EFFECTS OF INTRAJUGULAR GLUCOSE INFUSION ON FEED INTAKE, MILK YIELD AND METABOLIC RESPONSES OF EARLY POSTPARTUM COWS FED DIETS VARYING IN PROTEIN AND STARCH CONCENTRATION

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ABSTRACT

EFFECTS OF INTRAJUGULAR GLUCOSE INFUSION ON FEED INTAKE, MILK YIELD AND METABOLIC RESPONSES OF EARLY POSTPARTUM COWS FED DIETS VARYING IN PROTEIN AND STARCH CONCENTRATION

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Effects of glucose infusion on feed intake, milk production, and metabolic responses of early postpartum cows fed a high starch or high protein diet were evaluated utilizing a randomized complete block design experiment with a 2x2 factorial arrangement of treatments. Twenty-four multiparous Holstein cows were blocked by BCS and 305-ME milk production, and randomly assigned at parturition to one of four treatments. Treatments were continuous intrajugular infusion of glucose (GI; 4 L/d of 25% w/v dextrose) or isotonic saline (SI; 4 L of 0.9% sodium chloride), and diets containing high starch/low crude protein (HSLP) or high crude protein/low starch (HPLS) concentration. Treatments were initiated at the first feeding following parturition and lasted 12 d. GI, compared with SI, reduced cumulative DMI for HPLS (164.0 vs. 196.5 kg) but not HSLP (194.2 vs. 189.7 kg; interaction $P = 0.04$) by reducing meal size for HPLS (13.6 vs. 16.4 kg) but not HSLP (16.4 vs. 15.9 kg; interaction $P = 0.14$). HPLS increased plasma concentrations of NEFA (1184 vs. 895 µEq/L, $P = 0.01$) and BHBA (13.0 vs. 9.5 mg/dL, $P = 0.03$), liver triglyceride (5.7 vs. 3.5 mg/g wet liver, $P = 0.03$), milk fat concentration (5.71 vs. 4.72%, $P < 0.01$) and yield (2.14 vs. 1.78 kg/d, $P < 0.01$) and 3.5% fat-corrected milk (50.9 vs. 45.1 kg, $P = 0.02$), and tended to increase body condition loss (-0.82 vs. -0.65 BCS units/12 d, $P = 0.06$) compared with LPHS. HPLS increased BCS loss despite no difference in plasma insulin between dietary treatments ($P = 0.23$). The HPLS diet and GI interacted to reduce meal size and feed intake, possibly by affecting hepatic oxidation of fuels.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AA – amino acid

ATP – adenosine triphosphate

BCS – body condition score

BHBA – *beta*-hydroxybutyrate

CoA – coenzyme A

CLA – conjugated linoleic acid

CP – crude protein

DM – dry matter

DMI – dry matter intake

FCM – fat-corrected milk

GH – growth hormone

GI – glucose infusion

HOT – hepatic oxidation theory

HSLP – high-starch, low-protein diet

HPLS – high-protein, low-starch diet

ME – metabolizable energy

MFD – milk fat depression

MP – metabolizable protein

NEFA – non-esterified fatty acid

RUP – rumen-undegradable protein

SI – saline infusion

VLDL – very-low density lipoprotein

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

The transition period from pregnancy to lactation is widely recognized as one of the most critical periods in the life cycle of dairy cattle, marked by low dry matter intake (DMI) along with drastically increased energy demands due to the onset of lactation, resulting in a period of negative energy balance. The effect of this energy deficit is an increased likelihood of developing metabolic diseases, such as ketosis and hepatic lipidosis. Recently, many studies have focused on methods of increasing dry matter intake during the postpartum period in order to decrease the energy deficit, and thus decrease the prevalence of metabolic issues. This review of the literature will examine the mechanisms affecting dry matter intake in postpartum cows, metabolism of glucose and protein postpartum, and how glucose and protein status of the cow may affect feed intake and productive performance of the dairy cow in the postpartum period.

REGULATION OF DRY MATTER INTAKE IN FRESH COWS

Lipids

Transition dairy cows have decreased plasma insulin concentration and decreased cellular sensitivity to insulin (Bell and Bauman, 1997), resulting in increased lipolysis. Plasma concentration of non-esterified fatty acids (NEFA) increase shortly before and remain elevated for a short period after parturition (Grummer et al., 2004; Douglas et al., 2006). During this time, NEFA can be oxidized by the liver, producing reducing equivalents via β-oxidation (Drackley et al., 2001) for the production of adenosine triphosphate (ATP). However, rapid

elevation of plasma NEFA postpartum results in liver triglyceride accumulation (Vazquez-Añon et al., 1994) due to the poor ability of the ruminant liver to export lipid as very low density lipoprotein (Adewuyi et al., 2005). The degree of hepatic lipidosis may depend on the severity of lipid mobilization since the liver takes up lipid in proportion to the circulating blood concentration (Bell, 1979). In a review of the control of feed intake in ruminants, Allen et al. (2009) postulated that during lipolytic periods in ruminants, oxidation of fuels in the liver, such as NEFA, contribute to the satiety of ruminants by increasing the energy status of the liver.

Propionate

Starch degradation in the rumen produces propionic acid, which also plays a key role in dry matter intake depression in fresh cows as propionate has been shown to reduce intake when infused ruminally (Anil and Forbes, 1980, 1988; Oba and Allen, 2003; Bradford and Allen, 2007a). Propionate is taken up by the liver and converted to TCA cycle intermediates that can be used for gluconeogenesis in ruminants. During periods of high glucose demand, such as peak production, propionate is readily converted to glucose. Conversely, propionate supply that exceeds the glucose demand will create a 'bottleneck,' resulting in propionate conversion to acetyl coenzyme A (CoA) via pyruvate and subsequent oxidation in the TCA cycle. Gluconeogenic flux decreases as lactation proceeds beyond peak production, and excessive propionate production within the timeframe of meals might cause satiety sooner.

A unique situation arises in fresh cows, for example, when glucose demand is high but an accumulation of acetyl-CoA from lipid mobilization and breakdown of fatty acids has occurred. In this scenario, propionate stimulates oxidation of the existing pool of acetyl-CoA, producing energy and a hypophagic response as discussed above. Murondoti et al. (2004) noted that

hepatic expression of several key gluconeogenic enzymes was decreased in cows with hepatic lipidosis, and conversion of propionate to glucose *in vitro* decreased in hepatic cells with high concentrations of intracellular triacylglycerol (Cadórniga-Valiño et al., 1997). Propionate reduced DMI in fresh cows compared with acetate and the reduction in DMI was related to hepatic acetyl-CoA concentration; propionate infusion decreased DMI more with increasing acetyl-CoA concentration (Stocks and Allen, 2012). This interaction of hepatic lipid accumulation and propionate oxidation could explain the reduction in intake in fresh cows compared to cows in mid-lactation when infused ruminally with propionate (Oba and Allen, 2003). Based upon this knowledge of how lipid and propionate interact to decrease feed intake it may be beneficial target a lower body condition score (BCS) at calving than what is often recommended in order to decrease lipid mobilization; however, more definitive research is necessary.

Satiety signal mechanism

The mechanism by which increased energy status from hepatic fatty acid and propionate oxidation decreases feed intake may be transmitted by the hepatic vagus nerve, whereby increased oxidation sends a satiety signal to the brain to terminate a meal. Feed intake was reduced by administration of a variety of fuels in sham-vagotomized rats, but not in completely vagotomized rats (Langhans et al., 1985). Additionally, hepatic vagotomy and splanchnic nerve blocks eliminated the hypophagic responses of sheep infused intravenously with propionate (Anil and Forbes, 1980, 1988).

ROLE OF GLUCOSE IN FRESH COWS

Ruminants differ from non-ruminants mainly in their method of food digestion via utilization of microorganisms to break down carbohydrates to volatile fatty acids in the rumen. Starch consumed by the ruminant is converted primarily to propionic acid by amylolytic bacteria, and propionate is subsequently used as the primary substrate for glucose synthesis. The contribution of propionate to the synthesis of glucose is estimated to be between 43 and 77 percent of total glucose production in studies in sheep, beef steers and lactating dairy cows (Leng et al., 1967; Huntington et al., 1997). The amount of starch that escapes rumen microbial degradation and reaches the small intestine is variable, depending on rates of digestion and passage, which is influenced by the grain conservation method (Oba and Allen, 2003a), and the type of protein and its distribution around the starch in the endosperm (Rooney and Pflugfelder, 1986). Carbohydrate absorption in the small intestine is energetically more efficient than carbohydrate fermentation in the rumen (Owens et al., 1986), but, in comparison to nonruminants, the amount of starch that reaches the lower tract to be subjected to enzymatic degradation to glucose monomers available for absorption by the intestinal epithelia is less. Thirty to 63% of starch consumed was digested post-ruminally in cows fed corn-based diets (McCarthy et al., 1989; Oba and Allen 2003a; Taylor and Allen, 2005). Less than 10 percent of the glucose requirement is absorbed postruminally, meaning gluconeogenesis must account for 90 percent of the glucose needs in ruminants (Young, 1977). Young (1977) estimated that an extremely productive cow producing 89 kg of milk per day would need to synthesize 6.6 kg of glucose daily. For this reason, much research has been focused on improving the glucose status of cows in the postpartum period to determine if increased status can improve performance. In this section, the mechanisms of glucose metabolism in ruminants will be discussed, specifically

during late pregnancy and the transition to lactation. In addition, the effect of increasing the supply of glucose on dry matter intake, metabolic parameters and milk production performance will be reviewed.

Glucose metabolism prepartum

Glucose synthesis increases progressively throughout pregnancy in pregnant ewes compared to non-pregnant ewes fed a grass diet at restricted intake (Wilson et al., 1983). A similar increase was noted in ewes fed *ad libitum*, but this may have been confounded by increased feed intake as pregnancy continued (Steel and Leng, 1973). An increase in hepatic gluconeogenesis was not detected in Holstein cows prepartum, although this could be due to the short sampling window utilized in that study (Reynolds et al., 2003). The increase in gluconeogenesis in the feed-restricted ewes is presumably due to effects of lower insulin concentration on regulation of glucogenic enzymes and increased availability of glucose precursors from mobilization of peripheral tissues. Adipose tissue and muscle protein are the most prominent contributors to gluconeogenesis in this situation (Bell, 1995) considering that glycerol from triglycerides and certain amino acids are glucogenic (Drackley et al., 2001).

Insulin resistance of maternal peripheral tissues during pregnancy in ewes gives a competitive advantage to the uterus, which does not depend on insulin for glucose uptake (Petterson et al., 1993) and takes up approximately 50% of the maternal glucose supply (Bell, 1995). Guesnet et al. (1991) showed that the number of total insulin receptors in omental adipose tissue was not different between pregnant, lactating and control ewes, but that there were a greater number of high affinity receptors in pregnant ewes. However, high affinity receptors in subcutaneous tissue were higher in early pregnancy, and the number of high affinity and total

receptors per cell decreased beginning at 105 days of pregnancy (Vernon et al., 1981). While the number and affinity of insulin receptors in periparturient animals may still be debated, there is evidence to suggest that the ability of insulin to bind to receptors and cause autophorphorylation may not be changed (Vernon and Pond, 1997). If this scenario indeed holds true, insulin resistance must occur downstream from the cell membrane receptor (Wilson et al., 1996; Vernon and Pond, 1997).

Changes in glucose metabolism at parturition

Insulin resistance from late gestation continues during the first few weeks of lactation. Additionally, major changes in glucose metabolism occur during the transition from gestation to lactation in dairy cows; primarily an increase in hepatic gluconeogenesis and a decrease in oxidation of glucose by the peripheral tissues, thereby directing glucose to the mammary gland (Overton and Waldron, 2004). The requirement for glucose by the mammary gland increases greatly due to the initiation of lactation, and greatly exceeds that of the gravid uterus. Glucose uptake in the mammary gland four days postpartum in Holstein cows is estimated to be 2.7 times that of the uterus at day 250 of gestation (Bell, 1995). In dairy goats, mammary uptake of glucose four to six days postpartum was approximately 920% of the value recorded approximately one week prepartum (Davis et al. 1979). Sixty to 85 percent of the glucose entering the blood is used by the mammary gland at peak lactation (Annison and Linzell, 1964) demonstrating the high requirements of glucose in the lactating animal. Fifty to 90% of the glucose taken up by the mammary gland is utilized for lactose synthesis (Annison et al., 1974; Bickerstaffe et al., 1974), which serves as the main osmotic force for milk production.

To account for the increased glucose demand, more glucose must be synthesized from either the diet or endogenous sources. Net liver flux of glucose doubled between 9 days prepartum and 11 days postpartum in Holstein cows (Reynolds et al., 2003), and total body glucose entry rate nearly tripled as early as the day of parturition (Paterson and Linzell, 1974) suggesting that an increase in gluconeogenesis for lactation is an extremely rapid response. Using data from cows in varying stages of lactation from several studies, Paterson and Linzell (1974) demonstrated a highly significant positive correlation between milk production and glucose entry rate. Hepatic gluconeogenesis increases in response to increasing dietary metabolizable energy (ME) intake (Huntington et al., 2006) due to greater starch concentration providing glucose precursors for gluconeogenesis. It is reasonable to assume that a portion of the increased hepatic gluconeogenesis in a modern feeding regimen results from feeding 'fresh cow' diets postpartum that are higher in starch than prepartum diets. However, feed intake does not increase as rapidly as hepatic glucose output, suggesting that fresh cows must be mobilizing body tissues to support their glucose needs. In part, the increased hepatic glucose output despite a limited increase in energy intake could be from glucogenic amino acids, with alanine and glutamine contributing the largest percentage of all amino acids (Drackley et al., 2001). The major source of amino acids for gluconeogenesis likely arises from mobilization of labile protein reserves illustrated by a 25% reduction in muscle fiber diameter in transition cows (Reid et al., 1980) and elevated blood plasma 3-methylhistidine concentrations after parturition (Blum et al., 1985). To a smaller extent, glycerol from breakdown of triacylglycerol during lipid mobilization also will contribute to the hepatic glucose release (Drackley et al., 2001; Reynolds et al., 2003).

Despite increased hepatic gluconeogenesis after parturition, blood glucose concentrations are depressed compared to other points of the production cycle. Glucose demand in excess of

glucose production keeps plasma insulin concentrations low, leading to extended lipid mobilization and ketone body production, and resulting in a disorder commonly referred to as ketosis, usually occurring within the first 3 to 6 weeks postpartum (Ingvartsen, 2006). Ketosis preventative treatments involve administering propylene glycol, a gluconeogenic precursor, as an oral drench to the cow shortly after parturition to decrease NEFA and *beta*-hydroxybutyrate (BHBA) concentration (Overton and Waldron, 2004). The ability to improve metabolic indices by a simple drench mechanism postpartum demonstrates the critical role glucose plays in the postpartum performance of dairy cows.

Responses to exogenous glucose

Due to the large amount of blood glucose that is channeled to the mammary gland in the lactating cow and the unique system whereby ruminants must rely on gluconeogenesis for a majority of the glucose utilized, it is commonly thought that glucose availability limits milk yield of high producing dairy cows. Numerous studies have been undertaken to examine the effect of increasing the exogenous supply of glucose to the cow by infusions into the abomasum, duodenum and blood vessels to bypass the rumen environment. Two of the main considerations for examining these studies are the effects of exogenous glucose on feed intake and production performance.

Exogenous glucose administration demonstrates varied effects on feed intake, with some reporting an increase (Knowlton et al., 1998), no change (Frobish and Davis, 1978; Amaral et al., 1990; Abramson et al., 2005; Al-Trad et al., 2009) or a decrease in DMI (Rutter and Manns, 1986; Dhiman et al., 1993; Boudon et al., 2007; Larsen and Kristensen, 2009). Energy intake was generally not reported for glucose infusion studies, but Knowlton et al. (1998) reported

increased energy intake while Dhiman et al. (1993) cited a decrease in energy intake. Larsen and Kristensen (2009) and Rutter and Manns (1986) are the only two known studies to continuously infuse glucose during the postpartum period. In other studies where DMI was reduced or increased, the factor of the DMI change was normally small. However, in the two studies involving postpartum animals, the DMI of glucose infused animals was reduced by 40% (Larsen and Kristensen 2009) to 65% ($P < 0.06$; Rutter and Manns, 1986) by the end of the infusion period, demonstrating a drastically different response compared to animals further into lactation. These studies suggest that glucose may have different effects on metabolism and DMI in early lactation because of altered metabolic state in the postpartum period. One must be cognizant that the results in Larsen and Kristensen (2009) may be influenced by the low number of experimental units and the infusion device placed in the abomasum potentially affecting intake. Both early lactation studies are consistent with the hepatic oxidation theory (HOT) proposed by Allen et al. (2009).

Consistent with HOT (Allen et al., 2009), increased insulin concentration associated with glucose infusion may be responsible for the reduction in DMI noted in the early lactation studies. Insulin is recognized as decreasing the expression of regulatory gluconeogenic enzymes (Barthel and Schmoll, 2003) and reduces hepatic glucose output in ruminants (Brockman, 1985, 1990). The pathway for propionate conversion to glucose will be down-regulated by insulin as demonstrated by Donkin and Armentano (1994) whereby gluconeogenesis from propionate was decreased by increasing insulin in an *in vitro* culture of neonatal bovine liver cells.

