EFFECTS OF METHOD OF DELIVERY OF GLYCEROL ON PERFORMANCE AND METABOLISM OF DAIRY COWS DURING THE TRANSITION PERIOD

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ABSTRACT

Holstein cows (n=48) entering second or greater lactation were utilized to determine the effects of method of delivery of glycerol on performance and metabolism of dairy cows during the transition period. Beginning 21 d before expected parturition, cows were fed either a control diet or a diet containing glycerol (5% of DM). After parturition, cows were assigned to one of four treatments in a 2 (dietary glycerol; 3.3% of DM) X 2 (glycerol drench; 500 ml/d for 5 d beginning at parturition) factorial arrangement. From d 22 through 63 of lactation, cows were fed the same diet. Feeding glycerol during the prepartum period increased prepartum DMI, but feeding glycerol during the postpartum period tended to decrease postpartum DMI and drenching glycerol for the first 5 d of lactation decreased postpartum DMI. Milk yield was not affected by feeding glycerol during either the prepartum or postpartum periods or drenching glycerol during the first 5 d of lactation. Percentages and yields of milk fat and true protein were not affected by feeding glycerol during either the prepartum or postpartum periods; however, drenching glycerol tended to decrease milk protein content and decreased milk lactose content. Glycerol fed during the prepartum period resulted in no significant effects on plasma glucose, NEFA or BHBA concentrations during the prepartum period with no carry over effects on postpartum metabolites. Prepartum incorporation of glycerol in the diet resulted in no significant effects in liver triglycerides or glycogen content in liver samples collected d 1 after calving compared with control cows with no carry over effects on postpartum liver triglycerides or

glycogen content. Postpartum incorporation of glycerol in the diet resulted in no significant effects on postpartum liver composition. Short term (5-d) oral drenching of glycerol beginning at calving resulted in no significant effects on liver composition (d 10 and 21 postpartum) or on plasma glucose and NEFA. However, there was a trend for an increase in BHBA concentrations for cows drenched with glycerol. Intensive blood sampling performed on d 5 post calving demonstrated that a 500 ml oral bolus of crude glycerine significantly decreased plasma NEFA concentration with no overall significant effects on plasma glucose, BHBA, or insulin. Overall, incorporation of glycerol in to the diets of transition cows or the short-term oral drench of glycerol at calving resulted in few positive performance responses and only modest effects on metabolic variables studied.

BIOGRAPHICAL SKETCH

Kathleen Lynn Ogborn was born and raised in Massena, New York. Kathleen graduated from Massena Central High School in 1987. She then attended the State University of New York, Canton College of Technology and obtained an A.A.S. in Business Administration in 1989. Kathleen went to work for the American Morgan Horse Association in Shelburne, Vermont. In 1991 Kathleen returned to New York to work for I.L. Richer Company (Richer Feeds). After working for Richer Feeds for three years, Kathleen felt the need to be more involved with animal health and returned to SUNY Canton to complete an A.A.S. degree in Veterinary Technology and became a licensed veterinary technician in 1996. Kathleen went on to attend Cornell University to fulfill a B.S. degree in Animal Science in 1998. Kathleen continued working as a veterinary technician in private practice until she was offered a teaching position at the State University of New York, Delhi College of Technology in 1999. Kathleen's dedication to animal health and teaching in the veterinary field encouraged her to continue her education and pursue a Master of Science degree in animal science. Upon completion of this degree, Kathleen plans to continue teaching at SUNY Delhi where she is currently an assistant professor in veterinary technology.

In memory of Luther

In life and in death, you have been my inspiration to live life.

Your love and gentle touch will never be forgotten.

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LIST OF ABBREVIATIONS

ADF =	Acid detergent fiber
ADICP =	Acid detergent insoluble crude protein
BCS =	Body condition score
BHBA =	B-hydroxybutyric acid
BW =	Body weight
Ca-PFAD =	Calcium salts of palm fatty acids
CNCPS =	Cornell Net Carbohydrate and Protein System
CO_2 =	Carbon dioxide
CP =	Crude protein
DCAD =	Dietary cation-anion difference
DM =	Dry matter
DMI =	Dry matter intake
EE =	Ether extract
FCM =	Fat corrected milk
MP =	Metabolizable protein
MUN =	Milk urea nitrogen
NAHMS =	National Animal Health Monitoring System
NDF =	Neutral detergen fiber
NDFCP =	Neutral detergent fiber crude protein
NEB =	Net energy balance
NEFA =	Non esterified fatty acids
NE_L =	Net energy of lactation
NFC =	Nonfiber carbohydrates
NRC =	National Research Council

SE = Standard error

SEM = Standard error of the mean

TMR = Total mixed ration

VFA = Volatile fatty acids

VLDL = Very low density lipoproteins

CHAPTER ONE:

INTRODUCTION

The transition period of dairy cows, ranging from three weeks prepartum to three weeks postpartum, is a period marked with large changes in metabolic demands due to parturition and lactogenesis (Grummer, 1995; Drackley, 1999). Failure of the cow to properly coordinate her metabolism in support of lactation results in impaired performance and increased incidence of metabolic disorders. Ketosis and fatty liver are two metabolic disorders related to energy metabolism that occur in varying frequency and severity during the transition period of the dairy cow (Gummer, 1993). These conditions result in reduced milk production and poorer reproductive performance along with increased culling rates and veterinary costs. Developing and deploying methods to reduce the incidence of ketosis and fatty liver in the transition cow should improve the overall well being of the dairy cow along with increase productivity and profitability of the dairy industry.

One of the major metabolic challenges confronting the transition cow is gradually increasing demands for glucose to support the gravid uterus during late pregnancy followed by a dramatic increase in glucose demand at the onset of lactogenesis and copious milk secretion during early lactation (Bell, 1995; Reynolds, et al., 2003a). Concurrent with these increasing demands for glucose and other nutrients, the cow experiences a decline in voluntary feed intake as parturition approaches (Ingvartsen and Andersen, 2000), which results in the onset of a period of negative energy balance that begins prior to parturition and continues through early lactation. This results in mobilization of substantial amounts of adipose tissue reserves in the form of nonesterified fatty acids (NEFA) to meet overall energetic demands for lactation.

The liver is responsible for the majority of the increased gluconeogenesis that must occur to support this increased demand for glucose; however, the liver also takes up NEFA in proportion to their supply in the circulation (Reynolds, et al., 2003a). The liver uses these NEFA for energy; however, in excess they are accumulated as fat in liver tissue and it is believed that excessive accumulation of fat in liver tissue decreases the gluconeogenic capacity of the liver (Overton and Waldron, 2004). Therefore, strategies to manage metabolism of NEFA by the liver of dairy cows are of biological and economic interest. One opportunity to manage metabolism of NEFA and improve metabolic health of the transition cow exists through manipulations of energy supply. Increasing energy intake of the transition cow either through increased dry matter intake or increasing the energy density of the diet during the transition period can decrease circulating NEFA concentrations and potentially the amount of NEFA extracted by the liver (Grummer, 1995). In addition, oral administration of glucogenic compounds such as propylene glycol can increase circulating concentrations of glucose and insulin and decrease concentrations of NEFA, thereby potentially lowering triglyceride accumulation in the liver (Ingvartsen et al., 2003).

Increased understanding of the metabolism of glucogenic supplements and their application offers the opportunity to potentially improve metabolic health, productivity, and profitability of dairy farms. The purpose of the review of literature to follow is to overview the transition period and the dramatic metabolic changes that occur during this timeframe, and then to review the current state of knowledge regarding the biology and application of glucogenic supplements as tools to improve the metabolic health of transition dairy cows.

CHAPTER TWO:

REVIEW OF LITERATURE

TRANSITION PERIOD

The transition period has been defined as three weeks prepartum to three weeks postpartum (Grummer, 1995), and is a time period where cows are susceptible to most major metabolic disorders. Disorders such as milk fever, ketosis, retained placenta, metritis and displaced abomasum occur on farms with varying frequency and severity during early lactation along with fatty liver and ketosis. Health disorders that occur during the transition period result in reduced milk production (King, 1979; Rowlands and Lucey, 1986; Detilleux and Grohn, 1994) and reproductive performance (Gerloff, et al., 1986) along with increased culling rates and veterinary costs (Detilleux and Grohn, 1994). The success of the transition period has a significant impact on performance and health, and thus profitability of the transition cow.

Jordan and Fourdraine (1993) studied 61 of the highest producing herds in the United States and reported the incidences of metabolic disorders in these herds. They evaluated these herds for incidence of milk fever, displaced abomasum, ketosis, retained placenta and metritis. Their survey revealed that 20% of cows had at least one of these health disorders during the periparturient period. The mean incidence of ketosis, milk fever and metritis were 3.7%, 7.2%, 12.8%, respectively, with ranges of 0 to 20%, 0 to 44.1% and 0 to 66%, respectively. In addition to their clinical forms, milk fever, ketosis, and metritis can occur in subclinical forms. As a part of the USDA National Animal Health Monitoring System (NAHMS, 2002) dairy study, subclinical hypocalcemia was defined as serum calcium less than 8.0 mg/dl (Horst, et al., 2003). From this study it appears that those cows with serum

calcium levels higher than 8.0 mg/dl also had lower serum NEFA concentrations than those cows with calcium levels lower than 8.0 mg/dl. This evidence suggests that normocalcemic cows tend to have a better energy balance and are less susceptible to energy-related disorders such as displaced abomasum, fatty liver, and ketosis. Subclinical ketosis [defined as circulating concentration of B-hydroxybutyric acid (BHBA) greater than 1400 umol/L (Duffield, et al., 1998)] was present in approximately 25% of the control cows in a field study conducted on dairy farms in Ontario (Duffield, et al., 1998). Subclinical ketosis results in impaired milk production. Duffield (1997) estimated that cows having serum BHBA concentrations greater than 1600 μmol/L produced 1.8 kg less milk per day, cows having serum BHBA greater than 1800 μmol/L produced 3 kg/d less milk per day, and cows having serum BHBA greater than 2000 μmol/L produced 4 kg/d less milk.

When the transition period is affected by health disorders, milk production losses occur not only during the time of illness but often persist into lactation. In a study conducted by Wallace et al. (1996), mean daily milk yield for the first 20 d of lactation was 7.2 kg/d lower in cows that experienced a health disorder compared with cows that did not. Furthermore, DMI was decreased by 5.4 kg/d for cows that experienced a health disorder compared with those that did not. Rajala-Schultz et al. (1999) determined that milk losses due to ketosis began even before the diagnosis of clinical ketosis and that milk losses in mature cows persisted throughout lactation. Energy-related metabolic disorders such as ketosis often occur in a complex with hepatic lipidosis (Gummer, 1993). In addition to the correlation of triglyceride accumulation in the liver with increased circulating concentrations of BHBA and decreased capacity for gluconeogenesis from propionate (Piepenbrink

and Overton, 2003), hepatic lipidosis in varying degrees has been linked to decreased reproductive performance (Reid, 1980; Reid, 1982; Jorritsma, et al., 2003).

As is apparent from the discussion above, the transition period is a potentially challenging time for the dairy cow. Part of this challenge relates to the dynamic nature of nutrient demand and supply relationships together with the failure of nutritional management systems on many farms to adequately support the metabolic demands during this timeframe (Overton and Waldron, 2004). One of the hallmarks of this period is a large increase in demand for energy and all categories of nutrients on or about the day of parturition combined with inadequate DMI to satisfy these demands. Beginning two to three weeks prepartum, the transition cow decreases her DMI by approximately 30% while nutrient requirements for the gravid uterus and, subsequently the mammary gland, begin to increase dramatically (Bell, 1995; Grummer, 1995). Accordingly, virtually all cows enter a period of negative energy balance during the final week before parturition that continues through the first few weeks of lactation (NRC, 2001). During this same time period, the transition cow mobilizes adipose tissue in an attempt to meet this energetic demand. The following sections of this review of literature will overview aspects of energy metabolism of the transition dairy cow, together with the state of knowledge of the use of glucogenic supplements to positively affect energy metabolism and improve metabolic health of transition dairy cows.

ENERGY DEMANDS OF THE TRANSITION PERIOD

The dairy cow experiences dramatic increases in her demand for glucose during late pregnancy and early lactation. Whole-body glucose demand during the last 21 d prepartum has been estimated to be 1000 to

1100 g/d and the demand for glucose increases dramatically after calving to be 2.5 times greater at 21 d postpartum (Bell, 1995). Although glucose supply based upon dietary energy intake has been predicted to be insufficient by about 500 g/d during the first three weeks post-calving (Overton, 1998), it is clear that hepatic gluconeogenesis is substantially upregulated to supply sufficient glucose to the cow (Reynolds et al., 2003a).

During the transition period, metabolic changes must occur to meet the demand of the mammary gland during early lactation. In support of lactation, oxidation of glucose by peripheral tissues is reduced to conserve glucose for milk synthesis while liver metabolism must increase dramatically at calving to support glucose demands (Overton, 1998; Reynolds, et al., 2003a). Liver size does not increase significantly during the transition period (Reynolds, et al., 2003b); however, calculations of metabolic activity as indicated by oxygen uptake per unit of liver weight doubles during early lactation (Overton and Piepenbrink, 2001). This increased metabolic activity is attributed to the increase in gluconeogenesis to meet glucose demand.

Gluconeogenesis

Glucose is required as a source of fuel for the gravid uterus, mammary gland, some peripheral tissues, central nervous system, red blood cells, gastrointestinal tract, and also lactose synthesis by the lactating mammary gland. Very little net absorption of glucose occurs in the intestinal tract of ruminants; therefore, the cow is almost entirely dependent upon gluconeogenesis in the liver, and to a lesser extent the kidney, to support overall glucose needs (Seal and Reynolds, 1993). Ruminal fermentation of starch and other fermentable carbohydrates yields glucogenic substrates such as propionate, amino acids, and lactate. Maximal contributions to hepatic

gluconeogenesis from glucogenic substrates, based upon uptake/output relationships of these substrates with hepatic glucose release, have been estimated to range from 32 to 72% for propionate, 10 to 30% for amino acids, 15% for lactate and also a small amount from glycerol (Seal and Reynolds, 1993). Initial data suggest that the contributions of these substrates to hepatic gluconeogenesis in the transition dairy cow occur at similar proportions throughout the transition period (Reynolds et al., 2003).

As described above, propionate produced from ruminal and hindgut fermentation is the volatile fatty acid that makes a net contribution to glucose synthesis and is the most important precursor for hepatic gluconeogenesis. Increased availability of propionate from the rumen potentially increases gluconeogenesis in the liver, raising blood glucose levels, increasing insulin concentrations, which would then decrease adipose tissue mobilization (Grummer, 1995).

