% salt, and 8.3% ash. The amount of glycerol fed was determined from recent work by Goff and Horst (2001) who found 0.83 kg of glycerol delivered by esophageal drench to increase blood glucose by 16% within 30 min. Our highest glycerol treatment was to be similar to that used by Goff and Horst (2001) in order to provide a reference point in identifying the efficacy of glycerol in elevating blood glucose when fed. Dietary treatments were formulated using NRC (2001).

Pretrial NE_L estimates for glycerol and corn starch were 1.92 and 2.16 Mcal/kg according to estimates calculated by the computer software package provided by NRC (2001). The analyzed nutrient composition of corn starch and glycerol were substituted for the default values listed for corn grain, ground, dry displayed within the energy feeds category of the software program. Based upon analyzed diet composition upon completion of the experiment, the revised NE_L estimates of glycerol and corn starch was 1.90 and 2.13 Mcal/kg, respectively, when formulated into the pre- and postfresh total mixed ration (TMR). The corn starch (Cargill, Inc., Minneapolis, MN) was considered a traditional glucogenic agent as it is primarily fermented to propionate in the rumen. Furthermore, ruminal fermentation characteristics of steers fed glycerol indicate glycerol ferments in a manner similar to a carbohydrate (vs. a fat) in the rumen when formulated into typical high forage, dairy diet (Schröder and Südekum, 1999).

Table 6. Ingredient composition of close-up (offered 21 d prior to expected calving), fresh (offered 1 through 21 DIM), and high-group (offered 22 through 70 DIM) diets without topdressed ingredient(s)[1].

	Diet		
Ingredient	Close-up	Fresh	High-group
	---------- % of diet DM ----------		
Brome grass hay	19.5	-	-
Alfalfa hay	19.5	11.4	8.7
Alfalfa haylage	-	11.4	8.7
Corn silage	37.3	24.9	27.4
Wet distillers grains	-	10.0	11.1
Whole cottonseed	2.6	7.6	8.0
Dried distillers grains	7.2	-	-
Liquid whey[2]	-	-	3.9
Corn grain, ground	-	21.9	19.6
Soybean meal, 44%	4.77	6.6	5.9
SoyChlor 16-7[3]	7.18	-	-
SoyPLUS[3]	-	2.7	2.4

Limestone	-	1.15	1.00
Megalac[4]	-	-	1.20
Sodium bicarbonate	-	0.73	0.64
Calcium chloride	0.73	-	-
Dicalcium phosphate	-	0.33	0.31
Urea	0.34	0.31	0.28
Vitamin A, D, & E premix	0.69[a]	0.25[b]	0.23[b]
Diamond V XP yeast[5]	-	0.23	0.19
Magnesium oxide	-	0.21	0.19
Salt	-	0.21	0.19
Zinpro 4-plex[6]	0.08	0.06	0.05
Vitamin E premix[7]	0.11	0.02	0.02

[a]Dry Cow Supreme, Land O' Lakes, Fort Dodge, IA. Assay, DM: 1.0% Ca, 4.5% Mg, 1.0% K, 1.0% S, 3150 ppm Fe, 1890 ppm Cu, 38 ppm Co, 8280 ppm Mn, 8280 ppm Zn, 454,000 IU/kg Vit A, 90,900 IU/kg Vit D, 3636 IU/kg Vit E.
[b]Dairy Micro Premix, Land O' Lakes, Fort Dodge, IA. Assay, DM: 10% Mg, 9840 ppm Fe, 120 ppm Cu, 26,000 ppm Mn, 26,000 ppm Zn, 400 ppm I, 140 ppm Se, 909,000 IU/kg Vit A, 182,000 IU/kg Vit D, 2424 IU/kg Vit E.
[1]Close-up and Fresh diets were topdressed (kg/d DM basis) with treatments of 0.86 corn starch (CON), 0.43 corn starch + 0.43 glycerol (LG), and 0.86 glycerol (HG).
[2]Contains 7.8% CP, 70% lactose, 0.30% Ca, 0.79% P, 3.9% K on a DM basis.
[3]West Central Soy, Ralston, IA.
[4]Megalac calcium salts of palm oil (Church and Dwight Co., Inc., Princeton, NJ).
[5]Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA.
[6]4-Plex, Zinpro Corp., Eden Prairie, MN.
[7]Contains 44,000 IU of vitamin E per kg.

Table 7. Nutrient composition of close-up (offered 21 d prior to expected calving), fresh (offered 1 through 21 DIM), and high-group (offered 22 through 70 DIM) diets.

	Diet		
Nutrient	Close-up	Fresh	High-group
DM, % as fed	51.1	52.4	47.0
CP, % of DM	16.5	18.6	18.0
RUP[1], % of CP	35.6	35.5	35.7
NE_L[1], Mcal/kg	1.50	1.63	1.63
ADF, % of DM	25.5	19.0	18.8
NDF, % of DM	38.7	30.9	31.5
NFC[2], % of DM	31.8	39.0	37.0
Starch, % of DM	18.1	28.9	30.1
Ether extract, % of DM	3.39	4.31	6.35
Ash, % of DM	9.60	7.15	7.08
Ca, % of DM	1.26	1.20	0.93

P, % of DM	0.45	0.49	0.45
Mg, % of DM	0.49	0.43	0.40
K, % of DM	1.42	1.29	1.38
Na, % of DM	0.15	0.32	0.50
Cl, % of DM	1.40	0.39	0.47
S, % of DM	0.25	0.28	0.21
DCAD[3], meq/kg of DM	-122	185	310

[1]Estimates from NRC (2001).
[2]NFC = 100 – (% NDF + % CP + % Ether extract + % Ash).
[3]DCAD as [(Na + K) – (Cl + S)] in milliequivalents per kg of DM.

Cows were housed on a wheat-straw bedded pack prepartum and in a free-stall barn during the postpartum phase of the experiment. Diets were mixed and fed daily at 0600 h for ad libitum consumption using the Calan Broadbent feeding system (American Calan, Inc., Northwood, NH). All diets were offered as a TMR. Weighbacks were measured, recorded, and discarded prior to feeding each day and amounts fed were adjusted to ensure a 10% feed refusal. Samples of diets were collected weekly for analysis. Body weights and body condition scores (1 to 5 in 0.25 increments; Wildman et al., 1982) were recorded on two consecutive days, 4 h after feeding on d 21 prior to expected calving, at parturition, and at 21, 49, and 70 DIM. The same individuals recorded body condition scores during the entire experiment. In addition, calf birth weights and calving difficulty scores were recorded (1 = no problem; 2 = slight problem; 3 = needed assistance; 4 = considerable force; 5 = cesarean). Cows were milked at 0600, 1400, and 2100 h, and milk yield was recorded. Milk samples were collected on d 7, 14, and 21 of lactation from all three milkings each day. Samples were mixed by gentle inversion 6 times and composited in equal

volumes for each cow on each sampling day. Composited samples were then analyzed for protein, fat, lactose, MUN, and SCC. Solids-not-fat was calculated.

Urine ketones were measured from a mid-stream urine sample daily during the first 7 DIM using Keto-stix reagent strips sensitive to acetoacetic acid (Bayer Corporation Diagnostics Division, Elkart, IN). Cows with a urine Keto-stix value $\geq$ 80 mg/dL within the first 7 DIM were considered subclinically ketotic and were treated with 250 mL of dextrose (50%, Bio-Ceutic Laboratories, St. Joseph, MO) after a blood sample was collected. Incidences of milk fever, metritis, displaced abomasum, mastitis, and foot aliments were also recorded during the experiment.

Target day and actual day of blood sampling relative to calving were -14 and -14.5 (standard deviation [SD] = 2.2), -7 and -7.8 (SD = 1.8), -2 and -2.6 (SD = 1.0), 7 and 7.4 (SD = 1.0), 14 and 14.0 (SD = 1.1), and 21 and 21.1 (SD = 1.1), respectively. Approximately 4 h after feeding, blood was sampled from a coccygeal vessel into evacuated tubes (Becton Dickinson and Co., Franklin Lakes, NJ) containing K-EDTA. Samples were immediately placed on ice and transported to the laboratory. Samples were centrifuged (500 × g) and plasma was harvested and stored at -20°C until further analysis. Ruminal fluid was collected 7 d prior to expected calving date and 14 d postpartum 4 h after feeding by applying vacuum pressure to an esophageal tube fitted with a suction strainer. To minimize saliva contamination, approximately 250 mL of rumen fluid were discarded prior to sample collection. Collected fluid was immediately

analyzed for pH using a portable pH meter equipped with a combination electrode. Following pH determination, a 10-mL sample was mixed with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen at -20ºC until analyzed for concentrations of VFA and NH_3-N. Liver tissue was collected by trocar and aspiration between the 11th and 12th rib (Smith et al., 1997) approximately 4 h after feeding on d 7, 14, and 21 of lactation. Samples were blotted to remove any residual blood, split into two equal aliquots, placed into cryovials, immediately submerged in liquid nitrogen, and transported to the laboratory where they were frozen at -80°C until analysis.

Laboratory Analysis

Samples of diets were dried at 55ºC in a forced-air oven and allowed to air-equilibrate before being ground to pass a 1-mm screen (Brinkman ultracentrifuge mill, Brinkman Instruments Co., Westbury, NY). Samples were composited by diet (prepartum and postpartum) and month and analyzed for CP (AOAC, 1997) using a LECO-428 combustion analyzer (LECO Corp., St. Joseph, MI), ether extract (AOAC, 1997), and lignin (AOAC, 1997). Neutral detergent fiber and ADF were measured using the ANKOM A200 (ANKOM Technology Corp., Fairport, NY) filter bag technique. Determinations of ADF was according to AOAC (973.18 C, 1997) whereas NDF was according to Van Soest et al. (1991) with the addition of 4 mL of alpha amylase and 20 g sodium sulfite. Minerals were quantified according to AOAC methods (985.01, 1997) using inductively coupled plasma spectrometer (Thermo Garrell Ash, Franklin, MA).

Milk compositional analysis was conducted by Heart of America DHI Laboratory (Manhattan, KS) according to approved procedures of AOAC (1990). Milk true protein, fat, and lactose were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments).

Coccygeal plasma samples were thawed and concentrations of glucose were determined using glucose oxidase (Sigma Kit #315, Sigma Diagnostics, St. Louis, MO) according to the procedures of Trinder (1969). Concentrations of BHBA in plasma were determined (Sigma Kit 310-A, Sigma Diagnostics, St. Louis, MO) following the methods of Williamson et al. (1962), and plasma NEFA concentrations were determined using a colorimetric assay (NEFA-C Kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993). Insulin was quantified by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) with an intraassay CV of 2.3%. An attempt was made to quantify concentrations of glycerol in plasma using high performance liquid chromatography (Model 2410, Waters Corp., Milford, MA) and a refractive index detector. Using a mobile phase (0.6 mL/min) of 0.01 N H_2SO_4, samples of plasma (50 µl injection volume) were injected onto an organic acid column (300 $\times$ 7.8 mm, HPX87H, Bio-Rad Laboratories, Hercules, CA) with

a constant temperature and pressure of 65ºC and 4.62 MPa, respectively. Concentrations of glycerol were undetectable.

Samples of liver tissue were thawed and analyzed for total lipid (Mills, et al., 1986) and glycogen (Derling et al., 1987). For determination of NH_3-N and VFA, ruminal samples were thawed and centrifuged at 30,000 $\times$ g for 20 min at 4ºC. Ammonia concentrations were determined following the general protocol of Broderick and Kang (1980). Concentrations of VFA were measured by gas capillary chromatography (Model 6890, Hewlett-Packard) using a 0.25 mm i.d.×15 m column (Nukol, 17926-01C, Supelco, Inc., Bellfonte, PA) and a flame ionization detector. The split ratio in the injector port (250ºC) was 100:1 with the column described and a flow of 1.3 mL/min of He. Column and detector temperature were maintained at 130 and 225ºC, respectively.

Statistical Analysis

Data from one multiparous cow fed HG was omitted from the final data set because of an unsuccessful recovery from health complications not related to treatment. Postpartum data from two primiparous cows fed HG were omitted because of complications associated with abomasal displacements. Among the cows remaining, 4 were treated for metritis, 2 for ketosis, 6 for mastitis, and 1 for a foot aliment. Effects of treatments on incidences of health disorders were not analyzed for statistical differences because of insufficient replication; however, all cows experiencing disorders were included in the data set, excluding those mentioned.

Milk yield and DMI data were reduced to weekly means for statistical analysis. Milk production data collected on the day of calving was not included in the data set because of the inherent difficulties associated with data collected on the day of calving. With the exception of rumen fluid variables, data were analyzed as repeated measures using PROC MIXED (Littell et al., 1996) of SAS software, version 8.1 (1999). For each variable, cow was subjected to four covariance structures: autoregressive order one, toeplitz, variance component, and compound symmetry. The structure yielding the largest Akaike's information criteria was used. For variables measured over time, the model included treatment, parity, time (week or day depending on the variable), and 2- and 3-way interactions as fixed effects. For the rumen fluid data, the model included treatment, parity, and treatment × parity interaction. The random effect was diet and parity nested within cow. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom for *F*- tests. Covariates of initial BW and BCS and days on treatment were included for all data sets. Covariates and any interactions were dropped from the model one at time, starting with the least significant and continuing until all remaining interactions and covariates were significant. Prepartum and postpartum data were analyzed separately. Preplanned contrasts were glycerol versus no glycerol (CON vs. LG and HG) and LG versus HG. The purpose of comparing CON versus both LG and HG was to test the effect of glycerol supplementation, regardless of amount. The LG vs. HG contrast was used to determine the effect of glycerol inclusion level. Least

square means and standard error of means are reported. Statistical significance was declared at $P < 0.05$, with trends noted at $P = 0.05$ to $P < 0.15$.

Results

Cows used in the study are characterized in Table 8. Treatments were fed for an average of 36 d (SD = 4.5 d). Body weights and body condition scores 21 d prior to expected calving date were similar among treatments ($P > 0.15$). Calf birth weights were greater ($P = 0.04$) for cows fed LG and HG diets relative to those fed CON (43.0 and 44.0 vs. 39.6 kg, respectively); however, birthing difficulties were not affected by treatments. Placental weights were not measured.

Supplementing glycerol decreased ($P < 0.01$) prepartum DMI (Table 9). Cows fed LG and HG diets consumed approximately 17% less DM relative to cows fed CON (10.8 and 11.3 vs. 13.3 kg/d, respectively); however, prepartum DMI was similar for cows fed LG and HG. Postpartum DMI and body condition scores were not affected by diet. A tendency was observed for a diet × day interaction (Figure 1 B, $P = 0.11$) for BW, as cows fed LG gained more BW after 21 DIM relative to cows fed HG. Body weight increased by 17, 42, and 4 kg for cows fed CON, LG, and HG, respectively, from 21 to 70 DIM (Figure 1 B). Milk yield and milk composition data are presented in Table 10. Feeding glycerol did not affect milk yield; however, feeding LG and HG tended ($P = 0.09$) to decrease yields of ECM (Orth, 1992) relative to cows fed CON (35.2 and 35.0 vs. 38.7 kg/d, respectively). The decrease in ECM yield was largely driven by a

tendency (*P* = 0.13) for a decrease in milk fat yield by cows fed LG and HG compared with those fed CON (1.32 and 1.36 vs. 1.52 kg/d, respectively). Feeding LG and HG tended (*P* = 0.08) to decrease MUN relative to cows fed CON (13.72 and 14.05 vs. 15.25, respectively).

Table 8. Previous 305-d mature equivalent milk, days on treatment, BW, BCS, calf birth weights, and calving difficulty of cows fed CON, LG, and HG*.

	Diet				Contrast[1]	
Item	CON	LG	HG	SEM	1	2
n						
Prepartum	10	10	9	-	-	-
Postpartum	10	10	7	-	-	-
P 305 d ME[2], kg	11000	11274	10866	465	0.90	0.54
Days on treatment	37.1	35.7	34.1	1.55	0.26	0.42
BW[3], kg	723	737	751	20.3	0.40	0.61
BCS[3, a]	3.76	3.74	3.81	0.09	0.87	0.57
Calf BW, kg	39.6	43.0	44.0	1.52	0.04	0.65
Calving difficulty[4]	1.5	2.2	2.1	0.38	0.21	0.85

* CON = control, LG = glycerol at 430 g/d, HG = glycerol at 860 g/d.
[1]Contrasts: 1= CON vs. LG and HG, 2= LG vs. HG.
[2]Previous mature equivalent milk yield; multiparous cows only.
[3]Collected 21 d prior to expected calving
[4]Five point scale: 1= no assistance, 2= slight problem, 3= needed assistance, 4= considerable force, and 5= cesarean.
[a]Wildman et al., 1982.

Table 9. Dry matter intake, body weight, and body condition of cows and heifers fed CON, LG, and HG*.

	Diet				Contrast[1]	
Item	CON	LG	HG	SEM	1	2
Prepartum DMI, kg/d	13.3	10.8	11.3	0.50	<0.01	0.43
Postpartum DMI, kg/d	17.9	17.5	15.8	1.45	0.45	0.40
BW[2,a], kg	658	667	664	17.8	0.71	0.91
BCS	3.28	3.32	3.30	0.06	0.66	0.88

*CON = control, LG = glycerol at 430 g/d, HG = glycerol at 860 g/d.
[1]Contrasts: 1= CON vs. LG and HG, 2= LG vs. HG.
[2]Data collected on d –21, 0, 21, 49, and 70 relative to parturition.
[a]Diet × day interaction (*P* = 0.11).

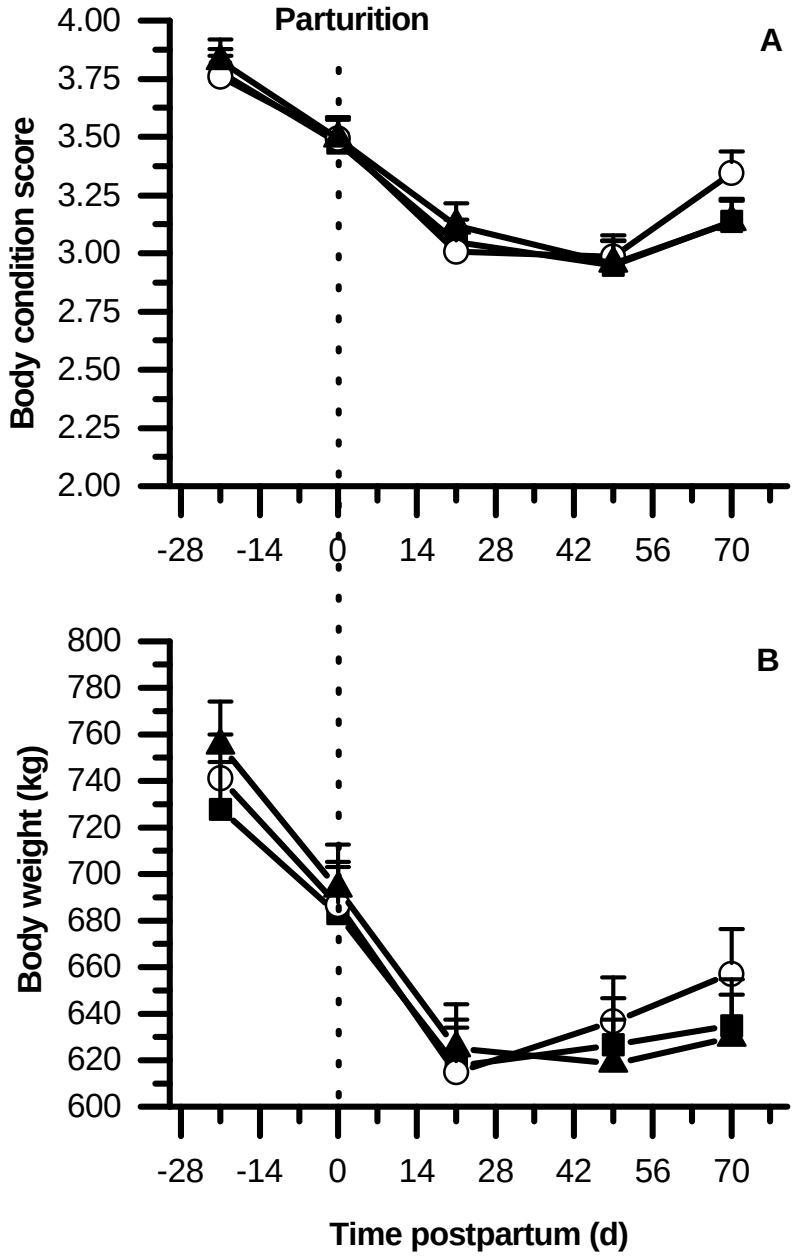

Figure 1. Body weight (A) and body condition score (B) of (pooled SEM = 19.2 and 0.095, respectively) of cows fed a control diet (CON, squares), 430 g/d glycerol (LG, open circles), and 860 g/d glycerol (HG, triangles).

Table 10. Milk yield and composition of cows and heifers fed CON, LG, and HG*.

	Diet				Contrast[1]	
Item	CON	LG	HG	SEM	1	2
Milk yield, kg/d	37.2	35.7	34.0	1.66	0.23	0.46
ECM[2] yield, kg/d	38.7	35.2	35.0	1.82	0.09	0.93
Fat, %	4.26	4.02	4.26	0.22	0.65	0.43
Protein, %	2.94	3.02	2.97	0.08	0.60	0.61
SNF, %	8.67	8.79	8.63	0.12	0.79	0.33
Lactose, %	4.81	4.80	4.72	0.06	0.55	0.39
Fat, kg/d	1.52	1.32	1.36	0.10	0.13	0.81
Protein, kg/d	1.03	0.99	0.95	0.04	0.17	0.36
SNF, kg/d	3.01	2.90	2.78	0.14	0.27	0.49
Lactose, kg/d	1.66	1.60	1.53	0.09	0.32	0.54
SCC x 10^3/mL	407	316	129	257	0.53	0.60
MUN, mg/dL	15.3	13.7	14.1	0.66	0.08	0.72

* CON = control, LG = glycerol at 430 g/d, HG = glycerol at 860 g/d.
[1]Contrasts: 1= CON vs. LG and HG, 2= LG vs. HG.
[2]ECM = [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)] Orth, 1992.

Effects of treatments on concentrations of plasma metabolites are shown in Table 11. Treatments did not affect prepartum concentrations of glucose, insulin, NEFA, and BHBA; however, postpartum concentrations of glucose in plasma tended (*P* = 0.12) to be greatest for cows fed CON relative to LG and HG (65.8 vs. 63.0 and 60.1 mg/dL, respectively). During the postpartum period, significant interactions of diet and day were observed for concentrations of glucose, insulin, NEFA, and BHBA. The postpartum diet × day interaction was observed for postpartum plasma glucose concentrations (*P* = 0.01, Figure 2 A). Plasma glucose was decreased in cows fed LG at 7 DIM; however, concentrations of glucose in cows fed HG decreased sharply from d 14 to d 21 postpartum. Similar to concentrations of glucose in plasma, from d 7 to 21 postpartum, concentrations of insulin in cows fed LG steadily increased whereas

plasma insulin concentrations in cows fed CON and HG remained constant (diet × day interaction, *P* = 0.02, Figure 2 B). Concentrations of BHBA in plasma decreased in cows fed LG and were unchanged in cows fed CON while BHBA steadily increased between 7 and 21 DIM for cows fed HG (diet × day interaction, *P* = 0.12, Figure 3 A). Non-esterified fatty acids were greater in cows fed CON and LG at 7 DIM relative to cows fed HG; however, concentrations became similar among treatments at 14 and 21 DIM (diet × day interaction, *P* = 0.03, Figure 3 B). No differences in liver lipid and glycogen composition were observed (Figure 4).

Table 11. Concentrations of plasma BHBA, glucose, NEFA, and insulin and liver lipid and glycogen content of cows fed CON, LG, and HG*.

	Diet				Contrast[1]	
Item	CON	LG	HG	SEM	1	2
Prepartum						
Glucose, mg/dL	71.4	73.3	70.5	1.8	0.79	0.25
Insulin, pg/mL	135.9	110.4	117.2	14.9	0.25	0.74
NEFA, µEq/L	172	174	200	37.5	0.74	0.61
BHBA, mg/dL	1.30	2.17	1.89	0.84	0.48	0.81
Postpartum						
Glucose[2], mg/dL	65.8	63.0	60.1	2.3	0.12	0.37
Insulin[2], pg/mL	69.9	73.2	73.7	5.2	0.56	0.95
NEFA[2], µEq/L	624	639	495	94.4	0.60	0.28
BHBA[2], mg/dL	4.31	6.18	5.43	1.50	0.40	0.72
Liver lipid[3]	6.15	9.80	9.81	4.99	0.64	0.99
Liver glycogen[3]	1.79	1.82	1.85	0.07	0.61	0.66

*CON = control, LG = glycerol at 430 g/d, HG = glycerol at 860 g/d.
[1]Contrasts: 1= CON vs. LG and HG, 2= LG vs. HG.
[2]Diet × day interaction (*P* < 0.05).
[3]Expressed as a percent of wet tissue weight.