Acetyl-CoA decreases pyruvate dehydrogenase activity (Roche et al., 2001) and allosterically activates pyruvate carboxylase (Jitrapakdee and Wallace, 1999). The effect of acetyl-CoA on these two enzymes channels fuels into the TCA cycle or gluconeogenesis through

pyruvate carboxylase. In the unusual scenario of fresh cows, where acetyl-CoA concentration from lipid breakdown is high, excess propionate from a meal not utilized for gluconeogenesis will provide TCA cycle intermediates that will promote oxidation of acetyl-CoA, generating ATP and a satiety signal to cease the meal. In exogenous glucose infusion studies, several laboratories have noted increased insulin concentration in fresh or early lactation animals along with a concomitant decrease in DMI (Boudon et al., 2007; Larsen and Kristensen, 2009), or a tendency for such (Rutter and Manns, 1986). It is possible that the high insulin concentration downregulates gluconeogenesis preventing propionate influx within meals from contributing to gluconeogenesis, and thus increasing TCA intermediates and the rate of oxidation of acetyl CoA withing the timeframe of meals.

The ewes used by Rutter and Manns (1986) were fed to either maintain (MAIN) or gain (GAIN) weight during gestation and infused with glucose (GLU) or saline (SAL). GAIN-GLU ewes consumed less than the other three treatments. It is well established that cows with higher BCS at parturition lose more condition and require more time to reach maximum feed intake (Garnsworthy and Topps, 1982). The ewes in Rutter and Manns (1986) did not differ in weight loss between treatments (possibly due to the short study length of 22 d); however, GAIN consumed less after lambing compared to MAIN and had higher insulin concentration. Due to a tendency for GLU to increase insulin and decrease DMI, HOT could explain the significantly lower intake of GAIN-GLU. Increased plasma insulin and potentially more hepatic acetyl-CoA could have decreased gluconeogenesis and lowered intake by propionate-stimulated oxidation of acetyl-CoA during meals.

To further support the theory that insulin plays a role in feed intake reduction, Bradford and Allen (2007) demonstrated insulin concentration was correlated negatively with DMI

response to a more fermentable diet. Cows with higher insulin concentration in the preliminary period experienced a greater reduction in feed intake in response to high moisture corn compared with dry corn. Additionally, hyperinsulinemic-euglycemic clamps depressed DMI by 33% in fresh cows, but there was no change in intake of non-lactating cows in late pregnancy (Leury et al., 2003). Fresh cows were in negative energy balance despite glucose infusion and had higher concentrations of NEFA compared to non-lactating cows.

Administration of exogenous glucose not only affects feed intake, and glucose and insulin concentrations, but also has effects on other blood metabolites in addition to milk production characteristics. Exogenous glucose decreased plasma concentrations of NEFA (Boudon et al., 2007), BHBA (Larsen and Kristensen, 2009), or both (Al-Trad et al., 2009) in studies that reported such measures, presumably due to the antilipolytic affects of insulin. Glucose consistently decreased milk fat yield, milk fat concentration, fat-corrected milk or a combination thereof (Frobish and Davis, 1977; Lemosquet et al., 1996; Hurtaud et al., 1998; Reynolds et al., 2001; Al-Trad et al., 2009; Larsen and Kristensen, 2009). The decrease is likely due to decreased lipid mobilization. Fat mobilization accounts for less than 10% of milk fatty acids in typical situations, but contributes more with increasing negative energy balance such as during early lactation (Bauman and Griinari, 2003). No consistent effects on milk protein are presented in the literature. Milk yield remains largely unchanged by glucose infusion, with only slight increases in yield when significant (1.9 kg/d; Frobish and Davis, 1977). However, it appears to be more common for cows fed grass silage based diets to have increased milk yield when receiving duodenal infusions of glucose (Boudon et al., 2007; Rigout et al, 2002). In the case of Boudon et al. (2007), this could be due to increased energy from the glucose infusion since no grain was provided in the diet.

PROTEIN METABOLISM IN FRESH COWS

Dietary protein concentration and the quality of dietary protein are widely recognized as two important aspects of balancing dairy cattle rations for the purpose of achieving optimal production performance. A large amount of research has been undertaken to determine the protein needs of dairy cows at differing stages of lactation, and how the degree of degradability in the rumen will further affect performance. Journet and Rémond (1981) identified protein feeding the first month after parturition as critical with the first two weeks postpartum being even more important. However, a paucity of information is available about the optimum concentration of metabolizable protein for newly parturient animals and for the first few weeks thereafter, and how protein is utilized by the postpartum cow. As such, this area remains a 'black box' of dairy nutrition, and is a topic that will surely receive more attention in the future with increased interest of reducing feed costs, improving efficiency and decreasing nitrogenous wastes. Therefore, this section will review periparturient protein metabolism and the effect of protein on production performance to the degree that is allowed by a small body of literature.

Protein metabolism prepartum

Considerable work has been completed on the effect of dietary protein concentration during the dry period and its impact on performance after parturition. Results were mixed, but a majority of studies showed a positive response in milk production to protein supplementation when the control diets contained less than 12% crude protein (CP; Bell et al., 2000). Prepartum diets have been implicated in the degree of nitrogen accretion in maternal tissues. McNeill et al. (1997) fed ewes diets containing 7.9, 11.6 or 15.7% CP. Ewes fed the 15.7% CP diet had

positive nitrogen accretion compared to the loss of nitrogen in ewes fed 7.9% CP, showing that mobilization of maternal tissues is a response to inadequate dietary protein prepartum. About five kg of protein was gained in cows during the last two months before parturition; however, it was not possible to verify how much of the gain was from the fetus and placenta, which may have contributed a significant portion (Belyea et al., 1978). Bell et al. (2000) suggested that high protein lactation diets may be masking the impact of insufficient protein in the prepartum diet.

Changes in protein metabolism at parturition

Similar to the requirements of glucose, the requirements for protein increase dramatically at calving. Bell et al. (2000) estimated that 2.3 times more protein is required the first week after calving compared to just prior to parturition. At this point the cow encounters not only a period of negative energy balance but also negative protein balance. Metabolizable protein (MP) balance reaches a nadir at 7 d postpartum with equilibrium being achieved at approximately 3-4 weeks postpartum (Bell et al, 2000).

To account for this negative energy balance, the body experiences metabolic changes, some of which were discussed earlier in regards to carbohydrate and lipid metabolism. Similar changes exist in protein metabolism as amino acids are mobilized from labile protein sources; up to 25% of the body protein can be mobilized (Botts et al., 1979). An estimated 8 to 9 kg of protein was lost in cows following parturition, but it was not possible to determine from what pool the protein was mobilized (Belyea et al., 1978). The ratio of 3-methyl histidine to creatinine increased at d 1 postpartum and slowly declined through 21 d postpartum indicating that skeletal muscle was degraded (Overton et al., 1998). In dairy goats, the hind-limb muscle and nitrogen composition was decreased three weeks postpartum compared to dry, non-lactating

goats (Baracos et al., 1991). The increased protein requirement at parturition causes not only increased protein degradation, but also decreased synthesis of muscle and skin thereby sparing amino acids (Baracos et al., 1991.) To further illustrate the effects of negative energy balance, blood albumin concentration consistently decreases postpartum for a period of one to three weeks (Little, 1974; Manston et al., 1975; Treacher et al., 1976), and long-term protein status has been shown to affect blood albumin concentration (Manston et al., 1975). The fall in albumin concentration postpartum may be a result of decreased liver synthesis due to lowered amino acid availability (Little, 1974). Despite mobilization of protein from muscle, there is an increase in the mass of liver and other visceral tissues postpartum (Gibb et al., 1992), which also may be responsible for the increasing amino acid demand postpartum and negative nitrogen balance.

Amino acid mobilization may be responsible for a small portion of gluconeogenesis, as discussed briefly earlier in this review. The net portal and splanchnic fluxes of essential amino acids (AA) were not sufficient to account for essential AA output by the mammary gland, suggesting endogenous contribution of AA by interorgan transfer and that, at least indirectly, protein may be limiting at 4 d postpartum (Larsen and Kristensen, 2009a). Alanine was cited as the largest contributor to hepatic gluconeogenesis in postpartum cows (Larsen and Kristensen, 2009a), and the contribution of alanine to total glucose output increased two to three weeks postpartum compared to three weeks before calving (Overton et al., 1998; Reynolds et al., 2003). In fasted cows, the contribution of alanine, glycine, serine and threonine increased from 8.6% to 36.8% of total hepatic glucose output by d 4 of feed restriction (Lomax and Baird, 1983).

Oxidation of amino acids for energy also occurs postpartum and generates ammonia, which has toxic effects on the body when present in excess. The urea cycle converts ammonia to non-toxic urea for excretion. The degree of lipid mobilization potentially decreases the

efficiency of ureagenesis in the liver. The rate of ureagenesis was reduced in bovine hepatic cells *in vitro* with increasing hepatic triglyceride accumulation (Strang et al., 1998) and *in vivo* liver triglyceride concentration was positively related to plasma ammonia concentrations in cows 22 h postpartum suggesting that decreased ureagenesis increases ammonia concentrations postpartum (Zhu et al., 2000).

Increasing ammonium chloride decreased propionate conversion to glucose *in vitro* using liver cells from lactating dairy goats (Aiello and Armentano, 1987), and decreased ureagenesis may indirectly reduce glucose production in fresh cows with high concentrations of hepatic triglyceride (Drackley, 1999). In agreement with HOT (Allen et al., 2009), the decreased rate of conversion of propionate to glucose may be partly to blame for the reduction in DMI in fresh cows due to increased oxidation of acetyl-CoA in the TCA cycle facilitated by propionate entry into the cycle increasing the capacity to oxidize acetyl-CoA. Furthermore, intraruminal infusions of ammonium propionate decreased DMI in mid-lactation cows by decreasing the number of meals compared to infusion of sodium acetate, ammonium acetate and sodium propionate (Oba and Allen, 2003b). Dry matter intake for the ammonium propionate-treated cows was less than half that for the ammonium acetate and sodium propionate treatments suggesting additive affects of ammonium and propionate. Considering that the amino acid aspartate is required for the urea cycle, the authors suggested that urea synthesis may have stimulated oxidation of amino acids thereby increasing the energy status of cells. Based upon the work of Aiello and Armentano (1987), if the ammonium chloride in Oba and Allen (2003b) decreased propionate conversion to glucose, then the addition of propionate to the treatment may have overwhelmed the liver's gluconeogenic capacity and caused oxidation of propionate

thereby increasing satiety. Additionally, the urea cycle produces the TCA cycle intermediate fumarate, which may serve as a conduit for oxidizing acetyl-CoA, thus decreasing intake.

Essential amino acids methionine and lysine may improve performance in early lactation as both have long been noted as limiting in lactating dairy cows (National Research Council, 2001). Methionine is an important component of apolipoprotein formation (Grummer, 1993), and it has been speculated that methionine may be important in the prevention of fatty liver and ketosis (McCarthy et al., 1968) by increasing very low density lipoprotein secretion. However, subsequent studies in the transition period or with induced fatty liver have not noted positive effects on hepatic metabolism as a result of methionine supplementation in the form of protected methionine or its analog 2-hydroxy-4(methylthio)-butanoic acid (Bertics and Grummer, 1999; Piepenbrink et al., 2004). Many studies, however, have shown a positive milk or FCM response to methionine supplementation (Overton et al., 1996; Piepenbrink et al., 2004). The reason for the lack of response on hepatic metabolism could be due to the fact that ruminants have inherently low very-low density lipoprotein (VLDL) production in the liver with little ability to increase VLDL synthesis (Grummer, 1993).

Dietary protein concentration and performance of fresh cows

For early lactation cows estimated to be consuming 14.5 kg dry matter (DM) at 11 DIM and producing 30 kg milk/d at 3.5% fat and 3% protein, the estimated requirement by the National Research Council (NRC, 2001) for dietary CP is 18.8%. Using data from 393 treatment means from 82 protein studies, the NRC (2001) determined that milk yield increases quadratically as CP content in a diet increases. Furthermore, Broster and Broster (1984) outlined numerous studies using cows in different stages of lactation demonstrating positive residual

effects from increasing the dietary protein concentration with a lesser number of studies demonstrating a negative residual effect. However, very few researchers have examined the performance of cows fed diets containing differing crude protein concentrations during the postpartum period, while a larger number have focused on the effects over the first several months of lactation. Without a doubt, protein nutrition postpartum plays a critical role during the fresh period and in subsequent lactation performance.

In support of the NRC (2001) prediction equation, increasing CP concentration increased milk yield for early lactation cows ((Roffler et al., 1978; Oldham et al., 1979; Sparrow et al., 1973; Journet and Rémond, 1981). This is in contrast to the results of Van Horn et al. (1976) where diets of 12, 14.5 or 17% CP fed from two to eight weeks postpartum did not affect intake, milk yield or milk components. Additionally, milk yield appears to respond rapidly to changes in dietary CP concentration. A reduction in dietary CP from 15.8 to 11.5% three weeks postpartum decreased milk yield significantly; however, milk yield increased to previous levels after returning to the high protein diet (Robinson et al., 1979).

Several studies have been conducted to examine the effects of increasing the protein supply to the lower intestine on performance during early lactation. Abomasal infusion of casein during feed restriction increased milk yield, milk fat concentration, FCM and milk protein yield (Ørskov et al., 1977). Abomasal infusions of casein or soy protein in cows fed alfalfa-based forage diets without concentrates increased milk yield, FCM, fat yield and protein yield (Dhiman et al., 1993). Similar studies conducted later in lactation noted increases in protein yield and protein concentration only (Clark et al., 1977) or no changes at all (Abramson et al., 2002). Increasing dietary CP content with fish meal increased milk yield by increasing non-ammonia nitrogen flow to the abomasum in postpartum ewes (Robinson et al., 1979), and ewes produced

more milk in weeks 2-3 postpartum at the same ME intake when fed a diet containing fish-meal compared with soybean meal or groundnuts (Robinson et al., 1979). Accordingly, models of protein nutrition demonstrate that milk yield increases with increasing RUP (NRC, 2001). Santos et al. (1998) note that, while only 17% of studies evaluating RUP effects on milk production have a positive milk production effect, heat-treated soybean meal and fish meal are most likely to increase milk production. Overall, it appears that RUP may have a significant impact on production performance in the postpartum period and warrants further investigation.

Negative energy balance and loss of body weight or BCS have been noted with increasing dietary protein or abomasal protein infusion, and a concomitant increase in milk yield also occurred (Sparrow et al., 1973; Robinson et al., 1974; Ørskov et al., 1977; Roffler et al., 1978; Journet and Rémond, 1981; Ørskov et al., 1987). Some reports showed negative energy balance despite no change in dry matter or energy intake (Robinson et al., 1974; Ørskov et al., 1977; Journet and Rémond, 1981; Ørskov et al., 1987). It is unclear why energy balance and body weight loss increase due to increased dietary protein, but if protein permits the cow to produce more milk it may increase lipid mobilization to support the concurrent increased energy requirements. Abomasal protein infusion increased plasma NEFA with decreasing glucose infusion (Ørskov et al., 1977), and in another study numerically increased plasma NEFA and BHBA concentrations despite no changes in plasma glucose or insulin concentrations (Dhiman et al., 1993). Fat mobilization accounts for an increasing portion of milk fatty acids during negative energy balance (Bauman and Griinari, 2003). Although not measured in other studies, higher circulating plasma NEFA concentration may have been responsible for increased milk fat production, milk fat concentration or FCM observed with greater dietary CP concentration

(Sparrow et al., 1973; Roffler et al., 1978; Oldham et al., 1979; Ørskov et al., 1987) or abomasal protein infusion (Ørskov et al., 1977; Dhiman et al., 1993).

Another reason for the greater negative energy balance could be due to fewer glucose precursors available from less starch in the diet; in most cases protein was added by substituting for a starch source. Unfortunately, there is little data in these papers focusing on blood metabolites to verify if diet affected metabolites and hormones. Plasma glucose was not different in week three postpartum for ewes when fish meal was substituted for barley where energy balance was unaffected (Cowan et al., 1981). Similarly, plasma glucose was unaffected despite diets of 10.5 and 22.3% CP where soybean meal replaced barley; however, cows on the low protein diet lost more weight over time and produced less milk fat than the cows fed high protein (Oldham et al., 1979). This situation of greater energy deficit and body weight loss from diets with greater dietary CP concentration may impact subsequent lactation and reproductive performance, and thus warrants more investigation.

RESEARCH OBJECTIVES AND HYPOTHESIS

The objective of the study described within this thesis is to determine DMI, milk production and metabolic responses to intravenous glucose infusion and a diet of high starch, low protein concentration, or a diet of high protein, low starch concentration. We hypothesize that a diet of high protein and low starch concentration combined with intrajugular glucose infusion will increase milk yield, resulting in increased glucose demand, and thus reduce plasma insulin concentration and the buildup of TCA cycle intermediates available to oxidize acetyl CoA. This will decrease satiety and increase dry matter intake.