The amount of propionate available for gluconeogenesis and oxidative metabolism is directly related to the amount of nonfiber carbohydrates (NFC) consumed by the cow, which is a function of both NFC concentration of the diet and dry matter intake. Available data suggest that the contribution of propionate to hepatic gluconeogenesis is related to its supply. Propionate contributed 43.3% of the carbon for gluconeognesis in steers fed a control diet; supplying additional propionate as sodium propionate increased its contribution to 67.1% (Veenhuizen, et al., 1988). Aiello et al. (1984) found the rate of conversion of [1-^{14}C]propionate to glucose was higher in liver slices from cows fed a high concentrate diet as compared to cows fed a high forage diet. In addition, Drackley et al. (2001) suggested that the capacity of liver slices to convert [1-^{14}C]propionate to glucose was increased as fat-free NE_L

intake increased during the first few weeks of lactation. However, this relationship was not evident in cows during the middle of the dry period and at 65 d postpartum, suggesting that overall glucose demand may also affect the relationship between propionate supply and its contribution to hepatic gluconeogenesis.

All amino acids except lysine, leucine and taurine can contribute to gluconeogenesis in ruminants. In ruminants, alanine and glutamine have been reported to make the greatest contribution to glucose synthesis, and may account for as much as 40 to 60% of all the gluconeogenic potential from amino acids (Bergman and Heitmann, 1978). The contribution of amino acids to glucose production may also be related to their supply (Lindsay and Williams, 1971; Danfaer, et al., 1995). Amino acids from skeletal muscle also apparently serve as a source of glucogenic precursor in support of gluconeogenesis (Bell, 1995). Overton et al. (1998) reported that the capacity of liver slices to convert [1-^{14}C]alanine to glucose was approximately doubled at one day postpartum compared to late pregnancy in dairy cows. These results were similar to those of Reynolds et al. (2003), who determined that the maximal contribution of alanine to glucose synthesis was doubled at 11 d postpartum compared to the prepartum period in dairy cows.

Lactate utilized for glucose production is a result catabolism of glucose by peripheral tissues (representing recycling of carbon) or propionate by ruminal epithelium. However, lactate is not produced in significant amounts during fermentation of typical diets fed to transition cows (Nocek, 1997). Some evidence suggests that lactate may make a greater contribution to gluconeogenesis during late gestation than early pregnancy due to the release of lactate by the gravid uterus and muscle in late gestation (Bell, 1995). Baird

et al. (1983) determined that the percentage of total glucose recycling and percentage contribution of lactate to glucose flux were lower during early lactation than during late pregnancy. Although the amount of lactate that was potentially converted to glucose by liver was increased during early lactation compared with late pregnancy, the maximal contribution of lactate to glucose synthesis expressed as a percentage of glucose release was not markedly altered during the transition period (Reynolds et al., 2003).

The availability of glycerol as a substrate for glucose production also can arise as a result of carbon recycling and may be an important gluconeogenic precursor as the cow adapts to lactation. Approximately 3.2 kg/d of triglycerides are mobilized at 4 d postpartum and may provide maximally 15 to 20% of the glucose demand (Bell, 1995). The contribution of glycerol to gluconeogenesis will depend on energy balance and the degree to which adipose tissue is mobilized (Overton, 1998; Drackley et al., 2001). Recent data (Reynolds et al., 2003) suggest that glycerol may be an important glucose precursor only during the immediate peripartum period, as glycerol uptake by liver at both 10 d prepartum and 11 d postpartum was low and accounted maximally for a small percentage of glucose release by liver.

Endocrine Regulation of Gluconeogenesis

Although the availability of propionate and, to some extent, amino acids for gluconeogenesis is controlled by dietary supplies of fermentable carbohydrates and protein, availabilities of other substrates (e.g., glycerol, lactate, and amino acids from catabolism of skeletal muscle protein) are partially under hormonal control, and overall hepatic gluconeogenesis has a component of endocrine regulation. Hormones that influence hepatic gluconeogenesis include insulin, glucagon, somatotropin, and cortisol.

Insulin is released by the beta cells of the pancreas in response to elevated circulating concentrations of glucose and, along with glucagon, is responsible for glucose homeostasis. Insulin both promotes glucose uptake and oxidation by tissues and can decrease hepatic gluconeogenesis in ruminants (Brockman, 1985; Brockman and Laarveld, 1986). Results of Brockman (1990) suggest that insulin also decreases the availability of other glucose precursors including amino acids by 30 -50% for gluconeogenesis in growing ruminants, in part by promoting anabolism in muscle tissue. Insulin concentrations decrease at parturition and remain low during early lactation (Bell, 1995), which promotes hepatic gluconeogenesis. The decreased contributions of lactate to gluconeogenesis described above during early lactation may be a function of decreased concentrations of insulin and decreased responses of peripheral tissues to insulin-dependent glucose uptake for oxidative metabolism during early lactation as a homeorhetic adaptation to lactation (Bauman and Currie, 1980; Bell, 1995).

Glucagon is released by alpha cells in the pancreas in response to low circulating concentrations of glucose and promotes both hepatic gluconeogenesis and the breakdown of glycogen in the liver. Glucose synthesis from amino acids is stimulated by glucagon and inhibited by insulin (Danfaer, et al., 1995). Intravenous infusion of glucagon into both dairy cows (She et al., 1999) and sheep (Brockman and Bergman, 1975) resulted in an increase in glucose synthesis rate. Hippen et al. (1999) found the intravenous administration of glucagon in early lactation cows increased plasma glucose and decreased plasma concentrations of β-hydroxybutyrate and NEFA. These researchers also found hepatic triglyceride content to be decreased by 71% at 35 d postpartum or after 14 d of glucagon treatment compared to control cows.

This research indicates that exogenous glucagon may play a role in increasing plasma glucose and decreasing fatty liver in the transition cow.

Somatotropin is a homeorhetic hormone that works to coordinate the metabolism of tissues to partition nutrients in the ruminant animal and appears to have a role in promoting hepatic gluconeogenesis in ruminants. Plasma concentrations of somatotropin increase during late gestation, peak at parturition and decrease slowly postpartum (Bell, 1995). This hormone functions in stimulation of gluconeogenesis by increasing the metabolism of propionate to glucose (Danfaer, et al., 1995) and the partitioning of nutrients to the mammary gland for milk synthesis (Bauman, 1992). Liver slices from cows treated with exogenous somatotropin showed an increase in the conversion of [1-^{14}C] propionate to glucose (Pocius and Herbein, 1989; Knapp, et al., 1992); therefore, elevated concentrations of somatotropin that persist from parturition through early lactation may help to promote hepatic gluconeogenesis in support of the increased glucose demand.

Glucocorticoids such as cortisol will promote gluconeogenesis and increase glucose blood supply. Plasma cortisol concentrations increase during the last 3 d before calving then peak at parturition and decrease to prepartum levels by 3 to 5 d of lactation (Goff et al., 1989). Glucocorticoids stimulate lipolysis and the mobilization of amino acids from extrahepatic tissues; the amino acids can then used by liver for gluconeogenesis.

FATTY ACID METABOLISM

As described above, dairy cows typically experience a period of negative energy balance during the immediate periparturient period and early lactation (Bauman and Currie, 1980). As a result, mobilization of adipose

tissue reserves occurs in essentially all clinically normal cows during this period. Net mobilization of adipose tissue, representing a balance of lipolysis and lipogenesis, results in the release of NEFA into the bloodstream for oxidative metabolism by peripheral tissues and incorporation into milk fat (Drackley, 1999). In addition, the liver takes up NEFA from the circulation in proportion to supply during the periparturient period (Reynolds et al., 2003) and either oxidizes them in the mitochondria or peroxisomes or reesterifies them into triglycerides for storage or export (Grummer, 1993; Drackley et al., 2001). The mobilization of body stores and accumulation of liver triglycerides begins prior to parturition, liver triglyceride concentrations peak at or following parturition, and hepatic lipidosis can precede ketosis in many cases (Grummer, 1993).

As described above, the liver takes up NEFA in proportion to their circulating supply and either oxidizes them or reesterifies them into triglycerides. Compared to nonruminants, ruminant liver has limited capacity to oxidize fatty acids and export triglycerides in VLDL (Gummer, 1993). This contributing factor predisposes the transition cow to the development of fatty liver. Low concentrations of liver triglyceride accumulation most likely have insignificant effects on liver metabolism (Overton and Waldron, 2004); however, as triglycerides accumulate in the liver, the capacity of the liver to produce glucose from propionate appears to decrease (Strang et al. 1998; Piepenbrink and Overton, 2003). Thus, it is uncertain whether the association of hepatic lipidosis with ketosis is simply a function of high circulating concentrations of NEFA that predispose cows to accumulate triglycerides in liver or a direct interference of triglyceride accumulation with hepatic carbohydrate metabolism that leads to the onset of ketosis.

GLUCOGENIC SUPPLEMENTS

Given the interest in increasing glucose availability and decreasing NEFA mobilization from adipose tissue during the transition period to facilitate the cow's metabolic adaptation to lactation and help to minimize occurrence of energy-related metabolic disorders, researchers have focused on strategies to increase the supply of glucogenic nutrients (Grummer, 1995; Overton and Waldron, 2004). This has typically been accomplished by either increasing the nonfiber carbohydrate content of the diet fed prepartum or by administering glucogenic supplements during the periparturient period (NRC, 2001; Overton and Waldron, 2004).

Glucogenic supplements are substances that are administered or fed to the cow that can subsequently be absorbed and converted to glucose by the liver, with the intent of increasing glucose availability to the cow. This increased glucose availability to the cow can promote insulin secretion, which in turn should decrease NEFA release from adipose tissue (Gummer, 1993; Overton and Waldron, 2004). Gluconeogenic supplements have been reported to decrease NEFA and β-hydroxybutyrate (BHBA, the predominant ketone body found in blood) and increase blood glucose. Bertics et al. (1992) proposed that glucose precursors administered prepartum would increase blood glucose which will elicit an insulin response and reduce mobilization of fatty acids from adipose tissue. Several gluconeogenic supplements including propylene glycol, sodium or calcium salts of propionate, and glycerol have been investigated and determined to be an effective means of preventing fatty liver and treating ketosis.

Propylene Glycol: Propylene glycol was first investigated in the 1950s (Johnson, 1953) as a treatment for clinical ketosis. As detailed below, many

studies have been conducted to investigate the effects of propylene glycol on metabolic parameters indicative of risk for ketosis and fatty liver and changes in ruminal volatile fatty acids in response to propylene glycol supplementation.

Johnson (1953) used lactating cows showing signs of clinical ketosis to evaluate the effects of oral administration of propylene glycol. Propylene glycol was administered in varying amounts from as much as 1800 g via rumen tube to as little as 225 g in the grain portion of the diet. Concerns with palatability were noted in this experiment; therefore, it was suggested that propylene glycol may be best administered in drinking water or as a drench versus in the diet. Clinical observations with concurrent monitoring of blood glucose concentrations suggested that oral administration of propylene glycol was an effective treatment for ketosis. A disadvantage of propylene glycol may be its toxic effects when administered in large quantities. Johnson found that propylene glycol given to ketotic cows in large quantities (> 800 g/d) may cause incoordination for several hours post treatment. Smaller quantities ranging from 200 – 500 g/d were effective in increasing plasma glucose without symptoms of toxicity. More intensive studies have been performed since this initial field trial. These studies have included evaluation of changes in rumen fluid, plasma concentrations of glucose, NEFA, BHBA, insulin, as well as liver triglycerides and milk production.

Several studies have evaluated the effects of oral administration of propylene glycol on ruminal parameters (Emery, et al., 1964; Fisher, et al., 1971; Grummer, et al., 1994; Christensen, et al., 1997). Propylene glycol leaves the rumen through three routes: absorption, fermentation, or passage to the lower intestinal tract. Emery et al. (1964) concluded that most of the propylene glycol escapes the rumen intact with a small portion metabolized to

propionate; however, Kristensen et al. (2002) recently determined that the majority of propylene glycol is metabolized in the rumen. Other reports also have indicated that the molar proportion of propionate in ruminal fluid is increased when ruminants have been fed propylene glycol (Emery et al., 1964; Fisher et al., 1971; Grummer et al., 1994; Christensen et al., 1997). Propylene glycol that escapes rumen fermentation reaches the liver where it is converted to glucose. Rumen fermentation of propylene glycol increases rumen concentrations of propionate which in turn is converted to glucose. Regardless of whether the liver receives propylene glycol in its native form or as propionate, peak circulating concentrations of glucose and insulin occur within the first 90 min after oral drenching, which indicates that propylene glycol is rapidly absorbed from the rumen and utilized in the liver for glucose production (Nielsen and Ingvartsen, 2004).

Propylene glycol decreases plasma concentrations of NEFA and BHBA while increasing concentrations of insulin and glucose. Grummer et al. (1994) compared 0, 300, 600, and 900 ml of propylene glycol administered once daily via oral drench to feed-restricted heifers. Increasing doses of propylene glycol demonstrated linearly increased plasma glucose and serum insulin concentrations and linearly decreased plasma NEFA and BHBA. Pickett et al. (2003) determined that once-daily drenching of 500 ml of propylene glycol during the first 3 d postpartum decreased concentrations of NEFA which concurred with data of Stokes and Goff (2001), who administered 300 ml of propylene glycol via oral drench for the first 2 d postpartum. Plasma BHBA also tended to be decreased by propylene glycol drench in the study by (Pickett et al., 2003); however, Stokes and Goff (2001) reported that BHBA was not affected by propylene glycol administration during the first 2 d

postcalving. Long term administration of propylene glycol to feed restricted heifers for 14 d did not affect plasma glucose or BHBA concentrations; however, insulin was increased and NEFA was decreased by propylene glycol administration (Christensen, et al., 1997).

The combined effects of propylene glycol on plasma glucose and serum insulin concentrations along with plasma NEFA and BHBA should in turn help to reduce hepatic triglyceride accumulation. Studer et al. (1993) evaluated propylene glycol as a 1 L oral drench given once daily beginning approximately 10 d before expected parturition on periparturient fatty liver. Liver triglycerides were reduced by 32 and 42% at 1 and 21 d postpartum along with decreased plasma NEFA and BHBA, and increased plasma glucose and insulin. Pickett et al. (2003) reported that propylene glycol administration did not affect liver triglyceride accumulation. These studies suggest that the effect of propylene glycol on liver triglyceride accumulation may vary depending upon the physiological state or overall opportunity to decrease liver triglyceride accumulation.

