THE EFFECT OF SUPPLEMENTING HIGH YIELDING HOLSTEIN COWS WITH BOTANICAL EXTRACTS, BACTERIAL INOCULANTS, OR DIETARY GLYCEROL DURING HEAT STRESS AND THE EFFECT OF DIETARY GLYCEROL IN TRANSITION COW DIETS ON SUBSEQUENT YIELD AND EFFICIENCY.

by

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(Under the Direction of JOE WEST)

ABSTRACT

A series of research trials were conducted to evaluate the effects of botanical additives, bacterial inoculants, and dietary glycerol on high yielding dairy cows during heat stress and measure the effects of glycerol in the diet of transition dairy cows. Trial 1 evaluated the effects of a botanical supplement (Thermal Care-D®) on apparent efficiency and milk yield. Thermal Care-D® had no effect on DMI or milk yield, but Thermal Care-D® alone exhibited greater ($P<0.05$) or numerically improved digestion of DM, NDF and ADF compared with Control and Thermal Care-D® with glycerol. Trial 2 evaluated the effects of the live bacterial inoculant Bovamine® ($4x10^9$ CFU/h/d combination of *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24*) and glycerol on milk yield, efficiency of yield, and nutrient digestibility. No effect on DMI or milk yield was observed, but improved efficiency (MY/DMI) [$P<0.06$] for Bovamine (1.5 ± 0.02) versus Control (1.42 ± 0.02) was observed. The addition of Bovamine® alone and with glycerol had a positive effect on apparent efficiency

compared with Control. Trial 3 evaluated levels of glycerol on rumen environment and nutrient digestibility. Six Holstein cows averaging 56 DIM and 37.9 kg/d of milk were used. The treatments were control (C), 200g glycerol h/d (G200), and 400g glycerol h/d (G400). A trend ($P<0.18$) for improved apparent efficiency (ECM/DMI) was noted for G200 and G400 versus Control, 1.73, 1.73, 1.68 (± 0.02) respectively. Data showed decreased acetate to propionate ratio with the addition of glycerol. G400 increased ruminal propionate and decreased acetate compared with C. Results suggest the addition of glycerol to the diet may alter ruminal VFA concentrations and improve efficiency in dairy cows. Trial 4 was a peripartum cow study to evaluate the effects of glycerol supplementation on postpartum lactation. No effect on milk yield or DMI was observed, but cows supplemented with glycerol during the prepartum and postpartum period did show an improvement in milk to feed conversion efficiency. Results from these trials suggest the addition of Thermal Care-D®, dietary glycerol, and Bovamine® may improve lactating cow performance under heat stress conditions by improving apparent efficiency of production and digestibility.

INDEX WORDS: HEAT STRESS, BOTANICAL EXTRACTS, DIETARY GLYCEROL, BACTERIAL INNOCULANTS, RUMINAL FERMENTATION, EFFICIENCY

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DEDICATION

Thanks for all the support and help Dad!! I never would have finished this without you!

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CHAPTER 1

INTRODUCTION:

The changing economy and the shift in public opinion over the past few years has created the need for new or modified approaches to dairy production in the United States and around the world. The need to find economical and efficient ways to meet the world demand for milk and satisfy the demand by some consumers for natural and organic production instead of conventional production practices has left producers, feed companies, and researchers searching for new natural supplements to replace products like rBST and monensin in the diet. The recent interest in producing "green" fuel with the ethanol industry's growth has presented farmers with high corn prices and left them looking for ways to supplement or replace expensive corn and energy ingredients in the ration.

Glycerol has been used on the farm for years, but the expense of glycerol due to its use in other industries has not made it a cost effective addition to the ration. Glycerol has been commonly used as a drench to treat ketosis in transition dairy cows. The growth of the ethanol industry has increased the amount of crude glycerol on the market. The improved availability of crude glycerol has reduced the cost, making it a potential substitute or supplement for corn in the diet. Glycerol has been observed by several researchers to have a similar or higher energy value then corn, further supporting its potential benefit to producers.

An area of recent interest has been the use of botanicals or bacterial supplements to improve production and efficiency in the dairy cow. The use of natural botanical products like plant extracts is particularly attractive to organic producers and consumers

demanding “natural” products. The use of bacterial inoculants is of similar interest because several promising studies have shown potential for improved rumen function and efficiency through the modification of the ruminal microbial profile.