CHAPTER 2

EFFECTS OF INTRAJUGULAR GLUCOSE INFUSION ON FEED INTAKE, MILK YIELD AND METABOLIC RESPONSES OF EARLY POSTPARTUM COWS FED DIETS VARYING IN PROTEIN AND STARCH CONCENTRATION

ABSTRACT

Effects of glucose infusion on feed intake, milk production, and metabolic responses of early postpartum cows fed a high starch/low protein diet or a high protein/low starch diet were evaluated utilizing a randomized complete block design with a 2x2 factorial arrangement of treatments. Twenty-four multiparous Holstein cows were blocked by BCS and 305-ME milk production, and randomly assigned at calving to one of four treatments. Treatments were continuous intrajugular infusion of glucose (GI; 1 kg/d as 4 L/d of 25% w/v dextrose) or isotonic saline (SI; 4 L of 0.9% sodium chloride), and diets containing high starch/low crude protein (HSLP) or high crude protein/low starch (HPLS) concentration. Treatments were initiated at the first scheduled feeding following parturition and lasted 12 d. Data for DMI were collected daily. Blood was collected every 2 d beginning on d 0, milk yield was collected at each milking, milk samples were obtained on d 4, 8, and 12, and liver samples were collected on d 0, 6, and 12. Data were analyzed by ANOVA, with repeated measures when applicable. The GI reduced cumulative DMI for HPLS (164.0 vs. 196.5 kg) but not HSLP (194.2 vs. 189.7 kg) compared with SI (interaction P = 0.04) and tended (interaction P = 0.12) to reduce daily DMI for HPLS (13.6 vs. 16.4 kg/d) but not HSLP (16.4 vs. 15.9 kg/d). Similarly, GI tended to decrease meal size for HPLS (1.21 vs. 1.52 kg) but not HSLP (1.55 vs. 1.49) compared with SI (interaction P = 0.14). GI did not affect cumulative milk yield for HPLS (441.6 vs. 437.8 kg) but increased milk yield for HSLP (469.1 vs. 395.0 kg) compared with SI (interaction P = 0.02). The GI increased cumulative milk yield 39 kg compared with SI (455 vs. 416 kg; *P* = 0.01). The HPLS treatment

tended to increase loss of body condition from 0.65 to 0.82 BCS units/12 d (P = 0.06) compared with HSLP. Consistent with this, HPLS increased plasma concentrations of NEFA (1184 vs. 895 μEq/L, P = 0.01) and BHBA (13.0 vs. 9.5 mg/dL, P = 0.03), liver triglyceride (3.5 vs. 5.7 mg/g wet liver, P = 0.03), milk fat concentration (5.71 vs. 4.72%, P < 0.01) and yield (2.14 vs. 1.78 kg/d, P < 0.01) and 3.5% fat-corrected milk (50.9 vs. 45.1 kg, P = 0.02) compared with HSLP. HPLS increased plasma NEFA, liver triglyceride and BCS loss despite no difference in plasma insulin between dietary treatments (P = 0.23). Elevated liver triglyceride from HPLS and elevated plasma insulin from GI may have interacted to reduce intake for GI-HPLS by reducing meal size.

INTRODUCTION

The transition period is widely recognized as the most critical period in the lactation cycle of dairy cattle, and is especially marked by suppressed dry matter intake (DMI) and a period of negative energy balance. Interest in understanding the mechanisms of feed intake around parturition is great due to the association of low feed intake with costly health disorders, such as ketosis and displaced abomasum. The hepatic oxidation of fuels in the liver was proposed as a mechanism by which feed intake is depressed during the transition period, and is broadly referred to as the hepatic oxidation theory (HOT; Allen et al., 2009). During the transition period, plasma insulin concentration is low (Douglas et al., 2006), and when combined with muscular and adipose tissue insulin resistance (Bell and Bauman, 1997), leads to increased lipid mobilization as non-esterified fatty acids (NEFA). The liver takes up circulating plasma NEFA, and beta-oxidation produces acetyl Coenzyme A (CoA). According to HOT, oxidation of acetyl CoA within a meal from propionate influx produces ATP very quickly, generating a

satiety signal and decreasing meal size and subsequently feed intake in cows immediately post-partum (Allen et al., 2009).

Larsen and Kristensen (2009) infused glucose into the abomasum of cows for 29 d after calving to determine if glucose was limiting milk production in early lactation. Unexpectedly, the infusion prevented the normal postpartum increase in DMI and milk yield. It is possible that the suppression of DMI was related to the increase in plasma insulin concentration; glucose infusion increased plasma insulin concentration three-fold (Larsen and Kristensen, 2009). Elevated plasma insulin concentration decreased hepatic glucose output in sheep (Brockman, 1985), and elevated liver acetyl CoA concentration from beta-oxidation decreases conversion of pyruvate to acetyl CoA by inhibiting pyruvate dehydrogenase complex (PDC; Roche et al., 2001). Propionate influx from a meal during down-regulation of gluconeogenesis and inhibition of PDC increases the pool size of tricarboxylic acid (TCA) intermediates, enhancing oxidation of acetyl CoA within meals, causing satiety sooner (Allen et al., 2009).

In a subsequent paper using liver flux data from the same study, Larsen and Kristensen (2009a) determined that essential amino acids (AA) may have been limiting milk yield in early lactation. The diet offered to the cows in that study contained only 15.6% crude protein (CP). According to the NRC (2001), milk yield increases quadratically with increasing dietary CP concentration. Furthermore, dairy cows are in a negative nitrogen balance for several weeks following parturition, highlighting the importance of adequate protein supply in early lactation (Bell et al., 2000). If dietary protein limited milk yield in the cows infused with glucose by Larsen and Kristensen (2009a), use of glucose for lactose synthesis might have been limited, thus increasing plasma insulin concentration and initiating the cascade resulting in lower DMI.

Objectives of this study were to determine if dietary CP concentration limits milk yield of cows in the immediate postpartum period in the presence of sufficient glucose when fed as a high protein, low starch diet, and to determine if a diet with high dietary protein and low starch concentration would attenuate the increase in plasma insulin concentration by glucose infusion and allow for greater feed intake. We hypothesized that a high protein, low starch diet with glucose infusion will increase milk yield, resulting in increased glucose demand, and thus reduce plasma insulin concentration and the buildup of TCA cycle intermediates available to oxidize acetyl CoA. This will decrease satiety and increase dry matter intake.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. The study was conducted from June 25 to September 18, 2011.

Animals, Experimental Design, and Treatments

Twenty-four multiparous Holstein cows from the Michigan State University Dairy Field Laboratory were assigned randomly to treatment in a randomized complete block design experiment with a 2x2 factorial arrangement of treatments. Cows were randomly assigned to block and treatment on the day of calving. Blocking criteria were body condition score (BCS; within one unit) and previous lactation ME milk production (within 4,500 kg). Treatments were continuous intrajugular drip infusion of glucose (GI; 1 kg dextrose/d as 4 L/d of 25% w/v dextrose in water) or isotonic saline (SI; 4 L of 0.9% NaCl), and isoenergetic diets containing 13.9% CP and 29.6% starch (HSLP), or 18.3% CP and 22.3% starch (HPLS). Both diets were

~33.5% neutral detergent fiber (NDF), and 29.6% forage NDF. CP and starch concentration of diets were adjusted by substituting soybean meal for dry ground corn (Table 1). Other diet ingredients were corn silage, grass hay, wheat straw, SoyPlus, and a mineral and vitamin mix (Table 1). Ingredients and nutrient composition of the prepartum diets are listed in Table 2. Mineral concentrations and NE_L listed in Tables 1 and 2 represent formulated values.

Treatments were initiated at 1200 h at the first scheduled feeding following parturition and lasted 12 d. Infusion bags were replaced every 6 h at 0600, 1200, 1800 and 2400 h daily.

Cows were fitted with indwelling catheters in each jugular vein 4 ± 3 d prior to expected calving date. Five milliliters of lidocaine hydrochloride was administered intradermally at the catheter insertion point. A stab incision was made with a #15 scalpel blade, and a pre-sterilized 10 gauge needle was inserted into the jugular vein. Pre-sterilized indwelling polypropylene catheters (0.24 cm o.d. x 0.17 cm i.d. tubing, MRE 085, Braintree Scientific, Braintree, MA) were inserted into the vein through the needle until approximately 30 cm of tubing was inside the jugular vein. Patency for catheters was checked daily prior to the initiation of infusion.

Data and Sample Collection

Throughout the experiment, cows were housed in tie stalls, fed once daily at 1200 h at 115% of expected intake. Cows were blocked from feed from 1000 to 1200 h daily, and the amount of feed offered and orts were weighed for each cow daily. Samples of all dietary ingredients and orts (12.5%) were collected daily. Dietary ingredients were composited weekly throughout the experiment and orts for each cow were composited for the 12-d treatment period. Feeding behavior was monitored continuously for 22 h daily for each cow while cows had access to feed (1200 to 1000 h) by a computerized data acquisition system (Dado and Allen, 1993).

Data of feed disappearance and voluntary water consumption were recorded for each cow every 5 s, and meal bouts, interval between meals, meal size, eating time, drinking bouts and water intake were calculated.

Cows were milked twice daily in their stalls at 0530 and 1700 h. Milk yield was recorded daily at each milking, and milk samples were collected at each milking on d 4, 8 and 12. Body weight was measured once at the beginning and conclusion of treatment administration. Body condition score (BCS) was measured 4 ± 3 d prior to calving and at the conclusion of treatment by 3 trained investigators, where 1 = thin and 5 = fat (Wildman, 1982).

Blood samples were collected at 1100 h on d 0 before treatment initiation and on d 2, 4, 6, 8, 10 and 12. Blood was sampled from coccygeal vessels and collected into 3 evacuated tubes, two containing potassium EDTA and one containing potassium oxalate with NaF as a glycolytic inhibitor. Tubes were centrifuged at 3,000 x *g* for 15 min immediately after sample collection, and plasma was harvested and frozen at -20˚C until analysis. A portion of the samples containing K_3EDTA were preserved with benzamidine (0.05 *M* final concentration) to reduce glucagon proteolysis.

Liver samples were collected between 1000 and 1200 h on d 0 before treatment initiation and on d 6 and 12 for analysis of gene expression and triglyceride content. After local anesthetization with 2% lidocaine hydrochloride, a 14-gauge biopsy instrument was inserted between the 11th and 12th ribs on a line between the olecranon and the tuber coxae on the right side. Five samples weighing approximately 20 mg each were collected. Samples were immediately (within 5 s) flash frozen in liquid nitrogen and stored on dry ice for transportation. Samples were promptly stored at -80˚C until further analysis.

Sample Analysis

Diet ingredients and orts were dried in a 55˚C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, CP, and starch. Ash concentration was determined after 5 h of oxidation at 500˚C in a muffle furnace. Concentrations of NDF were determined according to Mertens (2002). Crude protein was measured according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured with a glucose oxidase method (Glucose kit #510, Sigma Chemical Co., St. Louis, MO) and absorbance was determined with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp., Sunnyvale, CA). Concentrations of all nutrients except DM were expressed as percentages of DM determined by drying at 105˚C in a forced-air oven for more than 8 h.

Plasma samples were analyzed colorimetrically with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp., Sunnyvale, CA) to determine the plasma concentrations of metabolites. A glucose oxidase method was used to determine plasma glucose concentration (Sigma Chemical Co. St. Louis, MO), and a commercial kit was used for determination of plasma NEFA (HR Series NEFA-HR 2, Wako Chemicals USA, Richmond, VA), and BHBA (Procedure #2440, Stanbio Laboratory, Boerne, TX). An enzyme-linked immunosorbant assay kit was used to determine concentration of plasma insulin (Kit # 10-1201-01, Mercodia, Uppsala, Sweden). Plasma glucagon concentration was determined by a radioimmunoassay kit (Kit # GL-32K, Linco Research, Inc., St. Charles, MO).

Milk yield recorded at both milkings were summed for a daily total. Milk samples were analyzed for fat, true protein, lactose, solids, somatic cell count (SCC) and milk urea nitrogen

(MUN) using infrared spectroscopy by Michigan DHIA (East Lansing). Daily yields of energy-corrected milk (ECM; Dairy Records Management Service, 2011), 3.5% fat-corrected milk (FCM; Dairy Records Management Service, 2011) and milk components were calculated using milk yield and component concentrations for each milking on respective days. Gross feed conversion efficiency was calculated by dividing milk yield by DMI.

RNA Extraction and Real-Time Quantitative PCR

Total RNA was isolated from 20 mg frozen liver samples using a PerfectPure RNA Tissue Kit (5 PRIME, Inc., Gaithersburg, MD) and genomic DNA was removed using the RNase-Free DNase Set (5 PRIME, Inc.) and an isopropanol precipitation procedure. RNA quantity was determined using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and quality was verified by automated capillary gel electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA).

Gene expression of selected genes was determined using real-time quantitative PCR (qPCR) on a 7900 HT Fast-Real Time PCR System (Life Technologies Corporation, Carlsbad, CA). Primers for analysis of gene expression were developed using Primer Express 3.0 (Life Technologies Corp.) and primer sequences for each gene were commercially synthesized (Sigma-Aldrich, St. Louis, MO). Sequences, products sizes and accession numbers of primers are summarized in Table 7. cDNA was reverse transcribed from 900 ng total RNA as a template using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies Corporation, Carlsbad, CA). cDNA amplification efficiency was validated for endogenous control and target genes with pooled cDNA from all samples using a 5-fold dilution series. After validation of the endogenous control and target genes, relative gene expression was determined in triplicate for each sample. Each reaction was performed using 5 uL Power SYBR Green (Life Technologies

Corp.), 3 uL of a forward and reverse primer mix (1 uM) and 2 uL of sample (2.5 ng cDNA/uL). Amplification conditions for quantification were 2 min at 50˚C, 10 min at 95˚C, and 40 cycles of 15 sec at 95˚C and 1 min at 60˚C. The relative gene expression was determined using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) with HPRT1 as the endogenous control gene. Gene expression is reported as the fold difference of a gene compared to the expression on d 0, which was normalized to 1.

Statistical Analysis

Using the MIXED procedure of SAS (Version 9.2, SAS Institute, 2008) ANOVA was conducted using a randomized block design with repeated measures and an autoregressive covariance structure based upon the Bayesian Information Criterion. Block and cow were included as random effects. The effect of treatments on metabolic and milk responses and feeding behavior were analyzed with the following linear model:

$$Y_{ijm} = \mu + B_i + C(B_iG_kP_l)_j + G_k + P_l + G_kP_l + T_m + G_kT_m + P_lT_m + G_kP_lT_m + J + e_{ijm}.$$

Where μ = overall mean, B_i = random effect of block (i = 1 to 6), $C(B_iG_kP_l)_j$ = random effect of cow (j = 1 to 4) within block and infusion and dietary treatments, G_k = fixed effect of infusion treatment (k = 1 to 2), P_l = fixed effect of dietary treatment (l = 1 to 2), T_m = fixed effect of time (m = 1 to 12), G_kP_l = interaction of infusion and diet, G_kT_m = interaction of infusion and time, P_lT_m = interaction of diet and time, $G_kP_lT_m$ = three-way interaction of infusion, diet and time, J = random effect of Julian date, and e_{ijm} = residual. Julian date was included to account for variation from individual day effects.

Effects of treatments on gene expression were analyzed with a linear model, where:

$$Y_{ijm} = \mu + B_i + C(B_iG_kP_l)_j + G_k + P_l + G_kP_l + T_m + G_kT_m + P_lT_m + G_kP_lT_m + e_{ijm}$$

Julian was removed from the model and cow was no longer included as a random effect because the covariance parameter estimates for each were equal to 0.

Effects of treatment on body weight and BCS change, and cumulative DMI and milk were analyzed using a linear model without repeated measures, where:

$$Y_{ij} = \mu + B_i + C(B_iG_kP_l)_j + G_k + P_l + G_kP_l + J + e_{ij}.$$

Treatment effects were declared at $P < 0.05$ and tendencies for treatment effects at $P < 0.10$. Interactions were declared significant at $P < 0.10$ and tendencies for interactions at $P < 0.15$.

RESULTS

Feed Intake and Feeding Behavior

Infusion and diet interacted to affect cumulative DMI ($P = 0.04$); GI decreased cumulative DMI when HPLS was fed (164 vs. 197 kg) but not when HSLP was fed (194 vs. 190 kg; Table 3a). Similarly, a tendency existed for an interaction of infusion and diet on daily DMI ($P = 0.12$) whereby GI decreased DMI 2.8 kg when HPLS was fed (13.6 vs. 16.4 kg), but did not affect DMI with HSLP (16.4 vs. 15.9 kg; Table 3a). Infusion, diet and time interacted for meal size ($P = 0.07$) in which meal size increased more slowly over time for GI-HPLS compared to the other treatments (Figure 1). Infusion and time interacted for meal size ($P = 0.05$) whereby GI increased meal size more slowly over time than SI (Figure 1). Infusion and diet interacted for meal size ($P = 0.14$); GI decreased meal size 0.31 kg for HPLS (1.21 vs. 1.52 kg) but not for HSLP (1.55 vs. 1.49; Table 3a). There was a three-way interaction of infusion, diet and time

affecting meal frequency ($P = 0.1$) in which the response across time was different among the treatments (Figure 2). Infusion, diet and time tended to interacted for meal length ($P = 0.13$) whereby the response across time varied among the 4 treatments (Figure 3). Infusion and diet interacted for meal length ($P = 0.10$); GI decreased meal length for HPLS (24.5 vs. 28.4 min) but increased meal length for HSLP (27.7 vs. 24.5 min; Table 3a). A tendency for an interaction of infusion and time existed for meal interval ($P = 0.12$) because the response across time was different among the 4 treatments (Figure 4).

Infusion and time interacted to affect water intake ($P = 0.07$); the SI increased water intake over time (Figure 5). Accordingly, SI increased water intake 22.4% (76.5 vs. 62.5 L/d; $P < 0.01$; Table 3a) and the number of drinking bouts 30.7% compared with GI (14.9 vs. 11.4; $P < 0.01$; Table 3a). HPLS increased the number of drinking bouts/d 22.9% compared with HSLP (14.5 vs. 11.8; $P = 0.02$; Table 3a) but had no effect on water intake ($P = 0.57$).