Physiological state of the animal also appears to influence the overall effects of propylene glycol administration. In the 1970s, Canadian researchers (Fisher, et al., 1971; Fisher, et al., 1973; Sauer, et al., 1973) investigated propylene glycol as a feed additive. Results indicated that normal cows not subject to high lactational demands or inadequate feed intake during lactation did not benefit from adding propylene glycol to diet. However, when cows were both highly productive and had lower feed intake, dietary supplementation with propylene glycol showed reduced plasma BHBA, NEFA, and increased glucose concentrations. Early lactation cows and feed-restricted heifers are in negative energy balance and, therefore, mobilize more

adipose tissue resulting in higher concentrations of NEFA. Propylene glycol appears to have more notable effects on plasma BHBA in animals with higher NEFA concentrations (Nielsen and Ingvartsen, 2004).

Typically, milk yield and composition are not significantly affected by the administration of propylene glycol (Nielsen and Ingvartsen, 2004). Some studies do indicate that the supplementation of propylene glycol around calving or during early lactation may have a tendency to increase milk yield (Emery, et al., 1964; Fisher, et al., 1973; Studer, et al., 1993; Pickett, et al., 2003). It is logical that the effect of propylene glycol on milk yield may be dependent upon whether a metabolic condition existed before administration that resulted in decreased milk yield.

Although the concept of adding propylene glycol to the diet is attractive, available data suggest that propylene glycol is most effective if administered as an oral drench (Hutjens, 1996; Christensen et al., 1997; Overton and Piepenbrink, 2001). Propylene glycol has been described as being unpalatable and therefore when added to the diet may decrease DMI (Johnson, 1953; Fisher, et al., 1971; Fisher, et al., 1973; Sauer, et al., 1973). Christensen et al. (1997) compared the effects of method of delivery of propylene glycol. They reported that the administration of propylene glycol as an oral drench or in a concentrate resulted in more noticeable effect on blood parameters and ruminal fluid concentrations than when administered in a TMR. This is most likely due to more rapid uptake as an oral drench and in concentrate feeding versus the slow intake as part of a TMR. Therefore the use of propylene glycol as a dietary ingredient is less effective than when administered as an oral drench.

Sodium and Calcium Propionate: In ruminant animals, propionate is a known glucose precursor and propionate supplements complexed with Na, Ca, or trace minerals could be used to increase plasma glucose concentrations in the transition cow. When sodium propionate or calcium propionate is used as a dietary supplement, propionate is released from sodium or calcium and absorbed across the rumen wall into blood then transported to the liver where it is converted to glucose.

Early feeding studies in which (Schultz, 1958) sodium propionate was fed at a rate of 114 g per cow per day during the first 6 weeks of lactation indicated an increase in blood glucose along with reduced blood ketone concentration. Goff (1996) administered calcium propionate in the form of a paste and reported trends for decreased plasma concentrations of NEFA, and BHBA, which supports the theory that propionate supplements may improve energy status and prevent the development of ketosis and fatty liver. Other studies using propionate supplements have reported no significant effects on milk yield or plasma concentrations of NEFA and BHBA (Burhans and Bell, 1998; Stokes and Goff, 2001).

Glycerol: Glycerol has been reported to be a sweet-tasting liquid substance that, as a three-carbon molecule, enters the gluconeogenic pathway and can be used as a gluconeogenic supplement. It was evaluated as an aid for treatment of ketosis in the 1960s and 70s but not adopted due to high costs (Fisher et al., 1973; Sauer et al., 1973). New sources of glycerol as a byproduct of biodiesel have reduced the cost (Schroder and Sudekum, 1999) thus making glycerol more attractive and a potential supplement for addition to transition cow diets. Renewed interest of the use of glycerol is

evidenced by recent studies (Schroder and Sudekum, 1999; Goff and Horst, 2001; DeFrain et al., 2004; Linke et al., 2004).

Glycerol can be converted to glucose in the liver and enters the glucogenic pathway at the level of dihydroxyacetone phosphate and 3-phosphoglyceraldehyde. This is only true when glycerol is absorbed or administered directly into circulation. Oral administration of glycerol may result in fermentation in the rumen. Linke et al. (2004) found administration of 1 kg of glycerol as a dietary supplement, an oral drench, and via rumen tube increased rumen propionate compared to control cows (28.7, 30.4, 30.4 and 26.4 molar percent, respectively). This would suggest that glycerol is fermented in the rumen and provides propionate as a glucose precursor. This is supported by the observed increase in plasma glucose concentration expressed as area under the curve over baseline for 8 h (23.6, 54.6, 58.1, and 9.4 mg/dl*h, respectively). Goff and Horst (2001) administered 1, 2 or 3 L of glycerol via an esophageal pump and found an increase in plasma glucose by 16, 20 and 25% over pretreatment values respectively. Evaluation of ruminal contents by Linke et al. (2004) also indicated that glycerol administration increased butyrate concentrations in rumen fluid (20.0, 20.3, 21.5 and 14.1 molar percent) Increases in ruminal butyrate in effect can result in increased blood ketones such as BHBA, which can be used for energy by various tissues.

Early studies (Johnson, 1953) added glycerol to the grain portion of the diet to clinically ketotic cows and found improvements in appetite, milk production and plasma glucose as well as a decrease in plasma ketones. Fisher et al. (1971, 1973) added glycerol to the grain portion of the diet (3 and 6% of concentrate) of early lactation cows and found little difference in milk

production (25.8 and 24.3 vs. 25.5 kg/d) when compared to control cows. Fisher et al. (1971) determined that feeding a concentrate mixture containing 3.3% glycerol increased DMI (11.4 vs. 14.3 kg/d). The increase in DMI was not observed in their 1973 study comparing glycerol and propylene glycol; however, cows that consumed the diet containing 6% glycerol appeared to lose less body weight than control cows or cows fed a concentrate containing 3% glycerol or 3% propylene glycol.

Defrain et al. (2004) evaluated glycerol supplementation in the diet of transition cows. Glycerol (0, 0.5, or 1.0 kg/d) was topdressed on to the top one-third of a total mixed ration (TMR) and fed to transition cows from 14 d prepartum to 21 d postpartum. Glycerol treatments did not affect prepartum concentrations of glucose, insulin, NEFA or BHBA. However, postpartum concentrations of plasma glucose tended to be higher for the cows fed the control diet compared to those fed glycerol (65.8 vs. 63.0 and 60.1 mg/dL). Significant diet and day interactions were observed during the postpartum period for concentrations of glucose, insulin, NEFA, and BHBA. Plasma glucose decreased in cows fed 0.5 kg/d at 7 DIM while insulin steadily increased from d 7 to d 21. Those cows fed 1.0 kg/d glycerol decreased sharply from d 14 to d 21 and insulin remained constant. Between 7 and 21 DIM, plasma concentrations of BHBA decreased in cows fed 0.5 kg/d and did not change in the control cows but increased in cows fed 1.0 kg/d. At 7 DIM, NEFA were greater in cows fed the control diet and 0.5 kg/d compared to cows fed 1.0 kg/d; however, concentrations became similar among treatments at 14 and 21 DIM. Cows fed either 0.5 or 1.0 kg/d of glycerol had decreased prepartum DMI compared with the control cows, but postpartum DMI was not affected by treatment. Milk yield of multiparous cows was decreased by

feeding 1 kg/d of glycerol compared with the other two treatments. This current data indicates that dietary glycerol during the transition period may provide modest support to increasing postpartum plasma glucose concentrations and decreasing NEFA.

Glucogenic Comparison: Propylene glycol ($C_3H_8O_2$), glycerol ($C_3H_8O_3$) and propionate ($C_3H_6O_2$) are three carbon molecules. It appears that propylene glycol and glycerol may be fermented to propionate in the rumen, thus making a contribution to glucose production in the form of propionate. In theory, it would require 2 moles of each of these glucose precursors to provide 1 mole of glucose ($C_6H_{12}O_6$). There is approximately 6.8 moles in 500 ml of propylene glycol and glycerol and 500 g of propionate. Therefore, if 100 percent of a 500 ml or 500 g dose of each of these glucogenic substances were utilized for glucose production would provide approximately 3 moles of glucose. Due to effects of the rumen environment, it is likely that less than 100% of these substances are utilized for gluconeogenesis.

TABLE 2-1. Molecular weight, density and moles of glucogenic compounds.

Glucogenic Compound	Molecular Weight (g/mole)	Density (g/ml)	Moles
Propylene Glycol	76.10	1.036	6.8/ 500 ml
Glycerol	92.09	1.26	6.85/ 500 ml
Propionate	74.08		6.75/ 500 g

In summary, opportunities exist to influence transition cow metabolism and potentially productivity by administration of glucogenic supplements. As indicated above, glycerol administered in the form of crude glycerine has shown potential to be utilized as a glucogenic substance. In particular,

questions remain as to the appropriate time period for glycerol supplementation (prepartum vs. postpartum), its effectiveness as a dietary supplement compared to an oral drench, and whether there are additive effects of feeding and drenching glycerol. These questions will be the subject of the research described throughout the remainder of this thesis.

CHAPTER THREE:

EFFECTS OF METHOD OF DELIVERY OF GLYCEROL ON PERFORMANCE OF DAIRY COWS DURING THE TRANSITION PERIOD

INTRODUCTION

The transition period has been defined as three weeks prepartum to three weeks postpartum (Grummer, 1995). It is a period characterized by dramatic metabolic adaptations due to parturition and lactogenesis. During the late prepartum period, marked reductions in DMI occur concurrently with increased nutrient demands for the growth of the conceptus and initiation of milk synthesis (Bell, 1995; Grummer, 1995). This results in a period of negative energy balance that begins several days prior to parturition and continues through early lactation, resulting in mobilization of NEFA from adipose tissue and increased risk of development of energy-related metabolic disorders such as fatty liver, ketosis, and displaced abomasum in transition cows (Grummer, 1993). Furthermore, these changes occur concomitantly with a period of decreased immunocompetence during the transition period (Drackley, 1999).

Ketosis and fatty liver results in reduced milk production (King, 1979; Rowlands and Lucey, 1986; Detilleux and Grohn, 1994) and reproductive performance (Gerloff et al., 1986) along with increased culling rates and veterinary costs (Detilleux and Grohn, 1994). The degree of success of transition period management is considered to largely determine the profitability of the cow during that lactation (Drackley, 1999). When the transition period is affected by health disorders, milk production losses occur not only during the time of illness but often the entire lactation (Detilleux and Grohn, 1994; Wallace, et al., 1996; Geishauser, et al., 1998).

Ketosis is characterized by increased concentrations of ketone bodies in the blood, urine, and milk. Ketosis can be considered to occur in subclinical or clinical forms (Baird, 1982; Geishauser et al., 1998). The prevalence of sub-clinical ketosis has been reported to occur in the range of 8.9 to 34% (Kauppinen, 1983). Studies in Ontario herds reported the prevalence to be 12 – 14% of lactating dairy cows, with subclinical ketosis resulting in a loss of milk production of 1.0 to 1.4 kg/d (Dohoo and Martin, 1984). Duffield (1997) reported that cows with plasma concentrations of BHBA greater than 1600 μmol produced 1.8 kg less milk per day while cows with more than 1800 μmol produced 3 kg/d less per day and cows with >2000 μmol produced 4 kg/d less than cows with plasma concentrations of BHBA less than 1600 μmol.

One commonly used strategy to treat or perhaps prevent the development of fatty liver and subclinical ketosis has been the administration of glucogenic supplements such as propylene glycol (Nielsen and Ingvartsen, 2004; Overton and Waldron, 2004). Most of the studies conducted have evaluated administration of propylene glycol as an oral drench and commonly have reported decreased circulating concentrations of NEFA and BHBA following administration of propylene glycol (reviewed by Nielsen and Ingvartsen, 2004). Although most of the experiments in the literature evaluated administration of propylene glycol for 10 to 40 d, research conducted recently (Pickett et al., 2003) established that short-term (3 d) administration of propylene glycol beginning at parturition would result in carryover decreases in NEFA and BHBA during the first 21 d of lactation. Furthermore, although extensive research has not been conducted, available data suggest that the metabolic response to propylene glycol is greater if

administered as an oral drench instead of incorporated into the diet (Christensen et al., 1997).

Glycerol is another glucogenic compound has been evaluated for prevention and treatment of energy-related metabolic disorders. Early studies (Johnson, 1953) added glycerol to the grain portion of the diet to clinically ketotic cows and found that it increased appetite, milk production and plasma glucose concentrations and decreased plasma ketones. In the 1970s, Canadian researchers (Fisher, et al., 1971; Fisher, et al., 1973; Sauer, et al., 1973) evaluated glycerol as a feed additive in comparison to propylene glycol. These studies indicated that dietary glycerol may increase DMI, plasma glucose concentration and energy status. These researchers discontinued use of glycerol in their 1973 trial due to high costs of glycerol. Further research focused on glycerol was not conducted for many years because of the high cost of glycerol relative to propylene glycol.

Recently, new sources of glycerol as crude glycerine have become available as a coproduct of biodiesel production from soybeans (Schroder and Sudekum, 1999) and thus interest in glycerol as a glucogenic supplement has been renewed (Goff and Horst, 2001; DeFrain et al., 2004; Linke et al., 2004). Recent studies have evaluated glycerol both as an oral drench and as a dietary supplement. Goff and Horst (2001) evaluated glycerol for ketotic lactating cows and reported increased plasma glucose concentrations following administration by oral drench. DeFrain et al. (2004) investigated the addition of glycerol in transition cow diets and its effects on blood metabolites and lactation performance. This study revealed that dietary glycerol administered as a topdress during the transition period had transient and varied effects on circulating glucose and NEFA concentrations. Furthermore,

glycerol administered as a topdress decreased prepartum DMI compared to controls. Linke et al. (2004) compared effects of both oral drenching and feeding of glycerol and reported that glycerol increased plasma glucose and insulin concentrations during the 8-h period following administration when administered orally as a drench or via stomach tube.

To date, studies have not been conducted to evaluate whether incorporation of glycerol into the TMR in conjunction with short-term administration via oral drench beginning at parturition will improve performance of transition dairy cows. Hence, the objective of this study was to determine the effect of method of delivery of glycerol on DMI, milk production, milk composition, body weight and BCS during the transition period and early lactation.