To date, the effects of botanical or bacterial inoculants in combination with dietary glycerol on lactating dairy cows has not been examined. The objective of this research was to explore that area and to determine the effects of these products in high yielding dairy cows subjected to heat stress conditions, and the effects of varying levels of dietary glycerol on the postpartum performance of transition dairy cows.

CHAPTER 2

LITERATURE REVIEW:

Glycerol:

Glycerol is a by-product of the biodiesel industry. The growth of the biodiesel industry in the United States has increased the availability of crude glycerol generated by the transesterification of vegetable oils from 0.5 million in 1999 to 460 million gallons in 2007 (National Biodiesel Board, 2008). The primary by-product of biodiesel production is crude glycerol, which is approximately 10% weight of vegetable oil (Dasari et al., 2005). There are many uses of purified glycerol in the food, pharmaceutical and cosmetic industries as well as other fields, but the cost of refining crude glycerol to a high purity level is cost prohibitive to most small biodiesel plants (Pachauri and He, 2006). Because of this there may be large quantities of crude glycerol available as a potential energy source to livestock producers.

Increasing demand for renewable energy sources like soydiesel presents the opportunity for glycerol to become an affordable energy supplement in dairy cow diets. Glycerol is an odorless, colorless, hygroscopic, sweet tasting liquid that can be substituted in the diet in place of corn. During biodiesel production fatty acids are hydrolyzed from the glycerol backbone of the triglyceride molecule by a transesterification process using methanol. After the separation of the fatty acid esters, glycerol is removed, along with the excess methanol and salts from the reaction. The separation and purity of the glycerol by-product will depend on the refining process used

at that particular plant. Table 2.1 outlines the composition of glycerol by purity (Schroder and Sudekum, 1999).

The record increase in corn costs has caused farmers to look for alternative energy sources. Glycerol has a projected feed value of 100-120% of corn, making it a viable corn alternative (Hippen et al., 2008). The yield of glycerol from biodiesel is approximately 1 unit of glycerol for each 10 units of biodiesel produced. The biodiesel industry has a projected annual national production of over 2.2 billion gallons by the end of 2008, which would provide about 220 million gallons of glycerol of 80% purity annually (Feedstuffs, 2007).

Table 2.1: Composition of glycerol by degree of purity

	Purity of Glycerol		
	Low[1]	Medium[1]	High[1]
Water %	26.8	1.1	2.5
----------% of DM----------			
Glycerol	63.3	85.3	99.8
Ether extract	0.71	0.44	n/a
P	1.05	2.36	n/a
K	2.20	2.33	n/a
Na	0.11	0.09	n/a
Pb	0.0003	0.0002	n/a
Methanol[2]	26.7	0.04	n/a

(Schroder and Sudekum, 1999)

[1] Concentration of cadmium, mercury & arsenic were below the detection limit.

[2] FDA issued a statement that methanol levels above 150ppm are unsafe for animal consumption.

Glycerol has been recognized as safe when used in accordance with good manufacturing and feeding practices (FDA, 2007, 21 C.F.R. 582.1320) although an area of concern is the amount of contamination from residual methanol in the glycerol. Recently, the FDA issued a letter that methanol levels higher than 150 ppm could be considered unsafe for animal feed (Donkin and Doane, 2007). The office of the Texas State Chemist recently established guidelines for labeling with minimal levels of glycerol and maximal levels of moisture, sulfur, ash, and methanol. The Texas State Chemist guidelines state that the level of allowed methanol shall not exceed 1% in crude glycerol offered to ruminants (Feedstuffs, 2007).

Fermentation Characteristics of Glycerol:

Glycerol is reported to be rapidly fermented by rumen microbes. Garton et al, (1961) conducted in vitro incubations of glycerol and found that at 2 h, nearly 25% of the glycerol had disappeared and by 8 h nearly 90% of the glycerol was undetectable. Remond et al. (1993) reported that the addition of glycerol to fermentors decreased pH levels at a greater rate in fermentors fed starch compared with those fed cellulose. Remond et al. (1993) observed with the addition of glycerol the molar proportions of butyrate were higher in fermentors fed starch versus those fed cellulose. It can be concluded from both in vitro and in vivo studies that glycerol is rapidly fermentable in the rumen and that the effects of glycerol on propionate and butyrate levels is diet dependant.