Milk Production and Components

Infusion and diet interacted to affect cumulative milk yield ($P = 0.02$); HSLP, compared with HPLS, increased yield 6.1% for GI (469 vs. 442 kg) but decreased yield 9.8% for SI (395 vs. 438 kg; Table 4a). GI increased cumulative milk yield 39 kg compared with SI (455 vs. 416 kg; $P = 0.01$; Table 4a). Similar to cumulative milk yield, there was a tendency for an interaction of treatments for daily milk yield ($P = 0.12$) whereby HSLP, compared with HPLS, increased yield 2.1 kg for GI (38.9 vs. 36.8 kg) but decreased yield 3 kg for SI (33.5 vs. 36.5 kg; Table 4a). GI numerically increased daily milk yield 2.9 kg compared with SI (37.9 vs. 35.0 kg/d; $P = 0.08$; Table 4a). Infusion, diet and time interacted to affect daily milk yield ($P = 0.08$); SI-HSLP decreased milk yield the first 6 d of treatment and GI-HSLP yielded more milk the last

6 d of treatment (Figure 6). GI increased feed efficiency compared with SI (2.54 vs. 2.18; $P < 0.01$; Table 4a), and HPLS increased feed conversion efficiency compared with HSLP (2.48 vs. 2.22; $P = 0.03$; Table 4a).

The HPLS diet, compared with HSLP, increased milk fat concentration (5.71 vs. 4.72%; $P < 0.01$; Table 4a), milk fat yield (2.13 vs. 1.78 kg/d; $P < 0.01$; Table 4a), FCM (50.9 vs. 45.1 kg/d; $P = 0.02$; Table 4a), and tended to increase ECM (49.8 vs. 45.2 kg/d; $P = 0.06$; Table 4a). GI decreased milk fat concentration (4.84 vs. 5.59%; $P = 0.01$; Table 4a) but did not affect milk fat yield compared with SI ($P = 0.32$; Table 4a). There was no effect of treatment on protein yield, but GI decreased protein concentration compared with SI (3.27 vs. 3.50%; $P = 0.02$; Table 4a). GI, compared with SI, increased lactose concentration (4.78 vs. 4.59%; $P < 0.01$; Table 4a), and tended to increase lactose yield (1.87 vs. 1.69 kg/d; $P = 0.08$; Table 4a). HPLS doubled MUN concentration compared with LP (19.0 vs. 9.31 mg/dL; $P < 0.01$; Table 4a). There was no effect of treatment on SCC (Table 4a).

Plasma Metabolites and Hormones, Liver Triglyceride and Body Condition Loss

There was an interaction of treatments on plasma glucose concentration ($P = 0.07$); HPLS decreased plasma glucose concentration for SI (42.5 vs. 51.6 mg/dL; Table 5a) but not for GI (60.1 vs. 59.9 mg/dL; Table 5a). GI increased plasma glucose concentration 27.7% compared with SI (60 vs. 47 mg/dL; $P < 0.01$; Table 5a) and HSLP numerically increased plasma glucose 8.8% compared with HPLS (55.8 vs. 51.3 mg/dL; $P = 0.08$; Table 5a). Infusion and time interacted for plasma insulin concentration ($P < 0.01$); GI increased plasma insulin more over time compared with SI (Figure 7). Accordingly, GI increased plasma insulin concentration compared with SI (0.53 vs. 0.18 µg/L; $P < 0.01$; Table 5a). There was an interaction of infusion

and time on plasma glucagon concentration ($P < 0.01$) whereby GI increased plasma glucagon less over time compared with SI (Figure 8). GI decreased plasma glucagon concentration 17.8% compared with SI (93.1 vs. 113.2 pg/mL; $P = 0.01$; Table 5a). Infusion and time interacted for the insulin:glucagon ratio ($P < 0.01$); the ratio became greater over time for GI than for SI (Figure 9). GI increased the insulin:glucagon ratio (5.77 vs. 1.60; $P < 0.01$; Table 5a).

In agreement with the plasma hormone concentrations and ratios, infusion interacted with time to affect plasma NEFA concentration ($P < 0.01$; Figure 10) and plasma BHBA concentration ($P < 0.01$; Figure 11); plasma NEFA and BHBA increased over time for SI whereas GI prevented an increase in concentration of both metabolites. GI, compared with SI, decreased plasma NEFA concentration 46.5% (724 vs. 1354 µEq/L; $P < 0.01$; Table 5a), plasma BHBA concentration 52.3% (7.2 vs. 15.1 mg/dL; $P < 0.01$; Table 5a), and liver triglyceride concentration 49.7% (3.16 vs. 6.28 mg/g wet liver; $P < 0.01$; Table 5a) . Despite decreased plasma NEFA and BHBA and liver triglyceride for GI, there was no effect of infusion on BCS change ($P = 0.25$; Table 6a) or body weight change ($P = 0.16$; Table 6a).

HPLS, compared with HSLP, tended to increase BCS loss (-0.81 vs. -0.65 BCS units; $P = 0.06$; Table 6a), but there was no effect of treatment on body weight change (Table 6a). The increased BCS loss for HPLS resulted in increased plasma concentrations of NEFA 32.3% (1184 vs. 895 µEq/L; $P = 0.01$; Table 5a) and BHBA 34.4% for HPLS compared with HSLP (12.1 vs. 9.0 mg/dL; $P = 0.02$; Table 5a). Furthermore, HPLS increased hepatic triglyceride concentration 39.6% (5.71 vs. 3.56 mg/g wet liver; $P = 0.03$; Table 5a) compared with HSLP.

Gene Expression

There was no difference in the expression of HPRT1 across treatments and time ($P > 0.1$; results not shown), and thus it was deemed to be an acceptable endogenous control gene for this study.

The SI decreased expression of PC at d 6 compared with GI causing an interaction of infusion and time ($P = 0.1$; Figure 12). A three-way interaction of infusion, diet and time existed for PCK1 ($P = 0.04$); SI-HSLP increased expression of PCK1 less over time than the other treatments while GI-HSLP increased expression of PCK1 more over time (Figure 13). The GI increased the expression of PCK1 compared with SI ($P = 0.05$; 2.64 vs. 1.44 units; Table 8a). The HPLS diet decreased expression of G6PC after d 6, resulting in and interaction of diet and time ($P = 0.1$; Figure 14).

The GI increased the expression of PPARA over time, resulting in an interaction of infusion and time ($P = 0.05$; Figure 15). The GI increased expression of PPARA compared with SI ($P < 0.01$; 2.79 vs. 0.87 units; Table 8a). There was a three-way interaction of infusion, diet and time on HMGCS2 ($P = 0.01$; Figure 16); all treatments increased expression of HMGCS2 through d 6, but SI-HSLP and GI-HPLS decreased and SI-HPLS and GI-HSLP increased expression of HMGCS2 thereafter.

Infusion, diet and time interacted to affect gene expression for PDK4 ($P = 0.05$), whereby SI-HPLS increased expression of PDK4 on d 6 and 12, and GI-HSLP increased expression of PDK4 at d 12 (Figure 17). A tendency for an interaction between infusion and time existed for PDK4 ($P = 0.14$); the SI tended to increase expression of PDK4 more over time than GI (Figure 17). The HSLP diet decreased expression of CS after d 6, causing an interaction of diet and time ($P = 0.13$; Figure 18). The HPLS diet decreased expression of CS for SI (0.66 vs. 1.21 units) but not GI (1.54 vs. 1.56 units), resulting in an interaction of infusion and diet ($P =0.02$; Table 8a).

The GI increased the expression of CS compared with SI ($P < 0.01$; 1.55 vs. 0.90 units; Table 8a), and the HSLP diet increased expression of CS compared with HPLS ($P = 0.02$; 1.37 vs. 1.01 units; Table 8a).

The HSLP diet increased the expression of GLUD1 for GI (2.83 vs. 1.23 units) but not for SI (0.89 vs. 1.00 units) resulting in an interaction of infusion and diet ($P = 0.03$; Table 8a). The GI increased the expression of GLUD1 compared with SI ($P < 0.01$; 1.87 vs. 0.95 units; Table 8a), and HSLP tended to increase the expression of GLUD1 compared with HPLS ($P = 0.09$; 1.59 vs. 1.11 units; Table 8a). The HSLP diet increased the expression of ASS1 more over time than HPLS, resulting in a tendency for an interaction of diet and time ($P = 0.12$; Figure 19). There was a tendency for an interaction of infusion, diet and time for urea cycle enzyme ASL ($P = 0.14$); GI-HSLP increased expression of ASL over time, whereas GI-HPLS and SI-HSLP decreased expression of ASL over time (Figure 20). The GI increased expression of ASL for HSLP (1.67 vs. 0.62 units), but decreased the expression of ASL for HPLS (0.59 vs. 1.05 units) leading to an interaction of infusion and diet ($P = 0.03$; Table 8a).

DISCUSSION

Objectives of this study were to determine if dietary CP concentration limits milk yield of cows in the immediate postpartum period in the presence of sufficient glucose, and to determine if the HPLS diet would allow greater milk yield, thus attenuating the increase in plasma insulin concentration by GI and allow increased feed intake.

Contrary to our hypothesis, HPLS decreased DMI with GI. DMI is a function of meal size and meal frequency, and the reduction in DMI for GI-HPLS was because of a reduction in mean meal size over time for GI-HPLS with no effect on meal frequency compared with GI-

HSLP. The reduction in meal size suggests that satiety was increased within the timeframe of meals. Despite the reduction in intake for GI-HPLS, the present study failed to elicit a similar response to that observed by Larsen and Kristensen (2009) where abomasal glucose infusion reduced the overall treatment mean for DMI 6.2 kg ($P = 0.05$). In that study, GI increased plasma insulin concentration 2-3 fold, which may enhance hepatic oxidation of fuels by decreasing gluconeogenesis, consistent with the hepatic oxidation theory (HOT) of feed intake control (Allen et al., 2009). Hyperinsulinemic-euglycemic clamps decreased hepatic glucose output in feed-restricted ewes (Brockman, 1985), and hyperinsulinemic-euglycemic clamps decreased feed intake 33% in fresh cows by quadrupling plasma insulin concentration (Leury et al., 2003). In ruminants, a reduction in hepatic gluconeogenesis would force propionate to pursue a different biochemical pathway other than that for glucose synthesis. Propionate can be converted to pyruvate and then to acetyl-CoA by pyruvate dehydrogenase complex; however, this is greatly reduced for cows in the early post-partum period that have high concentrations of acetyl-CoA from beta-oxidation of NEFA as acetyl-CoA inhibits pyruvate dehydrogenase complex (Roche et al., 2001). Propionate flux to the liver increases greatly during meals (Benson et al., 2002) and propionate supply in excess of the enzymatic capability to convert it to glucose or acetyl-CoA will result in the buildup of TCA cycle intermediates. These TCA cycle intermediates provide an entry point for acetyl-CoA into the TCA cycle, which produces ATP and causes satiety. Stocks and Allen (2012) demonstrated the effect of propionate-stimulated oxidation of acetyl CoA on DMI by showing that hypophagic effects of propionate were enhanced with greater hepatic acetyl CoA concentration across cows.

In the present study, greater lipid mobilization by the HPLS diet combined with high insulin concentration from GI might have caused the reduction in meal size and feed intake in the

GI-HPLS treated cows. If HPLS increased the supply of acetyl-CoA to be oxidized while GI decreased gluconeogenesis, propionate flux within a meal could have increased the TCA intermediates and increased the rate of oxidation of acetyl CoA within meals. Over-conditioned cows at parturition generally have greater body weight loss postpartum, potentially contributing more to the pool of hepatic acetyl CoA. Ewes fed to gain weight during gestation weighed 8.1 kg more at parturition ($P < 0.05$), and had numerically greater weight loss postpartum (Rutter and Manns, 1986). Interestingly, the ewes that weighed more at parturition experienced a drastic reduction in intake postpartum when intrajugular glucose infusion was administered compared with ewes that did not gain weight during gestation.

Transition dairy cows experience an uncoupling of the somatotropic axis whereby growth hormone (GH) increases but IGF-1 decreases with a concomitant decrease in plasma insulin concentration (Rhoads et al., 2004). GH alters nutrient partitioning in a variety of ways, including an increase in basal adipose tissue lipolysis during negative energy balance (Bauman and Vernon, 1993). Administration of a hyperinsulinemic-euglycemic clamp 8 d postpartum tended to decrease plasma GH concentration and decreased plasma NEFA concentration (Butler et al., 2003). The elevated plasma insulin concentration by GI in the present study may have reduced the plasma GH concentration, potentially contributing to the reduced plasma NEFA for GI compared with SI.

While GI decreased plasma NEFA concentration, which might be expected to reduce the pool of acetyl CoA to be oxidized, we expect that the reduction in gluconeogenic capacity by insulin leading to a buildup of TCA cycle intermediates to be the largest determinant of oxidation of acetyl CoA. Fresh cows with elevated plasma insulin concentration from a

hyperinsulinemic-euglycemic clamp decreased feed intake despite 37-71% lower plasma NEFA concentration (Butler et al., 2003, Leury et al., 2003).

It is unclear why the HPLS diet in this study increased lipid mobilization despite no effect of diet on plasma insulin concentration. Propionate is a major end product of starch fermentation, and propionate increases insulin secretion (Sano et al., 1993). Therefore, it would be expected that the HSLP diet would produce more propionate compared with the HPLS diet and increase plasma insulin concentration. However, total glucose precursors provided by the two diets were likely similar because the HPLS diet also provided glucogenic AA. The discrepancy between the increased lipid mobilization for HPLS despite the lack of a diet effect on insulin concentration may be because the sampling time for blood was one hour before feeding compared with if postprandial samples had been obtained.

Plasma and ruminal ammonia concentration were not measured in this study; however, MUN concentration increased two fold for cows fed HPLS, suggesting increased ammonia production in the rumen. Increasing ammonium chloride decreased propionate conversion to glucose *in vitro* using liver cells from lactating dairy goats (Aiello and Armentano, 1987). If indeed ammonia synthesis increased in this study, resulting in decreased gluconeogenesis for HPLS, this could be another possible explanation for a buildup of TCA cycle intermediates to oxidize acetyl CoA, reducing DMI for GI-HPLS.

The increase in MUN concentration for HPLS also may have contributed to satiety by enhancing hepatic oxidation of amino acids. The amino groups of urea are derived from ammonia and the amino acid aspartate. Ammonia production increases with increasing dietary rumen undegradable protein, which results in greater α-amino nitrogen removal (Reynolds, 1992), and amino acid carbon skeletons may be oxidized rather than contribute to

gluconeogenesis (Parker et al., 1995). Infusion of ammonium with propionate interacted to reduce DMI, possibly by supplying additional carbon skeletons to the TCA cycle in the form of fumarate produced by the urea cycle (Oba and Allen, 2003b).

Intake was not likely influenced a large degree by milk production since intake did not follow the same pattern as that of milk yield. We hypothesized that GI-HPLS would increase milk yield, thus increasing feed intake. These results are inconclusive in regards to whether protein was limiting in early lactation; although SI-HSLP resulted in the lowest milk yield, GI-HSLP resulted in the greatest milk yield. Furthermore, GI increased cumulative milk yield compared with SI. Glucose is the precursor for lactose synthesis, with lactose being the main osmotic driver of milk production. The tendency for an increase in daily milk yield may be explained by the tendency for an increase in milk lactose yield. These results suggest that glucose may be more limiting than protein due to the increase in cumulative milk yield from glucose infusion compared with saline infusion.

The HPLS diet increased milk fat yield, and because there was only a tendency for GI to increase daily milk yield, HPLS increased milk fat concentration and fat-corrected milk. Elevated lipid mobilization can cause an increase in milk fat yield during negative energy balance (Bauman and Griinari, 2003). HPLS increased plasma NEFA concentration, which could have contributed to the increased milk fat. Alternatively, the HSLP diet may have altered ruminal biohydrogenation pathways causing milk fat depression (MFD; Bauman and Griinari, 2003). The HSLP diet offered was 29.6% starch compared with the 22.3% starch concentration of the HPLS diet. While HSLP was not considered a low forage diet at 29.5% forage NDF, the starch concentration was exceptionally high for early lactation cows and may have contributed to greater ruminal outflow of certain conjugated linoleic acid (CLA) isomers, inhibiting milk fat

yield. *Trans-10, cis-12* CLA inhibits milk fat yield in dairy cows by decreasing expression of genes in the mammary gland associated with milk fat synthesis (Baumgaurd et al., 2002). Furthermore, MFD induced by *trans-10, cis-12* CLA partitions energy toward fat stores by increasing the expression of lipogenic enzymes in adipose tissue (Harvatine et al., 2009). In the present study, the HSLP diet decreased milk fat yield, BCS loss and lipid mobilization, indicating that energy may have been partitioned toward adipose tissue. Unfortunately, because rumen volatile fatty acid and milk fatty acid samples were not obtained, it is impossible to determine definitively why HSLP decreased milk fat yield.

GI decreased milk fat concentration, which is consistent with previous research (Al-Trad et al., 2009). This could be because of the antilipolytic effect of insulin on adipose tissue. However, the reduction in milk fat concentration and protein concentration for GI is likely the result of a dilution factor; daily milk yield tended to increase for GI while milk fat yield and protein yield remained unchanged for GI.

The SI treatment increased water intake, which is consistent with infusion of salts into the rumen (Oba and Allen, 2003c). Unfortunately, no data exists with respect to the effect of water intake on the passage of ruminal liquid fractions during a drinking bout, so it remains possible that the increased water intake for SI may have altered the rumen ecosystem.