MATERIALS AND METHODS

Experimental Animals, Treatments, and Procedures

All procedures using experimental animals were approved by the Cornell University Institutional Animal Care and Use Committee. Forty-eight Holstein cows entering second or greater lactation were assigned to treatments in a completely randomized design. On one day per week prior to d 28 before expected parturition, cows were moved into tie stalls at the Cornell University Teaching and Research Center and fed a diet typical of that fed to close-up dairy cows in the Northeast and Upper Midwest (control diet, Table 3-1). Beginning 21 d before expected parturition, cows were assigned to receive either the control diet or the control diet with added glycerol (5% of DM). After parturition, cows were assigned in a balanced fashion to one of four treatments in a 2 (control vs. dietary glycerol; 3.3% of DM) X 2 (water vs. glycerol drench; 500 ml/d for 5 d) factorial arrangement. Glycerol was

supplied as crude glycerine, which contained 80.6% glycerol, 8.9% salt and 10.5% water (West Central Soy, Ralston, IA). From d 22 through 63 of lactation, cows were fed the postpartum control diet. Ingredient and nutrient composition of the prepartum and postpartum diets are listed in Table 3-1 and 3-2, respectively.

All cows were fed daily at approximately 1000 h. Amounts of feed offered and refused were recorded daily from 28 d prior to expected parturition until 63 d postpartum. Daily DMI were calculated based on the amounts offered and refused, corrected for DM content of the TMR. Individual forage and TMR samples were obtained on weekly basis and dried to a static weight

TABLE 3-1. Ingredient and nutrient composition (DM basis) of prepartum diets.

Ingredient	Control	Glycerol
Corn silage, %	43.7	41.5
Alfalfa silage, %	10.6	10.0
Grass hay, %	11.8	11.2
Straw, %	1.58	1.50
Citrus pulp, %	6.36	6.05
Wheat middlings, %	5.15	4.89
Soybean meal (47.5% CP), %	2.99	2.84
High moisture shell corn, %	2.88	2.74
Corn gluten feed, %	1.80	1.71
Corn gluten meal, %	1.31	1.24
Corn meal, %	1.12	1.06
Animal protein blend, %	0.72	0.68
SoyChlor[2], %	6.10	5.79
Mineral and vitamin mix, %	3.09	2.93
Ca-PFAD[1], %	0.76	0.72
Urea, %	0.13	0.12
Glycerine[3], %	----	5.0
Energy and nutrient		
NEL, Mcal/kg	1.59	1.61
NDF, %	37.1	35.9
NFC[4], %	39.2	40.5
Starch, %	22.6	19.8
Crude fat, %	3.46	3.98
CP, %	15.8	14.5
Lignin, %	3.43	3.37
Ca, %	1.02	0.96
P, %	0.38	0.37
K, %	1.29	1.24
Mg, %	0.35	0.33

[1]Ca-salts of palm fattty acid distillate (EnerGll;Bioproducts, Inc Fairlawn, OH)

[2]Anionic supplement (West Central Soy, Ralston, IA)

[3]80.6% glycerol, 8.9% salt and 10.5% water

[4]Calculated as 100 – [(NDF-NDFCP)+CP+EE+Ash] (NRC, 2001).

TABLE 3-2. Ingredient and nutrient (DM basis) composition of the postpartum diets.

Ingredient	Control	Glycerol
Corn silage, %	27.4	26.5
Alfalfa silage, %	17.1	16.5
Grass hay, %	4.71	4.55
Straw, %	1.03	1.00
Corn meal, %	10.7	10.3
High moisture shell corn, %	9.87	9.54
Soybean meal (47.5% CP), %	5.22	5.22
Soy Plus, %	4.95	4.95
Wheat middlings, %	3.94	3.94
Cottonseed, %	3.94	3.81
Citrus pulp, %	2.31	2.31
Soy hulls, %	1.84	1.84
Corn gluten meal, %	0.82	0.82
Fat-tallow, %	0.68	0.68
Animal protein blend, %	0.38	0.38
Mineral and vitamin mix, %	4.19	4.19
Ca-PFAD[1], %	0.99	0.99
Urea, %	0.12	0.12
Glycerol[2], %	---	3.30
Energy and nutrient		
NEL, Mcal/kg	1.69	1.70
NDF, %	30.7	30.2
NFC[3], %	41.7	41.3
Starch, %	26.9	26.1
Crude fat, %	4.73	4.79
CP, %	17.7	18.23
Lignin, %	3.58	3.49
Ca, %	0.91	0.96
P, %	0.35	0.35
K, %	1.12	1.12
Mg, %	0.26	0.26

[1]Ca-salts of palm fattty acid distillate (EnerGll;Bioproducts, Inc Fairlawn, OH
[3]80.6% glycerol, 8.9% salt and 10.5% water
[2]Calculated as 100 – [(NDF-NDFCP)+CP+EE+Ash] (NRC, 2001).

at 60ºC. A four-week composite sample was prepared from the individual weekly samples. Concentrate samples were obtained on a monthly basis. Prior to analysis, the four-week composites of forages and the monthly composites of concentrates were compiled into total experimental composites for each feedstuff. Composites of individual ingredients and TMR were prepared and subjected to the CNCPS nutrient profile analysis using wet chemistry techniques (Dairy One Laboratories, Ithaca, NY). Analysis of feed samples included CP, ADF, NDF, EE, ash, lignin, ADICP, NDICP, starch, soluble CP, and minerals. Nutrient composition of dietary ingredients is listed in Table 3-3.

The initial glycerol or water drench was administered within 36 h of calving and prior to feeding (between 0700 and 1000 h) and subsequent drenches were given at the same time each day for a total of 5 d. Drenches were administered using a 300 cc drench gun in two consecutive drenches to ensure the cow received a complete dose of 500 ml. A 500 ml dose of crude glycerine (80% glycerol on a weight basis) contained approximately 625 g of glycerol. Body weights and body condition scores were recorded on a weekly basis throughout the study. Two individuals evaluated and recorded body condition scores using a five-point scale (Wildman, et al., 1982). An average of the two individual scores was used as the assigned weekly value. General health records were maintained for each cow throughout the study.

After parturition cows were milked three times daily and individual milk weights were recorded at each milking through 63 d postpartum. Composites were prepared from all milkings on one day per week from each cow and analyzed for milk fat, true protein, lactose, MUN and somatic cell count using

TABLE 3-3. Nutrient composition of dietary ingredients (DM basis).

	Prepartum	Postpartum						High			Concentrates			
											Prepartum		Postpartum	
	Alfalfa silage	Alfalfa silage	BMR CS	Corn Silage	Grass Hay	Straw	Corn meal	moisture shell corn	Cottonseed	Soy Plus	Control	Glycerol	Control	Glycerol
CP, %	20.3	20.7	9.0	8.3	11.6	3.6	8.4	8.2	24.0	50.0	28.0	21.6	33.6	30.9
ADICP, %	1.6	1.7	0.4	0.5	1.0	0.9	0.4	0.2	2.5	1.0	1.3	1.1	1.2	1.0
Soluble CP, % of CP	57	77	63	66	34	49	15	51	55	10	35	28	30	29
NDICP, %	3.7	3.5	1.4	1.1	3.4	1.7	1.2	0.5	3.3	19.1	4.0	3.8	4.3	5.6
ADF, %	34.7	35.5	22.7	22.3	43.5	56.1	3.5	1.6	44.2	8.6	11.2	10.5	12.5	12.2
NDF, %	42.9	45.6	39.6	38.8	65.5	82.7	9.5	4.9	61.5	28.5	21.2	19.2	16.6	17.1
Lignin, %	7.6	8.5	1.9	3.4	7.9	9.7	1.3	0.6	15.7	3.1	2.4	2.3	0.8	0.9
NFC, %	27.8	24.9	45.9	47.5	17.7	8.6	77.5	80.7	...	27.2	38.0	44.5	29.7	31.9
Starch, %	4.1	3.3	36.3	36.7	2.2	2.4	72.2	75.5	1.1	1.7	10.8	9.5	4.0	2.8
Crude fat, %	2.7	3.0	3.2	3.4	1.7	1.6	4.2	5.1	15.2	6.9	4.8	6.2	6.6	7.4
Ash, %	9.9	9.3	3.7	3.1	6.9	5.2	1.5	1.6	4.0	6.6	12.0	12.2	17.8	18.4
NEL, Mcal/kg	1.32	1.28	1.72	1.69	0.97	0.37	2.09	2.18	1.67	2.02	1.76	1.83	1.78	1.80
Ca, %	1.29	1.22	0.22	0.2	0.77	0.23	0.02	0.02	0.14	0.36	2.44	1.94	2.35	2.71
P, %	0.33	0.33	0.29	0.28	0.35	0.11	0.3	0.34	0.61	0.81	0.58	0.51	0.42	0.49
K, %	2.3	2.2	1.1	0.9	2.4	1.1	0.3	0.4	1.1	2.1	0.9	0.8	0.9	1.0
Mg, %	0.26	0.25	0.19	0.18	0.20	0.11	0.11	0.12	0.34	0.31	0.73	0.60	0.45	0.60
Na, %	0.02	0.02	0.00	0.00	0.01	0.01	0.01	0.02	0.00	0.01	0.40	0.88	2.18	3.09
S, %	0.27	0.28	0.13	0.13	0.18	0.11	0.11	0.07	0.39	0.58	1.08	0.84	0.59	0.55
Fe, ppm	304	283	84	81	113	67	58	67	38	130	282	243	391	540
Zn, ppm	25	24	25	23	21	6	21	19	30	55	104	101	168	195
Cu, ppm	7	6	3	3	5	2	19	<1	5	15	31	27	43	48
Mn, ppm	33	30	13	11	35	20	11	5	11	31	98	81	147	145
Mo, ppm	0.7	0.7	0.5	0.4	0.8	0.2	0.6	0.2	0.1	2.7	2	2.1	1.5	2.6

mid infrared spectroscopy according to AOAC (2000) methods (DairyOne Laboratories, Ithaca, NY).

Statistical Analysis

Data for DMI and milk yield were reduced to weekly means prior to analysis. Effects of prepartum feeding of glycerol were evaluated separately for prepartum and postpartum variables using the MIXED procedure of SAS Version 8.2 (SAS Institute, Cary, NC) for a completely randomized design with repeated measures and specified error term. Terms included in the model used to evaluate prepartum effects were dietary treatment (control vs. glycerol), time, and their interaction. Effects of postpartum feeding or oral administration of glycerol (2 x 2 factorial arrangement of treatments) on postpartum variables were evaluated as above using SAS. Model terms were TMR, drench, their interaction, time, and two- and three-way interactions of main effects with time. Pretreatment values for DMI, BW and BCS were used as covariates during their respective analysis. Two cows were removed from the dataset as a result of problems at parturition and complications following the liver biopsy conducted at d 1 postpartum, one cow was fed glycerine prepartum, control diet postpartum and administered the glycerine drench and the second cow was fed the control diet prepartum, glycerine diet postpartum and administered the water drench. Significance was declared at $P < 0.05$ and trends toward a significant difference at $0.05 < P < 0.15$. Least squares means are presented throughout.

RESULTS AND DISCUSSION

The ingredient and nutrient composition of the prepartum control and glycerol-containing diets were similar with the exception of the incorporation of glycerol into the TMR at a rate of 5% of DM for cows assigned to the dietary

glycerol treatment (Table 3-1). Feeding glycerol during the prepartum period increased prepartum DMI (14.8 vs. 13.2 kg/d; P < 0.001; Figure 3-1); however, this did not carry over into a significant effect on postpartum DMI (21.4 vs. 21.4 kg/d; P > 0.15; Table 3-4). Cows fed glycerol during the prepartum period consumed on average 592 g/d of glycerol (740 g/d of crude glycerine). DeFrain et al. (2004) fed 430 g/d and 860 g/d on a DM basis from 14 d prepartum to 21 d postpartum and reported prepartum DMI to be lower in treated cows, consuming approximately 17% less DM (10.8 and 11.3 vs. 13.3 kg/d). Early feeding studies incorporating glycerol into the diet did not evaluate prepartum feeding (Johnson, 1953; Fisher, et al., 1971; Fisher, et al., 1973; Sauer, et al., 1973).

Milk yield averaged 42.1 kg/d during the experiment and was not affected (P > 0.15) by feeding glycerol during the prepartum period (Table 3-4 and Figure 3-2). Percentages and yields of milk components were not affected (P > 0.15) by feeding glycerol during the prepartum period (Table 3-4). DeFrain et al. (2004) evaluated the effects of feeding glycerol starting 14 d prior to expected calving to 21 d postpartum and reported no effect of feeding glycerol on milk yield. However, these researchers did observe a tendency for decrease in yield of energy-corrected milk (ECM) compared to control cows (35.2 and 35.0 vs 38.7 kg/d; P= 0.09), a tendency for a decrease in milk fat yield (1.32 and 1.36 vs. 1.52 kg/d; P = 0.13) and milk urea nitrogen (MUN) (13.7 and 14.0 vs. 15.2; P = 0.08).

Prepartum BW (637 kg vs. 639 kg; P > 0.25) and BCS (3.33 vs. 3.37; P > 0.25) were not affected by prepartum treatment. In accordance with the increased prepartum DMI of cows fed glycerol, cows fed glycerol had significantly greater calculated energy balance during the prepartum period

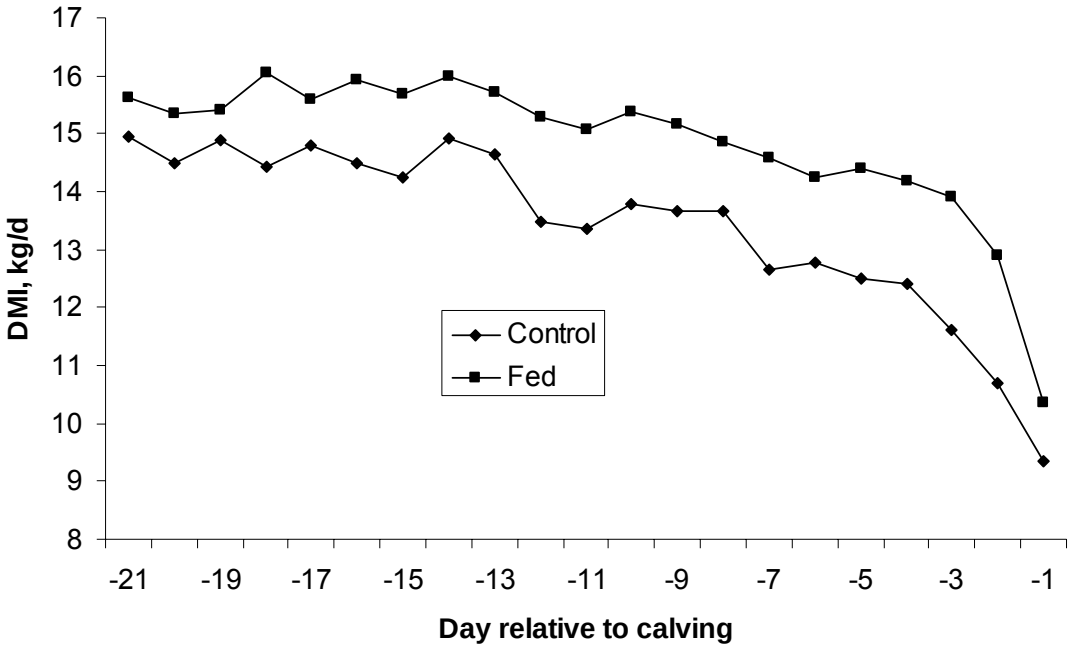

FIGURE 3-1. Least squares means for dry matter intake (DMI) during the prepartum period as affected by incorporation of glycerol into the diet. Effects: treatment, $P < 0.001$, SEM = 0.3; treatment by time, $P = 0.75$, SEM = 0.6.