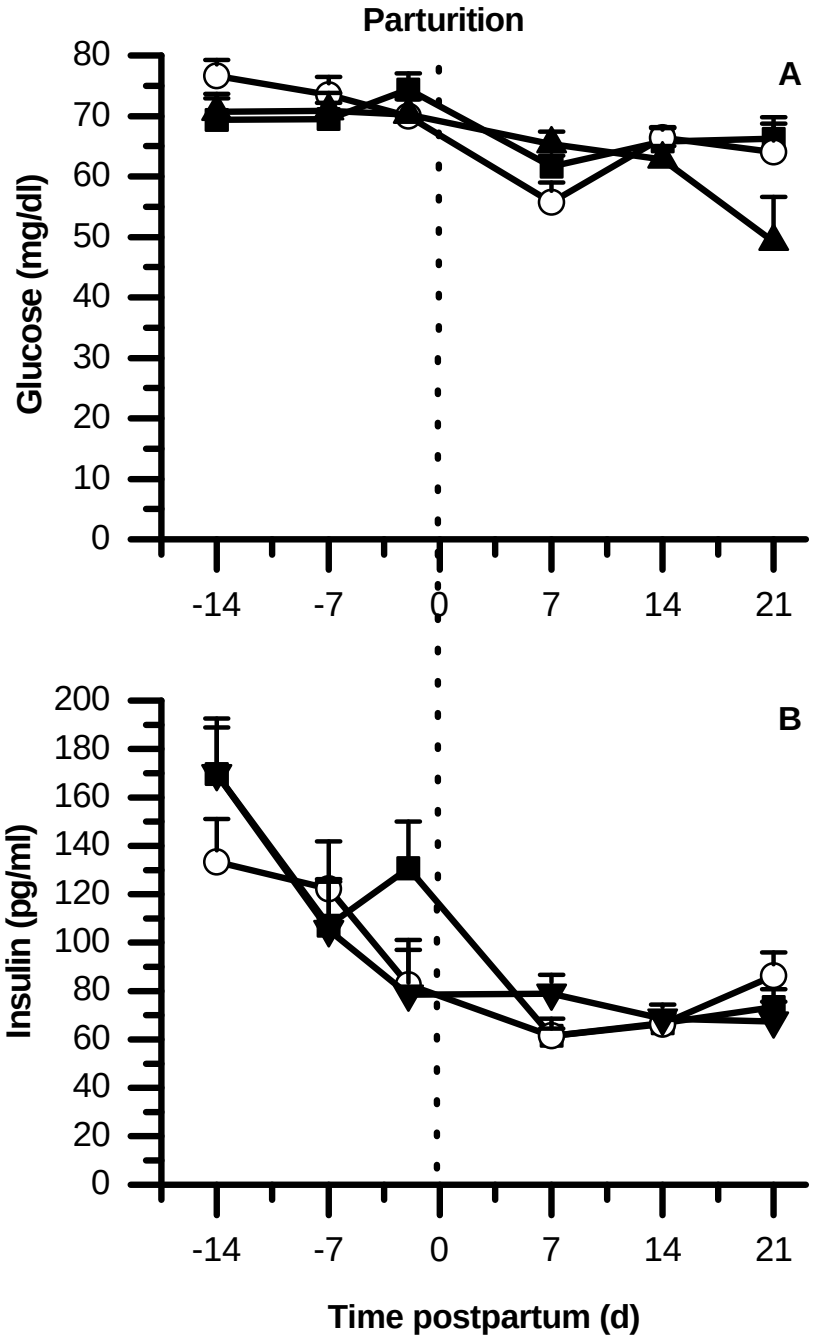

Figure 2. Plasma glucose (A) and insulin concentrations (B) (pooled SEM = 4.16 and 9.03, respectively) of cows fed a control diet (CON, squares), 430 g/d glycerol (LG, open circles), and 860 g/d glycerol (HG, triangles).

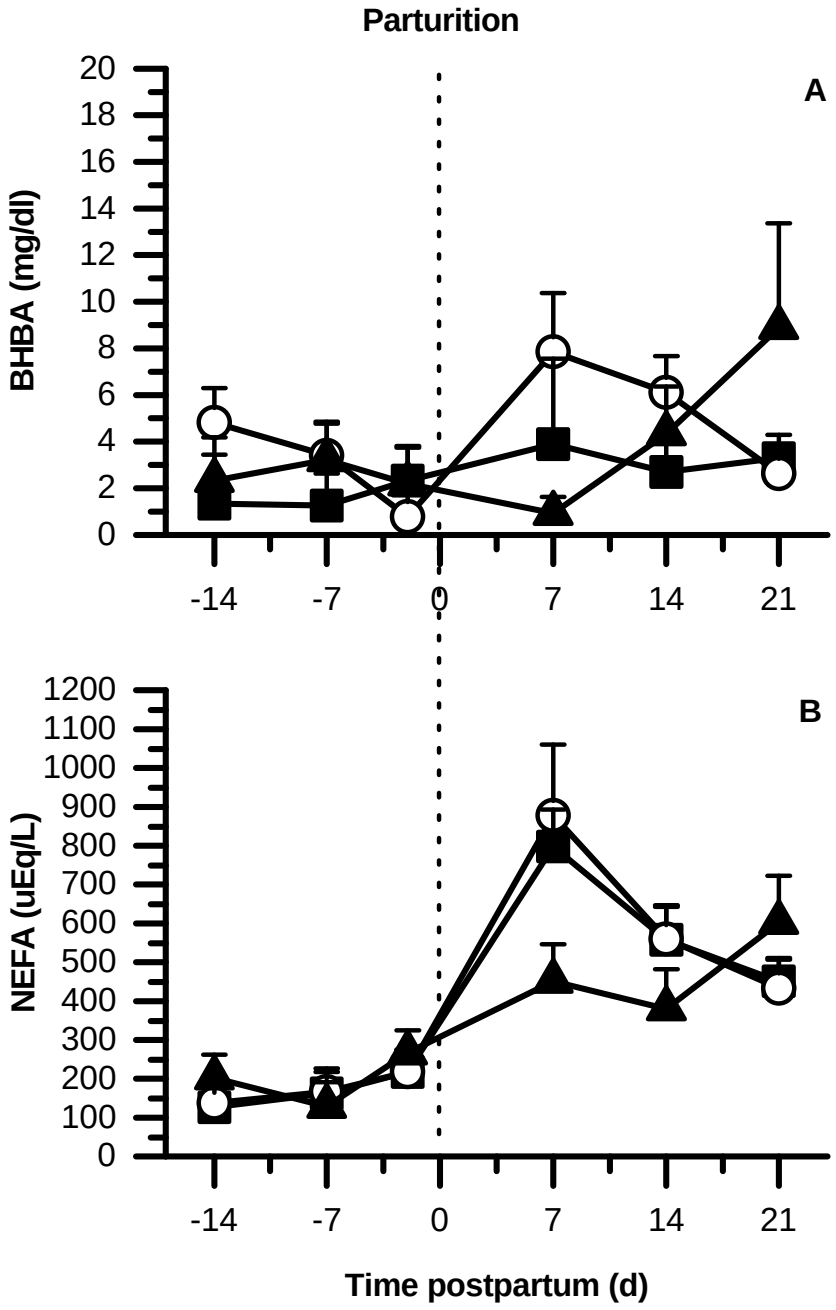

Figure 3. Plasma BHBA (A) and NEFA (B) concentrations (pooled SEM = 111 and 1.95, respectively) of cows fed a control diet (CON, squares), 430 g/d glycerol (LG, open circles), and 860 g/d glycerol (HG, triangles).

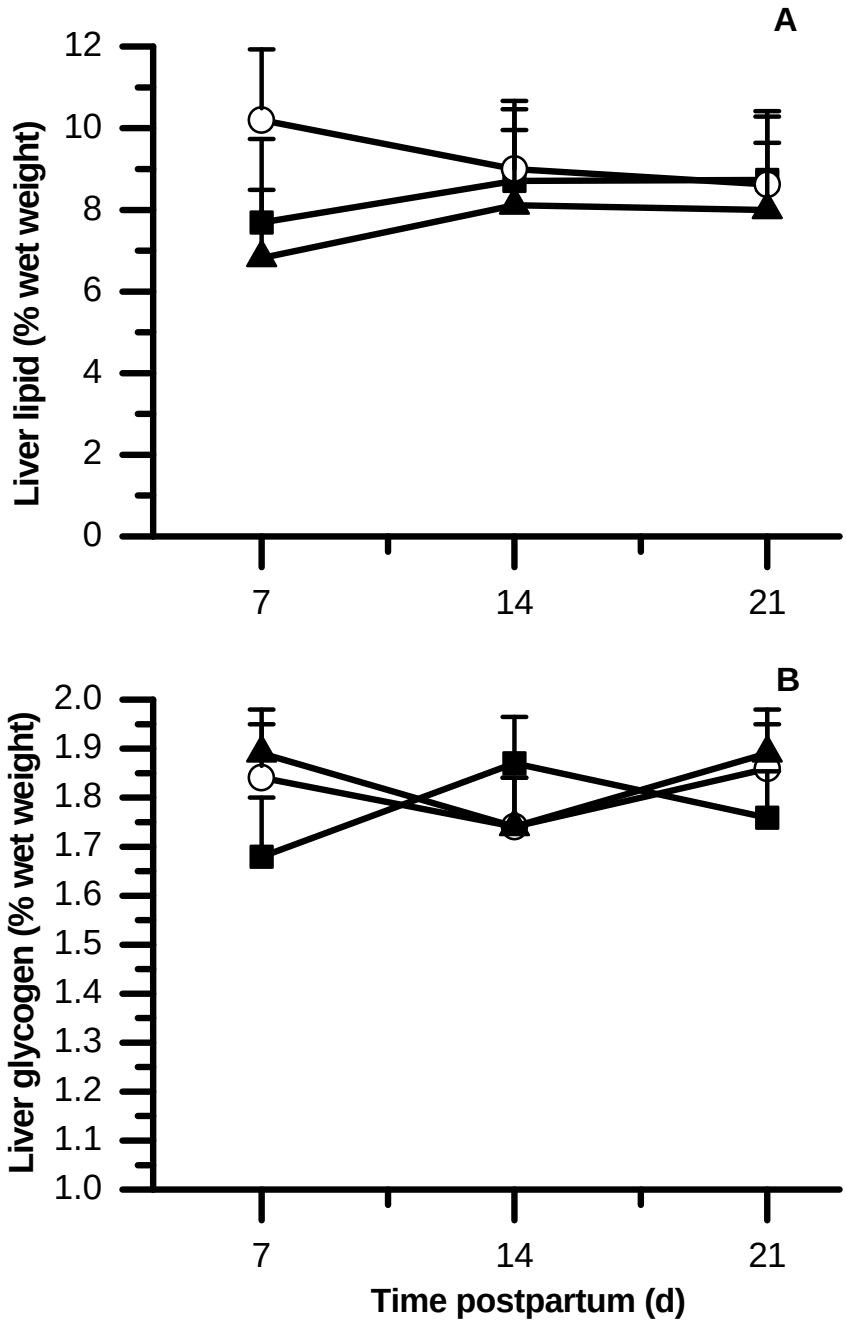

Figure 4. Liver lipid (A) and glycogen (B) composition (pooled SEM = 1.74 and 0.09, respectively) of cows fed a control diet (CON, squares), 430 g/d glycerol (LG, open circles), and 860 g/d glycerol (HG, triangles).

Rumen fluid characteristics are presented in Table 12. With the exception of NH_3-N, dietary treatments did not affect prepartum ruminal pH or molar proportions of VFA. It is important to note that data characterizing ruminal fluid represent samples collected via esophageal tube and might have been contaminated with saliva. Feeding HG tended ($P = 0.12$) to decrease prepartum concentrations of NH_3-N in rumen fluid (2.42 vs. 3.88 mg/dL, respectively). Postpartum ruminal pH tended ($P = 0.12$) to be greater for cows fed LG than those fed HG (6.89 vs. 6.61, respectively). Although molar proportions of butyrate were not affected by diets, cows fed LG and HG tended ($P = 0.06$) to have greater concentrations of ruminal butyrate relative to those fed CON at 7 DIM (9.94, 8.62, and 7.03 ± 0.99, respectively; data not shown). Feeding LG and HG also tended ($P = 0.06$) to increase postpartum total VFA concentrations relative to cows fed CON (70.2 and 61.4 vs. 56.2, respectively). The ratio of acetate to propionate in rumen fluid collected postpartum was greater ($P < 0.01$) in cows fed CON relative to cows fed LG and HG (2.92 vs. 2.07 and 2.40, respectively). Effects of diets on the ratio of acetate to propionate in rumen fluid was primarily a result of changes in proportions of propionate as feeding LG and HG increased ($P < 0.01$) proportions of propionate by nearly 20% relative to cows fed CON (27.1 and 24.7 vs. 21.7, respectively).

Table 12. Ruminal pH, NH_3-N, and VFA proportions of cows fed CON, LG, and HG*.

	Diet				Contrast[1]	
Item	CON	LG	HG	SEM	1	2
Prepartum						
pH	7.30	7.06	7.12	0.17	0.41	0.84
NH_3-N, mg/dL	3.73	3.88	2.42	0.67	0.48	0.12
Total VFA, mM	54.5	56.3	63.9	7.9	0.62	0.55
VFA, molar proportions						
Acetate	61.8	57.9	59.6	2.4	0.29	0.63
Propionate	22.9	25.2	24.2	1.7	0.36	0.67
Isobutyrate	0.72	0.77	0.77	0.14	0.77	0.98
Butyrate	12.4	14.0	12.9	1.12	0.42	0.49
Isovalerate	1.12	0.99	1.09	0.37	0.63	0.56
Valerate	1.09	1.05	1.39	0.20	0.59	0.24
BCFA[2]	1.85	1.76	1.87	0.24	0.91	0.75
Acetate:propionate	2.78	2.49	2.57	0.28	0.46	0.84
Postpartum						
pH	6.91	6.89	6.61	0.13	0.31	0.12
NH_3-N, mg/dL	4.11	4.66	5.29	1.09	0.67	0.82
Total VFA, mM	56.2	70.2	61.4	4.21	0.06	0.33
VFA, molar proportions						
Acetate	61.8	55.4	58.5	1.8	0.15	0.48
Propionate	21.7	27.1	24.7	1.1	0.005	0.31
Isobutyrate	1.07	0.86	0.89	0.11	0.20	0.74
Butyrate	12.3	14.4	13.2	1.1	0.28	0.44
Isovalerate	1.34	1.16	1.23	0.25	0.57	0.97
Valerate	1.35	1.44	1.20	0.11	0.94	0.33
BCFA[2]	2.39	2.02	2.13	0.36	0.40	0.91
Acetate:propionate	2.92	2.07	2.40	0.14	<0.001	0.30

*CON = control, LG = glycerol at 430 g/d, HG = glycerol at 860 g/d.
[1]Contrasts: 1= CON vs. LG and HG, 2= LG vs. HG
[2]Branched chain fatty acids.

Discussion

Demand for renewable energy resources will increase the availability of glycerol (Crandall, 2004), making it less costly as a preventative for ketosis in dairy cattle. Furthermore, incorporating glycerol into a TMR would decrease the likelihood of cows developing toxicity (Goff and Horst, 2001) as compared with other glucogenic agents such as propylene glycol. This report documents the most recent evaluation of glycerol as a glucogenic feed additive for dairy cows since the early 1970s (Fisher et al., 1971; 1973).

Decreased prepartum DMI in this study is contradictory to previous studies (Fisher et al., 1971; 1973). Relative to cows fed propylene glycol, Fisher et al. (1971) found glycerol to act as an appetite stimulant when fed at 472 g/d within the concentrate mix of a component-based diet. In a more extensive experiment using a larger number of cows and an 8 wk treatment period, Fisher et al. (1973) found feeding glycerol at 174 or 347 g/d to be ineffective at improving feed intake, milk yield, and energy balance relative to cows fed an unsupplemented control concentrate or propylene glycol. Differences in results between our study and the earlier work of Fisher et al. (1971; 1973) might be due to differences between component-fed diets versus a TMR. Because feeding LG and HG decreased prepartum but not postpartum DMI, one could speculate the glucogenic potential of glycerol may be most efficaciously used only during lactation; however, this hypothesis has yet to be tested.

Dietary treatments did not affect average BW or BCS; however, a tendency (diet × day interaction) was noted for BW (Figure 1 B). All treatments maintained (CON and HG) or increased (LG) BW from 21 to 70 DIM. Cows fed LG tended to increase BW at a greater rate relative to those fed the control diet or the greater quantity of glycerol. This response is similar to those of Fisher et al. (1973) who fed an amount of glycerol (374 g/d) similar to that used in cows fed LG in the current experiment (430 g/d). Fisher et al. (1973) found cows fed glycerol at 347 g/d during the first 8 wks of lactation lost less body weight ($P <$ 0.10) than cows fed glycerol at 174 g/d, 174 g/d propylene glycol, or a corn-based control concentrate.

With the exception of milk fat yield and milk urea N, there was no effect of treatments on milk yield or milk composition (Table 10). Other glycerol feeding studies (Fisher et al., 1971, 1973; Khalili et al., 1997) have found no effect of glycerol on milk yield. The tendency for greater ECM yields from cows fed CON was largely driven by yields of milk fat which tended to be lower for cows fed LG and HG. Relative to cows fed propylene glycol, Fisher et al. (1971) observed no effect of milk fat yield of cows fed 472 g/d glycerol. Effects of treatments on yields of milk fat agree with the decrease in the ruminal acetate to propionate ratio observed in in vivo glycerol fermentation studies (Table 12; Rémond et al., 1993; Schröder and Südekum, 1999). Although milk urea N tended (Table 10) to decrease in cows fed glycerol, a corresponding decrease in ruminal NH_3-N was not observed in glycerol-supplemented cows.

Feeding glycerol tended to decrease milk fat and milk urea N and decreased the ruminal acetate to propionate ratio. It is likely glycerol underwent ruminal fermentation to propionate similar to a fermentable carbohydrate source. Schröder and Südekum (1999) suggested glycerol of different purities could replace rapidly fermentable starches in diets for ruminants up to 10% of the diet DM. Our results corroborate the in vivo glycerol fermentation results of Schröder and Südekum (1999) and Khalili et al. (1997), where the ruminal acetate to propionate ratio decreased when feeding glycerol at 1.1 and 0.216 kg/d, respectively. With the exception of a tendency for a decrease in NH_3-N for cows fed glycerol, treatments did not affect prepartum ruminal measurements (Table 12); however, differences were notable postpartum. Perhaps differences between ruminal effects of glycerol in prepartum and postpartum diets were attributable to dietary forage composition (76 and 48% decreases, respectively).

Schröder and Südekum (1999) fed glycerol at 46, 98, 116, and 155 g/d (DM basis) in a low-starch concentrate diet and found either no effect or positive effects on digestibilities of organic matter, starch, and cell-wall components in sheep. Feeding similar levels of glycerol in high-starch concentrate diets, however, resulted in a reduction in cell-wall digestibility. Therefore, one possibility for the observed differences between dietary treatments could be attributed to the effects of glycerol on ruminal fermentation and diet digestibility when cows transitioned from the low concentrate prepartum diet to the high concentrate postpartum diet. Although not measured in this study, perhaps the

negative effects of glycerol on cell-wall digestibility were responsible for the unfavorable postpartum lactation performance of cows fed HG.

Relative to its traditional counterparts, most notably propionate and propylene glycol, glycerol is at a metabolic advantage because it enters the gluconeogenic pathway at the triose phosphate level (Leng, 1970), metabolically closer to glucose. Therefore, glycerol does not depend on the rate-limiting enzymes pyruvate carboxylase or phosphoenolpyruvate carboxykinase for its conversion to glucose via glycerol kinase. Glycerol kinase converts glycerol (K_m = 3 to 10 μM [Lin, 1977]) and ATP to glycerol-3-phosphate and ADP, an intermediate step where glycerol is directed toward either gluconeogenesis or glycolysis. These data are the first reported on the effects of feeding glycerol on plasma metabolites of transition dairy cows (Table 11). No differences were detected for prepartum concentrations of glucose, insulin, NEFA, or BHBA; however, feeding glycerol tended to decrease postpartum concentrations of glucose in plasma. In contrast, Goff and Horst (2001) drenched 0.83, 1.66, and 2.49 kg of glycerol via esophageal pump and observed concentrations of blood glucose increased by 16, 20, and 25% for cows treated with 0.83, 1.66, and 2.49 kg of glycerol, respectively, 30 m after dosing. Linke et al. (2004) compared delivery methods of glycerol (feeding vs. drenching 800 g) and found drenching to be more efficacious at increasing plasma glucose and insulin concentrations. The amount of glycerol flowing into the abomasum or absorbed across the rumen epithelium when drenched relative to the amount which is fermented

when fed appears to determine the gluconeogenicity of glycerol in peripartum dairy cows. Bearing in mind the observed changes in ruminal fermentation patterns of cows fed glycerol and the fact that glycerol was undetectable in plasma, it is likely the glycerol fed in the present study was predominately utilized as an energy substrate by the rumen microorganisms instead of entering the gluconeogenic pathway as hypothesized.

Given the diet × day interaction of plasma glucose and BHBA concentrations (Figures 2 A and 3 A) and the tendency for greater ruminal butyrate concentrations at 7 DIM, it is likely the ruminal fermentation of glycerol may have further increased ruminal butyrate beyond the sample of rumen liquor collected at that day. This is especially true with regard to the inverse relationship between plasma glucose and BHBA in cows fed HG at 21 d postpartum. Undoubtedly, molar proportions and concentrations of butyrate (and other ruminal VFA) do not necessarily represent ruminal production rates (Dijkstra et al., 1993). An extensive ruminal sampling regimen was not employed in the current study because the ruminal fermentation of glycerol was not a primary objective of the present experiment; however, countless other reports (Fisher et al., 1971; Rémond et al., 1993; Khalili et al., 1997; Schröder and Südekum, 1999) have found glycerol to increase concentrations of ruminal butyrate, downplaying the glucogenic ability of glycerol as described by others (Garton et al., 1961; Hobson and Mann, 1961; Czerkawski and Breckenridge, 1972). In addition, an intensive serial sampling protocol by Linke et al. (2004)

found both feeding and drenching 800 g of glycerol to increase the molar percentage of ruminal butyrate and plasma BHBA. Because dietary short-chain fatty acids, mainly butyrate, are primary contributors to alimentary ketogenesis (Bergman, 1970), perhaps the ruminal fermentation of glycerol to butyrate increased plasma BHBA and decreased concentrations of glucose in plasma.

Body weight and body condition loss during the first 21 DIM was similar among treatments (Figure 1). This is paralleled by the similar concentrations of NEFA in plasma found among treatments (Figure 3 B) as well as the similar liver lipid composition (Table 11). Feeding glycerol at levels used in this experiment did not have a significant impact on glucose and energy balance. Indeed, the predominant precursor giving rise to the postpartum increase in plasma BHBA in cows fed HG must have been derived from dietary short chain fatty acids (butyrate) as opposed to free fatty acids released from adipose tissue.

Conclusions

Feeding glycerol-supplemented diets to transition dairy cows did not appear to exhibit the glucogenic effect attributed to it by researchers delivering glycerol via esophageal drench (Johnson, 1955; Goff and Horst, 2001). This conclusion is based upon a group of key indicators known to be critical to a successful transition cow program. The only effect of glycerol-supplemented diets prepartum was on DMI, which was decreased by 17% regardless of amount of glycerol fed. In general, postpartum lactation performance was not affected by feeding glycerol, however, changes in ruminal profiles observed in

cows fed glycerol included increased molar proportions of propionate, concentrations of butyrate, and a decreased ratio of acetate to propionate, all of which are in agreement with Linke et al. (2004). Feeding HG decreased postpartum concentrations of glucose and increased plasma BHBA from 7 to 21 DIM, a time when cows are most susceptible to ketosis (Gröhn and Erb, 1989). Although only reported as a tendency because of limited sample numbers, it is hypothesized that glycerol altered ruminal fermentation toward an increase in butyrate as reported elsewhere (Fisher et al., 1971; Rémond et al., 1993; Khalili et al., 1997), prompting an increase in plasma BHBA. The data imply that glycerol should be delivered as a drench in hypoglycemic dairy cows and not fed as a component of transition dairy cow diets.

CHAPTER 4. Experiment 3. Impact of Ruminal Butyrate from Lactose Fermentation and Plasma Betahydroxybutyrate on the Glycemic Status of Transition Dairy Cows

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Abstract

Twenty-four multiparous Holstein cows (775 ± 24 kg body weight; 3.4 ± 0.11 body condition score) were used in a randomized complete block design to determine the impact of increased butyrate proportions from the rumen fermentation of lactose on key metabolic indicators and lactation performance. Cows were assigned to either a corn-based control diet (CON) or a diet containing lactose at 15.7% of diet DM (LAC) at the expense of corn grain. Experimental diets were fed from 21 d before expected calving through 21 DIM. From 22 to 70 DIM, all cows were fed a similar lactation cow diet. Intakes were recorded from 21 d pre- through 28 d postpartum. Energy density and CP were 1.66 and 1.69 Mcal/kg and 14.3 and 18.1% for pre- and postpartum diets, respectively. Milk composition was analyzed at 7, 14, and 21 DIM. Blood was sampled at 21, 14, 7, and 2 d prepartum and 2, 7, 14, and 21 DIM whereas rumen fluid was sampled 21 and 7 d pre- and at 7 d postpartum. Liver tissue was sampled via biopsy at 7 and 14 DIM. Pre- and postpartum DMI averaged 12.8 and 17.7 kg/d, respectively, and did not differ between treatments; however, cows fed LAC did not demonstrate a prepartum decrease in DMI. Milk yields

were unaffected by dietary treatments and averaged 45.7 kg/d during the first 70 DIM. Plasma glucose, insulin, and nonesterified fatty acids were not affected by treatments. Feeding LAC increased the proportion of rumen butyrate both pre- (9.2 vs. 11.3 ± 0.45%) and postpartum (10.3 vs. 13 ± 0.67%), which led to an increase in plasma betahydroxybutyrate (7.7 vs. 10.1 ± 1.0 mg/dL). Liver lipid content was decreased (14.7 vs. 8.6 ± 1.5% of wet weight) in cows fed LAC relative to those fed CON while liver glycogen was unaffected by dietary treatments. Results from this research demonstrated that substituting lactose for corn grain in diets of transition dairy cows increases the proportion of butyrate in the rumen increases concentrations of BHBA in plasma without resulting in hypoglycemia and without affecting lactational performance.