In the present study, the GI treatment increased plasma insulin concentrations; however, the expression of gluconeogenic enzyme PC was up-regulated on d 6 for the GI treatment compared with the SI treatment. This is in contrast to the results of Hammon et al. (2012) where hyperinsulinemic-euglycemic clamps decreased expression of PC in transition dairy cows. Glucose infusion tended to decrease expression of PC with increasing dose in mid-lactation cows (Al-Trad et al., 2009). Additionally, the hyperinsulinemic-euglycemic clamp decreased the

expression of cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (PEPCK) mRNA (Hammon et al., 2012), whereas GI in the present study increased expression of PCK1, a cytosolic isoform. Although diet and time interacted for G6PC, the lack of an effect of GI on G6PC mRNA in the present study contrasts with that of Al-Trad et al. (2009) where increasing dose of saline or glucose infusion both decreased expression of G6PC quadratically.

Increasing dietary CP concentration from 14.1% to 16.2% in prepartum cows through addition of a commercially available RUP product did not change the hepatic expression of PC or PEPCK mRNA (Hartwell et al, 2001). Accordingly, PC was not affected by diet in the present study; however, great differences occurred in the expression of PCK1 mRNA within diet, depending on the infusion treatment (Figure 13). Additionally, increasing RUP concentration did not affect expression of argininosuccinate synthetase in dry cows (Hartwell et al., 2001), but the HSLP diet increased expression of ASS1 mRNA over time in the present study. Compared with Harwell et al. (2001), the differences in dietary CP concentration between diets in the present study were not achieved through addition of a RUP product, but rather soybean meal was substituted for dry ground corn. Due to the greater CP concentration of soybean meal compared with corn grain, it can be assumed that the RUP content of the diet increased for HPLS compared with HSLP. However, comparison of the effects of RUP on gene expression between these two studies should be viewed with caution because ruminal protein digestibility of the diets was not determined.

Many studies have attempted to characterize hepatic gene expression over the transition period including several of the genes analyzed in this study. Unfortunately, there is a paucity of information available on the effects of dietary protein, starch concentration and glucose supply on gene expression during the early postpartum period, and we are not aware of other studies that

impose treatments to examine the effects of on gluconeogenic-, ureagenic-, and lipid-related gene expression during this period other than that recently reported by Hammon et al. (2012). As a result, this data is novel to the contribution of dairy nutrition. Because it does not necessarily support or confirm the results from the rest of this study, more research is necessary to understand the nutritional impacts of protein and glucose status on hepatic gene expression in the postpartum cow.

CONCLUSIONS

The GI treatment decreased cumulative DMI for cows fed HPLS. This might be attributed to the combined effects of elevated plasma insulin concentration for GI and increased plasma NEFA concentration and TCA cycle intermediates for HPLS resulting in increased oxidation of acetyl CoA within meals. The HPLS treatment increased MUN concentration possibly from greater ruminal ammonia production, which may have decreased gluconeogenesis from propionate and increased TCA cycle intermediates increasing oxidation of acetyl CoA. Additionally, elevated urea synthesis may have contributed to increased oxidation of amino acids in the urea cycle thus increasing satiety during meals. The GI-HPLS treatment combination resulted in the greatest milk yield while SI-HPLS resulted in the lowest milk yield; however, feed intake was likely not influenced a large degree by milk yield in this study. The HPLS treatment increased plasma NEFA concentration and liver triglyceride concentration despite no effect of diet on plasma insulin concentration. In addition, HPLS increased yield of milk fat, possibly from the greater plasma NEFA concentration, or less ruminal outflow of conjugated linoleic acid isomers known to inhibit milk fat yield. Future research should examine whether degree of lipid mobilization impacts feed intake postpartum during glucose infusion.

APPENDICES

APPENDIX A: TABLES

Table 1. Ingredients and nutrient composition of experimental diets

Item	HSLP	HPLS
Diet Ingredient, % of total DM		
Corn silage	50.0	50.0
Grass hay	7.3	7.3
Wheat straw	2.4	2.4
Dry ground corn grain	17.7	6.7
Soybean meal	10.5	21.4
SoyPlus	4.9	4.9
Mineral and vitamin mix	7.2	7.2
Chemical analysis, % of DM		
DM	54.8	53.9
NDF	33.4	33.6
Forage NDF	29.5	29.7
CP	13.9	18.3
Starch	29.6	22.3
Ash	6.02	6.61
NE_L, Mcal/kg[1]	1.59	1.59
Macro Minerals, % of DM[1]		
Ca	0.65	0.68
P	0.39	0.43
Mg	0.24	0.26
K	0.98	1.19
Na	0.30	0.30
Cl	0.25	0.25
S	0.21	0.25
Micro Minerals, ppm[1]		
Co	0.24	0.24
Cu	12.3	12.3
Fe	216	216
I	0.58	0.58
Mn	54.9	54.9
Se	0.29	0.29
Zn	50.7	50.7

[1]Formulated value

Table 2. Ingredients and nutrient composition of prepartum diets

Item	
Diet ingredient, % of total DM	
Corn silage	42.5
Grass hay	25.4
Dry ground corn grain	3.6
Soybean meal	14.7
SoyChlor	1.5
Mineral and vitamin mix	12.2
Chemical analysis, % of DM	
NDF	39.5
CP	14.1
Starch	20.0
Ash	7.6
Macro Minerals, % of DM[1]	
Ca	1.08
P	0.37
Mg	0.43
K	0.88
Na	0.08
Cl	0.77
S	0.22
Micro Minerals, ppm[1]	
Co	0.19
Cu	13.0
Fe	317.8
I	0.35
Mn	102
Se	0.3
Zn	46.65

[1]Formulated value

Table 3a. LSM of main effects of infusion and diet on feed intake and feeding behavior

	Saline		Glucose		
Item	HSLP[2]	HPLS[3]	HSLP	HPLS	SE
Feeding Behavior[1]					
DMI, kg	15.9	16.4	16.4	13.6	0.96
Meal Frequency	9.90	10.4	10.2	10.2	0.59
Meal Size, kg DM	1.49	1.52	1.55	1.21	0.12
Meal Length, min	24.5	28.4	27.7	24.5	2.07
Meal Interval, min	105	98.7	96.7	97.8	5.95
Water Intake, liters	72.9	80.2	63.9	61.2	4.29
Drinking bouts	13.4	16.4	10.3	12.5	1.10
Cumulative DMI, kg	190	197	194	164	10.1

[1]Recorded daily

[2]HSLP = high starch, low protein diet

[3]HPLS = high protein, low starch diet

Table 3b. Statistical probabilities of infusion and diet on feed intake and feeding behavior

	P-value					
Item	Inf[2]	Diet[3]	Inf*Diet[4]	Inf*Day[5]	Diet*Day[6]	Inf*Diet*Day[7]
Feeding Behavior[1]						
DMI, kg	0.26	0.25	0.12	0.34	0.41	0.44
Meal Frequency	0.91	0.64	0.73	0.65	0.59	0.10
Meal Size, kg DM	0.28	0.21	0.14	0.05	0.45	0.07
Meal Length, min	0.85	0.86	0.10	0.35	0.56	0.13
Meal Interval, min	0.43	0.65	0.52	0.12	0.66	1.00
Water Intake, liters	<0.01	0.57	0.23	0.07	0.49	0.80
Drinking bouts	<0.01	0.02	0.71	0.94	0.25	0.34
Cumulative DMI, kg	0.11	0.34	0.04	NA	NA	NA

[1]Recorded daily

[2]Inf = effect of infusion

[3]Diet = effect of diet

[4]Inf*Diet = interaction between infusion and diet

[5]Inf*Day = interaction of infusion and day

[6]Diet*Day = interaction of diet and day

[7]Inf*Diet*Day = 3-way interaction among infusion, diet, and day

Table 4a. LSM of main effects of infusion and diet on milk yield and components

	Saline		Glucose		
Item	HSLP[3]	HPLS[4]	HSLP	HPLS	SE
Yield (kg/d)					
Milk[1]	33.5	36.5	38.9	36.8	1.57
FCM (3.5%)[2]	44.9	51.4	45.2	50.3	2.58
ECM[2]	44.7	50.7	45.6	48.9	2.55
Fat[2]	1.84	2.18	1.72	2.09	0.12
Protein[2]	1.22	1.34	1.33	1.22	0.08
Lactose[2]	1.64	1.74	1.94	1.81	0.10
Solids[2]	1.96	2.08	2.32	2.15	0.12
Cumulative Milk, kg	395	438	469	442	17.2
Milk composition (%)[2]					
Fat	5.25	5.93	4.18	5.49	0.25
Protein	3.46	3.54	3.33	3.21	0.09
Lactose	4.62	4.56	4.83	4.74	0.06
Solids	5.53	5.47	5.77	5.65	0.07
MUN, mg/dL	9.31	17.78	9.30	20.1	1.11
SCC, x 1000[5]	82.5	51.0	85.1	48.8	0.41
Feed Efficiency[5]	2.09	2.24	2.35	2.75	0.47

[1]Recorded daily

[2]Recorded on d 4, 8, 12

[3]HSLP = high starch, low protein diet

[4]HPLS = high protein, low starch diet

[5]Data were transformed for analysis [xtrans = ln(x)]. LSM are back-transformed.

Table 4b. Statistical probabilities of infusion and diet on milk yield and components

	P-value					
Item	Inf[3]	Diet[4]	Inf*Diet[5]	Inf*Day[6]	Diet*Day[7]	Inf*Diet*Day[8]
Yield (kg/d)						
Milk[1]	0.08	0.79	0.12	0.96	0.68	0.08
FCM (3.5%)[2]	0.85	0.02	0.76	0.70	0.66	0.66
ECM[2]	0.86	0.06	0.56	0.70	0.63	0.69
Fat[2]	0.32	<0.01	0.84	0.63	0.72	0.58
Protein[2]	0.95	0.97	0.16	0.66	0.40	0.65
Lactose[2]	0.08	0.84	0.26	0.57	0.42	0.60
Solids[2]	0.09	0.84	0.25	0.57	0.42	0.63
Cumulative Milk, kg	0.01	0.70	0.02	NA	NA	NA
Milk composition (%)[2]						
Fat	0.01	<0.01	0.20	0.88	0.63	0.27
Protein	0.02	0.81	0.27	0.82	0.66	0.63
Lactose	<0.01	0.23	0.79	0.36	0.93	0.79
Solids	0.01	0.24	0.71	0.36	0.84	0.91
MUN, mg/dL	0.31	<0.01	0.29	0.29	0.69	0.96
SCC, x 1000[9]	0.99	0.18	0.92	0.69	0.45	0.32
Feed Efficiency[9]	<0.01	0.03	0.36	0.57	0.40	0.18

[1]Recorded daily; [2]Recorded on d 4, 8, 12

[3]Inf = effect of infusion; [4]Diet = effect of diet

[5]Inf*Diet = interaction between infusion and diet; [6]Inf*Day = interaction of infusion and day

[7]Diet*Day = interaction of diet and day

[8]Inf*Diet*Day = 3-way interaction among infusion, diet and day

[9]Data were transformed for analysis [xtrans = ln(x)]. LSM are back-transformed.

Table 5a. LSM of main effects of infusion and diet on blood plasma hormones and metabolites and liver triglyceride

	Saline		Glucose		
Item	HSLP[4]	HPLS[5]	HSLP	HPLS	SE
Blood[1]					
Glucose, mg/dL	51.6	42.5	59.9	60.1	2.63
Insulin, µg/L[12]	0.18	0.17	0.66	0.43	0.22
Glucagon, pg/mL	106	121	93.1	93.1	6.70
NEFA, µEq/L	1,194	1,514	595	853	97.7
BHBA, mg/dL[12]	12.6	18.2	6.41	8.02	0.12
Insulin:Glucagon ratio [12]	1.79	1.44	7.12	4.68	0.22
Liver					
Triglyceride, % [2, 3, 6]	5.01	7.86	2.41	4.14	0.24

[1]Recorded on d 0, 2, 4, 6, 8, 10, 12

[2]Recorded on d 0, 6, 12

[3]Reported as percentage of wet liver weight

[4]HSLP = high starch, low protein diet

[5]HPLS = high protein, low starch diet

[6]Data were transformed for analysis [xtrans = ln(x)]. LS Means are back-transformed.

Table 5b. Statistical probabilities of infusion and diet on blood plasma hormones and metabolites and liver triglyceride

	P-value					
Item	Inf[4]	Diet[5]	Inf*Diet[6]	Inf*Day[7]	Diet*Day[8]	Inf*Diet*Day[9]
Blood[1]						
Glucose, mg/dL	<0.01	0.08	0.07	0.24	0.80	0.90
Insulin, µg/L[10]	<0.01	0.23	0.46	<0.01	0.33	0.63
Glucagon, pg/mL	0.01	0.29	0.28	<0.01	0.45	0.20
NEFA, µEq/L	<0.01	0.01	0.76	<0.01	0.22	0.98
BHBA, mg/dL[10]	<0.01	0.02	0.56	<0.01	0.39	0.92
Insulin:Glucagon ratio [10]	<0.01	0.13	0.63	<0.01	0.27	0.72
Liver						
Triglyceride, % [2, 3, 10]	<0.01	0.03	0.83	0.22	0.20	0.86

[1]Recorded on d 0, 2, 4, 6, 8, 10, 12

[2]Recorded on d 0, 6, 12

[3]Reported as percentage of wet liver weight

[4]Inf = effect of infusion

[5]Diet = effect of diet

[6]Inf*Diet = interaction between infusion and diet

[7]Inf*Day = interaction of infusion and day

[8]Diet*Day = interaction of diet and day

[9]Inf*Diet*Day = 3-way interaction among infusion, diet and day

[10]Data were transformed for analysis [xtrans = ln(x)]. LS Means are back-transformed.

Table 6. LSM and statistical probabilities of main effects of infusion and diet on body condition score and body weight loss

	Saline		Glucose			P-value		
Item	HSLP[3]	HPLS[4]	HSLP	HPLS	SE	Inf[5]	Diet[6]	Inf*Diet[7]
BW Change, kg[1, 8]	-36.5	-42.8	-52.1	-60.6	0.23	0.16	0.51	0.98
BCS Change[2]	-0.61	-0.79	-0.69	-0.84	0.07	0.25	0.06	0.79

[1]Recorded on d 0, 12

[2]Recorded on d (-4 ± 3), 12

[3]HSLP = high starch, low protein diet

[4]HPLS = high protein, low starch diet

[5]Inf = effect of infusion

[6]Diet = effect of diet

[7]Inf*Diet = interaction between infusion and diet

[8]Data was transformed for analysis [xtrans = ln(x)]. LS Means are back-transformed.

Table 7. Primer sets used for real-time quantitative PCR analysis.

Gene[1]	Forward primer sequence (5' – 3') Reverse primer sequence (5' – 3')	Length (bp)	NCBI GenBank accession number
ARG1	TGTGTCATTTGGGTGGATGCT TCCCGGTTTTGGTTGTCAGT	60	NM_001046154.1
ASL	GTGGCCACTGGTGTCATCTCT GAGCCCGTCCCATGTTCTC	57	NM_001034428.1
ASS1	GCCAAAGCCCCCAACAG GGACCCCTTTCTTGAACTCGAT	54	NM_173892.3
CS	ACTAATGCATGTAGTGTGGGTTAGGT AAGAGCCAGATTCCCACTCTGA	65	NM_001044721.1
G6PC	TGGAGTGGAGTGGCACGAT ATCGCTTGAACCAGGAGGAA	57	NM_001076124.1
GLUD1	CGCTCTGCCAGGCAAATC GGTCCAGCCCCAGGTTATACTT	54	NM_182652.1
HMGCS2	CCTGCTGCAATCACTGTCATG TCTGTCCCGCCACCTCTTC	58	NM_001045883.1
HPRT1[2]	TGGCGTCCCAGTGAAATCA CAGCTGGCCACAGAACAAGA	58	NM_001034035.1
PC	AGGCAAGACGCTGCACATC GCCCGCCCGGTTGA	54	NM_177946.3
PCK1	CAGCCAAGCTGCCCAAGA CCGGCCTTGTCCTTTCG	54	NM_174737.2
PDK4	GAGGTGGTGTTCCCCTGAGA TTGGTGCAGTGGAGTACGTGTAA	62	NM_0011101883.1
PPARA	CAGCGCCGAGGAGTCATC TGTCCCCGCAGATCCTACAC	58	NM_001034036.1

[1] *ARG1* = arginase; *ASL* = arginosuccinate lyase; *ASS1* = arginosuccinate synthase 1; *CS* = citrate synthase; *G6PC* = glucose-6-phosphatase, catalytic subunit; *GLUD1* = glutamate dehydrogenase 1; *HMGCS2* = 3-hydroxy-3methylglutaryl-CoA synthase 2; *HPRT1* = hypoxanthine phosphoriboxyl-transferase 1; *PC* = pyruvate carboxylase; *PCK1* = phosphoenolpyruvate carboxykinase 1 (soluble); *PDK4* = pyruvate dehydrogenase kinase, isozyme 4; *PPARA* = peroxisome proliferator-activated receptor alpha

[2] endogenous control gene

Table 8a. LSM of main effects of infusion and diet on hepatic gene expression[1]