Glycerol Feeding Trials:

Early work by Fisher et al. (1973) evaluated the use of glycerol as a preventative for ketosis in dairy cows. Fifty-two peripartum Holstein cows were assigned randomly at calving to concentrates supplemented with 3% propylene glycol, 3% glycerol, 6% glycerol, or a control for 8 wk post-calving. Cows offered the 6% glycerol supplement lost less BW and remained in a more positive energy balance compared with other treatments.

Schroder and Sudekum (1999) determined the suitability of glycerol as an energy source in ruminant diets. They used wethers fed high and low starch concentrates with glycerol added at 10, 15, or 20% of the diet DM. For the low starch diet no effects on digestibility of OM, starch, or cell wall components were observed. For the high starch diets the addition of glycerol resulted in a decrease in cell-wall digestibility with no effect on OM or starch digestion. The workers concluded that glycerol acts more similarly to a carbohydrate than fat in the rumen when included in a typical dairy ration, and that the energy content of glycerol is 0.90 to 1.04 Mcal/kg NE_L.

Schroder and Sudekum (1999) used four ruminally cannulated steers to determine the ruminal effects of feeding glycerol. The steers consumed an average of 13.4 kg DM/d containing 2.1 kg/d of starch. Diets contained 0 or 1.1 kg/d glycerol with a resulting starch content of 2.1 or 1.4 kg/d respectively. These researchers observed that feeding glycerol did not affect diet digestibility but decreased the acetate: propionate ratio, increased molar ruminal butyrate concentrations, and stimulated greater water intake. They concluded that these changes would be beneficial to the dairy cow because it would lead to increased ruminal propionate, increasing the supply of gluconeogenic substrate to

the liver. Increased ruminal butyrate enhances the growth of ruminal epithelial tissue and possibly increase nutrient absorption from the rumen (Dirksen et al., 1985), and increased water intake could increase the supply of water available to the mammary gland for milk synthesis.

Donkin and Doane (2007) fed 0, 5, 10, and 15% glycerol (99.5% pure) of the dietary DM to lactating dairy cows replacing corn with glycerol and corn gluten feed. Feed intake was decreased by the 15% glycerol during the first 7d of the study but recovered thereafter. They observed that milk yield and composition was not affected by glycerol, but MUN decreased. Cows offered the 15% glycerol gained more weight after wk 8 compared with other treatments. The workers concluded that glycerol could be included at up to 15% of diet DM in lactating cow diets.

A transition cow study conducted at Pennsylvania State University used a dry glycerol product (food grade, 65% glycerol). The study lasted from calving to 21 d postpartum and used 39 multiparous Holstein cows (Chung et al., 2007). Two hundred and fifty grams of glycerol product was fed supplying 0.16 kg/d of glycerol. Feed intake, milk yield and composition, and serum insulin concentration were not affected by treatment. Glycerol supplemented cows had a more positive energy balance during wk 2 of lactation indicated by greater concentration of plasma glucose and lower concentrations of plasma ß-hydroxybutyrate (BHB) and urine ketones. The authors reported no difference in DMI or milk yield during the first 3 wk of lactation. They did observe a tendency for greater milk yield for glycerol supplemented cows during wk 6 of lactation (51.7 versus 45.8 kg/d) after the supplementation period had ended, suggesting a possible latent benefit of glycerol on energy status and subsequent milk production.

Ogborne (2006) used multiparous Holstein cows to determine the effects of method of delivery of glycerol on yield performance and metabolism during the transition period. Cows were fed either a control diet or a diet containing glycerol (5% of DM) starting 21 d prepartum. After calving, cows were offered glycerol at 3.3% of DM or given glycerol in a drench at 500 ml/d for the first five DIM. Feeding glycerol during the prepartum period increased DMI prepartum, but the addition of glycerol to the diet postpartum reduced intake levels. Church and Pond (1988) suggested that because cattle eat primarily to meet their energy requirement, providing additional ME to a cow in positive energy balance would theoretically decrease feed intake and thus improve efficiency. Ogborne (2006) reported that drenching glycerol for the first 5 d of lactation decreased DMI. Milk yield was not affected by either feeding or drenching glycerol. Glycerol fed during the prepartum period caused no significant effects on plasma glucose, NEFA, or BHB concentrations. There was a trend for increased BHB concentrations in cows drenched with glycerol but the difference was not statistically significant. Intensive blood sampling performed on d 5 post calving showed that a 500 ml oral bolus of glycerol decreased plasma NEFA concentration though not significant with no effect on plasma glucose, insulin, or BHB concentrations. Ogborne (2006) concluded that the use of glycerol in transition cow diets or short-term drenching of glycerol at calving resulted in few positive performance responses and only slight effects on metabolism.