TABLE 3-4 Postpartum DMI and milk yield and composition for the first 63 DIM as affected by incorporation of glycerol into the prepartum TMR.

	Prepartum treatment			
Item	Control TMR	Glycerol TMR	SEM	P-value
Number per treatment	23	23	---	---
DMI, kg/d	21.4	21.4	0.3	0.99
Milk, kg/d	42.7	41.5	1.4	0.55
3.5% FCM, kg/d	42.2	41.0	1.4	0.54
Fat %	3.50	3.50	0.06	0.70
Fat, kg/d	1.46	1.42	0.04	0.56
True protein, %	2.85	2.91	0.06	0.50
True protein, kg/d	1.18	1.17	0.05	0.91
Lactose, %	4.65	4.70	0.03	0.32
Lactose, kg/d	1.99	1.95	0.06	0.65
SCC (x 1000)	192	308	59	0.17
MUN, mg/dl	13.4	13.6	0.34	0.70

(7.73 vs. 10.63 Mcal/d; P < 0.05; Table 3-5). DeFrain et al. (2004) determined that BW and BCS were similar among treatments when glycerol was fed from 14 d prepartum to 21 d postpartum.

The ingredient and nutrient composition of the postpartum control and glycerol-containing diets were similar with the exception of the incorporation of glycerol to the TMR at a rate of 3.3% DM for treated cows (Table 3-2). Interactions of dietary supplementation with glycerol and oral drench with glycerol were not significant (P > 0.15) for any of the variables measured in this experiment; therefore, results from postpartum treatments will be reported and discussed as main effects.

Feeding glycerol during the postpartum period tended to decrease postpartum DMI (20.9 vs. 21.9 kg/d; P < 0.08; Table 3-6). Cows fed the glycerol diet during the postpartum period consumed on average 504 g/d (630 g/d of crude glycerine). DeFrain et al. (2004) fed 430 g/d and 860 g/d of glycerol on a DM basis from 14 d prepartum to 21 d postpartum and found postpartum DMI not be affected by diet. Fisher et al. (1971) reported that incorporation of glycerol in a concentrate mix (3.3% of concentrate mix) fed during early lactation increased postpartum DMI when compared to cows fed propylene glycol; however, in another study conducted by the same researchers (Fisher et al. 1973), they determined that the addition of glycerol in the concentrate mixture (3% and 6% of concentrate or 174 g/d and 347 g/d respectively) fed during the first 8 wk of lactation did not stimulate an increase in DMI.

Milk yield was not affected (42.8 vs. 42.7 kg/d; P > 0.15; Table 3-6) by the incorporation of glycerol into the postpartum diet. Percentages and yields of milk components were not affected (P > 0.15) by feeding glycerol during the postpartum period (Table 3-6). This is in agreement with other studies in

Table 3-5 Least squares means for BCS, BW and Net Energy Balance (NEB) during the prepartum period as affected by incorporation of glycerol into the prepartum[1] TMR.

	Treatment			
Item	Control TMR	Glycerol TMR	SEM	P-value
Number per treatment	23	23	---	---
DMI	13.2	14.8	0.30	<0.001
BCS	3.33	3.37	0.04	0.58
BW, kg	637	639	7	0.82
NEB[2], Mcal/d	7.73	10.63	0.58	0.001

[1]Prepartum diet included glycerol at a rate of 5% of DM.
[2] Net Energy Balance calculated according to NRC (2001).

TABLE 3-6 Postpartum DMI and milk yield and composition for the first 63 DIM as affected by incorporation of glycerol[1] into the postpartum TMR and short-term oral administration[2] of glycerol beginning at parturition.

	Treatment							
	Control TMR		Glycerol TMR			P-value		
Item	Water drench	Glycerol drench	Water drench	Glycerol drench	SEM	TMR	Drench	TMR x drench
Number per treatment	11	12	12	11	---	---	---	---
DMI, kg	22.5	21.2	21.9	19.9	0.5	0.08	0.001	0.48
Milk, kg/d	42.8	42.7	41.2	41.7	2.0	0.53	0.90	0.89
3.5% FCM, kg/d	42.3	42.3	40.7	41.1	2.0	0.50	0.91	0.90
Fat %	3.49	3.5	3.49	3.5	0.09	0.99	0.85	1.00
Fat, kg/d	1.46	1.47	1.41	1.42	0.07	0.48	0.93	0.97
True protein, %	2.9	2.85	2.98	2.79	0.08	0.91	0.15	0.42
True protein, kg/d	1.2	1.19	1.19	1.13	0.07	0.61	0.64	0.75
Lactose, %	4.73	4.70	4.73	4.56	0.05	0.18	0.04	0.16
Lactose, kg/d	2.01	2.00	1.95	1.91	0.09	0.36	0.79	0.87
MUN,mg/dl	13.8	13.2	13.6	13.3	0.5	0.85	0.36	0.76
SCC, x 1000	201	267	276	258	89	0.71	0.79	0.63

[1]Postpartum diet included glycerol at a rate of 3.3% of DM.
[2]500 ml of crude glycerine was administered for 5 consecutive days following parturition.

which glycerol was incorporated into the postpartum diet (Fisher et al., 1973; DeFrain et al., 2004).

Postpartum BW (590 vs. 590 kg; P > 0.15) and BCS (2.96 vs. 2.97; P > 0.15) were not affected by the incorporation of glycerol in the postpartum diet. These results concur with those of DeFrain et al. (2004). Fisher et al. (1973) found that cows fed glycerol at 347 g/d during the first 8 weeks of lactation lost less BW than cows fed glycerol at 174 g/d or the control diet. Energy balance during the postpartum period was not affected by feeding treatment (-0.10 vs. -0.18 Mcal/d; P >0.25).

Drenching glycerol for the first 5 d of lactation significantly decreased postpartum DMI (20.6 vs. 22.2 kg/d; P < 0.01; Table 3-6). The interaction for dietary addition and drench was not significant (P> 0.15), implying that administering a larger amount of glycerol simultaneously via two methods did not accentuate the negative effect of postpartum glycerol administration on postpartum DMI in this experiment. Drenching glycerol for the first 5 d of lactation did not affect milk yield or milk fat percentage during the first 63 d of lactation (Table 3-6); however, it tended to decrease milk protein content (2.82 vs. 2.94%; P < 0.15) and decreased milk lactose content (4.63 vs. 4.73%; P < 0.05). Despite these changes in composition, differences in yields of milk protein (1.19 kg/d vs. 1.20 kg/d; P > 0.25) and lactose (2.0 kg/d vs. 2.0 kg/d; P > 0.25) were not significant. Oral drenches of glycerol significantly decreased BW (590 vs. 545 kg; P < 0.05) and BCS (2.97 vs. 2.75 P >0.05) during the postpartum period (Table 3.7) and tended to decrease energy balance (-2.21 vs. -2.26 Mcal/d; P = 0.06).

Although we did not compare propylene glycol administration with glycerol administration in the diet or via oral drench in this experiment, we can

Table 3-7 Least squares means for DMI, BCS, BW and Net Energy Balance (NEB) as affected by incorporation of glycerol[1] in the postpartum TMR and short-term oral administration[2] of glycerol beginning at parturition.

	Treatment							
	Control TMR		Glycerol TMR			P-value		
Item	Water drench	Glycerol drench	Water drench	Glycerol drench	SEM	TMR	Drench	Treatment
Number per treatment	11	12	12	11	---	---	---	---
BCS	2.96	2.82	2.97	2.68	0.91	0.48	0.02	0.37
BW, kg	590	536	590	554	9	0.59	0.0005	0.60
NEB[3], Mcal/d	-0.10	-2.21	-0.18	-2.26	1.09	0.95	0.06	0.99

[1]Postpartum diet included glycerol at a rate of 3.3% of DM.
[2]500 ml of crude glycerine was administered for 5 consecutive days following parturition.
[3] Net Energy Balance calculated according to NRC (2001).

compare responses in DMI and milk yield from data available in the literature. Data from this experiment and others suggest that glycerol has varied effects on DMI during both the prepartum and postpartum effects, and that this variation occurs both in the magnitude and the direction of the response. Data from the literature suggest that propylene glycol administration via the diet will not increase DMI, and this failure to increase DMI is attributed to low palatability of propylene glycol (Johnson, 1953; Fisher, et al., 1973; Christensen, et al., 1997). In drenching studies using propylene glycol it is also evident that propylene glycol demonstrates no significant effect on DMI (Studer et al., 1993; Pickett et al., 2003). In contrast, oral drenching of glycerol significantly decreased postpartum DMI in this experiment. Propylene glycol administration as an oral drench and as a dietary supplement demonstrates no significant effects on BW or BCS (Studer et al., 1993; Formigoni et al., 1996; Pickett et al., 2003). Glycerol administered as a dietary supplement appears to not affect BW or BCS; however, in this experiment glycerol drench administered during the first 5 d of lactation significantly decreased BW and BCS.

Overall, oral administration of propylene glycol as a drench or as a dietary supplement has shown no significant effects on milk yield and milk composition (Fisher, et al., 1973; Studer et al., 1993; Formigoni, et al., 1996; Pickett, et al., 2003). Fisher et al. (1973) did indicate a tendency for dietary propylene glycerol to increase milk yield and decrease milk fat. Our study along with that of DeFrain et al. (2004) found glycerol administration as a dietary supplement does not significantly increase milk yield or increase milk components during early lactation.

Conclusions and implications

Although incorporation of glycerol at a rate of 5% of DM into the prepartum TMR resulted in potential benefits through increased DMI during the prepartum period, prepartum feeding of glycerol did not translate into increased DMI or yield of milk and milk components during the first 63 d postpartum. Glycerol incorporated into the postpartum TMR at a rate of 3.3% of DM tended to decrease postpartum DMI with no significant effect on milk yield and milk composition. Short-term (5 d) oral administration of glycerol beginning at parturition significantly decreased postpartum DMI and resulted in a trend for decreased milk protein percentage and a statistically significant decrease in milk lactose percentage; however, no effects on milk protein or milk lactose yield were observed. Given the overall lack of effect of prepartum or postpartum glycerol administration on yields of milk and milk components, change in net energy balance during both the prepartum and postpartum periods and BW and BCS during the postpartum period mirrored effects of glycerol administration on DMI. Overall, results suggest that glycerol administration as a dietary supplement or oral drench is not an effective strategy to improve performance of dairy cows during early lactation.

CHAPTER FOUR:

EFFECTS OF METHOD OF DELIVERY OF GLYCEROL ON METABOLISM OF DAIRY COWS DURING THE TRANSITION PERIOD

INTRODUCTION

The transition period of three weeks prepartum to three weeks postpartum is a period marked with dramatic metabolic demands due to parturition and lactogenesis in dairy cows (Bell, 1995). Decreases in nutrient intake during late gestation occur concurrently with dramatically increased nutrient demand; nutrient demand continues to outstrip dietary supply during early lactation. This results in the mobilization of adipose tissue, which increases plasma concentrations of non-esterified fatty acids (NEFA). The NEFA are used for energy by body tissues and as precursors for milk fat synthesis. In most situations, plasma NEFA concentrations are elevated beginning within 10 d prior to parturition and are associated with hepatic triglyceride accumulation and increased ketone production (Studer, et al., 1993; Vazques-Anon, et al., 1994). Available data suggest that liver uptake of NEFA is proportionate to portal blood supply (Reynolds et al., 2003). The liver does not have sufficient capacity to completely dispose of NEFA through export into the blood or catabolism for energy (Grummer, 1993; Drackley et al., 2001); therefore, when nutrient intake is insufficient and large amounts of NEFA are released into the blood, the liver begins to accumulate and store NEFA as triglycerides, resulting in fatty liver. Although likely benign if in small amounts, accumulation of increasing amounts of triglycerides in liver appears to impair other aspects of liver metabolism, including hepatic gluconeogenesis, ureagenesis, and endotoxin metabolism (reviewed by Drackley et al., 2001; Overton and Waldron, 2004).

Triglyceride accumulation in the liver frequently is accompanied by increased concentrations of circulating BHBA, which can result in the development of either subclinical or clinical ketosis (Grummer, 1993; Drackley, 1999). As a result of this complex of triglyceride accumulation in the liver and increased concentrations of ketone bodies in the circulation, researchers have sought to determine strategies to improve metabolic health of dairy cows during the transition period (Overton and Waldron, 2004).

In attempting to prevent or reduce the incidence of these metabolic disorders in the transition cow, management strategies should include ways to increase available glucose and decrease the supply of NEFA to the liver. Glucogenic supplements are substances that are administered or fed to the cow that can subsequently be absorbed and converted to glucose by the liver, with the intent of increasing glucose availability to the cow. This increased glucose availability to the cow can promote insulin secretion, which in turn should decrease NEFA release from adipose tissue (Gummer, 1993; Overton and Waldron, 2004). Administration of gluconeogenic supplements has been reported to decrease circulating concentrations of NEFA and BHBA, and increase blood glucose (Grummer, 1993; Nielsen and Ingvartsen, 2004). Bertics et al. (1992) proposed that glucose precursors administered prepartum would increase blood glucose which will elicit an insulin response and reduce mobilization of NEFA from adipose tissue.