	Saline		Glucose		
Item[2, 3]	HSLP[4]	HPLS[5]	HSLP	HPLS	SE
ARG1	0.79	1.07	0.78	0.75	0.29
ASL	0.62	1.05	1.67	0.59	0.49
ASS1	0.92	1.13	1.63	1.17	0.27
CS	1.21	0.66	1.56	1.54	0.18
G6PC	0.76	0.77	1.00	0.70	0.31
GLUD1	0.89	1.00	2.83	1.23	0.30
HMGCS2	2.99	3.62	2.47	1.23	0.52
PC	0.47	0.37	0.48	0.44	0.49
PCK1	1.80	1.15	2.66	2.61	0.44
PDK4	1.22	2.37	1.42	1.22	0.50
PPARA	0.81	0.94	3.51	2.23	0.53

[1]Expressed as the fold change relative to d 0

[2]*ARG1* = arginase; *ASL* = arginosuccinate lyase; *ASS1* = arginosuccinate synthase 1; *CS* = citrate synthase; *G6PC* = glucose-6-phosphatase, catalytic subunit; *GLUD1* = glutamate dehydrogenase 1; *HMGCS2* = 3-hydroxy-3methylglutaryl-CoA synthase 2; *PC* = pyruvate carboxylase; *PCK1* = phosphoenolpyruvate carboxykinase 1 (soluble); *PDK4* = pyruvate dehydrogenase kinase, isozyme 4; *PPARA* = peroxisome proliferator-activated receptor alpha

[3]Liver samples obtained on d 0, d 6, and d 12

[4]HSLP = low protein, high starch diet

[5]HPLS = high protein, low starch diet

Table 8b. Statistical probabilities of glucose infusion and diet on hepatic gene expression[1]

	P-value					
Item[2, 3]	Inf[4]	Diet[5]	Inf*Diet[6]	Inf*Day[7]	Diet*Day[8]	Inf*Diet*Day[9]
ARG1	0.23	0.39	0.26	0.89	0.24	0.48
ASL	0.54	0.46	0.03	0.92	0.93	0.14
ASS1	0.12	0.73	0.16	0.30	0.12	0.22
CS	<0.01	0.02	0.02	0.16	0.13	0.57
G6PC	0.67	0.41	0.39	0.59	0.10	0.23
GLUD1	<0.01	0.09	0.03	0.58	0.17	0.21
HMGCS2	0.30	0.86	0.67	0.95	0.50	0.01
PC	0.77	0.64	0.84	0.10	0.21	0.27
PCK1	0.05	0.44	0.48	0.55	0.30	0.04
PDK4	0.46	0.46	0.24	0.14	0.32	0.05
PPARA	<0.01	0.69	0.42	0.05	0.97	0.28

[1]Expressed as the fold change relative to d 0

[2]*ARG1* = arginase; *ASL* = arginosuccinate lyase; *ASS1* = arginosuccinate synthase 1; *CS* = citrate synthase; *G6PC* = glucose-6-phosphatase, catalytic subunit; *GLUD1* = glutamate dehydrogenase 1; *HMGCS2* = 3-hydroxy-3methylglutaryl-CoA synthase 2; *PC* = pyruvate carboxylase; *PCK1* = phosphoenolpyruvate carboxykinase 1 (soluble); *PDK4* = pyruvate dehydrogenase kinase, isozyme 4; *PPARA* = peroxisome proliferator-activated receptor alpha

[3]Liver samples obtained on d 0, d 6, and d 12

[4]Inf = effect of glucose infusion

[5]Diet = effect of diet

[6]Inf*Diet = interaction between infusion and diet

[7]Inf*Day = interaction of infusion and day

[8]Diet*Day = interaction of diet and day

[9]Inf*Diet*Day = 3-way interaction among infusion, diet and day

APPENDIX B: FIGURES

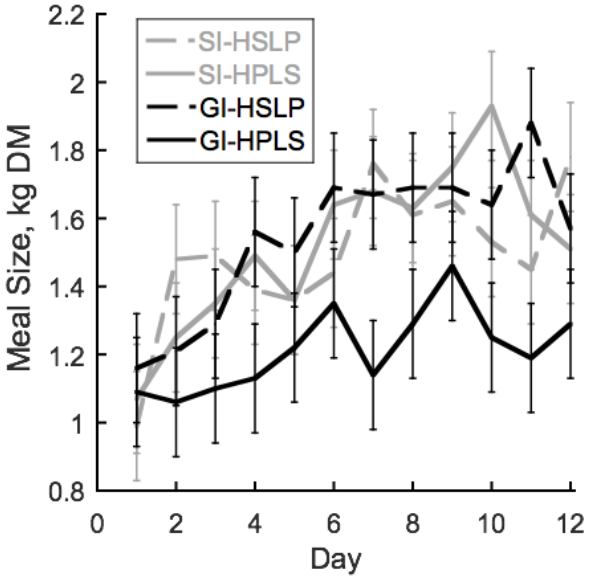

Figure 1. Interaction of infusion, diet and time on meal size. (P = 0.07; SEM = 0.12 kg DM)

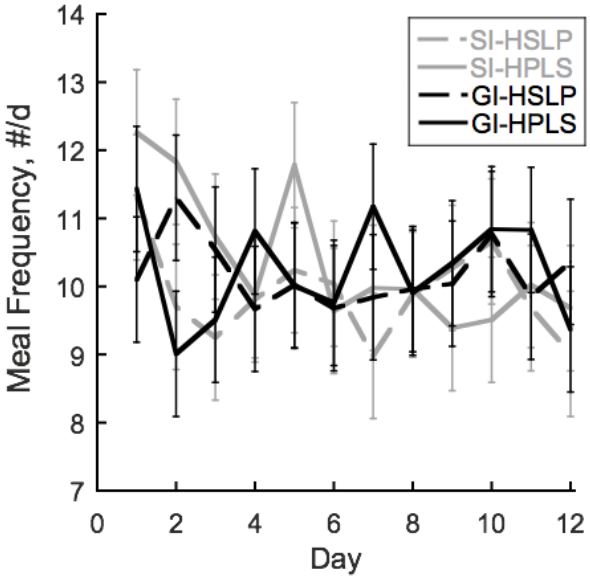

Figure 2. Interaction of infusion, diet and time on meal frequency. (P = 0.10; SEM = 0.59 meals/d)

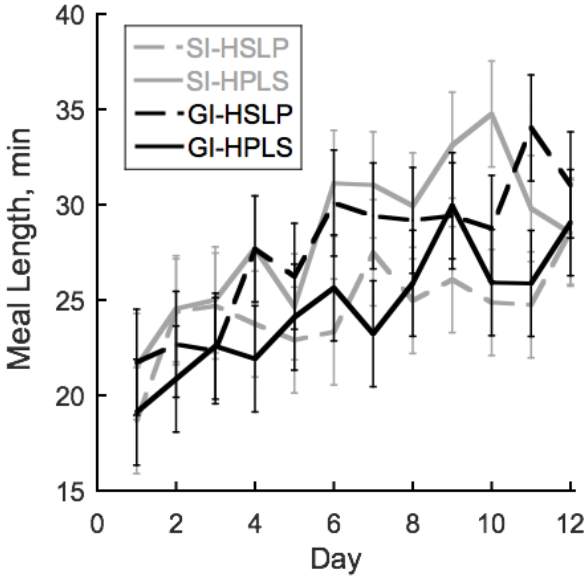

Figure 3. Interaction of infusion, diet and time on meal length. (P = 0.13; SEM = 2.07 min)

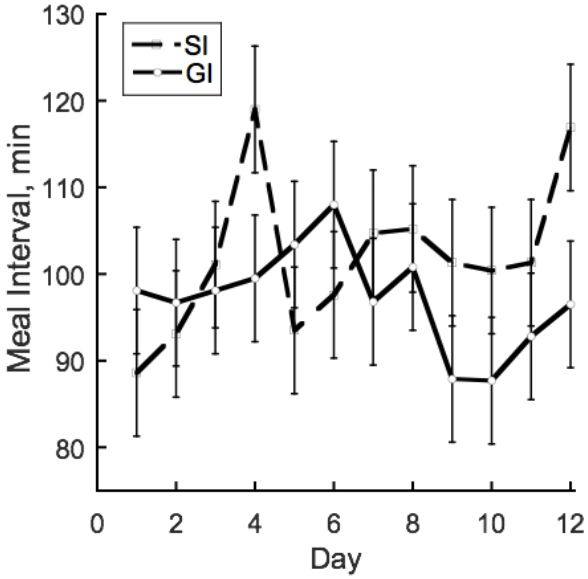

Figure 4. Interaction of infusion and time on meal interval. (P = 0.12; SEM = 7.30 min)

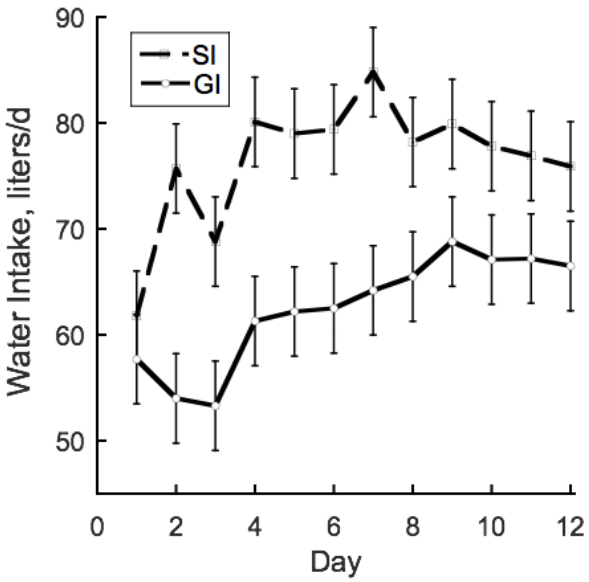

Figure 5. Interaction of infusion and time on water intake. (*P* = 0.07; SEM = 4.22 liters/d)

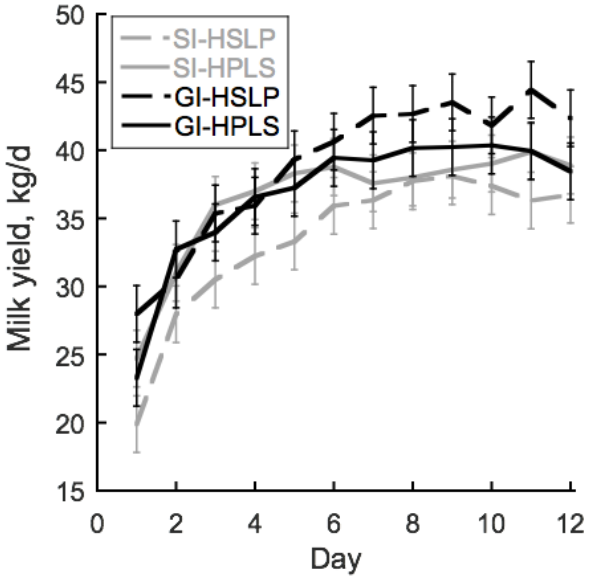

Figure 6. Interaction of infusion, diet and time on milk yield. (*P* = 0.08; SEM = 1.57 kg/d)

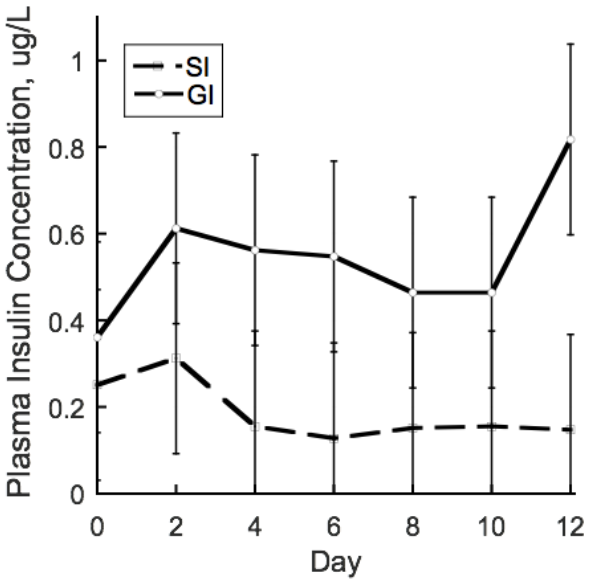

Figure 7. Interaction of infusion and time on plasma insulin concentration. (P <0.01; SEM = 0.21 µg/L)

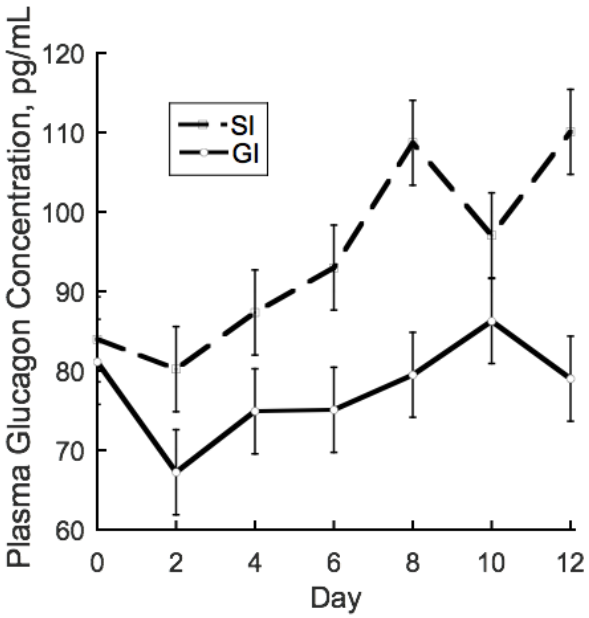

Figure 8. Interaction of infusion and time on plasma glucagon concentration. (P <0.01; SEM = 5.35 pg/mL)

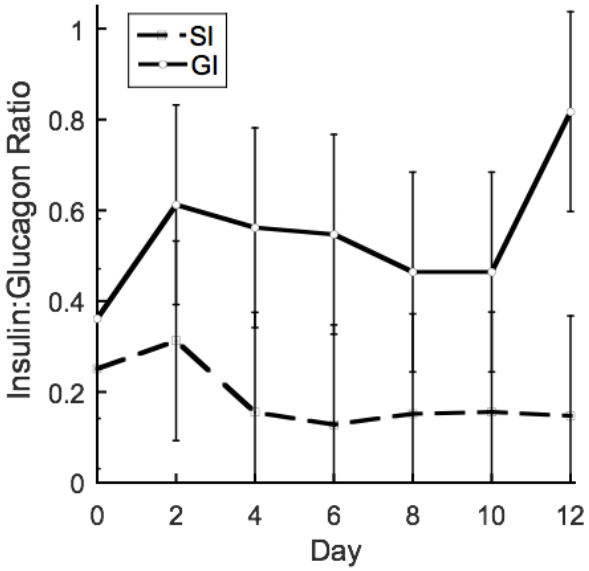

Figure 9. Interaction of infusion and time on the insulin:glucagon ratio. ($P < 0.01$; SEM = 0.23)

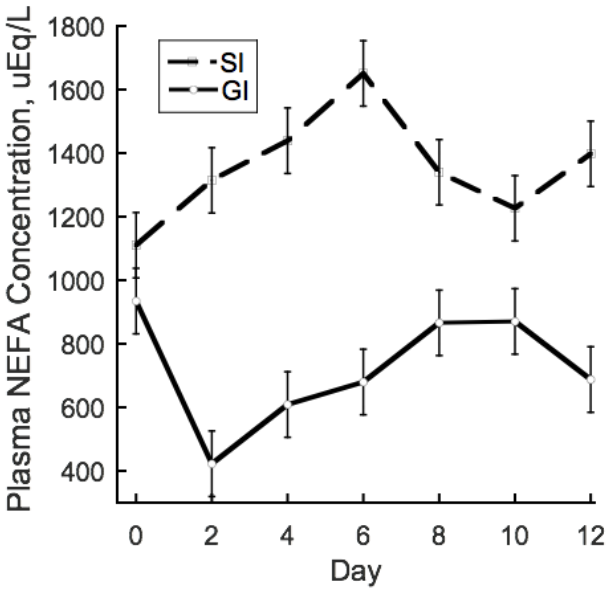

Figure 10. Interaction of infusion and time on plasma NEFA concentration. ($P < 0.01$; SEM = 103 µEq/L)

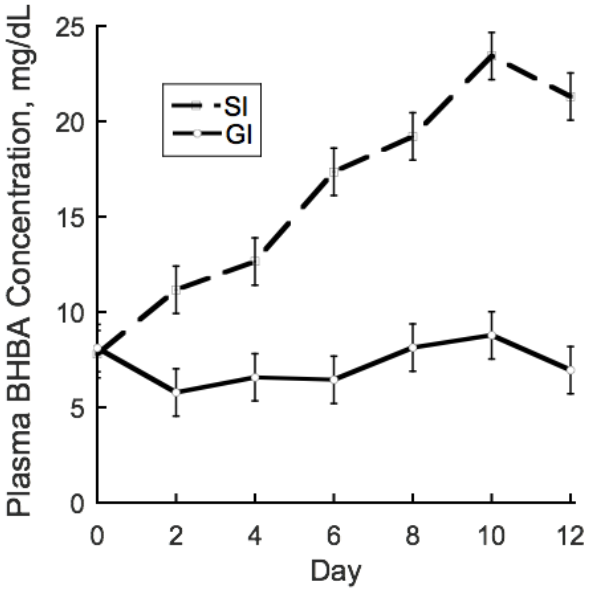

Figure 11. Interaction of infusion and time on plasma BHBA concentration. ($P < 0.01$; SEM = 0.12 mg/dL)