DeFrain et al. (2004) conducted a study with 21 multiparous and 9 primaparous Holstein cows offered diets top dressed with corn starch or glycerol. The treatments were composed of: 0.91 kg of corn starch, 0.45 kg corn starch + 0.45 kg of glycerol, or 0.91 kg

of glycerol. The diets were offered from 21d prepartum until 21d postpartum. Glycerol dosages were chosen based upon amounts shown to be effective in drenching studies by Goff and Horst (2001). Prepartum DMI was greater for control cows compared with cows offered the glycerol treatments (13.3, 10.7, and 11.2 ± 0.50 kg/d, for 0, 0.45, and 0.91 kg glycerol, respectively). No treatment effect was observed for prepartum plasma glucose, insulin, BHB, NEFA, or ruminal VFA profiles. Rumen fluid collected postpartum indicated that cows offered glycerol had greater total VFA concentrations, greater molar proportions of propionate and decreased acetate: propionate ratio compared with controls. Butyrate concentrations tended to be greater for cows receiving glycerol versus the control. Plasma glucose concentrations were higher for cows offered the control diet compared with those offered the glycerol treatments, discounting glycerol as a glycogenic precursor. No treatment effects were observed for DMI, BW, body condition score or liver lipids during the first 21 d postpartum. Plasma NEFA and BHB were decreased at 7 d postpartum for cows fed 0.91 kg/d of glycerol, but this effect disappeared by 14 d postpartum and by 21 d postpartum BHB levels were the greatest in cows offered glycerol compared to the controls. Yield of ECM tended to be greater for cows receiving the control diet compared with the glycerol treatments for the first 70d postpartum and cows fed glycerol had decreased MUN concentrations. Researchers concluded that increased energy in the glycerol supplemented diets may have been beneficial to the cows, but feeding glycerol did not provide an increase in gluconeogenic precursors.

Glycerol has been recognized for years as a way to alleviate the symptoms of ketosis when delivered as an oral drench. (Leng, 1970; Johnson, 1955; Fisher et al., 1973)

DeFrain et al. (2004) proposed that including glycerol in the diet would eliminate the need to restrain cows for drenching and still deliver a glucogenic substrate, thereby reducing fatty liver complex and improving lactational performance. These workers used 30 Holstein cows in a randomized block design from 14 d prepartum to 21 d postpartum. Treatments included a control, low glycerol (0.43 kg/d DM glycerol), and a high glycerol (0.86 kg/d dry matter basis glycerol). Prepartum DMI was higher for cows fed the control diet compared with the low glycerol or high glycerol diets (13.3, 10.8, and 11.3 ± 0.5 kg/d respectively). Prepartum plasma concentrations of glucose, insulin, BHB, NEFA, and ruminal profiles were not affected by treatment. However, rumen fluid collected postpartum from cows fed the high or low glycerol diets contained greater total VFA, greater molar proportions of propionate and decreased acetate to propionate ratio. Also, butyrate concentrations were higher in the high and low glycerol treatments compared with the control diet. Postpartum plasma glucose concentration was greatest in cows offered the control diet compared with the low glycerol or high glycerol treatments (66.0, 63.1, and 58.4 mg/dl, respectively). No effect was observed for DMI, BW, plasma NEFA, or liver lipids during the first 21 d postpartum among treatments. The yield of ECM during the first 70 d postpartum tended to be the greater for cows offered the control diet. The tendency for greater ECM yield for cows receiving the control diet can largely be attributed to the lower milk fat yield for the low and high glycerol diets compared with the control. It was noted that the low and high glycerol diets decreased MUN concentrations relative to the control diet. The inclusion of glycerol in the diet tended to decrease milk fat percentage, MUN and decreased the ruminal acetate to

propionate ratio. It is likely that the glycerol was fermented to propionate similar to a fermentable carbohydrate source.

Schroder and Sudekum (1999) suggested that glycerol with different purity levels may be used to replace rapidly fermentable starches in ruminant diets at up to 10% of the diet DM. The results reported by DeFrain et al. (2004) are in agreement with earlier work by Schroder and Sudekum (1999) and Khalili et al. (1997), where the ruminal acetate to propionate ratio decreased when feeding glycerol at 1.1 and 0.216 kg/d, respectively. The researchers concluded that based on the prepartum DMI, plasma glucose and BHB concentrations postpartum, feeding glycerol to dairy cows at the levels used in this experiment increased the serum indicators used to gauge the degree of ketosis in cattle and that glycerol should be delivered as a drench in hypoglycemic dairy cows and not fed as a component of transition dairy cow diets.