As discussed in the review of literature, extensive study has been performed using propylene glycol as a glucogenic supplement. Propylene glycol decreases plasma concentrations of NEFA and BHBA while increasing concentrations of insulin and glucose (Neilsen and Ingvartsen, 2004). Grummer et al. (1994) reported that the effects of propylene glycol

administration on these circulating metabolites and hormones were dose-dependent and linear within the dose ranges studied. Short-term administration of propylene glycol via oral drench beginning at parturition decreased circulating concentrations of NEFA in two studies (Stokes and Goff, 2001; Pickett et al., 2003) and tended to also decrease plasma BHBA concentrations in one of these studies (Pickett et al., 2003). Long-term administration of propylene glycol to feed-restricted heifers for 14 d did not result in effects on plasma glucose or BHBA concentrations; however, plasma insulin concentrations were increased and plasma NEFA was decreased (Christensen et al., 1997); these effects were only evident when propylene glycol was administered as an oral drench or topdress rather than TMR incorporation. These changes in circulating metabolites should decrease hepatic triglyceride accumulation. Studer et al. (1993) reported that propylene glycol administered as an oral drench given once daily beginning approximately 10 d before expected parturition decreased liver triglyceride content following parturition; however, Pickett et al. (2003) determined that short-term oral drenching of propylene glycol beginning at parturition did not affect subsequent concentrations of liver triglycerides.

Glycerol is another gluconeogenic supplement that was proposed as an aid for treatment of ketosis in the 1960s and 70s but not adopted due to high costs (Fisher, et al., 1973; Sauer, et al., 1973). New sources of glycerol have reduced the cost (Schroder and Sudekum, 1999), making glycerol a potential supplement that may be added to transition cow diets. Renewed interest of the use of glycerol is evidenced by recent studies (Schroder and Sudekum, 1999; Goff and Horst, 2001; DeFrain et al., 2004; Linke et al., 2004).

Early studies administering glycerol as a dietary supplement indicated an increase in plasma glucose and decrease plasma ketone concentrations (Johnson, 1953). More recently, DeFrain et al. (2004) reported that administration of glycerol as a topdress during the transition period did not affect circulating concentrations of glucose, insulin, NEFA and BHBA or liver concentrations of triglycerides and glycogen. Oral drenching of glycerol has increased plasma concentrations of glucose (Goff and Horst, 2001; Linke et al., 2004) and insulin concentrations (Linke et al., 2004). Linke et al. (2004) found both feeding and drenching of glycerol increased plasma BHBA. This finding may be due to increased ruminal production of butyrate from glycerol fermentation to butyrate.

Research has not been conducted to evaluate the prepartum and postpartum effects of glycerol incorporation into the TMR, or to determine whether short-term drenching of glycerol beginning at parturition will affect metabolism in a manner analogous to the study of Pickett et al. (2003) using propylene glycol. Furthermore, the potential for interaction between TMR administration and oral drench has not been evaluated. Therefore, the objectives of this study were to evaluate administration of glycerol in the prepartum and postpartum TMR and by short-term oral drench on metabolic parameters (blood metabolites and liver composition) important to metabolic health in transition dairy cows.

MATERIALS AND METHODS

Experimental Animals, Treatments, and Procedures

All procedures using experimental animals were approved by the Cornell University Institutional Animal Care and Use Committee. Full details of experimental design and cow management are provided in Chapter 3.

Briefly, 48 Holstein cows entering second or greater lactation were assigned to treatments in a completely randomized design. On d 28 prior to expected parturition, cows were moved into tie stalls at the Cornell University Teaching and Research Center and fed a diet typical of that fed to close-up dairy cows in the Northeast and Upper Midwest. Beginning 21 d before expected parturition, cows were fed either a prepartum control diet or a diet containing glycerol (5% of DM; Table 3-1). After parturition, cows were assigned in a balanced fashion, to one of four treatments in a 2 (postpartum control or dietary glycerol at 3.3% of DM; Table 3-2) X 2 (water or glycerol drench; 500 ml/d for 5 d) factorial arrangement. From d 22 through 63 of lactation, cows were fed the control diet (Table 3-2).

Plasma and Tissue Sampling

Blood was collected from the coccygeal vessels using sodium heparinized (100 U/ml) Vacutainer® tubes (Becton Dickinson, NJ) every 4 d from 21 d prepartum until parturition and every other day through 21 d postpartum. Samples were drawn prior to feeding and treatment administration (between 0700 and 1000 h). Samples were placed on ice immediately after collection, and plasma was prepared by centrifugation (2,800 x *g,* 15 min) in a refrigerated (4ºC) centrifuge. Aliquots of plasma were snap-frozen in liquid N_2 and stored at -20°C until analysis for glucose (glucose oxidase procedure, kit # 315, Sigma Diagnostics, St. Louis, MO), NEFA (NEFA-C kit; Wako Chemicals, Dallas, TX) and BHBA (BHBA dehydrogenase procedure, kit # 310, Sigma).

On d 5 after parturition, a additional series of blood samples was collected into heparinized Vacutainer® tubes to characterize changes in blood metabolites and also insulin during the immediate post-drench period.

Samples were drawn prior to drench and at 1, 2, 4, and 6 h post-drench. Plasma samples were prepared and stored as described above. Subsequently, samples were analyzed for glucose, NEFA, and BHBA as described above for routine blood samples. In addition, these samples were analyzed for insulin by radioimmunoassay.

Samples of liver tissue (3 to 5 g) were obtained by percutaneous trochar biopsy (Veenhuizen, et al., 1991) under local anesthesia on d 1, 10, and 21 postpartum. Liver samples were blotted dry to remove excess blood and connective tissue and frozen immediately in liquid nitrogen, stored at -80ºC and subsequently analyzed for concentrations of triglycerides (Rukkwamsuk, et al., 1999) and glycogen (Lo, 1970).

Statistical Analysis

Effects of prepartum feeding of glycerol were evaluated separately for prepartum and postpartum variables using the MIXED procedure of SAS version 8.2 (SAS Institute, Cary, NC) for a completely randomized design with repeated measures and specified error term. Model terms were treatment, time, and their interaction. Effects of postpartum feeding or oral administration of glycerol (2 x 2 factorial arrangement) on postpartum variables were evaluated as above using SAS. Model terms were TMR, drench, their interaction, time, and two- and three-way interactions of main effects with time. Pretreatment values for blood metabolites were used as covariates during their respective analysis. For analysis of the intensive blood sampling data collected on d 5 postpartum, statistical analysis was conducted initially using the same model applied to postpartum repeated measures data. However, neither the effects of postpartum diet nor the interactions of postpartum diet and drench were significant ($P > 0.15$) for any plasma variables considered;

therefore, data were reanalyzed to assess only the effect of drench, using the pre-drench samples as covariates. Data for all metabolic indices from the same two cows were removed from the data set in chapter 3 were also eliminated prior to analysis. Significance was declared at $P < 0.05$ and trends toward a significant difference at $0.05 < P < 0.15$. Least squares means are presented throughout.

RESULTS AND DISCUSSION

Glycerol fed during the prepartum period resulted in no significant effects on plasma glucose, NEFA or BHBA concentrations (Table 4-1 and Figures 4-1, 4-2 and 4-3) during the prepartum period with no carry over effects on postpartum metabolites (Table 4-2). DeFrain et al. (2004) determined that cows fed 1 kg/d of glycerol tended to have greater concentrations of plasma BHBA compared to control cows; effects on other plasma metabolites measured (glucose and NEFA) were not significant. In contrast, two studies reported that prepartum administration of propylene glycol significantly decreased plasma NEFA and BHBA concentrations along with a significant increase in plasma glucose (Studer et al., 1993; Formigoni et al., 1996).

Despite increased DMI by cows fed glycerol during the prepartum period in this experiment (Chapter 3), liver composition (triglyceride and glycogen content) in samples collected within 36 h following parturition were not affected by prepartum dietary treatment (Table 4-1). Furthermore, prepartum dietary treatment did not have carryover effects on liver composition measured at d 10 and 21 postpartum (Table 4-2). These results are similar to those of DeFrain et al. (2004), who reported that feeding glycerol during the transition period did not affect liver triglyceride or glycogen contents.

TABLE 4-1. Prepartum routine plasma metabolite concentrations and liver composition (d 1 only) as affected by incorporation of glycerol into the prepartum TMR.

	Treatment			
Item	Control	Glycerol	SEM	P value
Number per treatment	23	23	---	---
Glucose, mg/dl	60.4	60.3	0.7	0.96
BHBA, mg/dl	6.1	5.8	0.3	0.43
NEFA, µEq/L	206	170	23	0.27
Glycogen, % wet wt*	1.1	1.1	2.3	0.90
Triglyceride, % wet wt*	7.6	5.5	1.0	0.17

*Liver biopsy obtained within 36 h of calving.

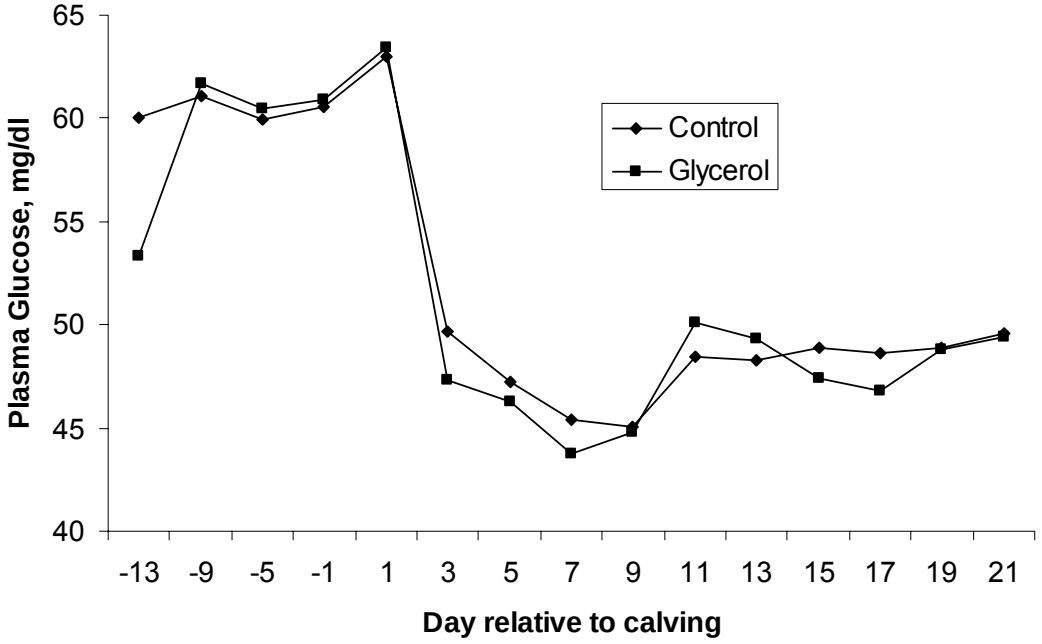

Figure 4-1. Least squares means for concentrations of glucose in plasma collected from cows as affected by incorporation of glycerol into the prepartum TMR. Feeding glycerol prepartum resulted in no significant effects on prepartum plasma glucose concentrations (60.4 mg/dl vs. 60.3 mg.dl, P = 0.96, SEM = 0.7) and no carry over effects on postpartum plasma glucose concentrations (49.4 mg/dl vs. 48.9 mg/dl, P = 0.69, SEM = 0.94).

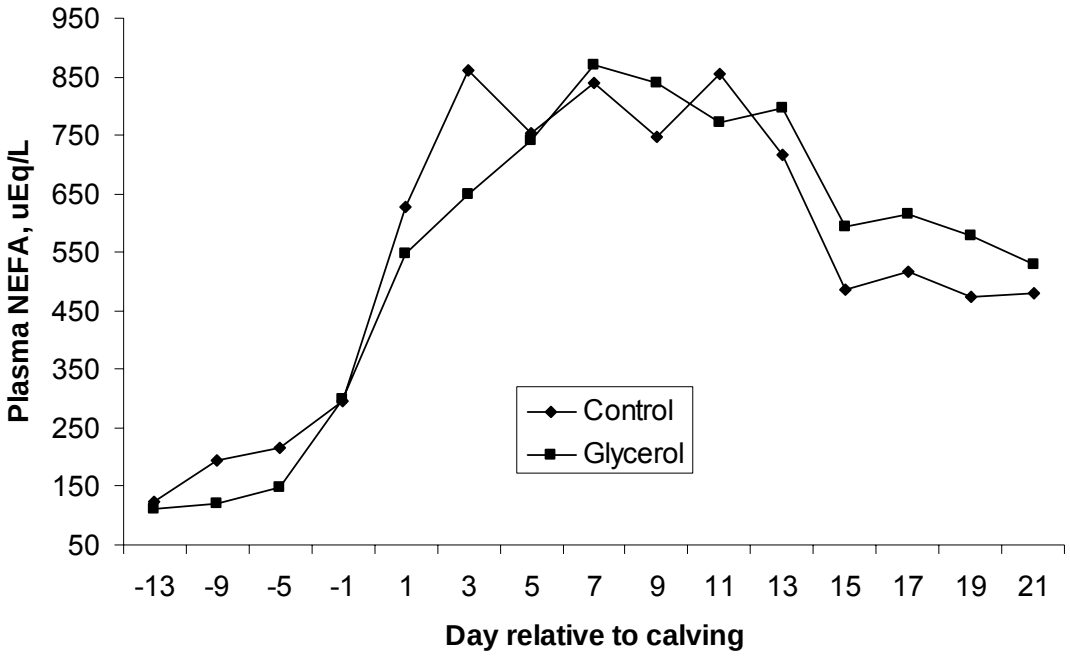

Figure 4-2. Least squares means for concentrations of NEFA in plasma collected from cows as affected by incorporation of glycerol into the prepartum TMR. Feeding glycerol prepartum resulted in no significant effects on prepartum plasma NEFA concentrations (206 µEq/L vs. 170 µEq/L, P = 0.27, SEM = 23) and no carry over effects on postpartum plasma NEFA concentrations (668 µEq/L vs. 685 µEq/L, P = 0.77, SEM = 39).