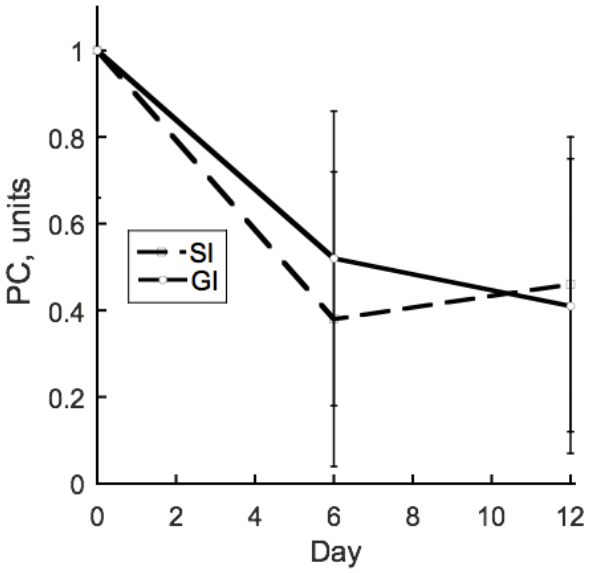

Figure 12. Interaction of infusion and time on expression of PC. ($P = 0.10$; SEM = 0.37 units)

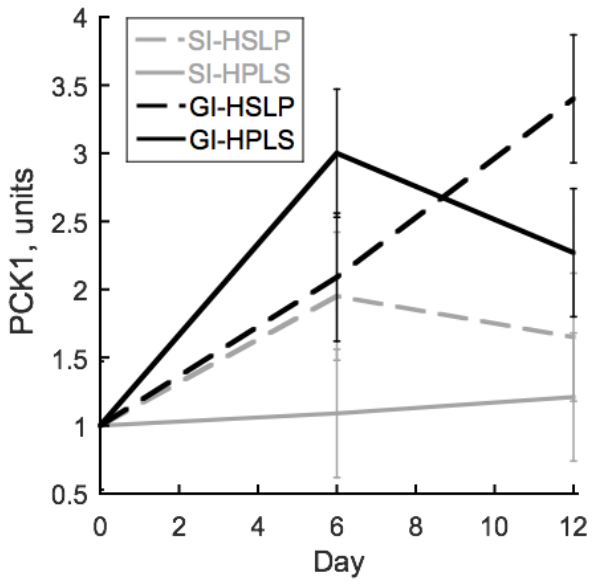

Figure 13. Interaction of infusion, diet and time on expression of PCK1. (*P* = 0.04; SEM = 0.44 units)

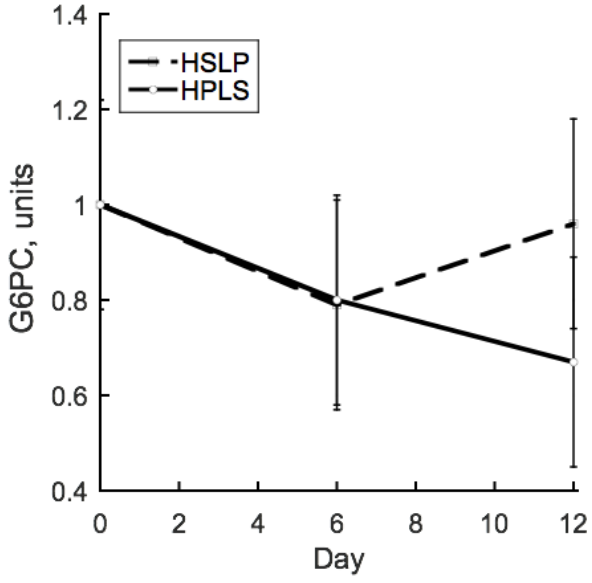

Figure 14. Interaction of diet and time on expression of G6PC. (*P* = 0.10; SEM = 0.24 units)

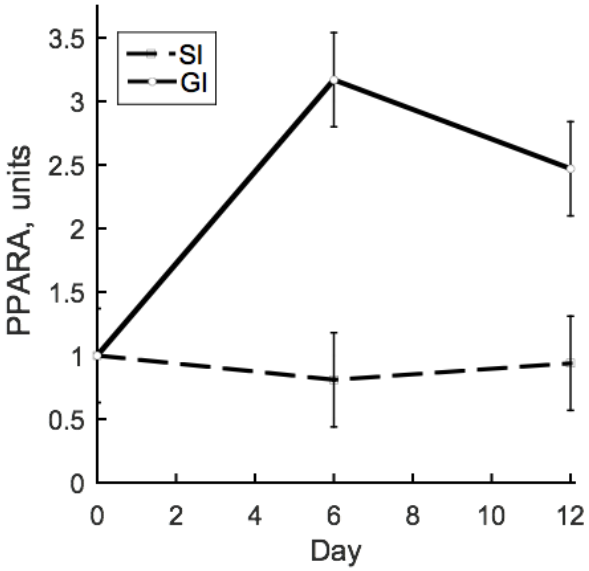

Figure 15. Interaction of infusion and time on expression of PPARA.
(*P* = 0.05; SEM = 0.39 units)

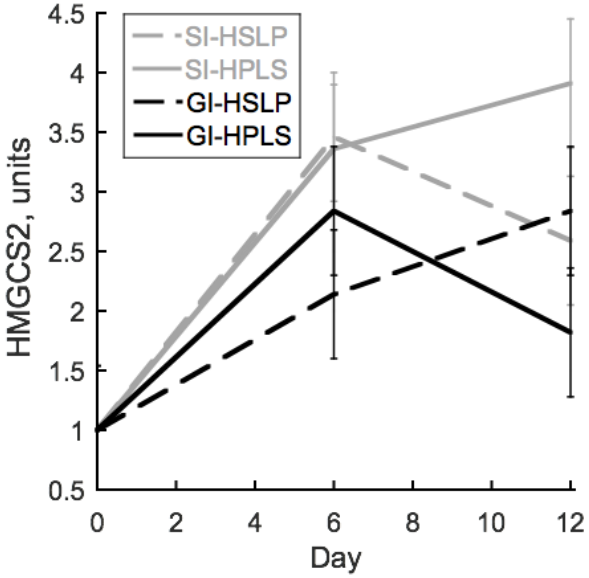

Figure 16. Interaction of infusion, diet and time on expression of HMGCS2.
(*P* = 0.01; SEM = 0.52 units)

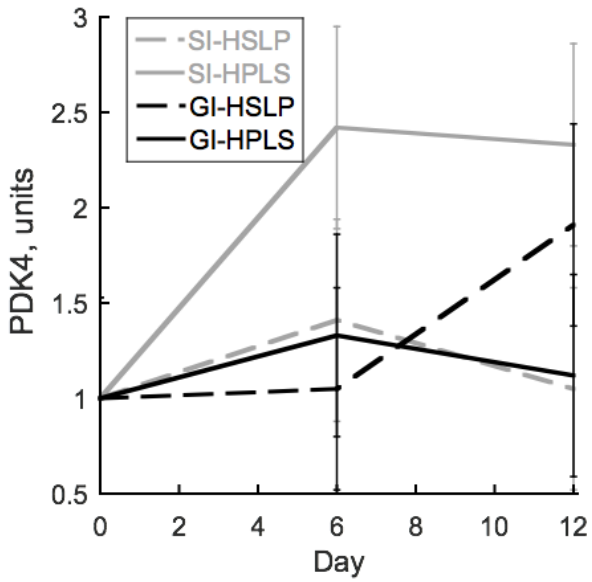

Figure 17. Interaction of infusion, diet and time on expression of PDK4. (*P* = 0.05; SEM = 0.50 units)

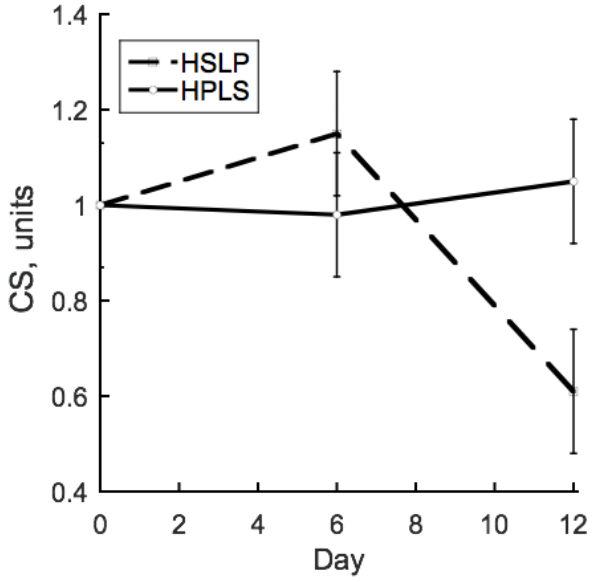

Figure 18. Interaction of diet and time on expression of CS. (*P* = 0.13; SEM = 0.17 units)

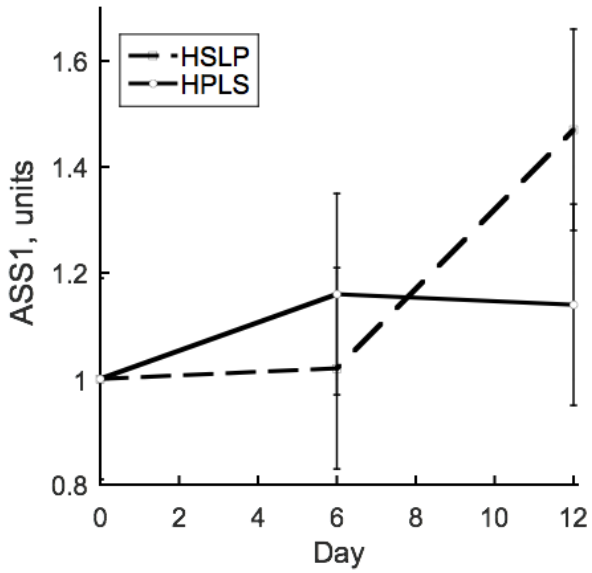

Figure 19. Interaction of diet and time on expression of ASS1. (P = 0.12; SEM = 0.22 units)

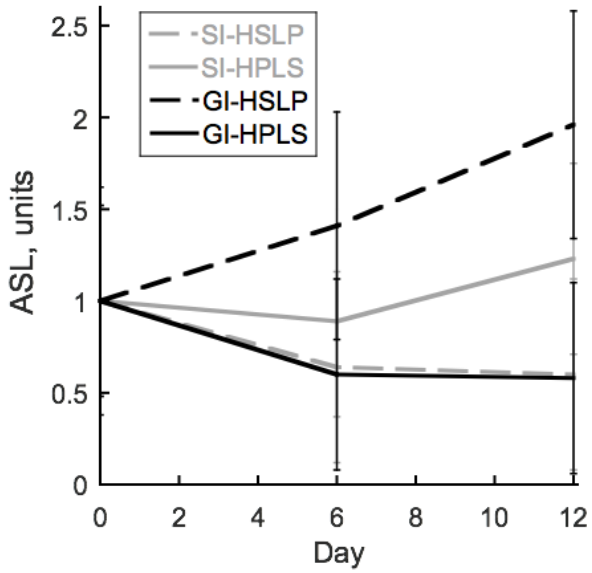

Figure 20. Interaction of infusion, diet and time on expression of ASL.
(P = 0.14; SEM = 0.49 units)

REFERENCES

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

Al-Trad, B., T. Wittek, G. B. Penner, K. Reisberg, G. Gäbel, M. Fürll, and J. R. Aschenbach. 2010. Expression and activity of key hepatic gluconeogenesis enzymes in response to increasing intravenous infusions of glucose in dairy cows. J. Anim. Sci. 88:2998-3008.

Abramson, S. M., I. Bruckental, S. Zamwel, and A. Arieli. 2002. Effect of abomasally infused casein on post-ruminal digestibility of total non-structural carbohydrates and milk yield and composition in dairy cows. Anim. Sci. 74:347-355.

Abramson, S. M., I. Bruckental, L. Lipshitz, U. Moalem, S. Zamwel, and A. Arieli. 2005. Starch digestion site: influence of ruminal and abomasal starch infusion on starch digestion and utilization in dairy cows. Anim. Sci. 80:201-207.

Adewuyi, A. A., Gruys, E., and F. J. C. M. van Eerdenburg. 2005. Non-esterified fatty acids (NEFA) in dairy cattle. A review. Veterinary Quarterly. 27:117-126.

Aiello, R. J., and L. E. Armentano. 1987. Gluconeogenesis in goat hepatocytes is affected by calcium, ammonia and other key metabolites but not primarily through cytosolic redox state. Comp. Biochem. Physiol. 88B:193-201.

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

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

Amaral, D. M., J. J. Veenhuizen, J. K. Drackley, M. H. Cooley, A. D. McGilliard, and J. W. Young. 1990. Metabolism of Propionate, Glucose, and Carbon Dioxide as Affected by Exogenous Glucose in Dairy Cows at Energy Equilibrium. J. Dairy Sci. 73:1244-1254.

Anil, M. H., and J. M. Forbes. 1980. Feeding in sheep during intraportal infusions of short-chain fatty acids and the effect of liver denervation. J. Physiol. 298:407-414.

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

Annison, E. F., and J. L. Linzell. 1964. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their over-all metabolism and to milk formation. J. Physiol. 175:372-385.

Bauman, D. E., and R. G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. Annu. Rev. Nutr. 13:437-461.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. *trans-10, cis-12* Conjugated Linoleic Acid Decreases Lipogenic Rates and Expression of Genes Involved in Milk Lipid Synthesis in Dairy Cows. J. Dairy Sci. 85:2155-2163.

Baracos, V. E., J. Brun-Bellut, and M. Marie. 1991. Tissue protein synthesis in lactating and dry goats. Br. J. Nutr. 66:451-465.

Barthell, A., and D. Schmoll. 2003. Novel concepts in insulin regulation of hepatic gluconeogenesis. Am J. Physiol. Endocrinol. Metab. 285:E685-E692.

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. and Neoplasia. 2(3):265-278.

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

Bell, A. W. 1995. Regulation of Organic Nutrient Metabolism During Transition from late Pregnancy to Early Lactation. J. Anim. Sci. 73:2804-2819.

Bell, A. W., W. S. Burhans, T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proc. Nutr. Soc. 59:119-126.

Belyea, R. L., G. R. Frost, F. A. Martz, J. L. Clark, and L. G. Forkner. 1978. Body Composition of Dairy Cattle by Potassium-40 Liquid Scintillation Detection. J. Dairy Sci. 61:206-211.

Benson, J. A., C. K. Reynolds, P. C. Aikman, B. Lupoli, and D. E. Beever. 2002. Effects of Abomasal Vegetable Oil Infusion on Splanchnic Nutrient Metabolism in Lactating Dairy Cows. J. Dairy Sci. 85:1804-1814.

Bertics, S. J., and R. R. Grummer. 1999. Effects of Fat and Methionine Hydroxy Analog on Prevention or Alleviation of Fatty Liver Induced by Feed Restriction. J. Dairy Sci. 82:2731-2736.

Bickerstaffe, R., E. F. Annison, J. L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids I lactating dairy cows. J. Agric. Sci., Cambr. 82:71-85.

Blum, J. W., T. Reding, F. Jans, M. Wanner, M. Zemp, and K. Bachmann. 1985. Variations of 3-Methylhistidine in Blood of Dairy Cows. J. Dairy Sci. 68:2580-2587.

Botts, R. L., R. W. Hemken, and L. S. Bull. 1979. Protein Reserves in the Lactating Dairy Cow. J. Dairy Sci. 62:433-440.

Boudon, A., P. Faverdin, R. Delagarde, P. Lamberton, and J. L. Peyraud. 2007. Effects of Rumen or Duodenal Glucose Infusions on Intake in Dairy Cows Fed Fresh Perennial Ryegrass Indoors. J. Dairy Sci. 90:4397-4410.

Bradford, B. J., and M. S. Allen. 2007. Depression in Feed Intake by a Highly Fermentable Diet is Related to Plasma Insulin Concentration and Insulin Response to Glucose Infusion. J. Dairy Sci. 90:3838-3845.

Bradford, B. J., and M. S. Allen. 2007a. *Short Communication*: Rate of Propionate Infusion Within Meals Does Not Influence Feeding Behavior. J. Dairy Sci. 90:2305-2308.

Brockman, R. P. 1985. Role of insulin in regulating hepatic gluconeogenesis in sheep. Can. J. Physiol. Pharmacol. 63:1460-1464.

Brockman, R. P. 1990. Effect of insulin on the utilization of propionate in gluconeogenesis in sheep. Br. J. Nutr. 64:95-101.

Broster, W. H., and V. J. Broster. 1984. Reviews of the progress of Dairy Science: Long term effects of plane of nutrition on the performance of the dairy cow. J. Dairy Res. 51:149-196.

Butler, S. T., A. L. Marr, S. H. Pelton, R. P. Radcliff, M. C. Lucy, and W. R. Butler. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-1 and GH receptor 1A. J. Endocrinol. 176:205-217.

Cadórniga-Valiño, C., R. R. Grummer, L. E. Armentano, S. S. Donkin, and S. J. Bertics. 1997. Effects of fatty acids and hormones on fatty acid metabolism and gluconeogenesis in bovine hepatocytes. J. Dairy Sci. 80:646-656.

Clark, J. H., H. R. Spires, R. G. Derrig, and M. R. Bennink. 1976. Milk Production, Nitrogen Utilization and glucose synthesis in lactating Cows Infused Postruminally with Sodium Caseinate and Glucose. J. Nutr. 107:631-644.

Cowan, R. T., J. J. Robinson, I. McHattie, and K. Penne. 1981. Effects of protein concentration in the diet on milk yield, change in body composition and the efficiency of utilization of body tissue for production in ewes. Anim. Prod. 33:111-120.