Key words: lactose, betahydroxybutyrate, transition dairy cow

Introduction

As dairy cows proceed through the lactation cycle, nutrient requirements change (NRC, 2001). These changes in nutrient requirements bring about necessary changes in diet ingredient and nutrient composition. Regrettably, numerous and often abrupt adjustments in diets are associated with the transition period, a period also coupled with profound physiologic and metabolic changes, most notably hypoglycemia. Manipulating the diet during this time causes significant changes in rumen function and fermentation patterns and necessitates paralleled changes in mechanisms of nutrient uptake and metabolism to satisfy glucose demands of the mammary gland immediately following parturition.

Mostly because of its contribution to gluconeogenesis, much recent research on the feeding and management of transition dairy cows has focused on dietary manipulations to increase propionate to maximize hepatic gluconeogenesis. These research efforts include feeding propionate-based supplements (Mandebvu, et al., 2003), decreasing the forage to concentrate ratio (Holcomb et al., 2001), and the administration of postpartum drenches (Pickett et al., 2003). These reports have provided much needed information on our understanding of hepatic metabolism instead of alleviating hypoglycemia in the early lactation dairy cow. Reports delineating effects of other ruminal VFA during this time are limited.

Ruminally-produced butyrate is considered a ketogenic VFA as most of it is absorbed as BHBA (Weigand et al., 1975). Effects of ruminal butyrate on blood glucose and ketone bodies appear to be somewhat dependent upon the origin of butyrate (fed vs. ruminally synthesized). Andersson and Lundström (1985) found a positive correlation between butyric acid intake from silage high in butyric acid and milk ketone bodies. Krehbiel et al. (1992) infused butyrate into the rumen and observed increases in plasma BHBA and decreases in plasma glucose concentrations. More recently, feeding lactose, an ingredient that consistently ferments to butyrate in the rumen (Schingoethe, 1976), at 0, 7, and 14% of diet DM, increased the molar proportion of butyrate (13.9, 16.3, and 18.0, respectively) but did not place cows at risk for developing ketosis as evidenced by changes in plasma BHBA and glucose concentrations (DeFrain et al., 2004).

Although ruminal butyrate is extensively metabolized to BHBA by the rumen epithelium, BHBA indirectly influences gluconeogenic activity because it is metabolized to acetyl-CoA, an allosteric activator of pyruvate carboxylase (Utter and Keech, 1963). Pyruvate carboxylase converts pyruvate to oxaloacetate during hepatic gluconeogenesis. Therefore, butyrate metabolism spares the oxidation of pyruvate, a glucogenic precursor, and enhances the conversion of pyruvate to oxaloacetate (Black et al., 1966). In support of conclusions from Black et al. (1966), tracer work by Anand and Black (1970) found butyrate injections to stimulate gluconeogenesis in cattle. In addition, ruminally produced butyrate, and therefore BHBA, could also be beneficial in the transition cow by

providing precursors for fatty acid synthesis (Palmquist et al., 1969) during colostrogenesis and act to spare glucose in some extramammary tissues (Holtenius and Holtenius, 1996).

The impact of diets fermenting to butyrate in the rumen of transition dairy cows is largely unexplored and requires attention, especially for dairy producers utilizing whey and products containing lactose. The objective of this experiment was to determine the impact of increased butyrate proportions from the rumen fermentation of lactose on key metabolic indicators and lactation performance. It was hypothesized that increasing ruminal butyrate will decrease the degree of hypoglycemia in transition dairy cows and improve performance.

Materials and Methods

Experimental Design and Feeding and Management of Cows

The experiment was conducted from September of 2003 through July of 2004 at the South Dakota State University Dairy Teaching and Research Facility (Brookings, SD). Animal care and use was according to a protocol approved by the South Dakota State University Institutional Animal Care and Use Committee. Twenty-four multiparous Holstein cows were used to examine the effects of feeding lactose on DMI, milk production and composition, blood metabolites, and liver composition. Treatments were arranged as a randomized block design and blocked by expected calving date.

At 28 d prior to expected calving date, cows were assigned to their respective treatment diet (Table 13), either control (CON) or lactose (First District

Ag Service, Litchfield, MN; LAC) at 15.7% of diet DM. The amount of lactose fed was determined from DeFrain et al. (2004) who fed lactose at 14.2% of the diet DM and increased ruminal butyrate proportions from 13.9 to 18.0%. Our intentions were to achieve a similar ruminal butyrate response to feeding lactose. The lactose was formulated into the diet at the expense of corn and extruded soybeans. Dried distillers grains were incorporated into LAC during the first 21 DIM to balance diet RUP. Cows were adapted to the Calan Broadbent feeding system (American Calan, Inc., Northwood, NH) for 1 wk prior to initiation of treatments. Experimental diets were fed from 21 d (SD = 5.2) before calving through 21 DIM. From 22 to 70 DIM, all cows were fed a similar lactation cow diet. Cows were moved to a box stall with their feed just prior to calving. Calves were immediately removed after parturition. Cows remained in the box stall until their milk was free of antibiotics and then moved to a free-stall barn equipped with the Calan Broadbent feeding system. Cows were housed on a wheat-straw bedded pack prepartum and in a free-stall barn during the postpartum phase of the experiment.

Table 13. Ingredient composition of Control (CON) and Lactose (LAC) diets.

	-21 to 0 DIM		1 to 21 DIM		
Ingredient, % of diet DM	CON	LAC	CON	LAC	22 to 70 DIM[1]
Brome grass hay	14.3	14.3	-	-	-
Alfalfa hay	14.5	14.5	8.3	8.3	15.3
Alfalfa haylage	-	-	14.5	14.5	6.6
Corn silage, processed	40.2	40.2	27.9	27.9	24.9
Whole cottonseed	2.0	2.0	8.6	8.6	7.9
Wet distillers grains	-	-	3.6	3.6	-
Dried distillers grains	-	-	-	3.8	4.6

Corn, high moisture	-	-	-	-	17.9
Corn grain, ground	17.7	-	25.2	5.4	7.6
Lactose[2]	-	15.7	-	15.7	-
Energizer 4-19W[3]	-	-	-	-	4.2
Soybean meal, 44%	1.81	1.65	4.84	4.30	2.43
SoyChlor 16-7[4]	6.63	6.64	-	-	-
SoyPlus[4]	1.79	3.60	4.26	4.77	4.53
Limestone	0.36	0.22	1.09	1.06	0.52
Dicalcium phosphate	-	0.18	0.19	0.33	-
Sodium bicarbonate	-	-	0.59	0.59	0.57
Fish meal, menhaden	-	-	-	-	0.40
Pork meat & bone meal	-	-	-	-	1.21
Yeast culture[5]	-	-	-	-	0.21
Magnesium oxide	-	-	0.24	0.24	0.17
Salt	-	-	0.24	0.24	0.50
Vitamin A, D, & E premix	0.65[a]	0.65[a]	0.31[b]	0.31[b]	0.31[b]
Urea	0.02	0.29	0.02	0.24	0.10
4-Plex[6]	0.07	0.07	0.07	0.07	0.05
Vitamin E premix[7]	-	-	0.05	0.05	0.02

[1]All cows were fed the same diet from 22 to 70 DIM.
[2]First District Ag Service, Litchfield, MN.
[3]Quality Liquid Feeds, Inc., Dodgeville, WI. Liquid mixture of cane molasses, condensed whey, and tallow (assay DM basis: 12.9% CP, 61% fat).
[4]West Central Soy, Ralston, IA. SoyChlor 16-7 is a heat-processed soybean meal treated with HCl (assay DM basis: 23% CP, 53% RUP, 10.3 % Cl). SoyPlus is a heat-processed soybean meal (assay DM basis: 50% CP, 60% RUP).
[5]Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA. *Saccharomyces cerevisiae* yeast and the media it was grown on.
[6]4-Plex, Zinpro Corp., Eden Prairie, MN. Zn and Mn methionine complex, Cu lysine complex, and Co glucoheptonate.
[7]Contains 44,092 IU of vitamin E per kg.
[a]Contains 454,000 IU of vitamin A, 90,900 IU vitamin D, and 3,636, IU of vitamin E per kg.
[b]Contains 909,000 IU of vitamin A, 182,000 IU vitamin D, and 2,424, IU of vitamin E per kg.

Table 14. Nutrient composition of Control (CON) and Lactose (LAC) diets[1].

	-21 to 0 DIM		1 to 21 DIM		
Nutrient	CON	LAC	CON	LAC	22 to 70 DIM[2]
DM, % as fed	49.3	55.1	54.2	55.0	48.7
CP, %	14.2	14.4	18.1	18.1	17.8
NE_L[3], Mcal/kg	1.63	1.68	1.70	1.68	1.68
ADF, %	24.0	24.5	21.1	20.1	19.2
NDF, %	37.3	36.3	32.6	31.9	31.7
NFC[4], %	37.0	36.7	37.2	37.3	36.5
Ether extract, %	4.3	5.3	5.5	4.6	6.6
Starch, %	22.7	24.4	25.7	18.3	29.0
Lactose, %	-	15.8	-	16.0	0.7
Ash, %	7.2	7.3	6.6	8.1	7.4
Ca, %	0.99	1.01	1.20	1.27	1.12
P, %	0.38	0.39	0.42	0.43	0.48
Mg, %	0.50	0.53	0.50	0.49	0.46
K, %	1.25	1.23	1.39	1.29	1.36
Na, %	0.16	0.17	0.35	0.36	0.48
Cl, %	0.89	1.09	0.33	0.41	0.58
S, %	0.21	0.20	0.27	0.27	0.29
DCAD[5], meq/kg of DM	8	-43	247	203	212

[1]Values are based upon nutrient analyses of diets and actual mean DMI by treatment.
[2]All cows were fed the same diet from 22 to 70 DIM.
[3]Calculated using NRC (2001).
[4]NFC = 100 – (% NDF + % CP + % Ether extract + % Ash).
[5]DCAD as [(Na + K) – (Cl + S)] in milliequivalents per kg of DM.

Measurements and Collection of Samples

Diets were mixed and fed daily at 0600 h for ad libitum consumption as a TMR. Weighbacks were measured, recorded, and discarded prior to feeding each day and amounts fed were adjusted to ensure a 10% feed refusal. Samples of diets were collected weekly and frozen and composited monthly. The composited samples were sent to Dairyland Laboratories (Arcadia, WI) for analysis. Body weight and BCS (1 to 5 in 0.25 increments; Wildman et al., 1982) were recorded on two consecutive days, 4 h after feeding on d 21 prior to

expected calving date, at parturition, and at 28 and 70 DIM. The same three individuals recorded BCS during the entire experiment. In addition, calf birth weights and calving difficulty scores were recorded (1 = no problem; 2 = slight problem; 3 = needed assistance; 4 = considerable force; 5 = cesarean). Cows were milked at 0600, 1400, and 2100 h, and milk yield was recorded. Milk samples were collected on d 7, 14, and 21 of lactation from all three milkings each day and were preserved using a tablet containing bronopol and natamycin (Broad Spectrum Microtabs II, D & F Control Systems, Inc., Dublin, CA). Incidences of milk fever, metritis, displaced abomasum, mastitis, and foot aliments were also recorded during the experiment.

Target day and actual day of blood sampling relative to calving were -21 and –20.8 (SD = 2.2), -14 and –14.6 (SD = 1.8), -7 and –8.0 (SD = 1.9), -2 and –2.5 (SD = 0.9), 2 and 2.2 (SD = 1.0), 7 and 7.0 (SD = 1.0), 14 and 14.1 (SD = 1.0), 21 and 21.3 (SD = 1.0), and 28 and 28.2 (SD =1.1), respectively. Approximately 4 h after feeding, blood was sampled from a coccygeal vessel into two evacuated tubes (Becton Dickinson and Co., Franklin Lakes, NJ) containing K-EDTA and sodium fluoride. Samples were immediately placed on ice and transported to the laboratory where they were centrifuged (500 × g) and plasma was harvested and stored at -20°C until further analysis. Ruminal fluid was collected at 21 (SD = 5.4) and 7 (SD = 4.7) d prior to expected calving date and 7 (SD = 1.2) d postpartum 4 h after feeding by applying vacuum pressure to an esophageal tube fitted with a suction strainer. To minimize saliva contamination,

approximately 250 mL of rumen fluid were discarded prior to sample collection. A 10-mL sample was mixed with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen at -20ºC until analyzed for concentrations of VFA and NH_3-N. Liver tissue was collected by trocar and aspiration between the 11th and 12th rib (Smith et al., 1997) approximately 4 h after feeding on d 7 and 14 (actual d were 7.2 [SD = 1.2] and 14.1 [SD = 1.0]) of lactation. Samples were blotted to remove any residual blood, split into two equal aliquots, placed into cryovials, immediately submerged in liquid nitrogen, and transported to the laboratory where they were frozen at -80°C until analysis.

Laboratory Analysis

Samples of diets were dried at 55ºC in a forced-air oven and allowed to air-equilibrate before being ground to pass a 2-mm screen of a standard Wiley mill (Model 3; Arthur H. Thomas Co., Philadelphia, PA). Samples were composited by diet (prepartum, postpartum, and lactation TMR) and month and analyzed for DM at 105ºC for 24 h, CP (AOAC, 1997) using a LECO-428 combustion analyzer (LECO Corp., St. Joseph, MI), NDF (Van Soest et al., 1991), ether extract (AOAC, 1997), minerals (AOAC, 1997; 985.01), and ADF (AOAC, 1997). Neutral detergent fiber and ADF were measured using the ANKOM A200 (ANKOM Technology Corp., Fairport, NY) filter bag technique. Determinations of ADF was according to AOAC (973.18 C, 1997) whereas NDF was according to Van Soest et al. (1991) with the addition of 4 mL of alpha-amylase and 20 g of sodium sulfite. Starch was measured as dextrose after

treating samples with glucoamylase using a YSI 2700 SELECT Biochemistry Analyzer (Yellow Springs, OH; Holm et al., 1986). Minerals were quantified according to AOAC methods (985.01, 1997) using an inductively coupled plasma spectrometer (Thermo Garrell Ash, Franklin, MA). Samples were also analyzed for lactose according to AOAC (974.06, 1990) using an HPLC (Waters Corporation, Milford, MA) equipped with a refractive index detector and a 300 mm $\times$ 7.8 mm column (HPX-87H, Bio-Rad Laboratories, Hercules, CA) using a flow rate of 0.6 mL/min of 0.01 N H_2SO_4.

Milk compositional analysis was conducted by Heart of America DHI Laboratory (Manhattan, KS) according to approved procedures of AOAC (1990). Samples were composited by day and analyzed for protein, fat, lactose, MUN, and SCC and SNF was calculated. Milk true protein, fat, and lactose were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments).

Plasma samples were thawed and concentrations of glucose were determined using glucose oxidase (Sigma Kit #315, Sigma Diagnostics, St. Louis, MO) according to the procedures of Trinder (1969). Concentration of BHBA in plasma was determined (Pointe Scientific, Inc., Lincoln Park, MI) following the methods of Williamson et al. (1962) and plasma NEFA

concentrations were determined using a colorimetric assay (NEFA-C Kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993). Insulin was quantified by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) with an intraassay CV of 1.6%. Samples of liver tissue were thawed and analyzed for total lipid and glycogen as described by Mills et al., (1986) and Derling et al., (1987), respectively.

Statistical Analysis

Effects of treatments on incidences of health disorders were not analyzed for statistical differences because of insufficient replication; however, all cows experiencing disorders were included in the data set. Two cows fed CON and three cows fed LAC gave birth to twins, all of which were natural deliveries. One of the cows fed CON was diagnosed with metritis at 10 DIM while the other was treated for a retained placenta. None of the cows fed LAC that twinned received any treatment for either metritis or retained placenta. One cow from each treatment was treated for mastitis and recovered quickly. Other than those aforementioned, no other health disorder events were noted.

The experiment was a randomized complete block design based upon expected calving date. Milk yield and DMI data were reduced to weekly means for statistical analysis. Milk production data collected on the day of calving was not included in the data set because of the inherent difficulties associated with data collected on the day of calving. Data were analyzed as repeated measures using PROC MIXED (Littell et al., 1996) of SAS software, version 8.1 (1999).

For each variable, cow was subjected to four covariance structures: autoregressive order one, toeplitz, variance component, and compound symmetry. On average, the structure yielding the Akaike's Information Criterion closest to zero was variance component and autoregressive order one. For variables measured over time, the model included treatment, time (week or day depending on the variable), and 2-way interactions as fixed effects. The random effect was diet nested within cow. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom for *F*- tests. Covariates of initial BW and BCS, days on treatment, and previous 305 d mature equivalent milk yield were included for all data sets. Covariates and interactions were dropped from the model one at a time, starting with the least significant and continuing until all remaining variables were significant. Prepartum and postpartum data were analyzed separately.

Statistical significance was declared at $P < 0.05$, with trends noted at $P > 0.05$ to $P \leq 0.15$. Least square means and SEM are reported for all data. Significance of interactions are reported when significant. When significant effects among dietary treatments existed, mean separation was conducted by the PDIFF option in SAS.

Results

Ingredient and nutrient composition of diets are shown in Tables 13 and 14, respectively. Diets were formulated using CPM (Cornell Pennsylvania Miner) Dairy (version 3.0.5; University of Pennsylvania, Kennett Square, PA; Cornell

University, Ithaca, NY; William H. Miner Agricultural Research Institute, Chazy, NY) and recommendations from NRC (2001). A BW of 680 kg and a targeted DMI of 12.2, 19.0, and 25.6 kg/d for pre-, postpartum, and lactation (29 to 70 DIM) TMR, respectively, were used during formulations. The ratio of forage to concentrate was 70:30, 50:50, and 46:54 for pre-, postpartum, and lactation diets, respectively. Pre- and postpartum of LAC contained 15.8% (SD = 0.79) and 16.0% (SD = 0.65), respectively.

Initial cow characteristics are shown in Table 15. Average previous 305 d mature equivalent milk yield was 11,632 ± 467 kg. Previous 305 d mature equivalent yield tended ($P < 0.07$) to be lower and initial BCS tended ($P < 0.06$) to be greater for cows fed LAC. Therefore, when significant, previous 305 d milk yield and initial BCS were included as covariates during statistical analysis of the data. Days on treatment and number of lactations prior to initiation of treatments were similar among treatments and averaged 40.5 ± 1.5 d and 1.8 ± 0.26, respectively. Average prepartum DMI was not affected by diet and averaged 12.8 ± 0.59 kg/d. Dry matter intake decreased from wk 2 to wk 1 prepartum for cows fed CON while intakes of cows fed LAC remained unchanged (Figure 5).

Table 15. Previous 305-d mature equivalent milk, days on treatment, BW, BCS, calf birth weights, calving difficulty, and prepartum DMI of cows fed Control (CON) and Lactose (LAC).

	Diet			
Item	CON	LAC	SEM	*P*[1]
n	12	12	-	-
Previous 305 ME[2], kg	12,254	11,010	467	0.07
Days on treatment	41.9	39.0	1.5	0.19
Lactation No.[3]	1.8	1.7	0.26	0.66
BW, kg				
d -21	777	775	11.7	0.92
d 0	704	702	11.7	0.92
d 28	657	643	12.3	0.43
d 70	678	669	12.7	0.61
BCS[4]				
d -21	3.34	3.47	0.06	0.17
d 0	3.33	3.20	0.06	0.12
d 28	3.02	2.89	0.07	0.16
d 70	3.09	3.05	0.07	0.71
Calf BW, kg	41.8	40.7	2.55	0.75
Calving difficulty[5]	1.4	1.8	0.28	0.36
Prepartum DMI, kg/d	12.2	13.3	0.59	0.22

[1]Significance of *F*-test.
[2]Mature equivalent.
[3]Lactation number before calving.
[4]Wildman et al., 1982.
[5]Five point scale: 1= no assistance, 2= slight problem, 3= needed assistance, 4= considerable force, and 5= cesarean.

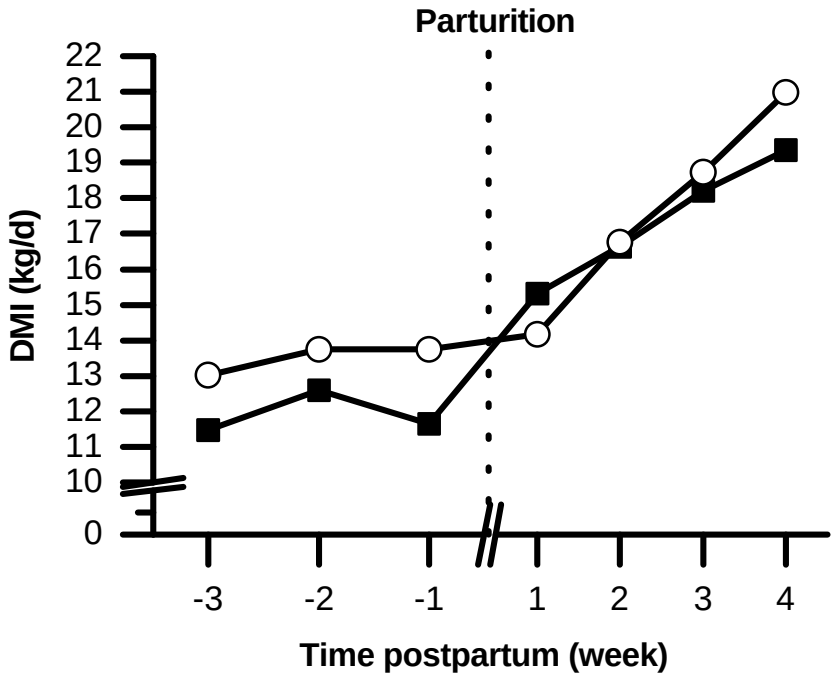

Figure 5. Dry matter intake (pooled SEM = 1.16) of cows fed Control (squares) and Lactose (circles).

Postpartum performance data are presented in Table 16. Average DMI during the first 28 DIM was 17.7 ± 0.89 kg/d and did not differ among treatments. Milk yield, ECM yield (Orth, 1992), and production efficiencies (ECM/DMI) were unaffected by dietary treatments during the first 21 DIM. With the exception of milk SCC, milk composition was not affected by dietary treatments. Cows fed CON tended ($P < 0.11$) to yield milk with a greater SCC than those fed LAC. Body weight and BCS collected at 28 and 70 DIM were not different among treatments.

Effects of treatments on plasma metabolites are presented in Table 17 and plotted over time in Figures 7, 8, and 9. Concentrations of plasma glucose and insulin were not different among treatments; however, differences were observed on specific days relative to calving. Cows fed LAC tended ($P < 0.11$) to have greater concentrations of glucose in plasma at 7 d pre- (74.1 vs. 63.5 mg/dL) and 14 d (66.7 vs. 59.6 mg/dL) postpartum relative to those fed CON. Plasma insulin concentrations tended ($P < 0.06$) to be greater in cows fed CON at 21 DIM compared to cows fed LAC (211 vs. 119 pg/mL). Concentrations of NEFA in plasma were similar among all cows; however, concentrations of BHBA in plasma of cows fed LAC tended ($P < 0.07$) to be greater than those fed CON

Table 16. Postpartum DMI, milk yield, milk composition, and milk SCC of cows fed Control (CON) and Lactose (LAC).

	Diet			
Item	CON	LAC	SEM	P^1
DMI, kg/d	18.2	17.2	0.89	0.43
Milk d 1-21, kg/d	40.7	38.6	2.46	0.54
ECM[2] d 1-21, kg/d	44.5	41.3	2.2	0.32
ECM/DMI d 1-21	2.97	2.60	0.28	0.37
Milk composition, d 1-21				
Fat, %	4.82	4.71	0.29	0.79
Fat kg/d	1.81	1.73	0.12	0.68
True protein, %	3.07	2.93	0.07	0.21
True protein, kg/d	1.17	1.07	0.07	0.36
SNF, %	8.42	8.42	0.13	0.99
SNF, kg/d	3.29	3.09	0.23	0.55
Lactose, %	4.63	4.72	0.11	0.55
Lactose, kg/d	1.86	1.69	0.12	0.33
SCC x 10^3/mL	618	68	245	0.11
Urea N, mg/dL	11.41	11.33	0.72	0.94
Milk d 1-70, kg/d	44.8	46.6	2.13	0.57

[1]Significance of *F*-test.
[2]ECM = [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)]; (Orth, 1992).