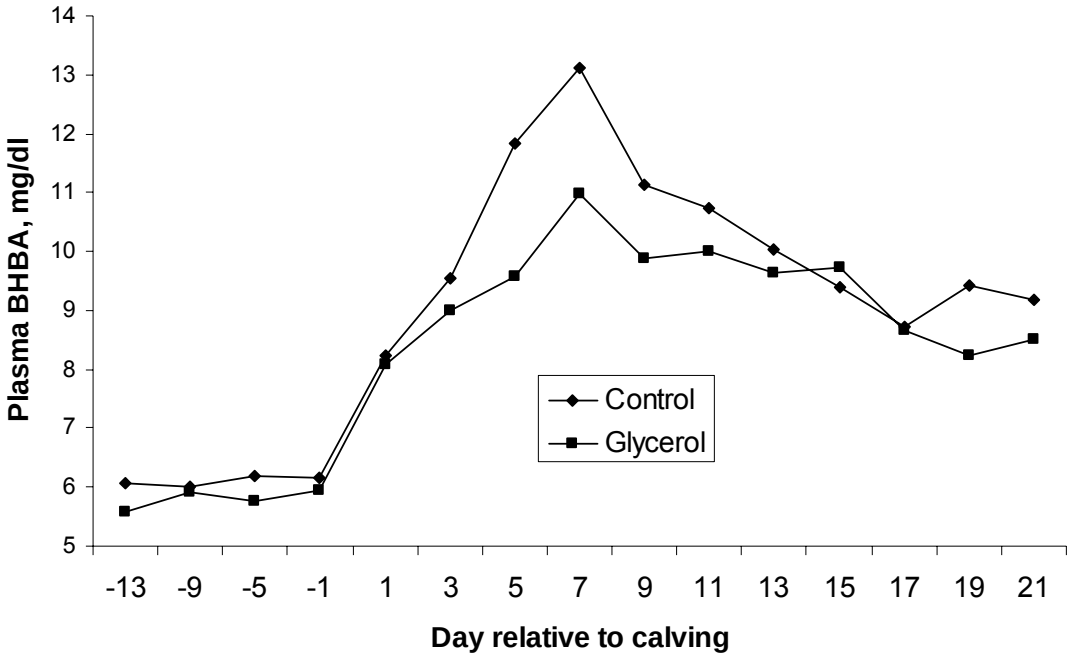

Figure 4-3. Least squares means for concentrations of BHBA in plasma collected from cows as affected by incorporation of glycerol into the prepartum TMR. Feeding glycerol prepartum resulted in no significant effects on prepartum plasma BHBA concentrations (6.1 mg/dl vs. 5.8 mg/dl, P = 0.43, SEM = 0.3) and no carry over effects on postpartum plasma BHBA concentrations (10.1 mg/dl vs. 9.3 mg/dl, P = 0.44, SEM = 0.75).

TABLE 4-2 Postpartum routine plasma metabolite concentrations and liver composition as affected by incorporation of glycerol into the prepartum TMR.

	Treatment			
Item	Control	Glycerol	SEM	P value
Number per treatment	23	23	---	---
Glucose, mg/dl	49.4	48.9	0.94	0.69
BHBA, mg/dl	10.1	9.3	0.75	0.44
NEFA, µEq/L	668	685	39	0.77
Glycogen, % wet wt	1.1	1.0	1.3	0.58
Triglyceride, % wet wt	15.1	12.6	1.9	0.36

* Liver biopsies obtained d 10 and d 21 postpartum.

Interactions of postpartum dietary glycerol supplementation and short-term oral drench of glycerol beginning at parturition were not significant ($P > 0.15$) for any of the plasma or liver variables studied (Table 4-3); therefore, results will be presented and discussed as the main effects of dietary inclusion of glycerol during the postpartum period and short-term oral drench of glycerol beginning at parturition. Postpartum incorporation of glycerol in the diet did not affect circulating concentrations of glucose, NEFA, and BHBA and liver concentrations of triglyceride and glycogen in this experiment (Table 4-3; Figures 4-4, 4-5, and 4-6). DeFrain et al. (2004) reported that feeding 1 kg/d of glycerol transiently decreased plasma NEFA concentrations at d 7 postpartum; however, effects on glucose and BHBA were not significant.

Short-term (5-d) oral drenching of glycerol beginning at parturition resulted in no significant effects on circulating concentrations of glucose, NEFA, and BHBA and liver concentrations of triglycerides and glycogen during the postpartum period (Table 4-3). Previous studies have shown oral drenching of glycerol to increase plasma glucose and insulin concentrations; however, these samples were collected several hours postdrench (Linke et al., 2004) compared to those in this study that were more representative of basal (predrench) concentrations. Daily prepartum oral drenching of propylene glycol beginning 10 d prior to expected calving demonstrated the increase in plasma glucose and decrease in NEFA and BHBA as noted by others along with reduced total liver triglyerides 1 d postpartum (Studer, et al., 1993). In a previous study conducted in our laboratory, Pickett et al. (2003) reported that short-term oral drenches of propylene glycol were effective in

TABLE 4-3. Postpartum routine plasma metabolites and liver composition as affected by incorporation of glycerol into the postpartum TMR and short-term oral administration of glycerol beginning at parturition.

	Treatment							
	Control TMR		Glycerol TMR			P value		
Item	Water drench	Glycerol drench	Water drench	Glycerol drench	SEM	TMR	Drench	Treatment
Number per treatment	11	12	12	11	---	---	---	---
Glucose, mg/dl	48.8	48.3	48.4	50.8	1.3	0.44	0.46	0.30
BHBA, mg/dl	9.3	10.7	8.7	10.1	1.0	0.56	0.20	0.96
NEFA, µEq/L	716	623	671	694	56	0.82	0.54	0.31
Glycogen, % wet wt*	9.8	10.2	11.1	11.0	1.9	0.61	0.94	0.89
Triglyceride, % wet wt*	14.8	12.9	14.7	13.2	2.8	0.97	0.57	0.95

* Liver biopsies obtained d 10 and d 21 postpartum.

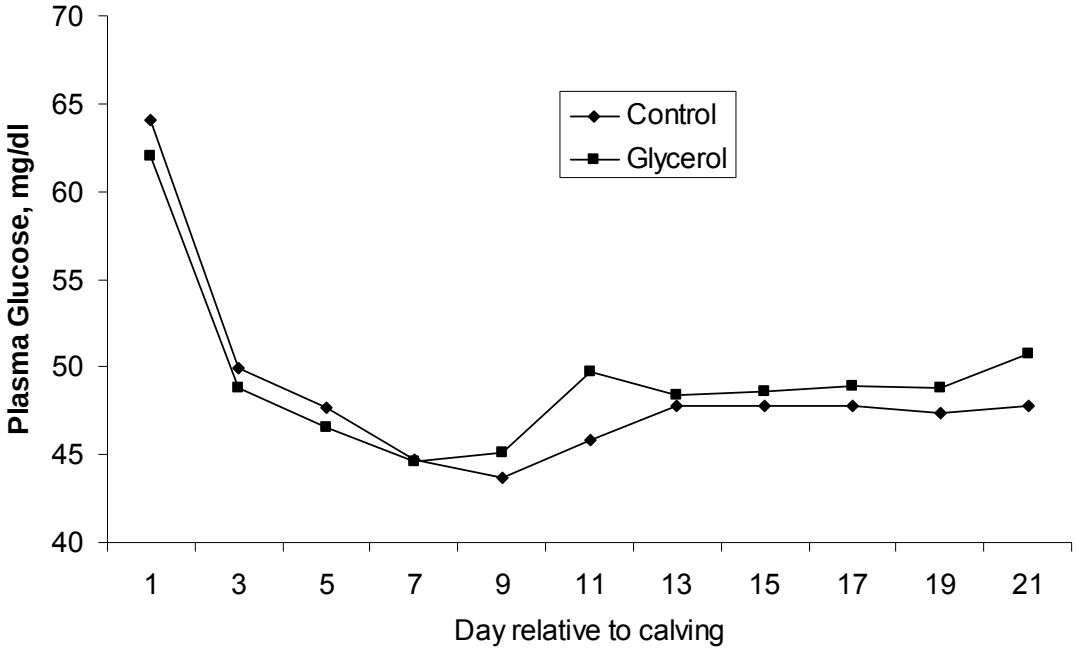

Figure 4-4. Least squares means for concentrations of glucose in plasma collected from cows as affected by incorporation of glycerol into the postpartum TMR. Feeding glycerol postpartum resulted in no significant effects on postpartum plasma glucose concentrations (48.5 mg/dl vs. 49.3 mg/dl, P = 0.44, SEM = 1.3)

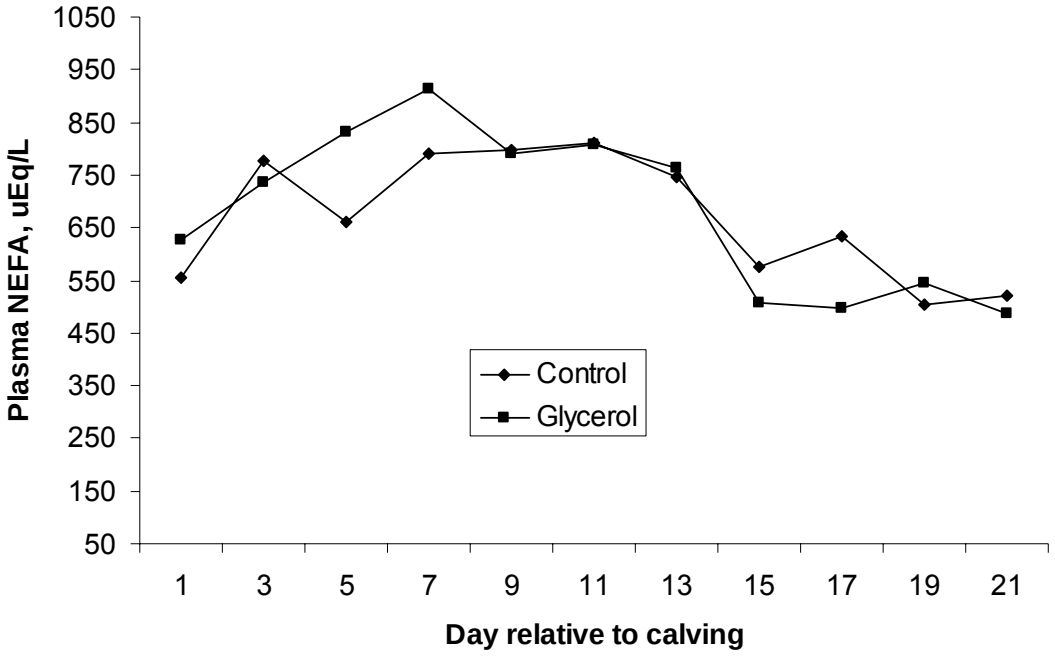

Figure 4-5. Least squares means for concentrations of NEFA in plasma collected from cows as affected by incorporation of glycerol into the postpartum TMR. Feeding glycerol postpartum resulted in no significant effects on postpartum plasma NEFA concentrations (670 µEq/L vs. 682 µEq/L, P = 0.82, SEM = 55.8)

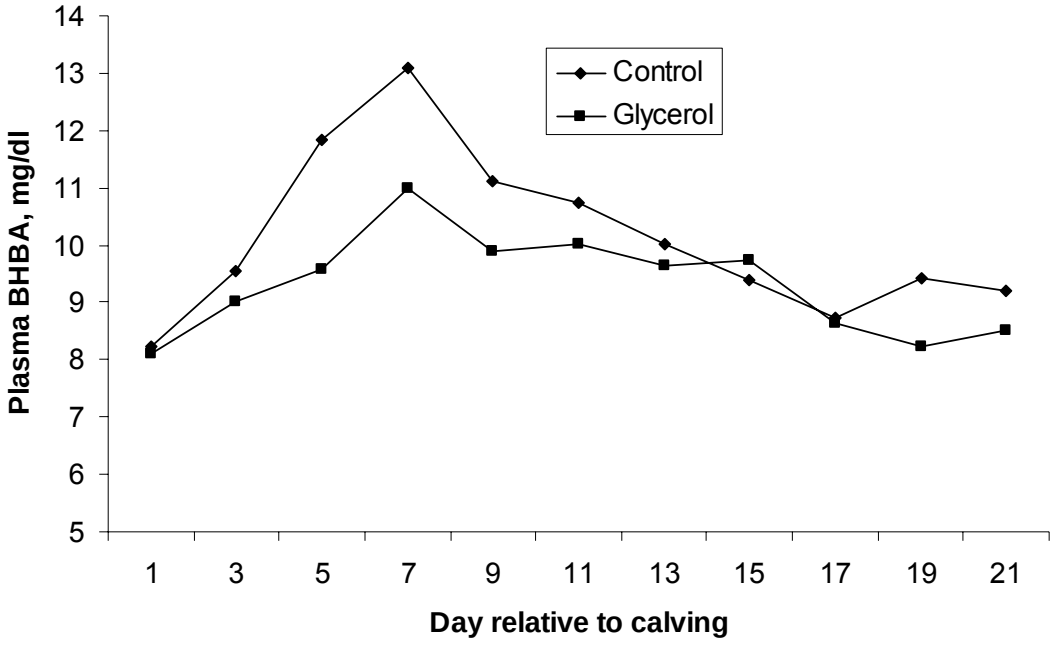

Figure 4-6. Least squares means for concentrations of BHBA in plasma collected from cows as affected by incorporation of glycerol into the postpartum TMR. Feeding glycerol postpartum resulted in no significant effects on postpartum plasma BHBA concentrations (10.1 mg/dl vs. 9.3 mg/dl, P = 0.56, SEM = 1.08).

decreasing plasma NEFA and tended to decrease plasma BHBA assessed using similar timing of blood sampling relative to the drench as that employed in the current study. These results suggest that the effects of glycerol drench are not as long-lasting as those from propylene glycol.

In addition to effects of glycerol drench on baseline circulating concentrations of glucose, NEFA, and BHBA, we also evaluated the pattern of change of these metabolites plus insulin following glycerol drench. Intensive blood sampling performed on d 5 post calving demonstrated that a 500 ml oral bolus of crude glycerine significantly decreased plasma NEFA concentration (312.6 vs. 206.2 μEq/L; $P < 0.05$) with no overall significant effects on plasma glucose, BHBA or insulin (Table 4-4). Data from our study indicated trends for drench X hour interactions for plasma glucose, NEFA and insulin concentrations (Figures 4-7, 4-8, 4-9, and 4-10) during the 6-h period following administration of glycerine or water. In our study, plasma glucose concentrations peaked at 1 h post drench while insulin was increasing by 1 h and peaked at 2 h postdrench. These results concur with those of Linke et al. (2004), who determined that plasma glucose and insulin concentrations increased and peaked at approximately 1.5 h post drench of 1 kg of glycerol. Goff and Horst (2001) administered glycerol as 1, 2 or 3 L oral drench and found glucose to be increased by 16, 20 and 25% over pretreatment values at 30 min post drench. These findings are also similar to patterns of plasma metabolites and insulin following oral drench of propylene glycol, in which plasma glucose and insulin peaked at approximately 100 min post treatment and NEFA concentrations were decreased (Christensen, et al., 1997)

TABLE 4-4. Effects of oral glycerol drench on plasma metabolites and insulin.