Linke et al. (2004) evaluated the addition of glycerol to the diet of mid-lactation cows. These researchers used 6 primaparous Holstein and 6 primaparous Brown Swiss cows in a 3x3 Latin square with 4 wk periods. Treatments were control (no glycerol), low glycerol (0.50 kg glycerol), and high glycerol (1.0 kg glycerol). Ruminal VFA profiles showed no change in molar proportions of acetate in the cows fed glycerol. Propionate tended to increase in cows offered glycerol and butyrate increased linearly as glycerol level increased in the diet. No effect was observed for DMI, milk, or FCM with the addition of glycerol to the diet. The researchers noted that feed efficiency increased with glycerol supplementation with milk to feed ratios of 0.66, 0.72, and 0.73 kg of FCM/kg of DMI for 0, 0.50, 1.0 kg of glycerol respectively. Other than a decrease in MUN with the addition of glycerol to the diet, no effect on milk components was noted.

They concluded based on the increased feed efficiency and decreased MUN that the addition of glycerol may improve rumen efficiency. Also, based on differences in feed efficiency, they calculated the energy value of glycerol to be approximately 20% greater then that of corn yielding an NE_L of about 1.06 Mcal/kg.

Linke et al. (2004) conducted a comparison of how feeding glycerol versus drenching impacted the ruminal environment. Four high yielding Holstein cows were used in a Latin square design with 1wk periods to evaluate the effect of methods of glycerol delivery on ruminal VFA, plasma concentrations of glucose, BHB, NEFA, and insulin. Treatments were: control (corn), 1.0 kg glycerol + corn, 1.0 kg glycerol solution in 1.1 qt of water and drenched with a drenching bottle and 1.0 kg of glycerol in 2.5 gallons of water and delivered with an esophageal tube and pump. After glycerol administration, concentrations of ruminal acetate decreased in all cows given glycerol regardless of method of delivery. Propionate and butyrate increased with all methods with peak concentrations seen at 4 h post-delivery. Glucose concentration increased in cows that were drenched or tubed compared with the control or fed glycerol treatments. Insulin concentration increased for the drenching and tubed treatments compared with control or fed glycerol diets. Also, BHB increased for all cows receiving glycerol, reaching peak concentrations at 1.5, 2.4, and 1.6 for drenching, tubing, and feeding, respectively. A potential explanation for these results is that because dietary short chain fatty acids, (mainly butyrate) are the principal contributors to alimentary ketogenesis (Bergman, 1970), the ruminal fermentation of glycerol to butyrate increased plasma BHB and decreased concentrations of glucose in the plasma with the addition of dietary glycerol.

The authors (Linke et al., 2004) concluded from this study, that in order to be gluconeogenic, glycerol must be delivered in water to associate with the liquid fraction of the rumen contents or be able to bypass the rumen in a form to be absorbed as glycerol and converted to glucose by the liver. Glycerol that is available to the rumen microbes will be converted to propionic and butyric acids. The portion converted to butyrate will be metabolized to BHB by the ruminal epithelium; therefore glycerol that is fed is actually ketogenic rather than glucogenic.

Glycerol is an efficient glucogenic substrate because it can enter the gluconeogenesis pathway at the triose phosphate level and is therefore not dependant on the rate limiting enzymes pyrvate carboxylase and phosphoenolpyruvate carboxykinase for its conversion to glucose via glycerol kinase (Leng, 1970). Glycerol kinase converts glycerol (K_m =3 to 10 uM) [Lin, 1977] and ATP to glycerol-3-phosphate and ADP, an intermediate step where glycerol is directed toward glycolysis or gluconeogenesis. A dairy cow in negative energy balance has pathways activated for the utilization of glycerol released by the mobilization and hydrolysis of triglycerides from body fat. This activity depends on the absorption of glycerol rather than the fermentation of glycerol to propionate and butyrate, which can be counterproductive with the ketogenic nature of butyrate (Hippen et al., 2008). Glycerol that bypasses ruminal fermentation may be a highly efficient glucogenic substrate.