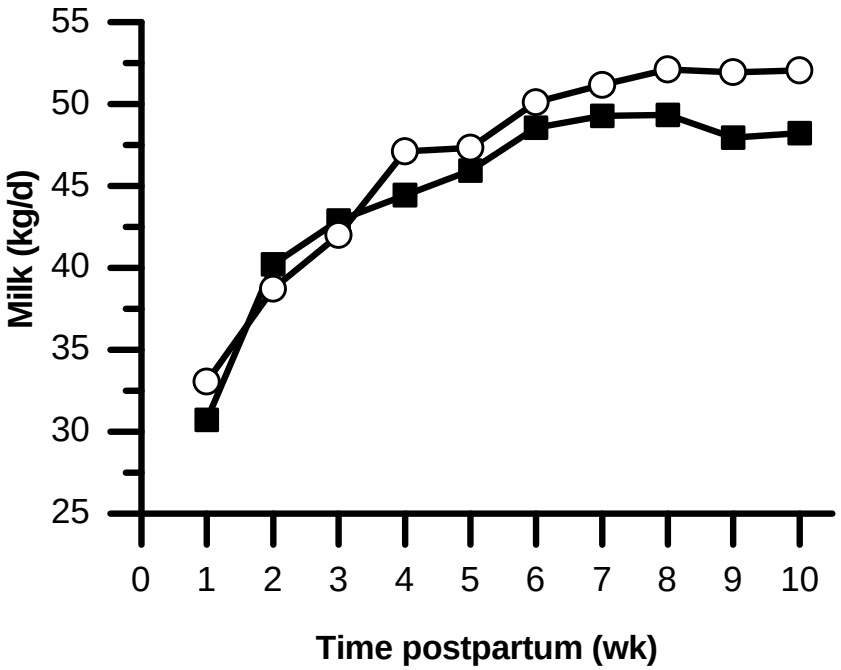

Figure 6. Milk yield (pooled SEM = 2.58) of cows fed Control (squares) and Lactose (circles).

Table 17. Plasma glucose, insulin, NEFA, and BHBA and liver composition of cows fed Control (CON) and Lactose (LAC).

	Diet			
Item	CON	LAC	SEM	P^1
Glucose, mg/dL				
All data	66.0	70.0	2.28	0.23
Prepartum	68.9	74.1	3.64	0.32
Postpartum	63.4	66.4	2.02	0.31
Insulin, pg/mL				
All data	258	319	37	0.26
Prepartum	367	526	84	0.19
Postpartum	170	145	12	0.18
NEFA, µEq/L				
All data	367	419	37	0.33
Prepartum	167	175	24	0.83
Postpartum	539	612	54	0.35
BHBA, mg/dL				
All data	7.65	10.06	1.02	0.07
Prepartum	4.25	6.14	0.31	< 0.01
Postpartum	8.34	14.61	1.67	0.01
Glucose + BHBA, mg/dL				
All data	72.8	80.8	2.70	0.04
Prepartum	73.0	80.0	3.64	0.19
Postpartum	71.5	81.4	3.13	0.04
Liver glycogen, % wet weight	3.12	2.98	0.41	0.81
Liver lipid, % wet weight	14.7	8.6	1.49	0.01

[1]Significance of *F*-test.

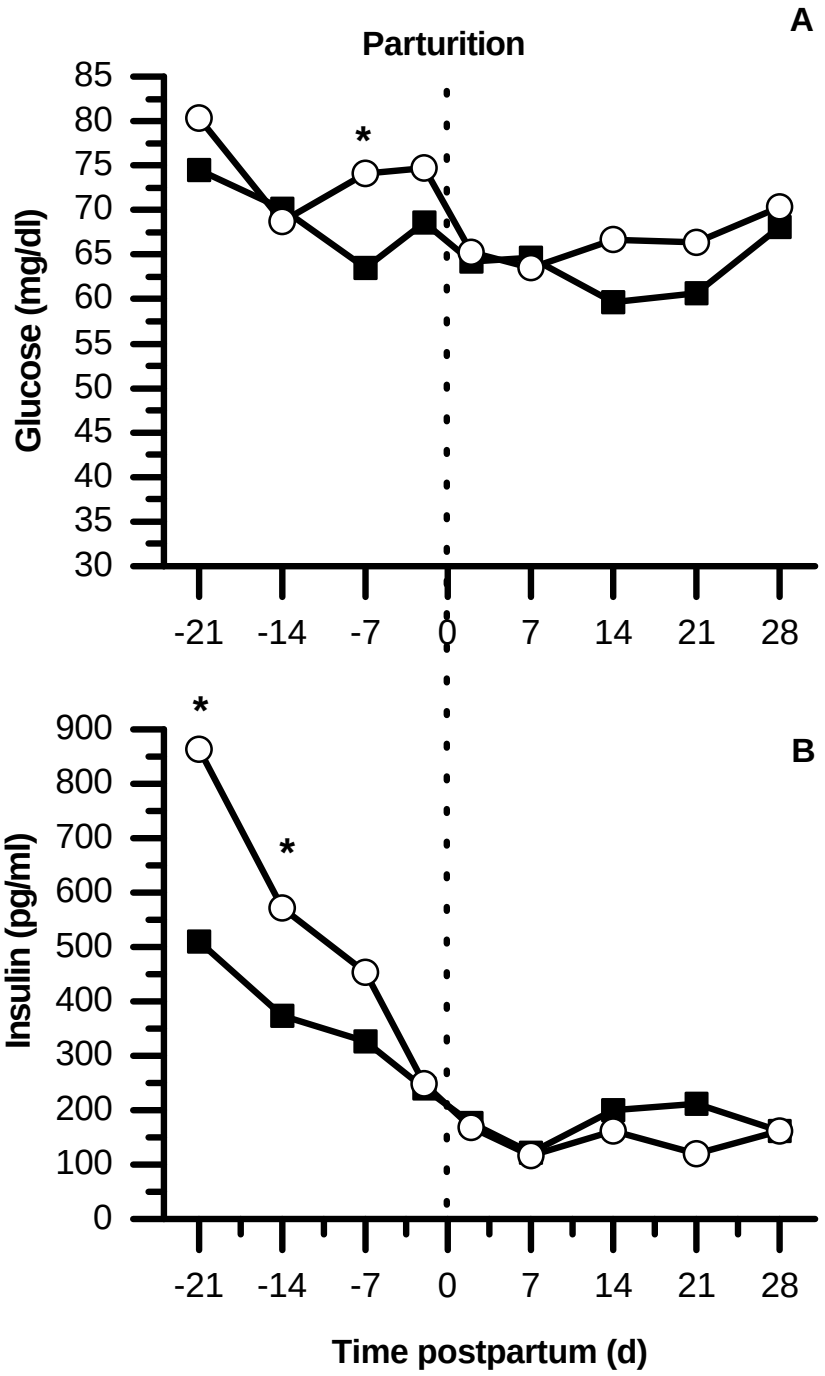

Figure 7. Plasma glucose (A) and insulin (B) concentrations (pooled SEM = 3.96 and 78.8, respectively) of cows fed Control (squares) and Lactose (circles). Differences at individual time points are indicated by * ($P < 0.05$).

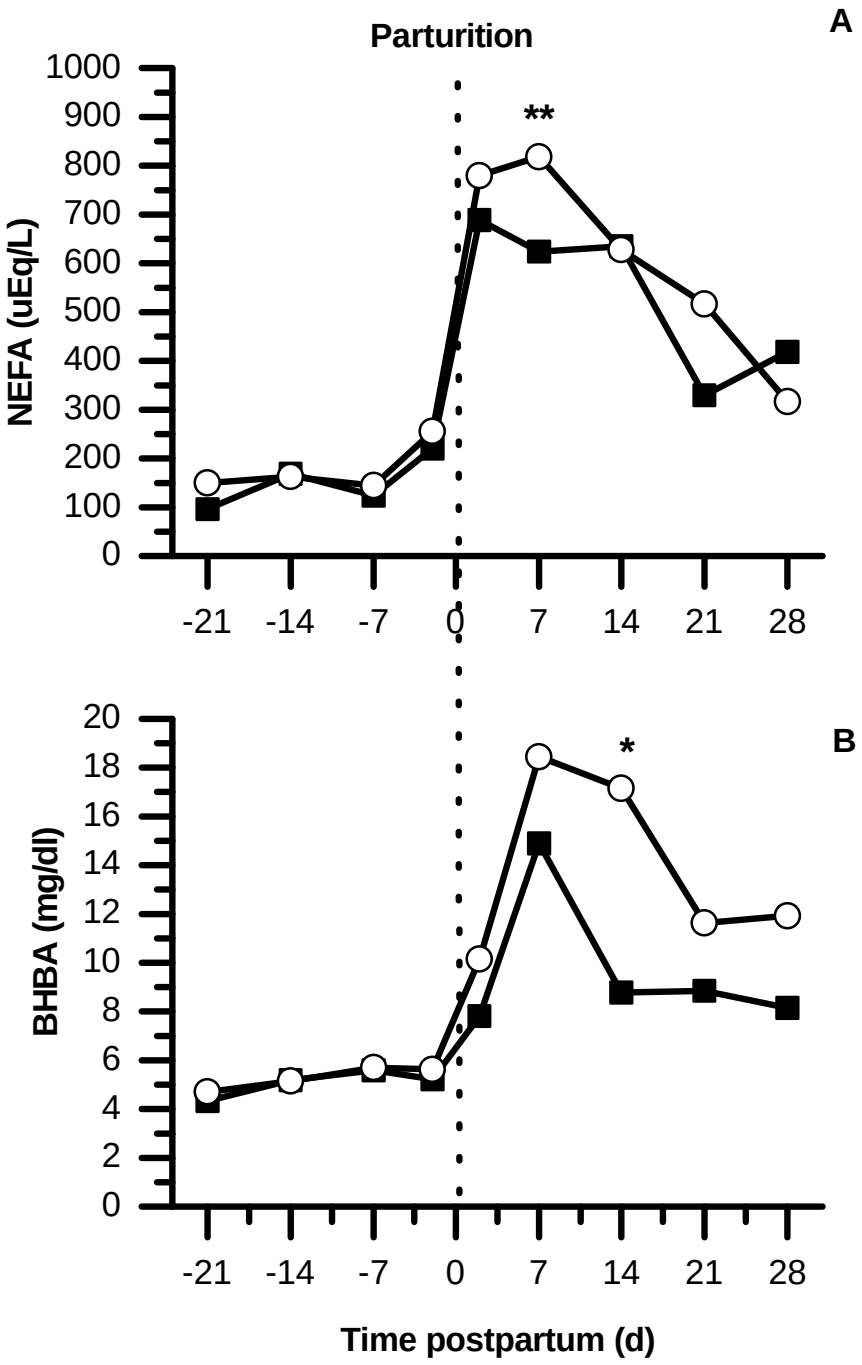

Figure 8. Plasma NEFA (A) and BHBA (B) concentrations (pooled SEM = 94 and 2.47, respectively) of cows fed Control (squares) and Lactose (circles). Differences at individual time points are indicated by * ($P < 0.05$) and ** ($P < 0.10$).

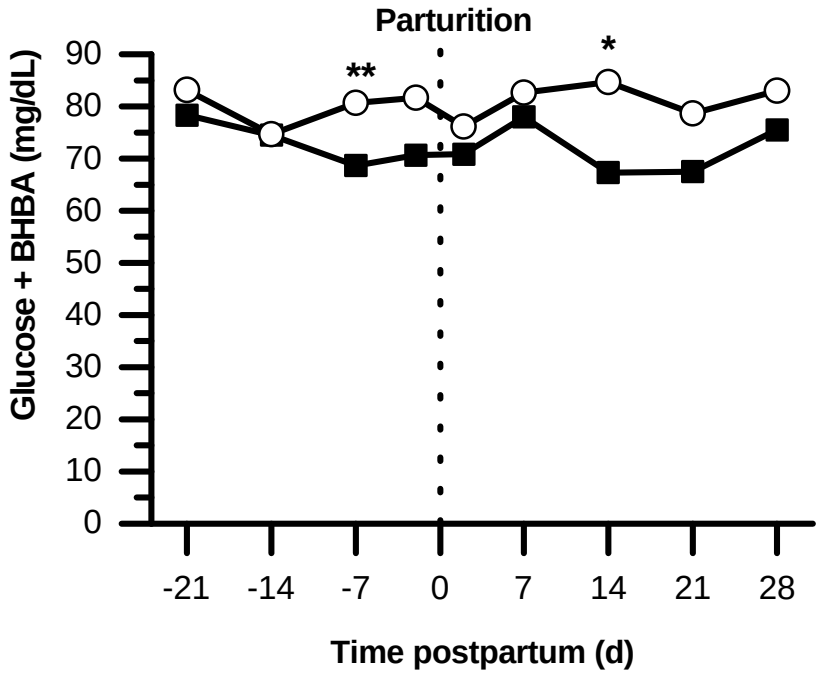

Figure 9. The sum of plasma glucose and BHBA concentrations (pooled SEM = 4.84) of cows fed Control (squares) and Lactose (circles). Differences at individual time points are indicated by * ($P < 0.05$) and ** ($P < 0.10$).

throughout the entire experiment. These differences were most pronounced at 14 DIM as concentrations of BHBA in plasma were nearly two-fold greater ($P < 0.01$) in cows fed LAC relative to those fed CON (17.1 vs. 8.8 mg/dL, respectively). Dietary treatments did not alter liver glycogen content which averaged 3.0% (wet weight; Table 17). Feeding LAC resulted in a 58% decrease ($P < 0.01$) in liver lipid content relative to cows fed CON (8.6 vs. 14.7, respectively).

Effects of diets on rumen NH_3-N concentrations and VFA proportions are presented in Table 18. Prepartum, but not postpartum, rumen NH_3-N

concentrations were greater (3.25 vs. 2.37 mg/dL; $P < 0.04$) for cows fed CON relative to those fed LAC. Feeding LAC tended to decrease proportions of rumen propionate prepartum. This treatment effect was more pronounced in samples collected postpartum ($P < 0.04$.). Rumen butyrate proportions increased ($P < 0.01$) an average of 2.4 percentage units both pre- (9.2 vs. 11.3%) and postpartum (10.3 vs. 13.0%) in cows fed LAC. The proportion of rumen VFA as branched chain fatty acids decreased by 0.85 percentage units in cows fed LAC relative to those fed CON. Total VFA and the ratios of acetate to propionate were not affected by treatments.

Table 18. Ruminal NH_3-N and VFA proportions of cows fed Control (CON) and Lactose (LAC).

	Diet			
Item	CON	LAC	SEM	P^1
Prepartum				
NH_3-N, mg/dL	3.25	2.37	0.30	0.04
Total VFA, mM	46.4	50.5	4.29	0.50
VFA, molar proportions				
Acetate	68.3	67.8	0.77	0.66
Propionate	19.6	18.8	0.38	0.15
Isobutyrate	0.80	0.44	0.13	0.06
Butyrate	9.2	11.3	0.45	< 0.01
Isovalerate	1.14	0.61	0.08	< 0.01
Valerate	1.05	1.09	0.10	0.78
BCFA[2]	1.94	1.05	0.17	< 0.01
Acetate:propionate	3.52	3.64	0.10	0.42
Postpartum				
NH_3-N, mg/dL	2.69	2.29	0.48	0.56
Total VFA, mM	40.8	34.3	4.58	0.33
VFA, molar proportions				
Acetate	63.7	64.7	2.8	0.62
Propionate	22.5	19.5	0.98	0.04
Isobutyrate	1.04	0.78	0.19	0.33
Butyrate	10.3	13.0	0.67	0.01
Isovalerate	1.24	0.68	0.22	0.08
Valerate	1.16	1.39	0.20	0.43

BCFA[2]	2.28	1.43	0.40	0.16
Acetate:propionate	2.98	3.38	0.25	0.28

[1]Significance of *F*-test.
[2]Branched chain fatty acids.

Discussion

The glucose deficit typically observed immediately postpartum exacerbates profit losses of a dairy enterprise as it influences the incidences of costly early postpartum health disorders, compromises lactation performance, and can even result in mortality. Much research has been directed toward developing interventions to overcome the physiologic and metabolic challenges observed during this phase of the lactation cycle, particularly the use of dietary manipulations to increase the supply of propionate to the liver. This research documents an attempt to use rumen butyrate and BHBA to manipulate the biochemistry and enzymes involved in glucose metabolism.

A successful transition program resides heavily on maintaining feed intake during the periparturient period because prepartum DMI is inversely related to concentrations of NEFA and BHBA in plasma and liver triglycerides (Bertics et al., 1992). Feeding lactose did not result in statistical differences in amounts of DMI; however, cows fed LAC steadily consumed approximately 13.5 kg of DM from three wk prior to calving through 7 DIM while those fed CON suffered an intake depression (nadir = 11.6 kg/d) prior to calving (Figure 5). Others have observed tendencies for an increase (DeFrain et al., 2004) or no effect (Doreau et al., 1987; Maiga et al., 1995) from diets containing lactose up to 14% of diet DM.

The consistency of prepartum DMI and the absence of a depression in DMI (Figure 5) suggest lactose may have improved rumen function and therefore nutrient supply and utilization. Allen and Xu (1998) demonstrated increased growth rates for length and width and overall greater surface area of ruminal papillae in nonpregnant, nonlactating dairy cows fed diets containing 43% lactose (DM basis) compared with 43% corn. Concentrations of rumen VFA were not reported by Allen and Xu (1998); however, there are numerous reports of increased rumen butyrate in diets containing lactose (Schingoethe, 1976; DeFrain et al., 2004) and butyrate has been shown to stimulate papillae development and growth. Papillae growth increases surface area and VFA absorptive capacity (Dirksen et al., 1985) which could have possibly prevented accumulation of VFA, decreased ruminal pH, and adverse affects on prepartum DMI in cows fed LAC.

The absence of treatment effects on milk composition is somewhat contradictory to effects of feeding lactose or whey on milk composition as discussed in a review by Schingoethe (1976). Early researchers found lactose and whey products to maintain or slightly increase milk fat when substituted for ground corn (Schingoethe et al., 1976; Boman and Huber 1967) presumably because the ruminal butyrate and plasma BHBA contribute to fatty acid synthesis in the mammary gland (Palmquist et al., 1969). The ability of lactose and whey products to affect milk fat may be dependent upon diet presentation and stage of lactation as cows used in our study were fed a TMR and were less than 21 DIM

while others have used component fed diets (Boman and Huber, 1967; Schingoethe et al., 1976) and/or cows that were farther into lactation (> 100 DIM; Schingoethe et al., 1976; DeFrain et al., 2004).

Feeding lactose did not affect milk true protein, which agreed with results from Pinchasov et al. (1982) and DeFrain et al. (2004) but not Schingoethe et al. (1976) who found substituting dried whole whey (5% diet DM) for shelled corn during late lactation (180 DIM) increased milk protein percent over control-fed cows (3.95 and 3.80%, respectively). In addition, feeding lactose did not affect MUN. This is contrary to known effects of lactose on N efficiency (Poncet and Rayssiguier, 1980; King and Schingoethe, 1983), particularly rumen NH_3-N which tended to be decreased in cows fed LAC prepartum but not postpartum. Feeding lactose has been observed to decrease concentrations of urea N in milk and NH_3-N in rumen fluid when substituted for corn starch (DeFrain et al., 2004). Relative to unsupplemented or corn and soybean meal-based controls, lactose has decreased concentrations of rumen NH_3-N nitrogen when fed in both high forage (Poncet and Rayssiguier, 1980) and high concentrate (King and Schingoethe, 1983) diets. The low rumen NH_3-N and MUN suggest there may have been an opportunity for greater rumen degradable protein supply and therefore greater microbial protein synthesis from lactose as previously reported by Susmel et al. (1995) based upon allantoin excretion rates.

The objectives of the experiment were met as the rumen fermentation of lactose led to an increase in the proportion of butyrate and a subsequent

increase in plasma BHBA in transition dairy cows. Feeding lactose has consistently increased rumen butyrate (Schingoethe, 1976; DeFrain et al., 2004) and plasma BHBA (Doreau et al., 1987; DeFrain et al., 2004). Our hypothesis, supported by Black et al. (1966) and Anand and Black (1970), was that increases in plasma BHBA would increase hepatic gluconeogenesis via allosteric activation of pyruvate carboxylase by the presence of acetyl CoA units created from BHBA metabolism. Concentrations of BHBA in plasma were greater for cows fed LAC than those fed CON, especially during 7 to 28 DIM, a time when pyruvate carboxylase mRNA expression is known to be greatest (Greenfield et al., 2000). Increases in plasma BHBA resulted from the increase in alimentary ketogenesis or a decrease in the uptake of ketone body utilizing enzymes because concentrations of NEFA in plasma were similar among treatments and liver lipid content was decreased. Concentrations of plasma BHBA peaked for all cows at 7 DIM and remained elevated through 14 DIM for cows fed LAC relative to cows fed CON. During this same time period (7 to 14 DIM) concentrations of glucose in plasma decreased in cows fed CON and increased and remained stable through 28 DIM in cows fed LAC (Figure 5), suggesting the BHBA in plasma may have upregulated pyruvate carboxylase and therefore hepatic gluconeogenesis, especially because liver glycogen content was also similar between treatments. DeFrain et al. (2004) found that feeding lactose decreased plasma glucose from 68.0 to 65.5 mg/dL, respectively, for cows fed 0 to 14% of the diet DM as lactose. Differences between studies are a result of

differences in stage of lactation, which is known to affect the expression of hepatic gluconeogenic enzymes (Greenfield et al., 2000). These will be used for supporting future investigations by our laboratory to characterize pyruvate carboxylase and phosphoenolpyruvate carboxykinase changes in periparturient cows fed lactose.

Although liver glycogen content was unaffected by treatments, liver lipid content of cows fed LAC was decreased by 58% relative to those fed CON. This effect likely resulted from the fermentation of lactose to butyrate and subsequent increase in plasma BHBA concentrations as lipolysis indicators (BW, BCS, and concentrations of NEFA in plasma) were unaffected by dietary treatments. According to Weigand et al. (1975), the enzyme system involved in rumen epithelial ketogenesis may become saturated; however, changes in concentrations of butyrate in plasma were undetectable in cows fed lactose at 14% of the diet DM (DeFrain et al., 2004). Therefore, it is possible that the increase in plasma BHBA observed in cows fed LAC in the current study mediated the effects of treatments on liver lipid content and metabolism. Two possible explanations are warranted. First of all, because of the similarities in postpartum plasma NEFA profiles (Figure 8), the BHBA in cows fed LAC may have been the result of increased ability to oxidize lipids to ketone bodies via increased pyruvate carboxylase. A second conjecture might be that elevated plasma BHBA in cows fed LAC increased the efficiency of assembly and/or secretion of liver triglycerides as VLDL. A review on ketone body utilization in

ruminants by Heitmann et al. (1987) reported BHBA infusions in sheep stimulated insulin secretion and production by the pancreas, decreased NEFA, decreased hepatic uptake of NEFA and subsequent hepatic ketogenesis. Therefore, effects of feeding LAC on liver lipid content may have been related to a decrease in hepatic uptake of NEFA or oxidation of lipids.

Conclusions

Formulating diets to increase the proportion of butyrate from rumen fermentation increases concentrations of BHBA in plasma without resulting in hypoglycemia. Feeding lactose from 21 d pre- to 21 d postcalving increased the proportion of butyrate in the rumen, resulted in more consistent prepartum DMI, improved prepartum rumen NH_3-N utilization, and decreased liver lipid content during the first 14 DIM. Although feeding lactose did not improve lactation performance per se, these data indicate substituting lactose for corn could possibly provide an economic advantage to producers located relatively close to cheese plants offering lactose or lactose-containing products such as whey. In addition, these data contribute to future research efforts planned in our laboratory designed to determine the effects of elevated plasma BHBA on rumen papillae and hepatic enzyme metabolism.

CHAPTER 5. Experiment 4. Feeding an Alpha-Amylase Enzyme Preparation to Improve the Glycemic Status and Performance of Transition Dairy Cows

J.M. DeFrain
September, 2004

Abstract

Twenty-four multiparous Holstein cows (759 ± 30 kg body weight; 3.2 ± 0.13 body condition score) were used in a randomized complete block design to determine the impact of feeding an alpha-amylase enzyme preparation during the transition period on rumen fermentation, key metabolic indicators, and lactation performance. Cows were assigned to either a control diet (CON) or the control diet supplemented with an alpha-amylase enzyme preparation (662 FAU/g fungal alpha-amylase, AMA) at 0.1% of diet DM. Experimental diets were fed from 21 d before expected calving through 21 DIM. From 22 to 70 DIM, all cows were fed a similar lactation cow diet. Intakes were recorded from 21 d pre- through 28 d postpartum. Energy density and CP were 1.62 and 1.68 Mcal/kg and 14.3 and 18.0% for pre- and postpartum diets, respectively. Milk composition was analyzed at 7, 14, and 21 DIM. Blood was sampled at 21, 14, 7, and 2 d prepartum and 2, 7, 14, and 21 DIM whereas rumen fluid was sampled 21 and 7 d pre- and at 7 d postpartum. Liver tissue was sampled via biopsy at 7 and 14 DIM. Average pre- and postpartum DMI were 12.4 and 17.8 kg/d, respectively, and did not differ between treatments; however, DMI increased in cows fed CON by 1.5 kg/d and decreased 1.8 kg/d in cows fed

AMA. Treatment differences were undetected for concentrations of insulin in plasma and lipid and glycogen in liver tissue. Concentrations of glucose and betahydroxybutyrate tended to be greater in cows fed AMA relative to cows fed CON. Prepartum plasma nonesterified fatty acid concentrations were greater in cows fed AMA. Supplementing diets with amylase tended to increase proportions of rumen butyrate prepartum but not postpartum. Increases in plasma betahydroxybutyrate and glucose, both of which are considered circulating energy metabolites, placed amylase-supplemented cows at an energetic advantage over unsupplemented controls.