	Drench			
Item	Glycerol	Water	SE	P-value
Number per treatment	23	23	---	---
Glucose, mg/dl	48.9	46.7	1.5	0.11
NEFA, uEq/L	206	313	25	0.06
BHBA, mg/dl	10.7	10.3	0.9	0.41
Insulin, ng/ml	0.11	0.12	0.01	0.098

Blood samples were obtained prior to drench and at 1, 2, 4, and 6 hours post drench.

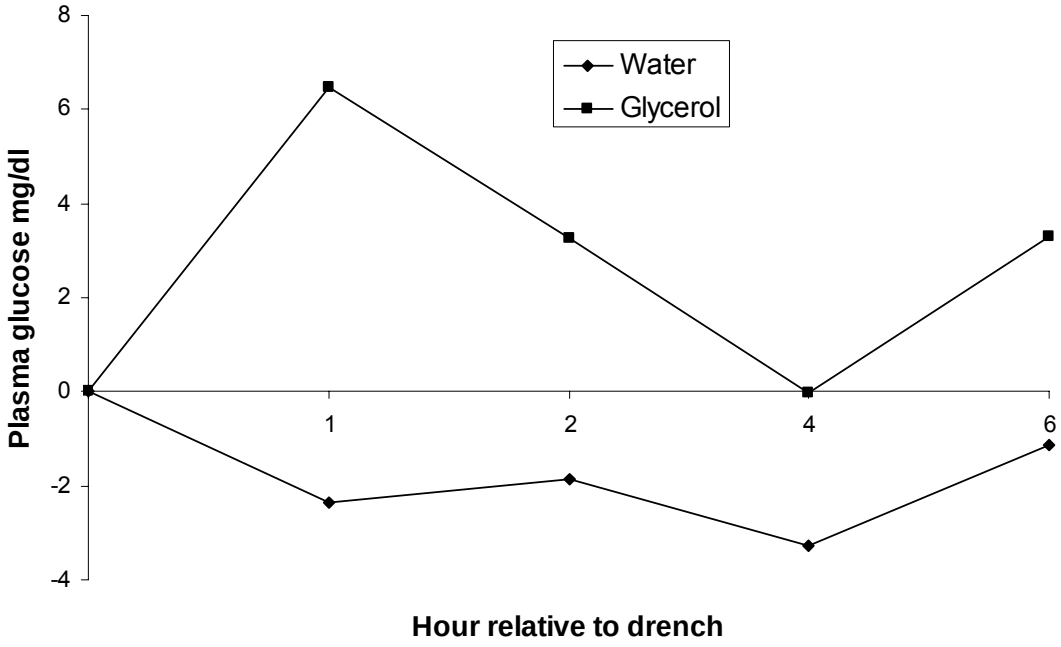

FIGURE 4-7. Least squares means for glucose concentrations in plasma immediately post oral drench of 500ml of crude glycerine. (Drench X hour interaction, P = 0.11; SEM = 1.6)

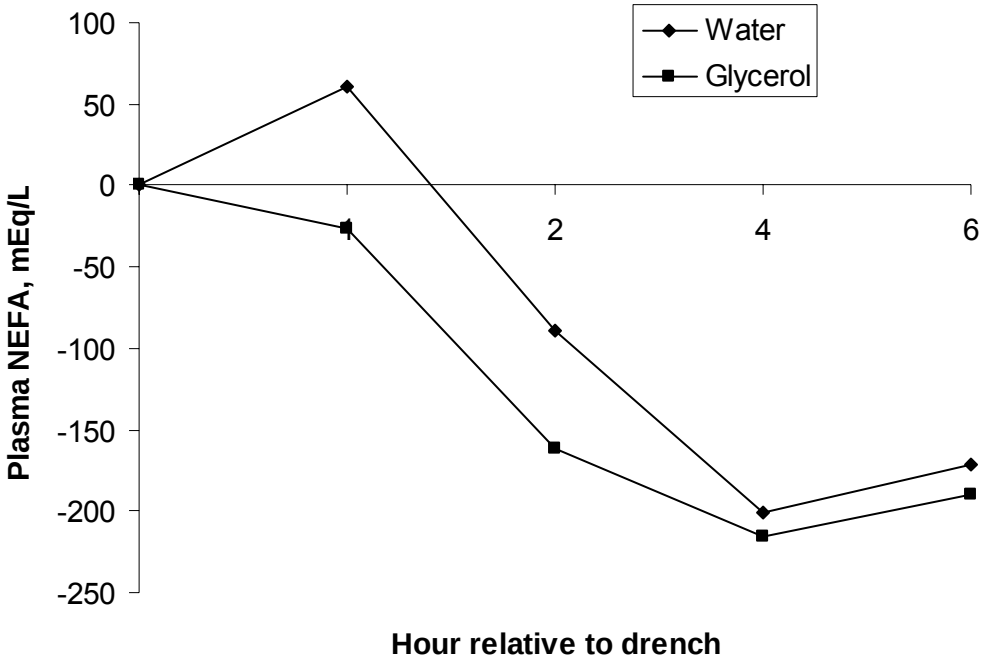

FIGURE 4-8. Least squares means for plasma concentration of NEFA immediately post drench of 500 ml of crude glycerine. (drench X hour interaction, P = 0.06; SEM = 32)

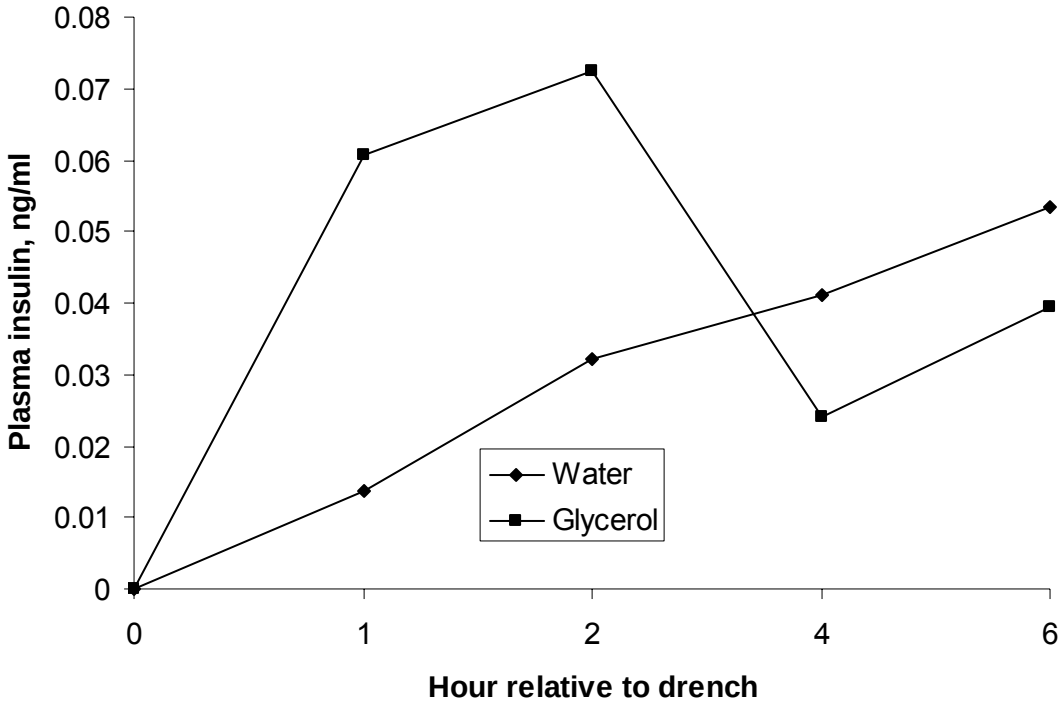

FIGURE 4-9. Least squares means for plasma concentration of insulin immediately post drench of 500 ml of crude glycerine. (drench X hour interaction, P = 0.098; SEM = 0.016)

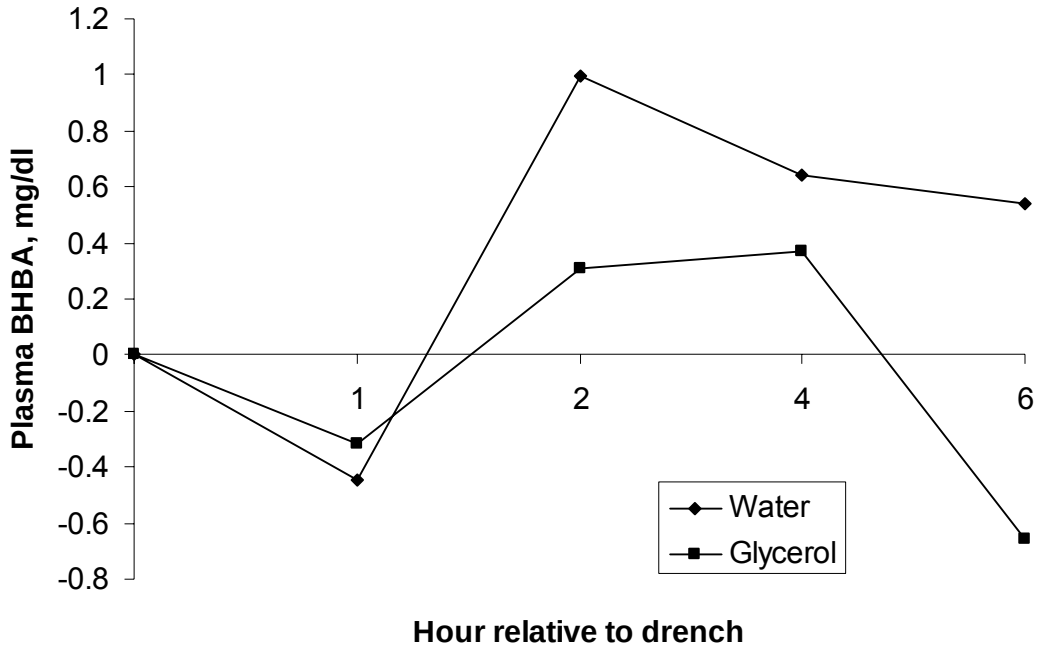

FIGURE 4-10. Least squares means for plasma concentration of BHBA immediately post drench of 500 ml of crude glycerine. (drench X hour interaction, P = 0.41; SEM = 0.63)

The incidence of health disorders are reported in Table 4-5 for information only. The number of cows assigned to each treatment in this experiment was too few to evaluate differences among treatments statistically. In general, incidences of metabolic disorders were similar across the prepartum and postpartum treatments.

Conclusions and implications

Glycerol addition to prepartum and postpartum diets did not significantly affect plasma concentrations of glucose, NEFA, and BHBA during either the postpartum or postpartum periods. In addition, short-term (5-d) administration of glycerol via oral drench beginning at parturition did not affect basal concentrations of these metabolites. Furthermore, glycerol administration either by dietary incorporation or oral drench did not affect concentrations of triglycerides or glycogen during the transition period. Although the pattern of change of metabolites such as glucose and NEFA and hormones such as insulin following oral drench change in a manner consistent with other glucogenic supplements such as propylene glycol, the changes appear to be transient. Collectively, these data suggest that glycerol has limited utility as a glucogenic supplement for administration to transition cows.

TABLE 4-5 Incidence of health-related disorders during first 63 d of lactation as affected by prepartum and postpartum treatment.

			Prepartum Feeding				Postpartum Feeding				Drench			
			Control		Glycerol		Control		Glycerol		Water		Glycerol	
Disorder	Total # cows	Total # episodes	# cows	# episodes	# cows	# episodes	# cows	# episodes	# cows	# episodes	# cows	# episodes	# cows	# episodes
Milk Fever	3	3	1	1	2	2	2	2	1	1	1	1	2	2
Retained Placenta	6	6	5	5	1	1	4	4	2	2	2	2	4	4
Displaced Abomasum	5*	5	3	3	2*	2	2	2	3*	3	1*	1	4	4
Ketosis	14	18	7	9	7	9	6	8	8	9	6	9	8	9
Mastitis	9	14	5	7	4	7	4	5	5	9	5	9	4	5

* One cow suffered a right displaced abomasum one week prior to calving.

CHAPTER FIVE:

INTEGRATED DISCUSSION AND SUMMARY

Data from this study indicate that the addition of glycerol to the diets of transitions cows does not demonstrate the meaningful glucogenic effects attributed by earlier studies (Johnson, 1953; Fisher et al., 1971). At dietary concentrations or drench amounts studied in this experiment, neither TMR incorporation nor short-term oral administration of glycerol as crude glycerine resulted in any apparent benefits on transition cow performance or metabolic indices.

Data from our study did indicate that the incorporation of glycerol into the prepartum TMR resulted in potential benefits through increased DMI during the prepartum period; however, prepartum feeding of glycerol did not translate into increased DMI or yield of milk and milk components during the first 63 d postpartum. Conversely, glycerol incorporated into the postpartum TMR tended to decrease postpartum DMI with no significant effects on milk yield and milk composition. Feeding glycerol during both the prepartum and postpartum period demonstrated no significant effects on plasma glucose, NEFA or BHBA concentrations. The negative effects of crude glycerine on postpartum DMI likely negated any potential for positive effects of glycerol on performance or energy metabolism. The decreased DMI by cows fed glycerol during the postpartum period likely negated any potential for beneficial effects of glycerol on metabolic indices.

Short-term (5 d) oral administration of glycerol beginning at parturition resulted in no apparent benefits to the transition cow. Short-term oral drenches of glycerol significantly decreased postpartum DMI and resulted in a trend for decreased milk protein percentage and a statistically significant

decrease in milk lactose percentage; however, no effects on milk protein or milk lactose yield were detected. In addition, oral drench of glycerol beginning at parturition did not affect liver composition or plasma metabolites.

Data from this study are consistent with another recently reported study in which glycerol was administered by dietary topdress during the transition period (DeFrain, et al., 2004; Linke, et al., 2004); however, these changes appear to be too transient to impact overall metabolism of the transition cow.

From extensive studies evaluating propylene glycol (as reviewed by (Nielsen and Ingvartsen, 2004), propylene glycol is a glucogenic supplement that may be more beneficial for the transition cow in preventing fatty liver and ketosis than glycerol. However, propylene glycol has been demonstrated to be most effective when administered as an oral drench rather than as a dietary component.

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