Dietary glycerol provides a supplement that is basically “pure energy” (Hippen et al., 2008). It can be used to enhance rumen fermentation and has been shown to improve feed efficiencies. In addition, glycerol is beneficial as a feed additive because it provides

"stick" to complete diets, enhances palatability in low doses, and increases water consumption.

Chung et al. (2007) conducted a study with 39 multiparous Holstein cows to determine the effects of dry glycerin in the postpartum ration. Researchers top dressed the ration with 250g/d (corresponding to 162.5 g of food grade glycerol) from parturition to 21 d in milk. The glycerin treatment tended to improve energy availability with higher blood glucose, lower blood BHB, and lower urine ketones compared with the control treatment during the wk 2 of lactation. No increase in DMI or milk yield was noted during the first 3 wk of lactation. Researchers did observe that during wk 6 of lactation glycerin-supplemented cows tended to have a higher milk yield (52 versus 46 kg/d; P=0.14) after the supplementation period had ended (glycerin supplementation ended at 3 wk postpartum). These authors suggested that a potential latent benefit on milk yield from glycerin supplementation may exist, perhaps due to changes in metabolism.

Propionibacterium:

Research has shown that various strains of *Propionibacterium* increase the molar proportion of ruminal propionate (Kim et al., 2000; Stein et al., 2006). Feeding *Propionibacterium* alone or in combination with other bacteria to dairy cows has been studied but results have been inconsistent. *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* are the primary bacterial organisms fed to ruminants. Feeding these organisms in combination may be beneficial, because *L. acidophilus* is a lactate producing bacteria and *P. freudenreichii* is a lactate utilizing bacteria that

produces propionate, a glucose precursor as a product of fermentation (Raeth-Knight et al., 2007).

Propionibacteria is a natural inhabitant of the rumen making up 1.4% of the ruminal microflora (Oshio et al., 1987) and is responsible for producing propionate, a major precursor for glucose production through hepatic gluconeogenesis (Sauer et al., 1998). The theoretical efficiency for propionate use as a source of energy for ATP is 108% compared with glucose (McDonald et al., 2002). So the direct feeding of propionibacteria may be a natural way to increase hepatic glucose production and positively influence metabolism (Francisco et al., 2002).

Direct fed microbials (DFM) are defined as a source of live, naturally occurring microorganisms (Krehbiel et al., 2003) and are used in the dairy industry to improve milk yield, feed efficiency, and health (Yoon and Stern, 1995). Increasing demand by the public for antibiotic free production practices has increased the interest in DFM. These products can be marketed as a "natural" feed additive and according to Nocek and Kautz (2006) the inclusion of DFM in dairy cow diets has become a generally accepted practice.

The mode of action for DFM is debated among researchers, though several mechanisms have been suggested. These include the modification of the rumen or lower gut microbial population, alteration of ruminal fermentation patterns, increased intestinal nutrient flow, improved diet digestibility, and improved immune function (Yoon and Stern, 1995; Krehbiel et al., 2003).

Normal dairy feeding standards may reduce ruminal pH to thresholds that can result in subclinical acidosis (Nocek et al., 2002). One popular theory is that DFM may prevent a decline in rumen pH by decreasing lactic acid production by some microbes

(Chaucheyras et al., 1996; Fulton et al., 1979). Specific strains of microbials have been identified, which when selected and combined could strategically manipulate and regulate ruminal metabolism. It may be possible to include certain combinations of lactic acid synthesizing DFM the diet, so that a tonic level of lactic acid may be maintained in the rumen that would be higher and less variable. This in turn should stimulate lactic acid utilizing bacteria, resulting in lower levels of total lactic acid available in the rumen and reduced total rumen acidity (Nocek et al., 2002b).

Francisco et al. (2002) reported that early lactation cows fed 17 g of *Propionibacterium* culture (strain identified as P169 and supplemented at approximately 6×10^{10} cfu/d)[Stein et al., 2006] consumed less DM and produced similar amounts of milk as the control group. A subsequent study by Stein et al. (2006) reported that early lactation, multiparous cows offered 6×10^{10} or 6×10^{11} cfu/d of *Propionibacterium* strain P169 produced about 8% more fat corrected milk than the control cows, but no difference were seen among the primiparous cows.

Raeth-Knight et al. (2007) conducted a feeding study using mid-lactation cows to determine effects of *Lactobacillus acidophilus* and *Propionibacteria freudenreichii* on milk yield, nutrient digestibility, and rumen fermentation. The authors concluded that supplementing midlactation dairy cows with these products had no effect on DMI or yield of milk or milk components. Also, no effect on apparent digestibility or rumen fermentation was observed.