Dado, R. G., and M. S. Allen. 1993. Continuous computer acquisition of feed and water intake, chewing reticular motility, and ruminal pH of cattle. J. Dairy Sci. 76:1589-1600.

Dairy Records Management Services. 2011. DHI Glossary. Accessed June 22, 2012. http://www.drms.org/PDF/materials/glossary.pdf.

Davis, A. J., I. R. Fleet, J. A. Goode, M. H. Hamon, F. M. Maule Walker, M. Peaker. 1979. Changes in mammary function at the onset of lactation in the goat: correlation with hormonal changes. J. Physiol. 288:33-44.

Dhiman, T. R., C. Cadorniga, and L. D. Satter. 1993. Protein and Energy Supplementation of High Alfalfa Silage Diets During Early lactation. J. Dairy Sci. 76:1945-1959.

Donkin, S. S., and L. E. Armentano. 1994. Regulation of gluconeogenesis by insulin and glucagon in the neonatal bovine. Am. J. Physiol. 266:R1220-R1228.

Douglas, G. N., T. R. Overton, H. G. Bateman, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J. Dairy Sci. 89:2141-2157.

Drackley, J. K. 1999. Biology of Dairy Cows During the Transition Period: the Final Frontier? J. Dairy Sci. 82:2259-2273.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of Glucose and Long-Chain Fatty Acid Metabolism in Liver of Dairy Cows during the Periparturient Period. J. Dairy Sci. 84(E. Suppl.): E100-E112.

Frobish, R. A., and C. L. Davis. 1978. Effects of Abomasal Infusions of Glucose and Propionate on Milk Yield and Composition. J. Dairy Sci. 60:204-209.

Garnsworthy, P. C., and J. H. Topps. 1982. The effect of body condition of dairy cows at calving on their food intake and performance when given complete diets. Anim. Prod. 35:113-119.

Gibb, M. J., W. E. Ivings, M. S. Dhanoa, and J. D. Sutton. 1992. Changes in body components of autumn-calving Holstein-Friesian cows over the first 29 weeks of lactation. Anim. Prod. 55:339-360.

Grummer, R. R. 1993. Etiology of Lipid-Related Metabolic Disorders in Periparturient Dairy Cows. J. Dairy Sci. 76:3882-3896.

Grummer, R. R., D. G. Mashek, and A. Hayirli. 2004. Dry matter intake and energy balance in the transition period. Vet Clin. Food Anim. 20:447-470.

Guesnet, P.M., M.J. Massoud, and Y. Demarne. 1990. Regulation of adipose tissue metabolism during pregnancy and lactation in the ewe: The role of insulin. J. Anim. Sci. 69:2057-2065.

Hach, C. C., B. K. Bowden, A. B. Lopelove, and S. V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. J. AOAC. 70:783-787.

Hammon, H. M., U. Kautzsch, C. Weber, B. Kuhla, M. Rö ntgen, and R. M. Bruckmaier. 2012. Insulin action on hepatic gene expression in dairy cows with different fat mobilization during early lactation. J. Dairy Sci. Vol. 95, Suppl. 2:319.

Hartwell, J. R., M. J. Cecava, and S. S. Donkin. 2001. Rumen Undegradable Protein, Rumen-Protected Choline and mRNA Expression for Enzymes in Gluconeogenesis and Ureagenesis in Periparturient Dairy Cows. J. Dairy Sci. 84:490-497.

Harvatine, K. J., J. W. Perfield, and D. E. Bauman. 2009. Expression of Enzymes and key Regulators of Lipid Synthesis Is Upregulated in Adipose Tissue during CLA-Induced Milk Fat Depression in Dairy Cows. J. Nutr. 139:849-854.

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84(E. Suppl.):E14-E24.

Hurtaud, C., H. Rulquin, and R. Verte. 1998. Effects of Graded Duodenal Infusions of Glucose on Yield and Composition of Milk from Dairy Cows. 1. Diets Based on Corn Silage. J. Dairy Sci. 81:3239-3247.

Ingvartsen, K. L. 2006. Feeding- and management-related diseases in the transition cow. Physiological adaptation around calving and strategies to reduce feeding-related diseases. Anim. Feed Sci. Technol. 126:175-213.

Jitrapakdee, S., and J. C. Wallace. 1999. Structure, function and regulation of pyruvate carboxylase. Biochem. J. 340:1-16.

Journet, M., and B. Rémond. 1981. Response of dairy cows to protein level in early lactation. Livest. Prod. Sci. 8:21-35.

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

Knowlton, K. F., T. E. Dawson, B. P. Glenn, G. B. Huntington, and R. A. Erdman. 1998. Glucose Metabolism and Milk Yield of Cows Infused Abomasally or Ruminally with Starch. J. Dairy Sci. 81:3248-3258.

Langhans, et al. 1985. Selective hepatic vagotomy eliminates the hypophagic effect of different metabolites. J. Auton. Nerv. Syst. 13:255-262.

Larsen, M., and N. B. Kristensen. 2009. Effect of abomasal glucose infusion on splanchnic and whole-body glucose metabolism in periparturient dairy cows. J. Dairy Sci. 92:1071-1083.

Larsen, M., and N. B. Kristensen. 2009a. Effect of abomasal glucose infusion on splanchnic amino acid metabolism in periparturient dairy cows. J. Dairy Sci. 92:3306-3318.

Lemosquet, S., N. Rideau, H. Rulquin, P. Faverdin, J. Simon, and R. Verte. 1996. Effects of a Duodenal Glucose Infusion on the Relationship Between Plasma Concentrations of Glucose and Insulin in Dairy Cows. J. Dairy Sci. 80:2854-2865.

Leng, R. A., J. W. Steel, and J. R. Luick. 1967. Contribution of Propionate to Glucose Synthesis in Sheep. Biochemical Journal. 103:785-790.

Leury, B. J., L. H. Baumgard, S. S. Block, N. Segoale, R. A. Ehrhardt, R. P. Rhoads, D. E. Bauman, A. W. Bell, and Y. R. Boisclair. 2003. Effect of insulin and growth hormone on plasma leptin in periparturient dairy cows. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285:R1107-1115.

Little, W. 1974. An Effect of the Stage of Lactation on the Concentration of Albumin in the Serum of Dairy Cows. Res. Vet. Sci. 17:193-199.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods. 25:402-408.

Lomax, M. A., and G. D. Baird. 1983. Blood flow and nutrient exchange across the liver and gut of the dairy cow. Br. J. Nutr. 49:481-496.

Manston, R., A. M. Russell, S. M. Dew, and J. M. Payne. 1975. The influence of dietary protein upon blood composition in dairy cows. Vet. Rec. 96:497-502.

McCarthy, R. D., G. A. Porter, and L. C. Griel, Jr. 1968. Bovine Ketosis and Depressed Fat Test in Milk: A Problem of Methionine Metabolism and Serum Lipoprotein Aberration. J. Dairy Sci. 51:459-462.

McCarthy, R. D., Jr., T. H. Klusmeyer, J. L. Vicini, J. H. Clark, and D. R. Nelson. 1989. Effects of Source of Protein and Carbohydrate on Ruminal Fermentation and Passage of Nutrients to the Small Intestine of Lactating Cows. J. Dairy Sci. 72:2002-2016.

McNeill, D. M., R. Slepetis, R. A. Ehrhardt, D. M. Smith, and A. W. Bell. 1997. Protein requirements of sheep in late pregnancy: partitioning of nitrogen between gravid uterus and maternal tissues. J. Anim. Sci. 75:809-816.

Mertens, D. R. 2002. Gravimetric Determination of Amylase-Treated Neutral Detergent Fiber in Feeds with Refluxing in Beakers or Crucibles: Collaborative Study. J. AOAC Int. 85:1217-1240.

Murondoti, A., R. Jorritsma, A. C. Beynen, T. Wensing, and M. J. H. Geelen. 2004. Activities of the enzymes of hepatic gluconeogenesis in periparturient dairy cows with induced fatty liver. J. Dairy Res. 71:129-134.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Ed. Natl. Acad. Sci., Washington, D.C.

Oba, M., and M. S. Allen. 2003. Dose-response Effect of Intraruminal Infusion of Propionate on Feeding Behavior of Lactating Cows in Early or Midlactation. J. Dairy Sci. 86:2922-2931.

Oba, M., and M.S. Allen. 2003a. Effects of Corn Grain Conservation Method on Ruminal Digestion Kinetics for Lactating Dairy Cows at Two Dietary Starch Concentrations. J. Dairy Sci. 86:184-194.

Oba, M., and M. S. Allen. 2003b. Hypophagic Effects of Ammonium Are Greater When Infused with Propionate Compared with Acetate in Lactating Dairy Cows. J. Nutr. 133:1100-1104.

Oba, M., and M. S. Allen. 2003c. Effects of intraruminal infusion of sodium, potassium, and ammonium on hypophagia from propionate in lactating dairy cows. J. Dairy Sci. 86:1398-1404.

Oldham, J. D., W. H. Broster, D. J. Napper, and J. W. Siviter. 1979. The effect of a low-protein ration on milk yield and plasma metabolites in Friesian heifers during early lactation. Br. J. Nutr. 42:149-162.

Ørskov, E. R., D. A. Grubb, and R. N. B. Kay. 1977. Effect of postruminal glucose or protein supplementation on milk yield and composition in Friesian cows in early lactation and negative energy balance. Br. J. Nutr. 38:397-405.

Ørskov, E. R., G. W. Reid, and C. A. G. Tait. 1987. Effect of fish meal on the mobilization of body energy in dairy cows. Anim. Prod. 45:345-348.

Overton, T. R., D. W. LaCount, T. M. Cicela, and J. H. Clark. 1996. Evaluation of a Ruminally Protected Methionine Product for Lactating Dairy Cows. J. Dairy Sci. 79:631-638.

Overton, T. R., J. K. Drackley, G. N. Douglas, L. S. Emmert, and J. H. Clark. 1998. Hepatic gluconeogenesis and whole-body protein metabolism of periparturient dairy cows as affected by source of energy and intake of the prepartum diet. J. Dairy Sci. 81:Suppl. 1, 295. Abstr.

Overton, T. R. and M. R. Waldron. 2004. Nutrition Management of Transition Dairy Cows: Strategies to Optimize Metabolic Health. J. Dairy Sci. 87:(E Suppl.):E105-E119.

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

Parker, D. S., M. A. Lomax, C. J. Seal, J. C. Wilton. 1995. Metabolic Implications of ammonia production in the ruminant. Proc. Nutr. Soc. 54:549-563.

Paterson, J. Y. F., and J. L. Linzell. 1974. Cortisol secretion rate, glucose entry rate and the mammary uptake of cortisol and glucose during pregnancy and lactation in dairy cows. J. Endocrinol. 62:371-383.

Pettersen, J. A., F. R. Dunshea, R. A. Ehrhardt, A. W. Bell. 1993. Pregnancy and Undernutrition Alter Glucose Metabolic Responses to Insulin in Sheep. J. Nutr. 123:1286-1295.

Piepenbrink, M. S., A. L. Marr, M. R. Waldron, W. R. Butler, T. R. Overton, M. Vásquez-Añon, and M. D. Holt. 2004. Feeding 2-Hydroxy-4(Methylthio)-Butanoic Acid to Periparturient Dairy Cows Improves Milk Production but not Hepatic Metabolism. J. Dairy Sci. 87:1071-1084.

Reid, I. M., C. J. Roberts, and G. D. Baird. 1980. The effects of underfeeding during pregnancy and lactation on structure and chemistry of bovine liver and muscle. J. Agric. Sci., Cambr. 94:239-245.

Reynolds, C. K. 1992. Metabolism of Nitrogenous Compounds by Ruminant Liver. J. Nutr. 122:850-854.

Reynolds, C. K., S. B. Cammmell, D. J. Humphries, D. E. Beever, J. D. Sutton, and J. R. Newbold. 2001. Effects of Postpartum Starch Infusion on Milk Production and Energy Metabolism in Dairy Cows. J. Dairy Sci. 84:2250-2259.

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic Metabolism of Dairy Cows During the Transition From Late Gestation Through Early Lactation. J. Dairy Sci. 86:1201-1217.

Rhoads, R. P., J. W. Kim, B. J. Leury, L. H. Baumgard, N. Segoale, S. J. Frank, D. E. Bauman, and Y. R. Boisclair. 2004. Insulin Increases the Abundance of the Growth Hormone Receptor in Liver and Adipose Tissue of Periparturient Dairy Cows. J. Nutr. 134:1020-1027.

Rigout, S., S. Lemosquet, J. E. van Eys, J. W. Blum, and H. Rulquin. 2002. Duodenal Glucose Increases Glucose Fluxes and Lactose Synthesis in Grass Silage-Fed Dairy Cows. J. Dairy Sci. 85:595-606.

Robinson, J. J., C. Fraser, J. C. Gill, and I. McHattie. 1974. The effect of dietary crude protein concentration and time of weaning on milk production and body-weight change in the ewe. Anim. Prod. 19:331-339.

Robinson, J. J., I. McHattie, J. F. Calderon Cortes, and J. L. Thompson. 1979. Further studies on the response of lactating ewes to dietary protein. Anim. Prod. 29:257-269.

Roche, T. E., J. C. Baker, X. Yan, Y. Hiromasa, X. Gong, T. Peng, J. Dong, A. Turkan, and S. Kasten. 2001. Distinct Regulatory Properties of Pyruvate Dehydrogenase Kinase and Phosphatase Isoforms. Prog. Nucl. Acid Res. Mol. Biol. 70:33-75.

Roffler, R. E., L. D. Satter, A. R. Hardie, and W. J. Tyler. 1978. Influence of Dietary Protein Concentration on Milk Production by Dairy Cattle during Early Lactation. J. Dairy Sci. 61:1422-1428.

Rooney, L. W., and R. L. Pflugfelder. 1986. Factors Affecting Starch Digestibility with Special Emphasis on Sorghum and Corn. J. Anim. Sci. 63:1607-1623.

Rutter, L. M., and J. G. Manns. 1986. Changes in Metabolic and Reproductive Characteristics Associated with Lactation and Glucose Infusion in the Postpartum Ewe. J. Anim. Sci. 63:538-545.

Sano, H., N. Hattori, Y. Todome, J. Tsuruoka, H. Takahashi, and Y Terashima. 1993. Plasma insulin and glucagon responses to intravenous infusion of propionate and their autonomic control in sheep. J. Anim. Sci. 71:3414-3422.

Santos, F. A. P., J. E. P. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of Rumen-Undegradable Protein on Dairy Cow Peformance: A 12-Year Literature Review. J. Dairy Sci. 81:3182-3213.

Sparrow, R. C., R. W. Hemken, D. R. Jacobson, F. B. Button, and C. M. Enlow. 1973. Three protein percents on nitrogen balance, body weight change, milk production and composition of lactating cows during early lactation. J. Dairy Sci. 56:664. (Abstr.)

Steel, J. W., and R. A. Leng. 1973. Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 1. The Kinetics of glucose metabolism. Br. J. Nutr. 30:451-473.

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

Strang, B. D., S. J. Bertics, R. R. Grummer, and L. E. Armentano. 1998. Effect of Long-Chain Fatty Acids on Triglyceride Accumulation, Gluconeogenesis, and Ureagenesis in Bovine Hepatocytes. J. Dairy. Sci. 81:728-739.

Taylor, C. C., and M. S. Allen. 2005. Corn Grain Endosperm Type and Brown Midrib 3 Corn Silage: Site of Digestion and Ruminal Digestion Kinetics in Lactating Cows. J. Dairy Sci. 88:1413-1424.

Treacher, R. J., W. Little, K. A. Collis, and A. J. Stark. 1976. The influence of dietary protein intake on milk production and blood composition of high-yielding dairy cows. J. Dairy Res. 43:357-369.

Van Horn, H. H., E. A. Olaloku, J. R. Flores, S. P. Marshall, and K. C. Bachman. 1976. Complete Rations for Dairy Cattle. VI. Percent Protein Required with Soybean Meal Supplementation of Low-Fiber Rations for Lactating Dairy Cows. J. Dairy Sci. 59:902-906.

Vazquez-Añon, M., S. Bertics, M. Luck, R. R. Grummer, and J. Pinheiro. 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. J. Dairy Sci. 77:1521-1528.

Vernon, R. G., R. A. Clegg, D. J. Flint. 1981. Metabolism of sheep adipose tissue during pregnancy and lactation. Biochem. J. 200:207-314.

Vernon, R. G., and C. M. Pond. 1997. Adaptations of Maternal Adipose Tissue to Lactation. J. Mammary Gland Biol. Neoplasia. 2:231-241.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. Journal of Dairy Science. 65:495-501.

Wilson, L. A., S. E. Mills, E. Finley, E Kilgour, and P. J. Buttery. 1996. Effect of lactation on insulin signal transduction in sheep adipose tissue and skeletal muscle. J. Endocrinol. 151:469-480.

Wilson, S., J. C. MacRae, P. J. Buttery. 1983. Glucose production and utilization in non-pregnant, pregnant and lactating ewes. Br. J. Nutr. 50:303-316.

Young, J. W. 1977. Gluconeogenesis in Cattle: Significance and Methodology. J. Dairy Sci. 60:1-15.

Zhu, L. H., L. E. Armentano, D. R. Bremmer, R. R. Grummer, and S. J. Bertics. 2000. Plasma Concentration of Urea, Ammonia, Gluatmine Around Calving, and the Relation of Hepatic Triglyceride, to Plasma Ammonia Removal and Blood Acid-Base Balance. J. Dairy Sci. 83:734-740.