Key words: amylase, betahydroxybutyrate, transition dairy cow

Introduction

The use of feeding exogenous enzymes to alter rumen fermentation and improve animal performance has increased in response to demands for using more “natural” growth-promoting additives. The use of exogenous enzymes has primarily been directed toward improving ruminal fiber digestion. In most cases, improvements in animal performance have been realized due to the ability of fibrolytic enzymes to increase NDF digestion and DMI (Lewis et al., 1999; Zinn and Salinas, 1999). Both of these factors are known to be critical to achieving success in nutrition of the transition dairy cow. The ability of exogenous enzyme preparations to alter rumen microbial activity suggests their mode of action might

be through improvements in efficiency of nutrient utilization. These exogenous enzymes have been shown to increase the attachment of microbes to feed (Yang et al., 2000), increase the hydrolytic activity of the rumen (Morgavi et al., 2000), and increase the number of rumen bacteria (Nsereko et al., 1999). In addition, Hristov et al. (1998) suggested some exogenous enzymes might pass through the rumen and affect the utilization of nutrients in the small intestine. The inclusion of fiber-digesting enzyme preparations in diets for dairy cattle has been shown to be especially useful during early lactation (Rode et al., 1999; Schingoethe et al., 1999).

Investigations on the incorporation of starch-digesting enzyme preparations have not been recently delineated. Early reports on the application of enzymes in ruminants involved amylases (Burroughs et al., 1960; Ralston et al., 1962). The ruminal digestion of starch is not considered to be limiting and rapid digestion of starch in the rumen can result in ruminal acidosis and decrease intake and production (Owens et al., 1998). Huntington (1997) indicated the digestion of starch in the rumen is more beneficial than postruminal digestion because it increases microbial protein outflow from the rumen, which subsequently stimulates the release of pancreatic enzymes, increasing the duodenal digestion of starch. Paradoxically, the digestion of starch in the rumen (vs. intestinal digestion) can cause a loss of energy through heat, CH_4, and H_2 (Rowe et al., 1999). Considering corn represents approximately 50% of the total dietary energy, the use of an alpha-amylase enzyme preparation might be more

valuable as an aid in extracting more energy in the transition dairy cow and decrease the degree of negative energy balance observed during early lactation.

Alpha-amylase (Tricarico et al., 2002) or amylase-containing (Hristov et al., 2000) supplements have increased the proportion of ruminal butyrate. Although ruminal butyrate is extensively metabolized to BHBA by the rumen epithelium, BHBA indirectly influences gluconeogenic activity because it is metabolized to acetyl-CoA, an allosteric activator of pyruvate carboxylase (Utter and Keech, 1963). Pyruvate carboxylase converts pyruvate to oxaloacetate during hepatic gluconeogenesis. Black et al., (1966) indicated butyrate metabolism spares the oxidation of pyruvate, a glucogenic precursor, and enhances the conversion of pyruvate to oxaloacetate. In support of conclusions from Black et al. (1966), tracer work by Anand and Black (1970) found butyrate injections to stimulate gluconeogenesis in cattle. Effects of feeding an alpha-amylase enzyme preparation on plasma glucose were reported by Tricarico et al. (2002) who found cows (117 DIM) fed 0 or 12 g/d alpha amylase to have greater concentrations of glucose in plasma compared to those fed 24 or 36 g/d. Increasing rumen butyrate proportions have also increased parameters related to nutrient absorption and affect rumen growth in calves supplemented with amylase (Gehamn et al., 2003). Ruminally produced butyrate, and therefore BHBA, could also be beneficial in the transition cow by providing precursors for fatty acid synthesis (Palmquist et al., 1969) during colostrogenesis and act to spare glucose in some extramammary tissues (Holtenius and Holtenius, 1996).

Improvements in ruminal digestion and animal performance of dairy cattle fed fibrolytic enzyme preparations during early lactation advocate the need for investigating starch-digesting enzyme supplements. This is of particular interest considering the rumen is forced to adapt to diet changes typically associated with the transition period. These diet changes are known to affect the morphology of the rumen papillae (Dirksen et al., 1985). Therefore, our objectives were to determine the impact of feeding an alpha-amylase enzyme preparation during the transition period on rumen fermentation, key metabolic indicators, and lactation performance. It was hypothesized that the amylase enzyme preparation would alter rumen function through changes in microbial metabolism which would increase rumen butyrate. These changes in rumen fermentation profiles would improve the glycemic status and performance of transition dairy cows.

Materials and Methods

Experimental Design and Feeding and Management of Cows

The experiment was conducted from September of 2003 through July of 2004 at the South Dakota State University Dairy Teaching and Research Facility (Brookings, SD). Animal care and use was according to a protocol approved by the South Dakota State University Institutional Animal Care and Use Committee. Twenty-four multiparous Holstein cows were used to examine the effects of feeding an alpha-amylase enzyme preparation (AMAIZE^{TM}, Batch No. 224638-8, Alltech, Inc., Nicholasville, KY) on DMI, milk production and composition, blood

metabolites, and liver composition. Treatments were arranged as a randomized block design and blocked by expected calving date.

At 28 d prior to expected calving date, cows were assigned to their respective treatment diet (Table 19), either control (CON) or an alpha-amylase enzyme preparation (AMA) at 0.1% of diet DM. The alpha-amylase preparation is a fermentation extract from Saccharomyces cerevisiae fermentation solubles and *Aspergillus oryzae* and was analyzed to contain (DM basis) 28.6% crude protein, 14.5% ether extract, 3.8% crude fiber, 8.1% ash and 662 FAU/g fungal alpha-amylase. One FAU is the amount of enzyme required to dextrinize soluble starch at the rate of 1 g/h at 30ºC and pH 4.8. The amount of enzyme preparation fed was determined from Tricarico et al. (2002) who supplemented cows with 0, 12, 24, and 36 g/d of the preparation and found milk yield and ruminal starch digestibility to respond quadratically, being greatest in cows fed 12 g/d, while DMI was unaffected. In addition, Tricarico et al. (2002) found concentrations of glucose in plasma to be greater for cows fed 0 and 12 g/d relative to those fed 24 and 36 g/d of the enzyme preparation. Cows were adapted to the Calan Broadbent feeding system (American Calan, Inc., Northwood, NH) for 1 wk prior to initiation of treatments. Experimental diets were fed from 21 d (SD = 4.9) before calving through 21 DIM. From 22 to 70 DIM, all cows were fed a similar lactation cow diet. Cows were moved to a box stall with their feed just prior to calving. Calves were immediately removed after parturition. Cows remained in the box stall until their milk was free of antibiotics

and then moved to a free-stall barn equipped with the Calan Broadbent feeding system. Cows were housed on a wheat-straw bedded pack prepartum and in a free-stall barn during the postpartum phase of the experiment.

Table 19. Ingredient composition of diets[*].

Ingredient, % of diet DM	-21 to 0 DIM	1 to 21 DIM	22 to 70 DIM[1]
Brome grass hay	14.3	-	-
Alfalfa hay	14.5	8.3	15.3
Alfalfa haylage	-	14.5	6.6
Corn silage, processed	40.2	27.9	24.9
Whole cottonseed	2.0	8.6	7.9
Wet distillers grains	-	3.6	-
Dried distillers grains	-	-	4.6
Corn, high moisture	-	-	17.9
Corn grain, ground	17.7	25.2	7.6
Energizer 4-19W[3]	-	-	4.2
Soybean meal, 44%	1.81	4.88	2.45
SoyChlor 16-7[4]	6.63	-	-
SoyPlus[4]	1.80	4.24	4.55
Limestone	0.36	1.07	0.52
Dicalcium phosphate	-	0.19	-
Sodium bicarbonate	-	0.59	0.57
Fish meal, menhaden	-	-	0.40
Pork meat & bone meal	-	-	1.21
Yeast culture[5]	-	-	0.21
Magnesium oxide	-	0.24	0.17
Salt	-	0.24	0.50
Vitamin A, D, & E premix	0.65[a]	0.31[b]	0.31[b]
Urea	0.02	0.02	0.10
4-Plex[6]	0.07	0.07	0.05
Vitamin E premix[7]	-	0.05	0.02

[*]Amylase was fed at 0.10% of diet DM to cows fed AMA.
[1]All cows were fed the same diet from 22 to 70 DIM.
[2]First District Ag Service, Litchfield, MN.
[3]Quality Liquid Feeds, Inc., Dodgeville, WI. Liquid mixture of cane molasses, condensed whey, and tallow (assay DM basis: 12.9% CP, 61% fat).
[4]West Central Soy, Ralston, IA. SoyChlor 16-7 is a heat-processed soybean meal treated with HCl (assay DM basis: 23% CP, 53% RUP, 10.3 % Cl).

SoyPlus is a heat-processed soybean meal (assay DM basis: 50% CP, 60% RUP).
[5]Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA. *Saccharomyces cerevisiae* yeast and the media it was grown on.
[6]4-Plex, Zinpro Corp., Eden Prairie, MN. Zn and Mn methionine complex, Cu lysine complex, and Co glucoheptonate.
[7]Contains 44,092 IU of vitamin E per kg.
[a]Contains 454,000 IU of vitamin A, 90,900 IU vitamin D, and 3,636, IU of vitamin E per kg.
[b]Contains 909,000 IU of vitamin A, 182,000 IU vitamin D, and 2,424, IU of vitamin E per kg.

Table 20. Nutrient composition of Control (CON) and an alpha-amylase enzyme preparation (AMA)[1].

Nutrient	-21 to 0 DIM	1 to 21 DIM	22 to 70 DIM[2]
DM, % as fed	50.3	48.9	48.7
CP, %	14.3	18.0	17.8
NE_L[3], Mcal/kg	1.62	1.68	1.68
ADF, %	23.7	21.2	19.2
NDF, %	36.8	32.3	31.7
NFC[4], %	37.6	37.5	36.5
Starch, %	23.0	25.0	29.0
Ether extract, %	4.1	5.3	6.6
Ash, %	7.3	7.0	7.4
Ca, %	1.02	1.19	1.12
P, %	0.38	0.42	0.48
Mg, %	0.51	0.50	0.46
K, %	1.25	1.39	1.36
Na, %	0.16	0.34	0.48
Cl, %	0.96	0.40	0.58
S, %	0.21	0.27	0.29
DCAD[5], meq/kg of DM	-13	223	212

[1]Values are based upon nutrient analyses of diets and actual mean DMI by treatment.
[2]All cows were fed the same diet from 22 to 70 DIM.
[3]Calculated using NRC (2001).
[4]NFC = 100 – (% NDF + % CP + % Ether extract + % Ash).
[5]DCAD as [(Na + K) – (Cl + S)] in milliequivalents per kg of DM.

Measurements and Collection of Samples

Diets were mixed and fed daily at 0600 h for ad libitum consumption as a TMR. Weighbacks were measured, recorded, and discarded prior to feeding each day and amounts fed were adjusted to ensure a 10% feed refusal. Samples of diets were collected weekly and frozen and composited monthly. The composited samples were sent to Dairyland Laboratories (Arcadia, WI) for analysis. Body weight and BCS (1 to 5 in 0.25 increments; Wildman et al., 1982) were recorded on two consecutive days, 4 h after feeding on d 21 prior to expected calving date, at parturition, and at 28 and 70 DIM. The same three individuals recorded BCS during the entire experiment. In addition, calf birth weights and calving difficulty scores were recorded (1 = no problem; 2 = slight problem; 3 = needed assistance; 4 = considerable force; 5 = cesarean). Cows were milked at 0600, 1400, and 2100 h, and milk yield was recorded. Milk samples were collected on d 7, 14, and 21 of lactation from all three milkings each day and were preserved using a tablet containing bronopol and natamycin (Broad Spectrum Microtabs II, D & F Control Systems, Inc., Dublin, CA). Incidences of milk fever, metritis, displaced abomasum, mastitis, and foot aliments were also recorded during the experiment.

Target day and actual day of blood sampling relative to calving were -21 and –20 (SD = 1.6), -14 and –13.4 (SD = 1.6), -7 and –7.3 (SD = 1.8), -2 and –2.8 (SD = 1.1), 2 and 1.8 (SD = 0.9), 7 and 7.0 (SD = 0.9), 14 and 14.1 (SD = 0.9), 21 and 20.9 (SD = 1.0), and 28 and 27.8 (SD = 0.7), respectively.

Approximately 4 h after feeding, blood was sampled from a coccygeal vessel into two evacuated tubes (Becton Dickinson and Co., Franklin Lakes, NJ) containing K-EDTA and sodium fluoride. Samples were immediately placed on ice and transported to the laboratory where they were centrifuged (500 × g) and plasma was harvested and stored at -20°C until further analysis. Ruminal fluid was collected at 21 (SD = 4.6) and 7 (SD = 4.1) d prior to expected calving date and 7 (SD = 1.0) d postpartum 4 h after feeding by applying vacuum pressure to an esophageal tube fitted with a suction strainer. To minimize saliva contamination, approximately 250 mL of rumen fluid were discarded prior to sample collection. A 10-mL sample was mixed with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen at -20ºC until analyzed for concentrations of VFA and NH_3-N. Liver tissue was collected by trocar and aspiration between the 11th and 12th rib (Smith et al., 1997) approximately 4 h after feeding on d 7 and 14 (actual d were 7.3 [SD = 1.0] and 14.1 [SD = 0.9]) of lactation. Samples were blotted to remove any residual blood, split into two equal aliquots, placed into cryovials, immediately submerged in liquid nitrogen, and transported to the laboratory where they were frozen at -80°C until analysis.

Laboratory Analysis

Samples of diets were dried at 55ºC in a forced-air oven and allowed to air-equilibrate before being ground to pass a 2-mm screen of a standard Wiley mill (Model 3; Arthur H. Thomas Co., Philadelphia, PA). Samples were composited by diet (prepartum, postpartum, and lactation TMR) and month and

analyzed for DM at 105ºC for 24 h, CP (AOAC, 1997) using a LECO-428 combustion analyzer (LECO Corp., St. Joseph, MI), NDF (Van Soest et al., 1991), ether extract (AOAC, 1997), minerals (AOAC, 1997; 985.01), and ADF (AOAC, 1997). Neutral detergent fiber and ADF were measured using the ANKOM A200 (ANKOM Technology Corp., Fairport, NY) filter bag technique. Determinations of ADF were according to AOAC (973.18 C, 1997) whereas NDF was according to Van Soest et al. (1991) with the addition of 4 mL of á-amylase and 20 g of sodium sulfite. Starch was measured as dextrose after treating samples with glucoamylase using a YSI 2700 SELECT Biochemistry Analyzer (Yellow Springs, OH; Holm et al., 1986). Minerals were quantified according to AOAC methods (985.01, 1997) using an inductively coupled plasma spectrometer (Thermo Garrell Ash, Franklin, MA). Samples were also analyzed for lactose according to AOAC (974.06, 1990) using an HPLC (Waters Corporation, Milford, MA) equipped with a refractive index detector and a 300 mm $\times$ 7.8 mm column (HPX-87H, Bio-Rad Laboratories, Hercules, CA) using a flow rate of 0.6 mL/min of 0.01 N H_2SO_4.

Milk compositional analysis was conducted by Heart of America DHI Laboratory (Manhattan, KS) according to approved procedures of AOAC (1990). Samples were composited by day and analyzed for protein, fat, lactose, MUN, and SCC and SNF was calculated. Milk true protein, fat, and lactose were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was

determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments).

Plasma samples were thawed and concentrations of glucose were determined using glucose oxidase (Sigma Kit #315, Sigma Diagnostics, St. Louis, MO) according to the procedures of Trinder (1969). Concentration of BHBA in plasma was determined (Pointe Scientific, Inc., Lincoln Park, MI) following the methods of Williamson et al. (1962) and plasma NEFA concentrations were determined using a colorimetric assay (NEFA-C Kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993). Insulin was quantified by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) with an intraassay CV of 1.6%. Samples of liver tissue were thawed and analyzed for total lipid and glycogen as described by Mills et al., (1986) and Derling et al., (1987), respectively.

Statistical Analysis

Effects of treatments on incidences of health disorders were not analyzed for statistical differences because of insufficient replication; however, all cows experiencing disorders were included in the data set. Two cows fed CON and two cows fed AMA gave birth to twins. Only one of these cows (fed CON) required a cesarean section. In addition, one of the cows fed CON was diagnosed with metritis at 10 DIM while the other was treated for a retained placenta. None of the cows fed AMA that twinned received any treatment for

either metritis or retained placenta. One cow fed CON was treated for mastitis at 65 DIM. Other than those aforementioned, no other health disorder events were noted.

The experiment was a randomized complete block design based upon expected calving date. Milk yield and DMI data were reduced to weekly means for statistical analysis. Milk production data collected on the day of calving was not included in the data set because of the inherent difficulties associated with data collected on the day of calving. Data were analyzed as repeated measures using PROC MIXED (Littell et al., 1996) of SAS software, version 8.1 (1999). For each variable, cow was subjected to four covariance structures: autoregressive order one, toeplitz, variance component, and compound symmetry. On average, the structure yielding the Akaike's Information Criterion closest to zero was variance component and autoregressive order one. For variables measured over time, the model included treatment, time (week or day depending on the variable), and 2-way interactions as fixed effects. The random effect was diet nested within cow. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom for *F*- tests. Covariates of initial BW and BCS, days on treatment, and previous 305 d mature equivalent milk yield were included for all data sets. Covariates and interactions were dropped from the model one at a time, starting with the least significant and continuing until all remaining variables were significant. Prepartum and postpartum data were analyzed separately.

Statistical significance was declared at $P < 0.05$, with trends noted at $P > 0.05$ to $P < 0.15$. Least square means and SEM are reported for all data. Significance of interactions are reported when significant. When significant effects among dietary treatments existed, mean separation was conducted by the PDIFF option in SAS.

Results

Ingredient and nutrient composition of diets are shown in Tables 19 and 20, respectively. Diets were formulated using CPM Dairy (version 3.0.5; University of Pennsylvania, Kennett Square, PA; Cornell University, Ithaca, NY; William H. Miner Agricultural Research Institute, Chazy, NY) and recommendations from NRC (2001). A BW of 680 kg and a targeted DMI of 12.2, 19.0, and 25.6 kg/d for pre-, postpartum, and lactation TMR (29 to 70 DIM), respectively, were used during formulations. The ratio of forage to concentrate was 70:30, 50:50, and 46:54 for pre-, postpartum, and lactation diets, respectively.

Initial cow characteristics are shown in Table 21. Average previous 305 d mature equivalent milk yield was 11,952 kg and was not different among treatments. Body weights and condition at 21 d prior to expected calving date were similar among all animals. Days on treatment and number of lactations prior to initiation of treatments were similar among treatments and averaged 42.1 ± 1.5 d and 1.9 ± 0.30, respectively. Average prepartum DMI was not affected by diet and averaged 12.2 ± 0.58 kg/d. Prepartum decrease in DMI (calculated

as average DMI during wk 3 prepartum – average DMI during wk 1 prepartum) was greater ($P < 0.03$) for cows fed AMA relative to those fed CON. Cows fed CON increased DMI 1.5 kg/d during this time period while those fed AMA decreased 1.8 kg/d (Figure 10).

Table 21. Previous 305-d mature equivalent milk, days on treatment, BW, BCS, calf birth weights, calving difficulty, and prepartum DMI of cows fed Control (CON) and an alpha-amylase enzyme preparation (AMA).

	Diet			
Item	CON	AMA	SEM	P[1]
n	12	12	-	-
Previous 305 ME[2], kg	12,254	11,650	424	0.32
Days on treatment	42.1	42.0	1.5	0.97
Lactation No.[3]	1.9	1.9	0.30	0.99
BW, kg				
d -21	765	754	12.6	0.54
d 0	692	688	13.6	0.84
d 28	645	643	12.9	0.92
d 70	667	673	14.3	0.77
BCS[4]				
d -21	3.23	3.20	0.07	0.80
d 0	3.21	3.06	0.07	0.15
d 28	2.91	2.95	0.08	0.70
d 70	2.97	3.02	0.08	0.65
Calf BW, kg	41.8	45.8	2.5	0.28
Calving difficulty[5]	1.6	1.7	0.35	0.90
DMI, kg/d	12.5	11.9	0.58	0.41
Prepartum DMI decrease[6], kg/d	-1.48	1.77	0.95	0.03

[1]Significance of *F*-test.
[2]Mature equivalent.
[3]Lactation number before calving.
[4]Wildman et al., 1982.
[5]Five point scale: 1= no assistance, 2= slight problem, 3= needed assistance, 4= considerable force, and 5= cesarean.
[6]Calculated as (average DMI during wk 3 prepartum – average DMI during wk 1 prepartum).

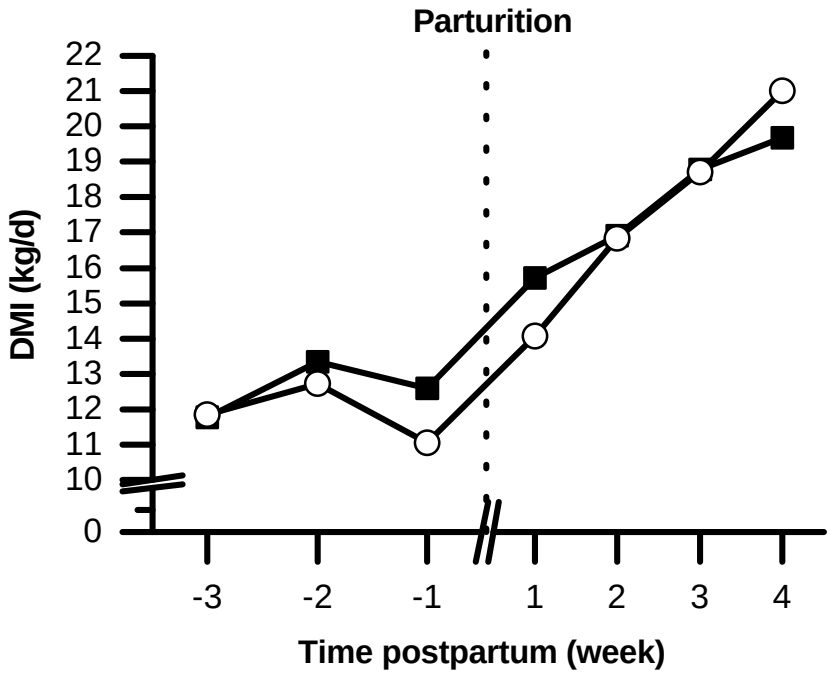

Figure 10. Dry matter intake (pooled SEM = 1.16) of cows fed Control (squares) and an alpha-amylase enzyme preparation (circles).

Postpartum performance data are presented in Table 22. Postpartum DMI was unaffected by the addition of the alpha-amylase enzyme preparation, averaging 17.8 ± 0.74 kg/d during the first 28 DIM. Milk, ECM (Orth, 1992), and production efficiencies (ECM/DMI) were similar among treatments during the first 28 DIM. With the exception of milk fat percent, milk composition was unaffected by dietary treatments. Milk from cows fed CON tended ($P < 0.13$) to have a greater milk fat percent (4.78 vs. 4.18) while fat yield (kg/d) was similar among treatments. Milk yield through 70 DIM averaged 43.9 ± 1.9 kg/d and was not affected by dietary treatments.

Effects of treatments on plasma metabolites are presented in Table 23 and plotted over time in Figures 12 and 13. Concentrations of glucose in plasma tended ($P < 0.07$) to be greater in cows fed AMA in all samples collected. This trend ($P < 0.08$) was also noted in samples collected postpartum, but not prepartum ($P < 0.56$). Concentrations of glucose in plasma of cows fed CON reached nadir at 14 DIM (64.6 mg/dL), which was decreased ($P < 0.04$) relative to cows fed AMA (71.0 mg/dL). Paradoxically, cows fed AMA reached nadir at 21 DIM (67.1 mg/dL). Although differences in plasma glucose existed, average pre- and postpartum concentrations of insulin in plasma were unaffected by dietary treatments.

Table 22. Postpartum DMI, milk yield, milk composition, and milk SCC of cows fed Control (CON) and an alpha-amylase enzyme preparation (AMA).

	Diet			
Item	CON	AMA	SEM	P^1
DMI, kg/d	17.8	17.7	0.74	0.92
Milk d 1-21, kg/d	38.5	38.1	1.97	0.91
ECM[2] d 1-21, kg/d	41.3	40.2	2.51	0.76
ECM/DMI	2.85	2.41	0.22	0.17
Milk composition, d 1-21				
Fat, %	4.78	4.18	0.26	0.13
Fat kg/d	1.69	1.52	0.11	0.32
True protein, %	3.06	3.12	0.11	0.72
True protein, kg/d	1.16	1.07	0.07	0.39
SNF, %	8.42	8.65	0.16	0.33
SNF, kg/d	3.07	3.18	0.14	0.62
Lactose, %	4.80	4.74	0.13	0.76
Lactose, kg/d	1.82	1.74	0.09	0.52
SCC x 10^3/mL	415	125	226	0.37
Urea N, mg/dL	11.45	10.51	0.80	0.42
Milk d 1-70, kg/d	43.6	44.2	1.90	0.83

[1]Significance of *F*-test.
[2]ECM = [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)]; (Orth, 1992).