Weiss et al. (2008) conducted a study to determine the effect of a DFM, *Propionibacterium* strain P169, on rumen fermentation, milk yield, and health of periparturient and early lactation cows. *Propionibacterium* strain P169 was fed at a rate

of 6 x 10^{11} cfu/d and the authors reported that dairy cows fed the *Propionibacterium* strain had similar milk yield and composition as the control cows. The calculated energy expenditures for maintenance, milk yield, and BW change were similar among treatments, but cows offered the microbial supplement had a lower DMI resulting in a 4.4% improvement in the efficiency of conversion of dietary DM to NE_L. This result was attributed to the alteration of ruminal fermentation patterns.

Lehloenya et al. (2008) studied the effect of feeding *Propionibacterium* strain 169 to ruminally cannulated steers. In this study, the P169 strain altered ruminal metabolism toward increased propionate production without affecting DMI, duodenal flow, microbial N synthesis, or ruminal kinetics.

Nocek et al. (2006) supplemented DFM products to transition dairy cows and fed 2g of DFM product/cow per d. The microbial supplement contained approximately 5 x 10^9 cfu of yeast and 5 x10^9 cfu of bacteria (*Enterococcus faecium*) with a cornmeal carrier. Cows consuming the DFM produced more milk and consumed more DM during the pre and postpartum periods. However treated cows exhibited a lower milk fat percentage compared with non-supplemented cows. Ruminal digestion of forage DM increased in cows supplemented with direct fed microbials. Increased concentrations of blood glucose and milk lactose and milk greater yield was noted for DFM treatment, but no effect on blood NEFA or BHB levels was observed.

West et al. (2009) compared a combination *Propionibacterium freudenreichii* Strain NP24 and *Lactobacillus acidophilus* Strain NP51 at 2 dosage levels with a control (Level 1: 2 x 10^9 cfu/d *Propionibacterium freudenreichii* Strain NP24 and 1 x 10^9 cfu/d *Lactobacillus acidophilus* Strain NP51 or Level 2: 2 x 10^9 cfu/d *Propionibacterium*

freudenreichii Strain NP24 and 5 x 10^8 cfu *Lactobacillus acidophilus* Strain NP51 and Strain NP45). Cows supplemented with the live bacterial inoculants had higher yield of fat, FCM, and ECM compared with controls. There was no effect of treatments on DMI, but a trend for improved efficiency (ECM/DMI) was noted for the supplemented cows versus controls. The authors concluded that inclusion of live bacterial inoculants containing *P. freudenreichii* and *L. acidophilus* improved milk yield and apparent efficiency of nutrient utilization.

Plant Extracts:

Essential oils are naturally occurring secondary metabolites and volatile components that can be extracted from plants by distillation methods, mainly through steam distillation (Benchaar et al., 2006). Essential oils are complex mixtures of mono- and sesquiterpenes and biogenetically related phenolics or monophenols (Hummelbrunner and Isman, 2001).

Some essential oils have antimicrobial tendencies against gram-negative and gram- positive bacteria (Conner, 1993) which have been related to a number of small terpenoid and phenolics compounds (Helander et al., 1998). Essential oils can inhibit activity of ruminal bacteria that are sensitive to monensin action (McIntosh et al., 2003; Newhold et al., 2004). Benchaar et al. (2006) reported that feeding essential oils alone decreased DMI whereas feeding essential oils in the presence of monensin increased DMI, which resulted in an interaction ($P<0.06$) when DMI was expressed in kg per d, and the interaction was significant ($P<0.04$) when DMI was expressed as a percentage of BW.

Several gram positive bacteria are involved in ruminal biohydrogenation of fatty acids (Bauman et al., 1999), suggesting that feeding essential oils could decrease biohydrogenation of fatty acids because of a reduced number of bacteria involved in the process. It may be possible to alter the fatty acid profile of milk by feeding essential oils to dairy cows. Benchaar et al. (2006) reported that feeding monensin had a slightly greater effect on the milk fatty acid profile than feeding essential oils. Cows offered monensin produced milk with greater concentrations of trans-10 18:1 (+17%) and trans-11 18:1 (+16%). Jenkins et al. (2003) observed that the addition of 25 ppm of monensin in continuous cultures of ruminal bacteria increased the concentration of trans-10 18:1 but did not affect the concentration of trans-11 18:1. Increased concentrations of trans-10 18:1 in milk has been associated with decreased milk fat concentration (Griinari et al., 1998), in agreement with Benchaar et al. (2006) who reported a lower milk fat content for cows on diets containing monensin. Benchaar et al. (2006) concluded that although essential oils may alter ruminal fermentation favorably *in vitro* effects *in vivo* need further research. Also, the addition of essential oils did not alter milk composition, including fatty acid profiles, ruminal total VFA, or molar proportions of individual VFA.