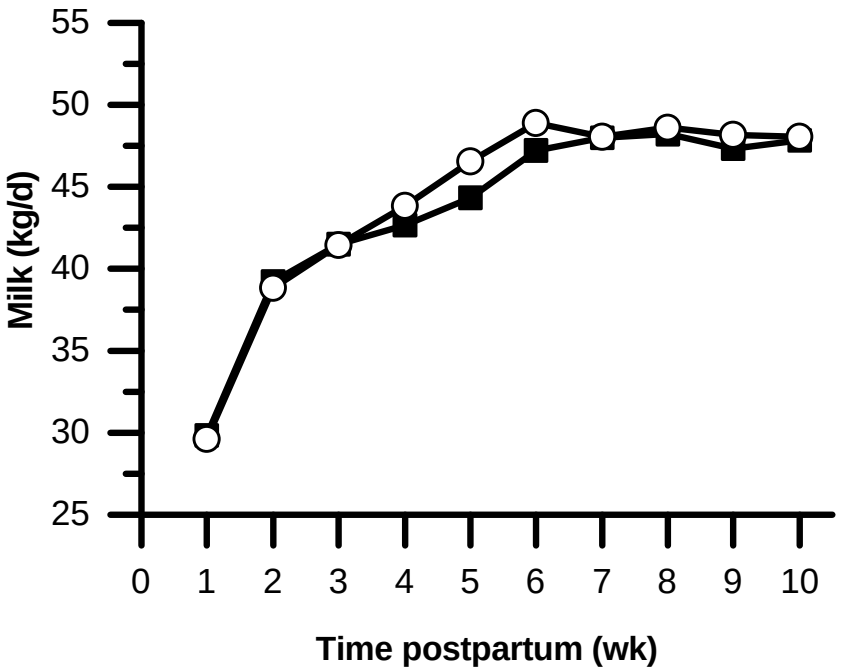

Figure 11. Milk yield (pooled SEM = 2.3) of cows fed Control (squares) and an alpha-amylase enzyme preparation (circles).

Table 23. Plasma glucose, insulin, NEFA, and BHBA and liver composition of cows fed Control (CON) and an alpha-amylase enzyme preparation (AMA).

	Diet			
Item	CON	AMA	SEM	P^1
Glucose, mg/dL				
All data	65.5	70.8	1.97	0.07
Prepartum	68.9	71.5	3.04	0.56
Postpartum	64.0	69.3	2.01	0.08
Insulin, pg/mL				
All data	258	246	24	0.71
Prepartum	366	329	56	0.64
Postpartum	171	177	17	0.81
NEFA, µEq/L				
All data	364	412	40	0.42
Prepartum	115	373	63	0.01
Postpartum	535	474	50	0.41
BHBA, mg/dL				
All data	6.58	8.91	1.08	0.11
Prepartum	3.91	6.28	0.46	< 0.01
Postpartum	9.32	10.31	1.43	0.64
Glucose + BHBA, mg/dL				
All data	73.2	78.5	2.25	0.11
Prepartum	72.3	78.1	3.05	0.19
Postpartum	73.4	79.4	2.71	0.13
Liver lipid, % wet weight	13.71	12.54	1.82	0.65
Liver glycogen, % wet weight	3.25	3.98	0.66	0.45

[1]Significance of *F*-test.

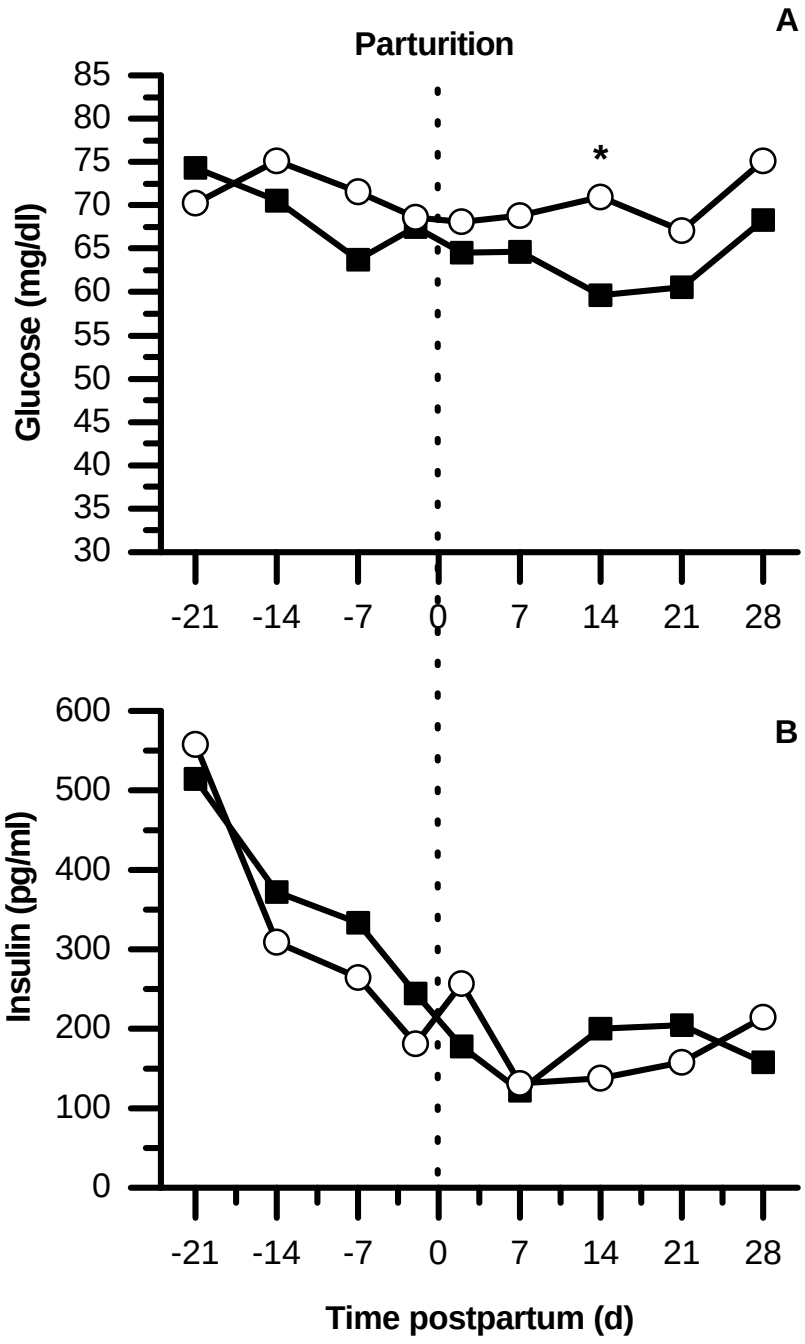

Figure 12. Plasma glucose (A) and insulin (B) concentrations (pooled SEM = 4.15 and 56.1, respectively) of cows fed Control (squares) and an alpha-amylase enzyme preparation (circles). Differences at individual time points are indicated by * ($P < 0.05$).

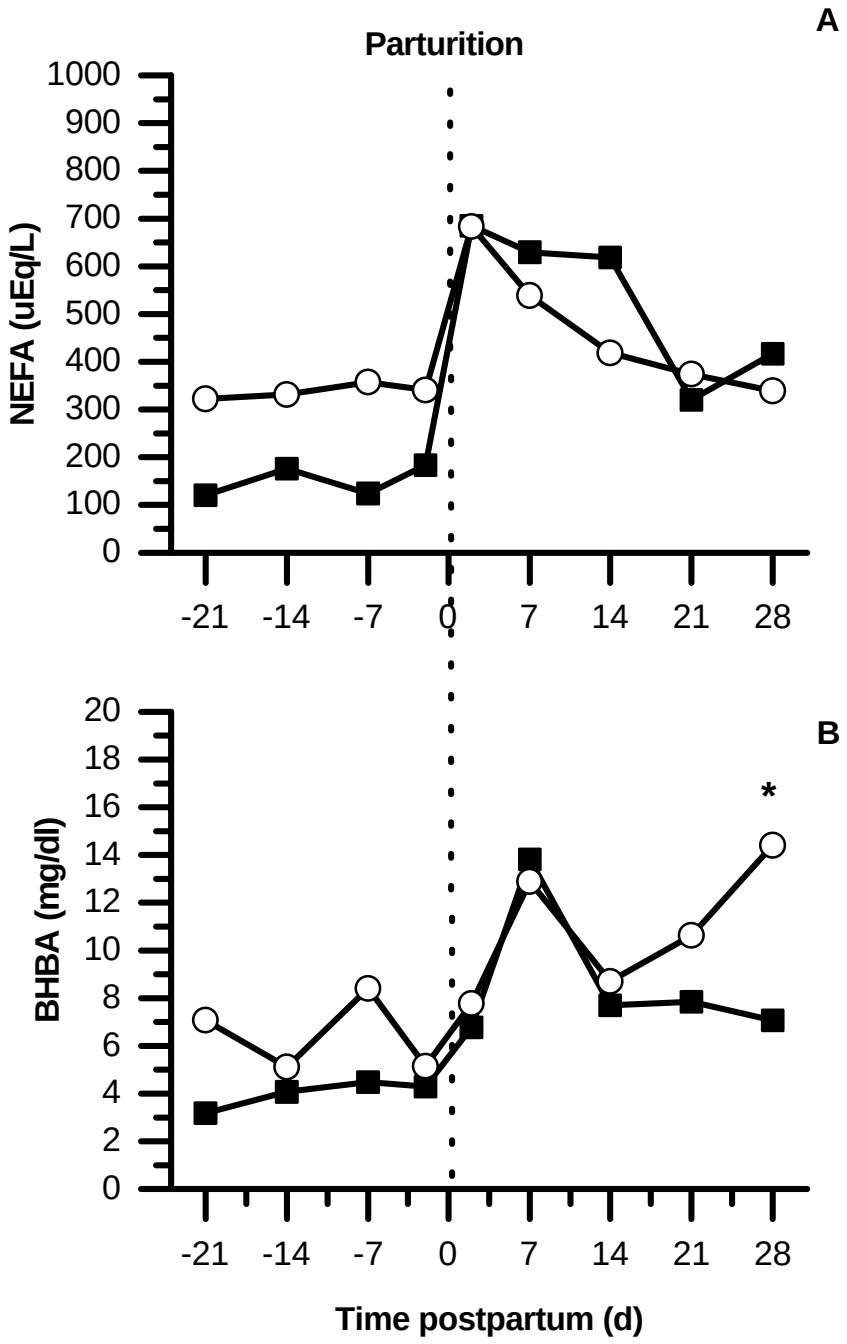

Figure 13. Plasma NEFA (A) and BHBA (B) concentrations (pooled SEM = 103 and 2.39, respectively) of cows fed Control (squares) and an alpha-amylase enzyme preparation (circles). Differences at individual time points are indicated by * ($P < 0.05$).

Prepartum, but not postpartum, concentrations of NEFA and BHBA in plasma were greater (3.2 and 1.6 times greater, respectively; $P < 0.01$) in cows fed AMA relative to those fed CON. Plasma NEFA concentrations peaked at 2 DIM for both treatments; however, concentrations in cows fed CON remained elevated through 14 DIM while concentrations in cows fed AMA had decreased to prepartum concentrations by this time (Figure 13 A). Feeding cows AMA tended ($P < 0.11$) to increase concentrations of BHBA in plasma at all time points compared to cows fed CON. Relative to cows fed CON, increases in plasma BHBA concentrations in cows fed AMA were particularly evident from 14 to 21 DIM, a time when concentrations of NEFA were decreasing for both treatments. The increased plasma BHBA in conjunction with the increased concentrations of glucose in cows fed AMA resulted in a tendency ($P < 0.11$) increased sum of glucose and BHBA in plasma for cows fed AMA compared to cows fed CON. Dietary treatments did not alter liver lipid or glycogen content, which averaged 13.1 and 3.62%, respectively (wet weight; Table 23).

Effects of diets on rumen NH_3-N concentrations and VFA proportions are presented in Table 24. Cows fed AMA tended ($P < 0.14$) to have greater molar proportions of rumen butyrate prepartum but not postpartum. Diet by day (21 vs. 7 d prepartum) interactions were noted for prepartum NH_3-N concentrations ($P < 0.05$), molar proportions of rumen propionate ($P < 0.04$), and the acetate to propionate ratio ($P < 0.04$). From 21 to 7 d prepartum NH_3-N concentrations and proportions of propionate decreased in cows fed AMA (4.19 to 2.44 mg/dL and

20.4 to 18.4%, respectively) relative to those fed CON (3.09 to 3.41 mg/dL and 19.4 to 19.7%, respectively). The ratio of ruminal acetate to propionate increased from 21 to 7 d prepartum in cows fed AMA (3.33 to 3.74) compared to cows fed CON (3.60 to 3.46). All other ruminal fermentation profiles were similar among treatments with the exception of proportions of valerate postpartum which tended to be decreased (1.16 vs. 1.46%; $P < 0.14$) in cows fed CON relative to those fed AMA.

Table 24. Ruminal NH_3-N and VFA proportions of cows fed Control (CON) and an alpha-amylase enzyme preparation (AMA).

	Diet			
Item	CON	AMA	SEM	P[1]
Prepartum				
NH_3-N, mg/dL[2]	3.25	3.31	0.36	0.90
Total VFA, m*M*	45.9	52.9	4.2	0.24
VFA, molar proportions				
Acetate	68.3	68.0	0.68	0.73
Propionate[2]	19.6	19.4	0.38	0.76
Isobutyrate	0.78	0.73	0.11	0.75
Butyrate	9.14	10.0	0.41	0.14
Isovalerate	1.13	0.91	0.11	0.18
Valerate	1.05	0.95	0.11	0.55
BCFA[2]	1.92	1.65	0.21	0.37
Acetate:propionate[3]	3.53	3.53	0.09	0.97
Postpartum				
NH_3-N, mg/dL	2.69	2.76	0.52	0.93
Total VFA, m*M*	40.8	46.2	4.11	0.36
VFA, molar proportions				
Acetate	63.7	62.4	1.25	0.48
Propionate	22.5	23.0	0.95	0.75
Isobutyrate	1.04	0.88	0.15	0.46
Butyrate	10.3	11.0	0.67	0.50
Isovalerate	1.24	1.30	0.16	0.82
Valerate	1.16	1.46	0.14	0.14
BCFA[2]	2.28	2.18	0.30	0.81
Acetate:propionate	2.99	2.75	0.22	0.46

[1]Significance of *F*-test.
[2]Branched chain fatty acids.
[3]Diet by day effect ($P < 0.05$).

Discussion

The inordinate amount of physiological and metabolic challenges encompassing the transition period in dairy cattle has been described in numerous review papers. The transition period in dairy cattle is typically plagued by an enormous energy deficit as lactation is initiated. According to Beauchemin et al. (2003), the amount of response observed in ruminants fed exogenous enzymes is maximal during times of compromised fiber digestion and energy-limiting situations, both of which often accompany the transition period of dairy cattle. This forms the basic premise behind the present investigation in characterizing the effects of an alpha-amylase preparation in diets of transition dairy cows.

Decreases in prepartum feed intake should be avoided to minimize the likelihood of elevated plasma NEFA and liver triglycerides (Bertics et al., 1992). Based on this concept, the decrease in DMI observed from wk 3 to wk 1 prepartum in cows fed AMA should have negatively affected metabolic indicators and lactation performance. The absence of any negative effects on NEFA and liver lipids in cows fed AMA suggest the decrease in DMI may have resulted from the effects of the alpha-amylase enzyme preparation on nutrient digestion and absorption. The alpha-amylase enzyme preparation fed in the present study is known to increase starch digestibility (Tricarico et al., 2002) and therefore could

have provided an improvement in nutrient supply in cows fed AMA. This is further supported by Allen (2000) who indicated that the site of starch digestion determines the form (glucose vs. propionate) of metabolic fuel absorbed, which affects DMI as propionate is more hypophagic than glucose. Others researchers have fed amylase-containing enzyme preparations and observed only small increases (< 1%; Burroughs et al., 1960) or no effect (Rust et al., 1963; Tricarico et al., 2002) on DMI. Therefore, it is likely that improvements in metabolic indicators, such as plasma glucose, in the present study are a result of the effect of the added enzyme on rumen fermentation.

Tricarico et al. (2002) observed increases in yields of milk, milk fat, and milk protein in cows supplemented with 12 g/d of an alpha-amylase enzyme preparation relative to an unsupplemented control group. Field trials conducted by Harrison and Tricarico (2004) also found supplementing cows with 12 g/d of an amylase preparation to increase the yields of milk and milk protein, but not milk fat. The absence of any effect on milk composition in the study under discussion is contradictory to Tricarico et al. (2002) and Harrison and Tricarico (2004), especially considering the effects of the enzyme preparation on rumen fermentation profiles. Alpha-amylase (Tricarico et al., 2002) or amylase-containing (Hristov et al., 2000) supplements have increased the proportion of rumen butyrate, giving rise to precursors necessary for fatty acid synthesis by the mammary gland. We observed a tendency for an increase in the molar proportion of rumen butyrate prepartum, but not postpartum. The lack of a

postpartum rumen butyrate response might be a result of the dietary change from pre- to postpartum. Alternatively, differences between our study and the increases in milk fat reported by Tricarico et al. (2002) and Harrison and Tricarico (2004) could have been due to differences in stage of lactation.

With the exception of plasma glucose, changes in plasma metabolites observed in the present study are in general agreement with the findings of Tricarico et al. (2002). Cows fed AMA tended to have greater concentrations of glucose in plasma while Tricarico et al. (2002) found incremental increases (0, 12, 24, or 36 g/d) in the amount of an alpha-amylase enzyme preparation to linearly ($P < 0.01$) decrease plasma glucose and a trend ($P < 0.08$) for a decrease in proportions of ruminal propionate. Differences between our study and that of Tricarico et al. (2002) can be ascribed to differences in experimental diets. Diets used in the current study contained half as much haylage and twice as much corn silage as diets fed by Tricarico et al. (2002). These differences resulted in a greater calculated NE_L (1.72 vs. 1.60 Mcal/kg) for cows fed AMA relative to the diet fed by Tricarico et al. (2002). Combining the differences in dietary structure with the fact that our cows were earlier in lactation and consuming nearly 5 kg/d less DM likely resulted in differences in rumen fermentation profiles and therefore differences in plasma metabolites. The postpartum increase in plasma BHBA without an increase in ruminal butyrate in cows fed AMA might be a result of the limited number of postpartum samples of rumen fluid.

Others have found cows fed supplements containing alpha-amylase enzyme preparations to increase plasma BHBA (Tricarico et al., 2002). Plasma BHBA represents an energy source for body tissues, especially for skeletal and cardiac muscle tissue and as a precursor for fatty acid synthesis in adipose tissue and the mammary gland (Church, 1988). Increases in plasma BHBA and glucose, both of which are considered circulating energy metabolites, provide amylase-supplemented cows with a metabolic advantage over unsupplemented controls. Increased plasma BHBA in amylase-supplemented diets is largely due to the increased proportions of rumen butyrate typically observed in cows fed amylase-containing supplements (Hristov et al., 2000; Tricarico et al., 2002) because of the extensive conversion of butyrate carbon to ketone bodies (mainly BHBA and acetoacetate) prior to release into portal circulation (Weigand et al., 1975). Without a doubt, the greater plasma NEFA observed in cows fed AMA prepartum also contributed to the greater plasma BHBA concentrations. According to data from Nielen et al. (1994), plasma BHBA concentrations greater than 12.5 mg/dL indicate cows with subclinical ketosis. The greatest concentration of BHBA (14.4 mg/dL) was observed in cows fed AMA at 28 DIM, one week after all cows began receiving a common TMR. During this time period, milk yield continued to increase in cows fed AMA and continued to increase through 7 wk of lactation (Figure 11). These data suggest cows fed AMA may have received additional benefit from consuming the alpha-amylase enzyme preparation beyond 21 DIM.

The extensive metabolism of butyrate by the rumen epithelial tissue plays a significant role in rumen epithelial growth in the young calf (Sakata and Tamate, 1978). Butyrate-stimulating feed ingredients, such as lactose, have also increased rumen papillation (Allen and Xu, 1998). Allen and Xu (1998) demonstrated increased growth rates for length and width and overall greater surface area of ruminal papillae in nonpregnant, nonlactating dairy cows fed diets containing 43% lactose (DM basis) compared with 43% corn. It is proposed that the effects of feeding an alpha-amylase enzyme preparation are at least partially mediated through morphological changes in the rumen epithelium. This has been documented in calves but not in mature animals. Gehamn et al. (2003) found that feeding amylase at 6 g/d to calves from birth to 5 wks of age increased rumen papillae length and width. Dirksen et al. (1985) observed proliferative and reductive processes in peripartum dairy cows and suggested these adaptive changes function to stabilize the pH of the rumen and provide the body with energy, two factors critical to the health and performance of the transition dairy cow. Future investigations will be necessary to elucidate effects of feeding amylase-based enzyme preparations on ruminal morphology and fermentation.

Conclusions

Supplementing diets for transition dairy cows with an alpha-amylase enzyme preparation resulted in differences in key metabolic indicators while effects on lactation performance were minimal. The magnitude of DMI

depression from 3 to 1 wk prior to parturition was greater in cows fed AMA relative to cows fed CON; however, cows performed similarly during the postpartum phase of the experiment. Plasma glucose profiles suggest supplementing cows with an alpha-amylase enzyme preparation is beneficial, especially considering the glucose demand at the initiation of lactation. Although concentrations of plasma BHBA were greater in cows fed AMA compared to cows fed CON, these ketone bodies were apparently used as an additional source of energy for milk synthesis. The sum of plasma BHBA and glucose, both of which are considered circulating energy metabolites, place amylase-supplemented cows at an energetic advantage over unsupplemented controls.

CHAPTER 6. Experiment 5. Effects of Feeding Propionate and Calcium Salts of Long-Chain Fatty Acids on Transition Dairy Cow Performance

J.M. DeFrain
September, 2004

Abstract

Multiparous Holstein cows (n = 40) were used in a randomized complete block design to determine the effects of feeding Ca and Na salts (1:1, wt/wt) of propionate and Ca salts of long-chain fatty acids (LCFA) on transition cow performance. All cows were fed the same basal diet once daily for ad libitum intake. Treatments (g/d) were 320 corn starch (C), 120 propionate (P), 120 propionate and 93 LCFA (PF1), and 178 propionate and 154 LCFA (PF2). Treatments were hand-mixed into the upper 1/3 of the TMR from 2 wk pre- through 3 wk postpartum. Intakes were recorded from 21 d pre- through 21 d postpartum. Energy density and CP were 1.54 and 1.65 Mcal/kg and 14.4 and 18.8% for pre- and postpartum diets, respectively. All cows received a common diet from 22 to 70 DIM. Milk composition was analyzed on d 7, 14, and 21. Blood was sampled at 14, 7, and 2 d prepartum and 2, 7, 14, and 21 DIM. Pre- and postpartal DMI averaged 11.9 and 16.4 kg/d, respectively, and did not differ among treatments. A diet by week interaction for postpartal DMI was observed as cows fed PF2 consumed 2 kg/d less DM during wk 2 relative to other treatments. Milk yields from 22 to 70 DIM were 48.8, 48.5, 47.8, and 51.3 kg/d for C, P, PF1, and PF2, respectively, and were not significantly affected by treatments. Milk true protein (3.32 vs. 3.16%) was increased and MUN (12.5 vs.

14.4 mg/dL) was decreased for C relative to other treatments. Milk fat yield from cows fed P tended to be greater than those fed PF1 (1.58 vs. 1.29 kg/d). Plasma glucose, insulin, and BHBA were not affected by treatments. The PF2 treatment tended to decrease NEFA in plasma relative to PF1 over all times measured (492 and 670 µEq/L) and significantly decreased plasma NEFA relative to PF1 postpartum (623 and 875 µEq/L). Relative to PF1, feeding propionate and LCFA at the greater level in this experiment improved energy balance postpartum as evidenced by decreased concentrations of plasma NEFA.

Key words: propionate, fat, transition dairy cow

Introduction

The transition from late gestation to early lactation is regarded as one of the most challenging elements of the production cycle. This transition imposes a plethora of profound physiologic and metabolic changes. These changes often disrupt the homeostatic mechanisms of the cow because they occur in a matter of days, perhaps hours, as the liver adapts from a minimal glucose demand to an overwhelming demand for glucose. The 3 wk before and after calving is the most important 6 wks of the production cycle as it imparts the level of success realized during the subsequent lactation.

Ruminal propionate is the single most important substrate for gluconeogenesis (Drackley et al., 2001); however, depressed feed intake at parturition is likely limiting ruminal propionate supply to the liver. Estimates by Seal and Reynolds (1993) indicate propionate supplies 32 to 73% of glucose demands. Feeding propionate has increased blood glucose (Schultz, 1958; Schmidt and Schultz, 1958) and decreased blood BHBA (Schultz, 1958; Schmidt and Schultz, 1958; Goff et al., 1996). Both drenching (Grummer et al., 1994) and feeding propylene glycol (Christensen et al., 1997) have decreased NEFA and increased insulin in plasma and increased ruminal propionate concentrations, all of which are beneficial to combating the extent and duration of ketosis and fatty liver. Effects of propylene glycol are partially mediated through the observed increases in ruminal propionate (Grummer et al., 1994; Christensen et al., 1997), providing reasons to feed propionate during the transition period.

Reasons for feeding fat during the transition period are several. Kronfeld (1982) introduced the glucogenic to lipogenic ratio concept, suggesting the mammary gland may be deficient in precursors for triglyceride synthesis, particularly at a time when milk fat content is the greatest. In a review of lipid-related metabolic disorders in peripartum dairy cows, Grummer (1993) hypothesized dietary fat could decrease fatty acid mobilization, sparing mammary glucose oxidation by decreasing amounts of NADPH necessary for mammary fatty acid synthesis. Reported effects of feeding either tallow (960

g/d; Skaar et al., 1989) or prilled fat (350 g/d; Salfer et al., 1995) during the transition period have been few. Fat escaping rumen fermentation leaves the small intestine as chylomicrons to be absorbed by extrahepatic tissues and does not contribute to hepatic lipidosis because of the absence of lipoprotein lipase in bovine liver (Emery et al., 1992).

Of particular interest in our experiment was the role of fatty acids on the carbohydrate status of peripartum dairy cows. Previous in vitro and in vivo work demonstrated the synergism of fatty acids and propionate metabolism during gluconeogenesis. Ferre et al. (1978) speculated the hepatic oxidation of fatty acids could provide the ATP, acetyl CoA, and NADH cofactors necessary for maximal gluconeogenic capacity. Following a 16 h fast, they found circulating glucose concentrations of neonatal rats injected with glucogenic substrates to increase twofold (from 1.1 to 2.6 m*M*) whereas a similar injection into rats previously fed triglycerides increased blood glucose fourfold (from 1.1 to 4.7 m*M*; Ferre et al., 1978). Based on in vitro work with sheep hepatocytes, Lomax et al. (1986) proposed that reducing equivalents from fatty acid metabolism affect the transport of carbon atoms across the mitochondrial membrane. This is of particular importance when shuttling malate carbon during gluconeogenesis in the transition dairy cow. Blood glucose and insulin increased and NEFA decreased in cows fed glucose precursors in combination with Ca salts of fatty acids relative to cows fed the same amount and type of lipids in combination with barley during the transition period (Patton et al., 2004). What is unclear from

Patton et al. (2004) is the impact of feeding glucose precursors alone compared with glucose precursors plus fat and whether or not dosage of lipids and glucose precursors could elicit a greater response. Therefore, our objectives were to use blood metabolites and lactation performance data to determine 1) the effect of feeding propionate with and without Ca salts of fatty acids and 2) whether the synergistic effects of propionate and Ca salts of fatty acids were dose dependent. Our hypothesis was that feeding propionate in combination with fatty acids would increase blood glucose, decrease blood NEFA, and improve lactation performance greater than feeding propionate alone.

Materials and Methods

Cows and Sampling

The experiment was conducted from January through October of 2003 at the South Dakota State University Dairy Teaching and Research Facility (Brookings, SD). Animal care and use was according to a protocol approved by the South Dakota State University Institutional Animal Care and Use Committee. Forty multiparous Holstein cows were blocked by parity and expected calving date and arranged into a randomized block design 28 d prior to expected calving until 70 DIM. The treatment groups consisted of four topdresses which were hand-mixed into the upper one-third of the prefresh (starting 14 d prior to expected calving) and postfresh (calving through 21 DIM) TMR. The topdresses were (kg/d, DM basis): 0.32 corn starch (C), 0.73 soyhull-propionate pellet + 0.21 corn starch (P), 0.73 soyhull-propionate pellet + 0.13 Ca soaps of long chain

fatty acids (LCFA, Megalac, Church and Dwight Co., Princeton, NJ; PF1), and 1.08 soyhull-propionate pellet + 0.19 LCFA (PF2). The corn starch (Cargill, Inc., Minneapolis, MN) was considered a traditional glucogenic agent as it is primarily fermented to propionate in the rumen. The amounts of propionate fed were determined from the earlier work of Schultz (1958) and Schmidt and Schultz (1958) who fed 114 and 228 g/d Na propionate, respectively, whereas amounts of LCFA fed were based upon the recent work of Patton et al. (2004). The PF2 treatment was formulated to contain approximately 50% more propionate and LCFA relative to PF1. Therefore, treatments were formulated to provide 120 g/d of propionic acid for P and PF1 and 178 g/d for PF2 and LCFA at 93 g/d for PF1 and 154 g/d for PF2. A treatment of fat alone was not included in our experimental design because effects of feeding fat alone during the transition period have been few (Skaar et al., 1989; Salfer et al., 1995; Patton et al., 2004). All cows received a common diet from 22 to 70 DIM. The ingredient and nutrient composition of diets are shown in Tables 1, 2, and 3.

The soyhull-propionate pellet fed to cows receiving P, PF1, and PF2 was manufactured at the South Dakota State University feed mill using a 4.76 × 31.75 mm (hole diameter × effective die thickness) die. The propionate used was a 1:1 (wt/wt) mixture of Na (Calprona NA, Verdugt, The Netherlands) and Ca (Calprona C/CA, Verdugt) salts containing 76 and 78% propionate, respectively. The mash of ingredients was not conditioned with steam prior to pelleting. Soyhulls were used because of their ease of pelleting. The addition of porcine

choice white grease was necessary to reduce die friction and optimize pellet quality. In addition, pelleting the soyhulls and propionate provided a homogeneous mixture to ensure consistent delivery of propionate to assigned treatments. The final pelleted product contained 21.1% salts of propionate, 69.5% soyhulls, 3.2% porcine choice white grease, 3.1% Na, and 3.1% Ca on a DM basis. Nutrient analyses of the soyhull-propionate pellet were: 85.9% DM, 8.8% CP, 32.0% ADF, 42.8% NDF, 3.9% ether extract, 0.11% P, 1.17% K, and 0.17% Mg on a DM basis.

Table 25. Ingredient composition of basal diets[1].

Ingredient	-21 to 0 DIM	1 to 21 DIM	22 to 70 DIM[2]
	----------% of diet DM----------		
Brome grass hay	17.3	-	-
Alfalfa hay	17.5	15.1	12.7
Alfalfa haylage	-	11.3	11.8
Corn silage, processed	37.0	25.3	26.5
Whole cottonseed	3.3	7.9	6.6
Wet distillers grains	-	6.9	6.9
Dried distillers grains	1.7	-	-
Corn, high moisture	-	12.1	16.4
Corn grain, ground	12.7	8.5	7.1
Soybean meal, 44%		5.8	4.8
SoyChlor 16-7[3]	7.7	-	-
SoyPlus[3]	-	3.04	2.53
Megalac-R[4]	-	-	1.25
Limestone	1.39	1.21	1.07
Sodium bicarbonate	-	0.75	0.63
Fish meal, menhaden	-	0.54	0.42
Pork meat & bone meal	-	0.50	0.42
Yeast culture[5]	0.40	0.24	0.20
Magnesium oxide	-	0.22	0.18
Salt	-	0.22	0.18
Vitamin A, D, & E premix	0.65[a]	0.28[b]	0.23[b]
Urea	0.18	-	-
4-Plex[6]	0.07	0.06	0.05
Vitamin E premix[7]	0.11	0.04	0.04

[1]Treatments applied as topdressed ingredients (fed from 14 d prepartum to 21 DIM) were (kg/d, DM basis):

Control (C): 0.32 corn starch

Propionate (P): 0.73 soyhull-propionate pellet + 0.21 corn starch

Propionate + Fat 1 (PF1): 0.73 soyhull-propionate pellet + 0.13 LCFA

Propionate + Fat 2 (PF2): 1.08 soyhull-propionate pellet + 0.19 LCFA

[2]All cows were fed the same diet from 22 to 70 DIM.

[3]West Central Soy, Ralston, IA.

[4]Church and Dwight Co., Princeton, NJ.

[5]Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA.

[6]4-Plex, Zinpro Corp., Eden Prairie, MN.

[7]Contains 44,092 IU of vitamin E per kg.

[a]Contains 454,000 IU of vitamin A, 90,900 IU vitamin D, and 3,636, IU of vitamin E per kg.

[b]Contains 909,000 IU of vitamin A, 182,000 IU vitamin D, and 2,424, IU of vitamin E per kg.

Table 26. Nutrient composition[1] (DM basis) of Control (CON), Propionate (P), Propionate + fat 1 (PF1) and Propionate + fat 2 (PF2).

	-14 to 0 DIM				1 to 21 DIM				22 to 70
Nutrient	CON	P	PF1	PF2	CON	P	PF1	PF2	DIM[2]
DM, % as fed	49.7	49.7	49.7	49.9	52.8	52.8	52.8	52.9	49.0
CP, %	14.7	14.4	14.3	14.0	19.4	18.8	18.7	18.3	18.1
NE_L[3], Mcal/kg	1.53	1.51	1.54	1.56	1.64	1.63	1.65	1.66	1.64
ADF, %	26.4	27.2	27.2	27.2	21.2	21.8	21.8	22.0	20.6
NDF, %	38.4	39.3	39.2	39.1	31.5	32.2	32.1	32.3	30.8
NFC[4], %	34.5	33.9	33.2	33.0	36.5	36.5	36.0	35.8	37.6
Ether extract, %	4.1	4.1	5.0	5.4	4.6	4.5	5.2	5.5	6.3
Ash, %	8.3	8.3	8.3	8.5	8.0	8.0	8.0	8.1	7.2
Ca, %	1.40	1.58	1.68	1.78	1.35	1.45	1.53	1.61	1.19
P, %	0.39	0.37	0.37	0.35	0.47	0.45	0.45	0.43	0.42
Mg, %	0.49	0.47	0.47	0.46	0.49	0.47	0.47	0.46	0.44
K, %	1.25	1.26	1.26	1.25	1.46	1.44	1.44	1.43	1.24
Na, %	0.16	0.37	0.36	0.47	0.35	0.50	0.50	0.57	0.31
Cl, %	1.08	1.02	1.02	0.98	0.45	0.42	0.42	0.41	0.37
S, %	0.24	0.23	0.23	0.22	0.26	0.25	0.25	0.24	0.22
DCAD[5]	-62	55	49	108	238	313	310	347	210

[1]Includes topdressed ingredients. Values are based upon nutrient analyses of basal diets and topdressed ingredients and actual mean DMI by treatment.
[2]All cows were fed the same diet from 22 to 70 DIM.
[3]Calculated using NRC (2001).
[4]NFC = 100 – (% NDF + % CP + % Ether extract + % Ash).
[5]DCAD as [(Na + K) – (Cl + S)] in milliequivalents per kg of DM.

Cows were housed on a wheat-straw bedded pack prepartum and in a free-stall barn during the postpartal phase of the experiment. Diets were mixed and fed daily at 0600 h for ad libitum consumption using the Calan Broadbent feeding system (American Calan, Inc., Northwood, NH). All diets were offered as a TMR. Weighbacks were measured, recorded, and discarded prior to feeding each day and amounts fed were adjusted to ensure a 10% feed refusal. Samples of TMR were collected weekly for analysis. Body weights and BCS (1 to 5 in 0.25 increments; Wildman et al., 1982) were recorded on two consecutive days, 4 h after feeding on d 21 prior to expected calving, at parturition, and at 21 DIM. The same three individuals recorded BCS during the entire experiment. In addition, calf birth weights and calving difficulty scores were recorded (1 = no problem; 2 = slight problem; 3 = needed assistance; 4 = considerable force; 5 = cesarean). Cows were milked at 0600, 1400, and 2100 h, and milk yield was recorded. Milk samples were collected on d 7, 14, and 21 of lactation from all three milkings each day. Samples were composited by day and analyzed for protein, fat, lactose, MUN, and SCC and SNF was calculated. Incidences of milk fever, metritis, displaced abomasum, mastitis, and foot aliments were also recorded during the experiment.

Target day and actual day of blood sampling relative to calving were -14 and -13.7 (SD = 2.1), -7 and -7.1 (SD = 1.6), -2 and -2.4 (SD = 1.0), 2 and 2.0 (SD = 0.6), 7 and 7.1 (SD = 1.0), 14 and 14.0 (SD = 1.6), and 21 and 21.0 (SD = 1.0), respectively. Approximately 4 h after feeding, blood was sampled from a

coccygeal vessel into two evacuated tubes (Becton Dickinson and Co., Franklin Lakes, NJ) containing K-EDTA and sodium fluoride. Samples were immediately placed on ice and transported to the laboratory where they were centrifuged (500 × g) and plasma was harvested and stored at -20°C until further analysis.

Laboratory Analysis

Samples of diets were dried at 55ºC in a forced-air oven and allowed to air-equilibrate before being ground to pass a 2-mm screen of a standard Wiley mill (Model 3; Arthur H. Thomas Co., Philadelphia, PA). Samples were composited by diet (prepartum and postpartum) and month and analyzed for DM at 105ºC for 24 h, CP (AOAC, 1997) using a LECO-428 combustion analyzer (LECO Corp., St. Joseph, MI), NDF (Van Soest et al., 1991), ether extract (AOAC, 1997), minerals (AOAC, 1997; 985.01), and ADF and lignin (AOAC, 1997). Neutral detergent fiber and ADF were measured using the ANKOM A200 (ANKOM Technology Corp., Fairport, NY) filter bag technique. Determinations of ADF were according to AOAC (973.18 C, 1997) whereas NDF was according to Van Soest et al. (1991) with the addition of 4 mL of alpha-amylase and 20 g of sodium sulfite. Starch was measured as dextrose after treating samples with glucoamylase using a YSI 2700 SELECT Biochemistry Analyzer (Yellow Springs, OH; Holm et al., 1986). Minerals were quantified according to AOAC methods (985.01, 1997) using an inductively coupled plasma spectrometer (Thermo Garrell Ash, Franklin, MA).

Milk compositional analysis was conducted by Heart of America DHI Laboratory (Manhattan, KS) according to approved procedures of AOAC (1990). Milk true protein, fat, and lactose were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments).

Plasma samples were thawed and concentrations of glucose were determined using glucose oxidase (Sigma Kit #315, Sigma Diagnostics, St. Louis, MO) according to the procedures of Trinder (1969). Concentration of BHBA in plasma was determined (Pointe Scientific, Inc., Lincoln Park, MI) following the methods of Williamson et al. (1962) and plasma NEFA concentrations were determined using a colorimetric assay (NEFA-C Kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993). Insulin was quantified by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) with an intraassay CV of 1.5%.

Statistical Analysis

Two cows suffered displaced abomasums: one fed C at 10 DIM and one fed PF1 at 15 DIM. Both cows were surgically treated and continued on trial. Dry matter intake collected during their recovery period (1 wk) was excluded from the data set. Two cows also suffered from foot ailments which subsequently

affected their DMI. Data from one of these cows (fed PF1) was already excluded due to her abomasal displacement. Feed intake data from 15 to 21 DIM were excluded from the second cow (fed C). Among the cows remaining, 6 were treated for metritis, 2 for ketosis, and 2 for mastitis. Effects of treatments on incidences of health disorders were not analyzed for statistical differences because of insufficient replication; however, all cows experiencing disorders were included in the data set, excluding those aforementioned.

The experiment was a randomized complete block design based upon expected calving date. Milk yield and DMI data were reduced to weekly means for statistical analysis. Milk production data collected on the day of calving was not included in the data set because of the inherent difficulties associated with data collected on the day of calving. Data were analyzed as repeated measures using PROC MIXED (Littell et al., 1996) of SAS software, version 8.1 (1999). For each variable, cow was subjected to four covariance structures: autoregressive order one, toeplitz, variance component, and compound symmetry. The structure yielding the largest Akaike's information criteria was used. For variables measured over time, the model included treatment, time (week or day depending on the variable), and 2-way interactions as fixed effects. The random effect was diet nested within cow. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom for *F*- tests. Covariates of initial BW and BCS, days on treatment, and previous 305 d mature equivalent milk yield were included for all data sets. Covariates and interactions

were dropped from the model one at time, starting with the least significant and continuing until all remaining variables were significant. Prepartal and postpartal data were analyzed separately. Preplanned contrasts were C vs. P, PF1, and PF2, P vs. PF1, and PF1 vs. PF2. The purpose of comparing C versus all other dietary treatments was to test the effect of glucogenic supplementation, regardless of type or amount. The P vs. PF1 contrast was used to determine the effect of feeding propionate with or without supplemental fat. The contrast of PF1 vs. PF2 was useful in determining if the combination of propionate and fat was dependent upon amount fed. Least square means and standard error of means are reported. Treatment effects at specific days were determined by use of the PDIFF option within the LSMEANS statement. Statistical significance was declared at $P < 0.05$, with trends noted at $P > 0.05$ to $P \leq 0.15$.

Results

Ingredient and nutrient composition of diets are shown in Tables 25 and 26, respectively. Crude protein decreased and ether extract increased with the addition of propionate and LCFA to P, PF1, and PF2. As expected, the addition of Na and Ca salts of propionate increased diet Ca and Na both pre- and postpartum. The greatest concentration of ether extract (PF2 at 5.5% of diet DM) was formulated and analyzed to be less than the maximal dietary level of 6 to 7% of diet DM recommended by NRC (2001). The LCFA source was assayed and found to contain 94.6% DM, 0.82% CP, 1.7% ADF, 3.7% NDF, 81.6% fat via acid-hydrolysis, 9.66% Ca, 0.01% P, 0.13% K, and 0.09% Mg on a DM basis.

Diets were formulated according to recommendations by NRC (2001) using a BW of 650 kg and targeted DMI of 12.9, 21.4, and 25.6 kg/d for pre-, postpartum, and high group (22 to 70 DIM) phases of the experiment.

Cows used in this study are described in Table 27. Average previous 305 d mature equivalent milk yield was 11,805 kg for all treatments. Previous 305 d mature equivalent yield was greater ($P < 0.01$) for cows fed PF1 relative to cows fed P. Therefore, when significant, previous 305 d milk yield was included as a covariate during statistical analysis of data. Days on treatment were similar among treatments and averaged 35.7 ± 1.4 d. Calf birth weights were greater ($P < 0.02$) for cows fed PF1 relative to those fed PF2; however, calving difficulty scores were similar among treatments. Average prepartum DMI was not affected by diet. With the exception of cows fed PF2, DMI decreased for all treatments from wk 2 to wk 1 prepartum (Figure 14).

Average DMI (Table 28) during the first 21 DIM was 16.4 kg/d and did not differ among treatments; however, a diet by week interaction ($P < 0.02$) for postpartal DMI was observed as cows fed PF2 consumed 2 kg/d less DM during wk 2 relative to other treatments (Figure 14). Milk (Figure 15) and ECM (Orth, 1992) yields were unaffected by dietary treatments. Actual 305 d milk yield (Table 28) averaged 12,931 kg and was not different among treatments. Feed conversion (ECM/DMI) tended ($P < 0.13$) to be greater for cows fed P compared to those fed PF1. Cows fed P tended ($P < 0.10$) to yield more milk fat than did those fed PF1 (1.58 vs. 1.29 kg/d, respectively). Percent true protein in milk was

greater for cows fed C relative to all other treatments ($P < 0.04$, 3.31 vs. 3.16%, respectively). A similar effect of treatments was observed for milk solids-not-fat, as percentages were greater for cows fed C than the average of those fed P, PF1, and PF2 ($P \leq 0.05$, 9.01 vs. 8.82%, respectively). Feeding C decreased MUN compared with cows fed other treatments ($P < 0.04$, 12.5 vs. 14.4 mg/dL, respectively). In addition, MUN was greater for cows fed PF2 relative to cows fed PF1 ($P < 0.02$, 16.4 vs. 13.8 mg/dL, respectively). Although BW were similar 3 wk prior to parturition (Table 27), BW tended to be greater for cows fed C at 21 DIM relative to all other treatments ($P < 0.15$, 678 vs. 654 kg, respectively).

Table 27. Previous 305-d ME milk, days on treatment, BW, BCS, calf birth weights, calving difficulty, and prepartum DMI of cows fed Control (CON), Propionate (P), Propionate + fat 1 (PF1) and Propionate + fat 2 (PF2).

	Diet					Contrast[1]		
Item	CON	P	PF1	PF2	SEM	1	2	3
n	10	10	10	10	-	-	-	-
Previous 305 ME, kg	11,732	10,975	12,452	12,063	396	0.83	0.01	0.49
Days on treatment	36.8	35.8	34.3	36.0	1.36	0.35	0.48	0.38
BW[2], kg	772	776	763	741	19	0.62	0.66	0.42
BCS[3]	3.37	3.41	3.40	3.20	0.11	0.77	0.95	0.19
Calf BW, kg	47.5	47.8	49.0	43.2	3.8	0.50	0.19	0.02
Calving difficulty[4]	1.6	1.8	2.6	1.3	0.38	0.85	0.85	0.31
DMI d –21 to 0, kg/d d	11.3	11.9	12.2	12.1	0.99	0.52	0.80	0.91

[1]Contrasts: 1= Control vs. all other diets, 2= P vs. PF1, 3= PF1 vs. PF2.
[2]Measured 21 d prior to expected calving.
[3]Wildman et al., 1982.
[4]Five point scale: 1= no assistance, 2= slight problem, 3= needed assistance, 4= considerable force, and 5= cesarean.

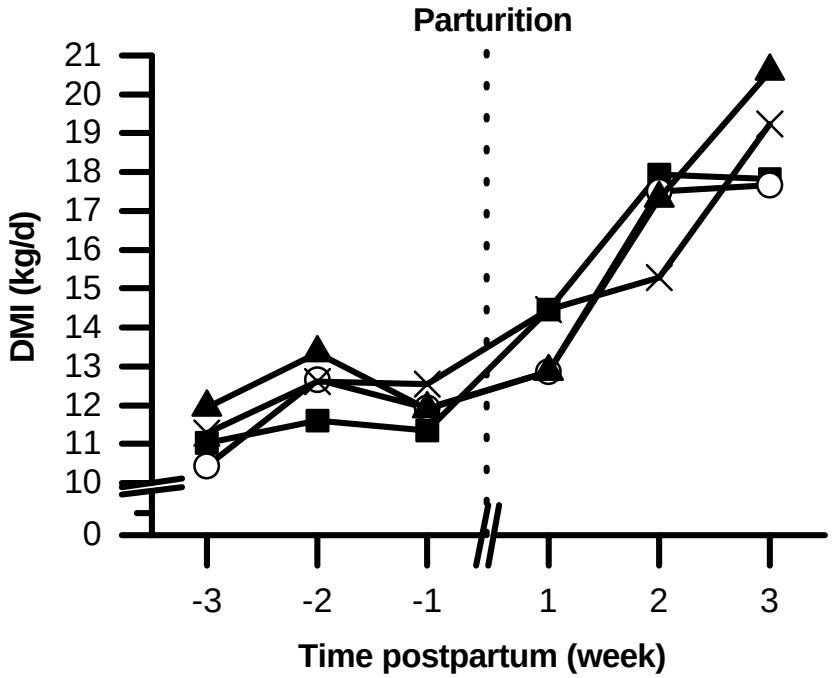

Figure 14. Dry matter intake (pooled SEM = 1.34) of cows fed Control (squares), Propionate (open circles), Propionate + fat 1 (triangles), and Propionate + fat 2 (crosses). There was a diet × week interaction (*P* = 0.02) for DMI.

Table 28. Postpartum DMI, milk yield, milk composition, milk SCC, BW, and BCS of cows fed Control (CON), Propionate (P), Propionate + fat 1 (PF1) and Propionate + fat 2 (PF2).

	Diet					Contrast[1]		
Item	CON	P	PF1	PF2	SEM	1	2	3
DMI[2] d 1 to 21, kg/d	16.4	16.2	16.6	16.3	1.56	0.99	0.85	0.87
Milk d 1 to 21, kg/d	33.5	35.8	32.3	32.9	2.75	0.96	0.41	0.89
Actual 305 d milk, kg	13,115	12,824	13,130	12,653	725	0.78	0.74	0.63
ECM[3] d 1 to 21, kg/d	36.3	40.1	34.2	35.1	2.87	0.95	0.18	0.82
ECM/DMI, d 1 to 21	2.48	3.79	2.54	2.97	0.575	0.36	0.13	0.60
Milk composition, d 1to 21								
Fat, %	3.99	4.48	4.22	4.08	0.18	0.22	0.32	0.57
Fat kg/d	1.34	1.58	1.29	1.31	0.11	0.67	0.10	0.91
True protein, %	3.31	3.16	3.15	3.18	0.06	0.04	0.87	0.67
True protein, kg/d	1.10	1.10	1.00	1.03	0.08	0.54	0.42	0.82
SNF, %	9.01	8.81	8.80	8.86	0.08	0.05	0.89	0.60
SNF, kg/d	3.03	3.13	2.85	2.91	0.24	0.82	0.45	0.85
Lactose, %	4.80	4.75	4.75	4.78	0.05	0.44	0.97	0.69
Lactose, kg/d	1.63	1.71	1.55	1.58	0.14	0.94	0.47	0.87
SCC x 10^3/mL	176	378	263	279	116	0.34	0.49	0.92
Urea N, mg/dL	12.5	13.0	13.8	16.4	0.80	0.04	0.49	0.02
Milk d 1 to 70, kg/d	44.3	45.4	42.8	44.4	2.35	0.96	0.46	0.62
BW[4], kg	678	654	648	660	15	0.15	0.78	0.54
BCS[4, a]	2.99	2.92	2.97	3.02	0.08	0.78	0.64	0.60

[1]Contrasts: 1= Control vs. all other diets, 2= P vs. PF1, 3= PF1 vs. PF2.
[2]Diet × week (P = 0.02).
[3]ECM = [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)]; (Orth, 1992).
[4]Measured at 21 DIM.
[a]Wildman et al., 1982.

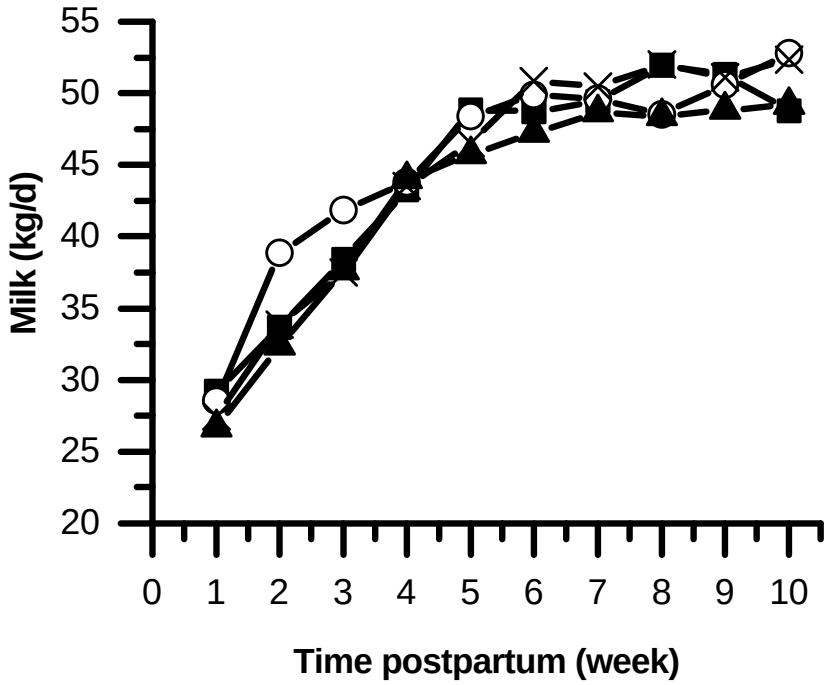

Figure 15. Milk yield (pooled SEM = 1.20) of cows fed Control (squares), Propionate (open circles), Propionate + fat 1 (triangles), and Propionate + fat 2 (crosses).

Effects of treatments on plasma glucose and insulin are presented in Table 29 and plotted over time in Figure 16. Overall, concentrations of plasma glucose and insulin were not different among treatments; however, differences were observed on specific days relative to calving. Concentrations of glucose in plasma of cows fed PF2 tended to be lower than for cows fed PF1 at 7 d prior to parturition (Figure 16 A, $P < 0.06$, 72.0 vs. 80.8 mg/dL, respectively). At 14 d prior to parturition, concentrations of insulin in plasma of cows fed P or PF2 were two-fold greater than those fed PF1 (Figure 16 B, $P < 0.06$, 211 and 202 vs. 95 pg/mL, respectively). Similarly, plasma insulin concentrations of cows fed PF2 and C tended to be greater than for cows fed PF1 at 14 d prepartum (Figure 16 B, $P < 0.08$, 202 and 211 vs. 95 pg/mL, respectively).

Effects of treatments on plasma NEFA and BHBA are shown in Table 29 and Figure 17. Concentrations of NEFA in plasma increased prepartum for cows fed C, P, and PF1 and were decreased ($P < 0.05$) 2 d prior to parturition for PF2 compared with PF1 (759 vs. 426 µEq/L, respectively). Overall concentrations of NEFA in plasma averaged 623 µEq/L and tended to be lower in cows fed PF2 compared to those fed PF1 ($P < 0.07$, 675 vs. 515 µEq/L, respectively). More importantly, concentrations of NEFA in plasma were greater during the first 21 DIM for cows fed PF1 relative to those fed PF2 ($P < 0.02$, 623 vs. 873 µEq/L, respectively). This lesser concentration of NEFA was particularly evident at 2 DIM as concentrations of NEFA in plasma of cows fed PF1 were two-fold greater than cows fed PF2 ($P < 0.01$, 552 vs. 1111 µEq/L, respectively). Effects of

treatments on NEFA in plasma did not translate into differences in concentrations of BHBA in plasma which were unaffected by dietary treatments.

Table 29. Plasma glucose, insulin, NEFA, and BHBA of cows fed Control (CON), Propionate (P), Propionate + fat 1 (PF1) and Propionate + fat 2 (PF2).

	Diet					Contrast[1]		
Item	CON	P	PF1	PF2	SEM	1	2	3
Glucose, mg/dL								
All data	73.3	71.8	72.2	70.6	2.0	0.48	0.64	0.54
Prepartum	80.8	81.2	81.3	77.2	2.49	0.74	0.72	0.29
Postpartum	67.7	64.5	65.2	65.2	2.46	0.36	0.40	0.87
Insulin, pg/mL								
All data	96.1	98.0	82.4	99.4	15.1	0.87	0.47	0.43
Prepartum	134.5	141.4	95.2	146.2	29.6	0.84	0.31	0.22
Postpartum	69.4	65.8	71.3	63.9	8.7	0.79	0.65	0.53
NEFA, µEq/L								
All data	633	668	675	515	62	0.85	0.93	0.07
Prepartum	383	579	414	377	92	0.49	0.24	0.77
Postpartum	810	720	873	623	82	0.43	0.18	0.02
BHBA, mg/dL								
All data	8.43	8.99	7.50	7.13	1.57	0.76	0.51	0.87
Prepartum	3.75	3.49	3.32	3.64	0.27	0.37	0.25	0.32
Postpartum	12.07	13.55	10.53	10.02	2.74	0.82	0.44	0.89

[1]Contrasts: 1= Control vs. all other diets, 2= P vs. PF1, 3= PF1 vs. PF2.

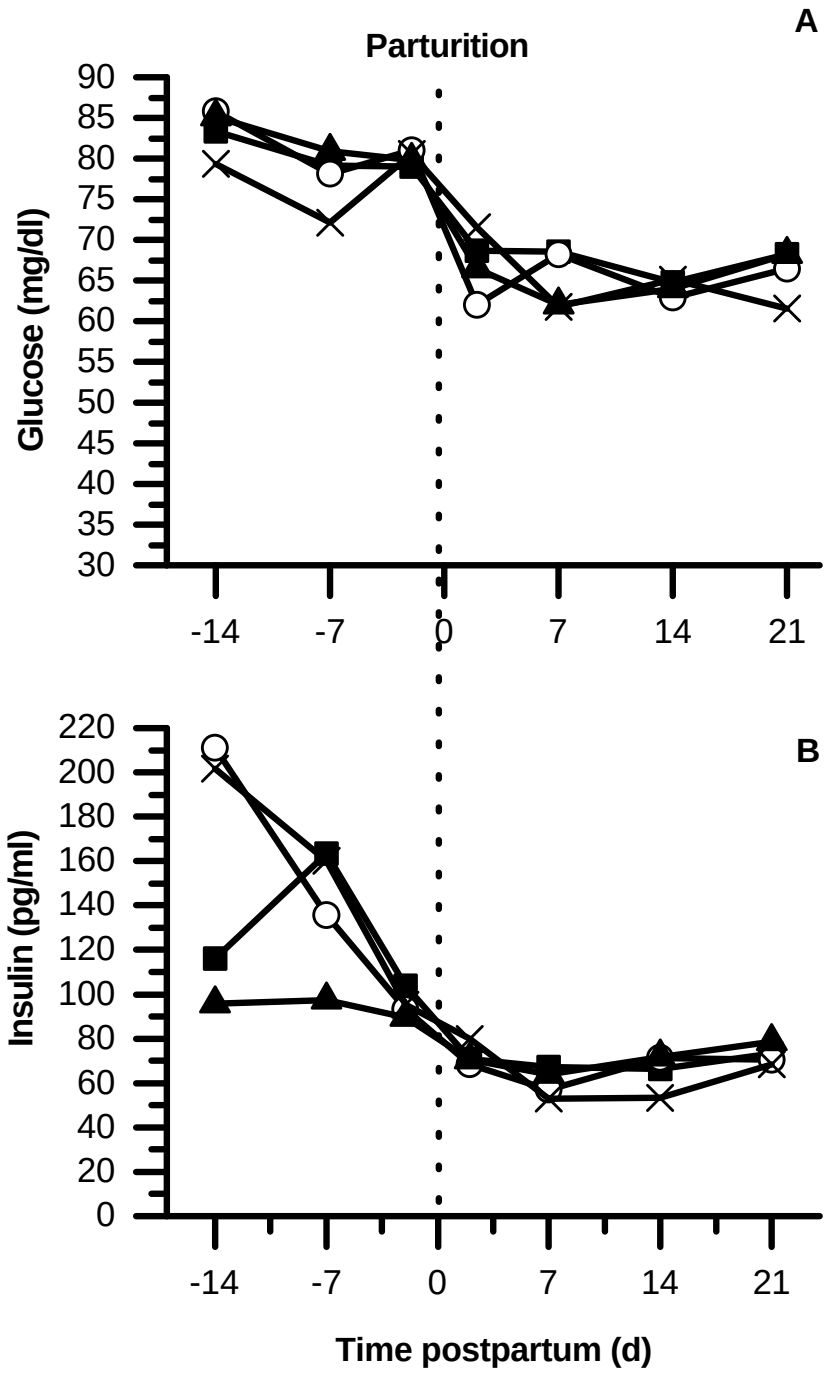

Figure 16. Plasma glucose (A) and insulin (B) concentrations (pooled SEM = 3.43 and 20.9, respectively) of cows fed Control (squares), Propionate (open circles), Propionate + fat 1 (triangles), and Propionate + fat 2 (crosses).

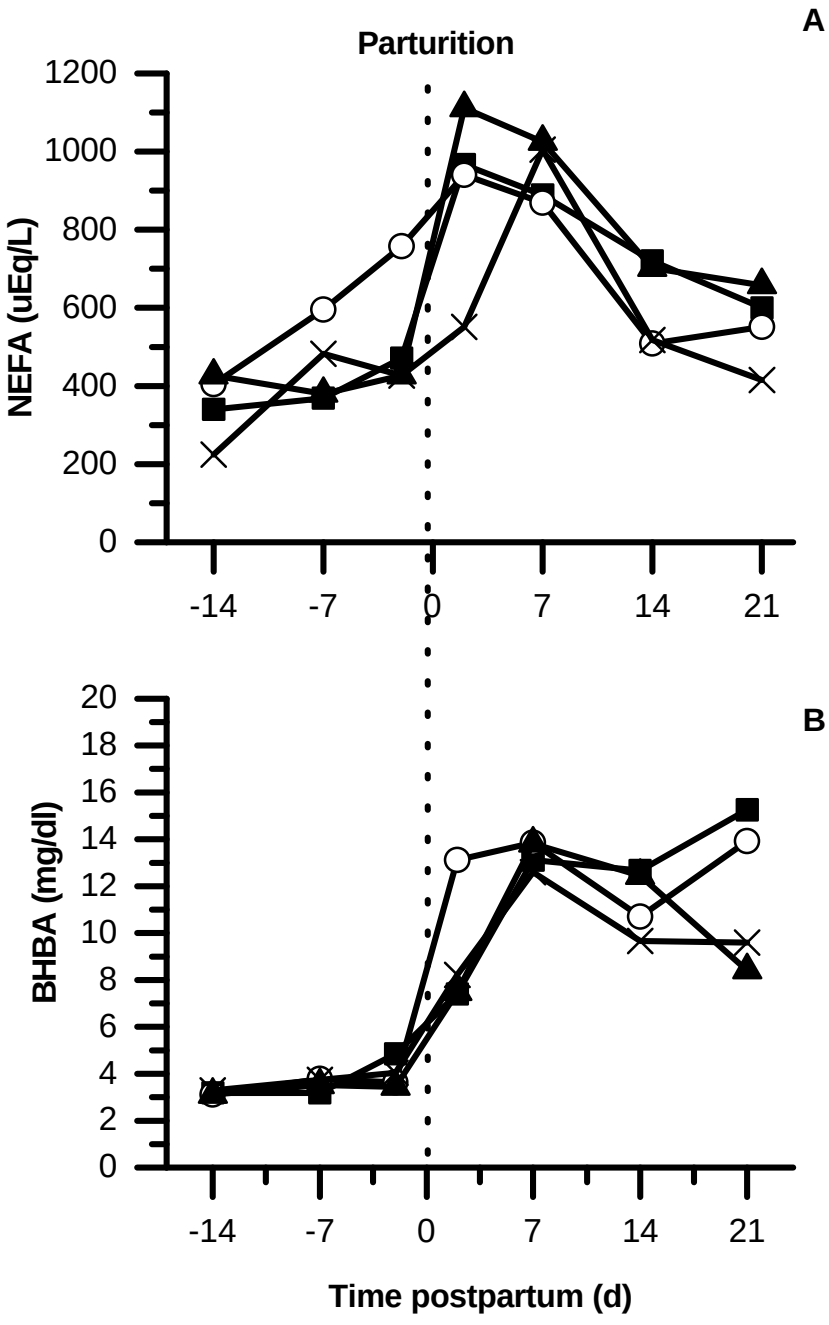

Figure 17. Plasma NEFA (A) and BHBA (B) concentrations (pooled SEM = 126 and 1.86, respectively) of cows fed Control (squares), Propionate (open circles), Propionate + fat 1 (triangles), and Propionate + fat 2 (crosses).

Discussion

A glucose deficit, estimated at 500 g/d (Drackley et al., 2001) is typical of the early postpartal period of the lactation cycle. Failure to address this metabolic challenge results in early postpartal health disorders, compromised lactation, and potential mortality. The objective of feeding glucose precursors is to alleviate this glucose deficit. Several studies document the effects of feeding propionate or fat either pre- (Grum et al., 1996; Beem, 2003) or postpartum (Schultz, 1958; Jerred et al., 1990); however, only one experiment (Patton et al., 2004) described the effects of supplementing these ingredients both prepartum and postpartum.

Minimizing prepartal feed intake depression is recommended (Grummer, 1995) as the focal point of the transition cow management. For any feed supplement to be effective as a glucose precursor, feed intake should not be depressed because prepartal DMI is inversely related to concentrations of NEFA and BHBA in plasma and liver triglycerides (Bertics et al., 1992). In the study under discussion, feeding propionate with or without LCFA did not significantly affect DMI. An exhaustive review by Allen (2000) found LCFA to decrease DMI 2.5% for each percentage unit of added Ca salts of palm fatty acids in the diet over control. Accordingly, we should have observed a decrease in DMI of nearly 3% pre- and postpartum for cows fed PF1 and PF2. According to Allen (2000), these decreases were likely to be minimized by hand-mixing the LCFA into the TMR versus applied as a top dress. Dry matter intake was decreased prepartum

(Grum et al., 1996) and postpartum (Jerred et al., 1990) when fat was fed at greater levels (0.6 and 1.1 kg/d, respectively) than those used in the present study. We intended to supply sufficient Ca salts of fatty acids without negatively affecting DMI. Results indicated DMI was not negatively affected in cows fed 93 or 154 g/d fat as Ca salts compared with cows fed a control diet free of supplemental fat, especially DMI of cows fed 154 g/d during the week prior to parturition (Figure 14).

Effects of feeding propionate on DMI are limited because most reports were either field studies or were conducted using propionate-based drenches in combination with pen research. Beem (2003) recently fed Ca-propionate at 113 g/d for 21 d prior to expected calving and found no effect on DMI. Although we fed greater amounts of propionate (120 and 178 g/d for PF1 and PF2, respectively), our results are in agreement with those of Beem (2003). The possibility of observing a decrease in DMI in cows fed P was likely because ruminal infusions of propionate are known to elicit hypophagia via effects on both satiety and hunger (Oba and Allen, 2003). Because DMI of cows fed propionate alone (P) or combinations of propionate and LCFA (PF1 and PF2) were similar to cows fed C, it is doubtful that the amounts of propionate fed in this study elicited hypophagic effects as reported by Oba and Allen (2003); however, it should be noted that these effects may have decreased DMI of cows fed the greater quantity of propionate found in PF2 during the second week of lactation.

Similar to Patton et al. (2004), results reported here of feeding propionate in combination with LCFA did not affect yields of milk or ECM. Milk fat yields (kg/d) and yields of ECM per kg of DMI tended to be greater in cows fed P relative to cows fed PF1. Similar to our results, the addition of LCFA has increased production efficiency when fed at 0.5 kg/d (Canale et al., 1990). Because DMI was similar between cows fed P and PF1, one could argue cows fed PF1 were experiencing a less negative energy balance and therefore mobilizing less triglycerides; however, concentrations of NEFA in plasma do not support this claim as plasma NEFA were numerically greater ($P < 0.18$) for cows fed PF1. According to Rigout et al. (2003), a negative relationship exists between milk fat yield and energy provided by glucogenic precursors ($R^2 = 0.89$) and one can expect a 0.14% decrease in milk fat for every 1 Mcal of glucose precursor ingested. Using the energy value of 0.233 Mcal/mole propionate from Rigout et al. (2003), cows fed propionate (P, PF1, PF2) ingested 0.62, 0.62, and 0.92 Mcal of glucose precursor, respectively. Based on these calculations, a 0.09, 0.09, and 0.13% decrease in milk fat would be expected, but was not observed.

The greater milk protein percent and solids-not-fat of cows fed C relative to cows fed all other diets may be attributable to the greater dietary CP supply (19.4 vs. 18.6%, respectively, Table 26) in combination with a highly fermentable carbohydrate (topdress of 0.32 kg/d of corn starch). Treatments did not affect DMI suggesting the decrease in milk protein percent and greater MUN could also

be attributed to effects of propionate and/or LCFA on ruminal pH and fermentation profiles, and therefore microbial protein synthesis and outflow to the intestine. Hoover and Stokes (1991) indicated the rate of ruminal carbohydrate digestion is critical in determining the energy available for microbial growth. Our aim was to supply propionate and Ca salts of fatty acids to improve the energy status of the peripartum dairy cow; however, these ingredients do not necessarily contribute to the energy pool of the rumen. Grummer (1988) fed 680 g/d LCFA, three times the quantity used in this experiment, and observed no effect on ruminal pH, total VFA, or VFA profiles and a decrease (0.13 percentage units; $P < 0.06$) in milk protein. Canale et al. (1990) fed 0 or 0.5 kg/cow/d of LCFA and observed a decrease in milk protein percentage from 3.10 to 3.03% ($P < 0.01$). Our results concur with those of Grummer (1988) and Canale et al. (1990) and the recent results of Patton et al. (2004) who observed a tendency for a decrease in milk protein concentration at 7 and 14 DIM in cows fed both propionate and LCFA.

Effects of treatments on plasma glucose and insulin concentrations showed no effect, which is in contrast to Patton et al. (2004) who found the combination of glucose precursors and LCFA to increase blood glucose and insulin when fed both pre- and postpartum. Differences between studies could be attributed to differences in prepartal DMI, diet ether extract content, and the type of glucose precursor used. Prepartal intakes averaged 11.9 kg/d whereas those of Patton et al. (2004) were > 15 kg/d. Although not apparent here,

Littledike et al. (1981) explained propionate may be less palatable than propylene glycol. Perhaps the greater dietary ether extract contributed to decreased intakes in our study. Pre- and postpartal diets of Patton et al. (2004) were 0.6 to 1.9 and 0.5 to 1.4 percentage points lower in ether extract, respectively. In addition, we fed propionate as the sole glucose precursor as opposed to a combination of propionate and propylene glycol used by Patton et al. (2004). We anticipated similar results because, when fed, propylene glycol contributes to the ruminal propionate pool (Grummer et al., 1994; Christensen et al., 1997). An excess of glucogenic nutrients relative to lipogenic nutrients has been proposed (Kronfeld, 1982) as one of the reasons for the onset of ketosis because glucose does not provide acetyl-CoA units necessary for milk fatty acid synthesis which must therefore be derived from depot fat. It is likely our treatments did not alter blood glucose as treatments did in Patton et al. (2004) because our diets provided sufficient lipogenic nutrients, a speculation supported by Kronfeld (1982).

The minimal effects of treatments on plasma metabolites are indicators that the degree of ketosis observed in the present study was not severe enough to require cows to rely heavily upon an exogenous supply of glucose precursors. This is evidenced by the fact that the greatest plasma BHBA concentration observed was 13 mg/dL at 2 DIM for cows fed P. This is slightly greater than the suggested plasma BHBA threshold of 12.5 mg/dL that Nielen et al. (1994) used to classify cows as experiencing subclinical ketosis. Blood ketone

concentrations in cows used by Schultz (1958) were also quite low. Schultz (1958) found feeding 113.5 g/d propionate during the first 6 wk of lactation to decrease ketone bodies from 13.5 to 9.7 mg/dL. Similar to our results, Patton et al. (2004) found concentrations of BHBA in plasma to be unaffected by feeding calcium salts of fatty acids in combination with glucose precursors.

Despite the limited degree of ketosis observed, a series of observations are noteworthy in evaluating the effects of feeding LCFA with and without propionate on lipolysis. First of all, concentrations of plasma NEFA continually increased from 21 d prepartum to 2 DIM for cows fed P relative to cows fed PF1 and PF2. Given DMI was similar between cows fed P and PF1, differences in prepartal plasma NEFA profiles (Figure 17) suggest an energetic advantage when 130 g/d of corn starch is replaced by LCFA in diets supplemented with propionate salts at 198 g/d. This is especially true considering the uptake and oxidation of NEFA by hepatic and extrahepatic tissue is directly related to their plasma concentrations (Katz and Bergman, 1969) and accumulation of NEFA in liver exacerbates production losses (Grummer, 1993). Secondly, the absence of an increase in plasma BHBA with elevated prepartal NEFA indicates the capacity to oxidize fatty acids to CO_2 was not exceeded in cows fed P (Grummer and Carroll, 1991). Thirdly, prepartal differences in NEFA profiles plotted against time between cows fed P vs. PF1 suggest LCFA ingested by cows fed PF1 may have been utilized so as to reduce the need for release of NEFA by adipose tissue. This is supported by the fact that Ca salts of fatty acids escaping rumen

fermentation enter the blood as chylomicrons and are readily extracted by the mammary gland which is rich in lipoprotein lipase (Havel, 1987). Lastly, it appears that feeding greater quantities of propionate in conjunction with LCFA (PF1 vs. PF2) may be justified because of the observed decrease ($P \leq 0.02$) in concentrations of NEFA in plasma for cows fed PF2 relative to those fed PF1. This response is most evident at 2 DIM where concentrations of NEFA in plasma were twice as great in cows fed PF1 relative to those fed PF2 (1111 vs. 552 µEq/L).

Conclusions

Supplementing diets for transition dairy cows with propionate and LCFA had minimal effects on lactational performance and blood metabolites. The addition of LCFA to diets of cows receiving propionate tended to decrease feed efficiency (ECM/DMI) during the first 21 DIM. Plasma NEFA profiles suggest a benefit to supplementing propionate and LCFA at greater than 154 and 93 g/d prepartum. This indication of improved energy balance is supported by maintenance of DMI during the prepartal period, as cows fed PF2 did not experience a decrease in DMI from week 2 to 1 prepartum (Figure 14). Based upon the glucogenic to lipogenic ratio proposed by Kronfeld (1982) and previous studies (Patton et al., 2004), the magnitude of the response to feeding propionate in combination with LCFA to transition dairy cows may be dependent upon the amount of dietary fat present in the basal diet.

OVERALL CONCLUSIONS

Ruminants rely upon absorbed VFA to satisfy energy requirements. The negative energy balance in transition dairy cows, most of which is characterized by hypoglycemia, has prompted researchers to delineate the influence of ruminal propionate while few studies have described the impact of formulating diets to increase ruminal butyrate proportions. The profiles of fermentation acids synthesized during rumen fermentation are largely dependent on dietary ingredients. Increasing ruminal butyrate would be contrary to current transition cow management recommendations because of the potential for ruminal ketogenesis to cause ketosis. The metabolism of ketones and ketone precursors and glucose synthesis are interrelated. It is obvious that the origin of ketones and their precursors affects animal responses. For example, cows fed diets high in butyric acid, such as poorly fermented forages or cows ruminally infused with butyrate are more susceptible to ketosis.

Substituting dietary lactose for corn starch in an alfalfa hay and corn silage-based diets increased proportions of ruminal butyrate and concentrations of BHBA in plasma. Plasma glucose was statistically decreased in cows fed lactose but the decrease is not likely to be biologically significant as evidenced by the similar levels of lactation performance among treatments. The increases in plasma BHBA observed were not great enough to place cows at risk for developing ketosis. These data indicate that lactose may be substituted for corn starch in diets of late-lactation dairy cows without leading to the onset of

physiological conditions favoring ketosis and may improve nutrient utilization as evidenced by the observed decreases in MUN.

Additional experiments were conducted to determine the effects of diets formulated to increase the supply of ketones or ketone precursors on transition dairy cows. Feeding glycerol increased ruminal butyrate and resulted in similar effects on blood chemistry profiles as plasma glucose decreased and plasma BHBA increased; however, these changes were more pronounced relative to cows fed lactose. It is hypothesized that the palatability of glycerol resulted in the decreased prepartum intake, which may have resulted in the increased indication of ketosis. Feeding lactose to transition dairy cows increased proportions of ruminal butyrate and plasma BHBA without affecting glucose concentrations. Paradoxically, feeding an alpha-amylase enzyme preparation to transition dairy cows tended to increase plasma glucose while other rumen and plasma metabolites responded similar to cows fed lactose. The enzyme preparation is known to affect rumen microbial populations, which may have resulted in the greater plasma glucose response. The final experiment found transition dairy cows fed glucose precursors (propionate) in combination with ketone precursors (fat) in sufficient quantities to decrease fat mobilization as reflected by decreased NEFA concentrations in plasma postpartum without affecting plasma BHBA or glucose.

Although the ketone-promoting diets investigated in these experiments did not improve animal performance, this research clearly demonstrates that plasma

BHBA derived from ruminal ketogenesis minimally affects the glycemic status of transition dairy cows. Because of the documented effects of elevated ruminal butyrate on rumen papillation, future research is warranted to study the effects of feeding diets stimulating rumen epithelial ketogenesis on rumen morphology and absorptive capacity of transition dairy cows.

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