The European Union banned the use of antibiotics in animal feeds in January 2006 and growing public opinion in the United States against rbST and antibiotics in dairy rations may accentuate the need to find natural alternatives to conventional additives. The industry is evaluating alternative additives among plant extracts that are recognized as safe for human and animal consumption. Several researchers have reported the potential for extracts and secondary plant metabolites to modify ruminal fermentation *in vitro* including saponins, anise oil, capsicum extract, eugenol, and

cinnamaldehyde (Cardoza et al., 2004, 2005; Busquet et al., 2005a,b, 2006; Klita et al., 1996; Hristov et al., 1999).

The addition of 1 g of 15% capsicum increased total DMI, concentrate intake, and water intake compared with controls in ruminally cannulated cows (Cardozo et al., 2006). Work by Zafra et al. (2003) demonstrated that capsicum, the active component of capsicum oil, increased DM and water intake in rats and stimulated an appetite increase in humans (Calixto et al., 2000).

Cardozo et al. (2004) reported no effects of cinnamaldehyde (containing 59% cinnamaldehyde) and pepper (containing 12 % of capsicum) extracts on total VFA concentration from *in vitro* experiments under a dairy-type environment (high forage diet at pH 6.4). The authors observed that the effects of essential oils on ruminal microbial fermentation appear to be diet and pH dependant (Cardozo et al., 2005; Castillejos et al., 2005). In comparison, when a 10:90 forage: concentrate diet with a pH of 5.5 was used, cinnamaldehyde and capsicum (doses from 0.3 to 30 mg/L) increased total VFA concentration. Capsicum (doses 3 to 30 mg/L) decreased acetate and increased propionate proportions suggesting that the changes in fermentation profile may be beneficial in beef feedlot systems.

Aspergillus Oryzae:

Beharka et al. (1998) reported that the supplementation of *Aspergillus oryzae* fermentation extract (Amaferm®) in young calf diets resulted in earlier weaning and higher total ruminal VFA, propionate, and acetate concentrations over controls. Calves offered supplemented diets had or exhibited increased DMI during the first 5 wk of the

study compared with controls regardless of dosage level (0.5, 1.0, or 3.0 g/calf/d). An increase in BW gain was noted throughout the entire study for the calves offered high and low supplement diets compared with controls. Beharka et al. (1998) also reported that total anaerobic, cellulolytic, hemicelluloytic, and pectinolytic bacterial counts tended to be higher in the *Aspergillus oryzae* supplemented calves than in controls. The authors observed that *Aspergillus oryzae* supplementation increased ruminal microbial activity. This is consistent with previous work by Wiedmeier et al. (1987) and Frumholtz et al. (1989) who reported that *Aspergillus oryzae* stimulated microbial bacterial activity *in vivo* and *in vitro*.

Beharka and Nagaraja (1993) used *Aspergillus oryzae* fermentation extract (Amaferm®) to determine the effects on *in vitro* fiber degradation in eight different types of fibrous feedstuffs. Amaferm® was added to the rumen fluid at four levels (0, 0.4, 0.8, or 1.2 g/L) of fermentation mixture. The researchers observed that effects on NDF and ADF degradation varied by dosage level and by feedstuff. They concluded that the *Aspergillus oryzae* fermentation extract (Amaferm®) improved NDF and ADF digestibility of some feedstuffs including both legumes and grasses. The authors attributed increased digestibility to a stimulation of bacterial activity and not to changes in fungal or protozoal activity. This theory is supported by the work of Newbold et al. (1990), who reported that *Aspergillus oryzae* had no effect on the growth of pure cultures of the rumen fungi *Neocallimastix frontalis, Neocallimastix patriciarum*, and *Piromonas communis*, concluding that increased fiber digestion with the use of *Aspergillus oryzae* can be attributed to stimulation of bacterial activity not fungal or protozoal activities.

Beharka and Nagaraja (1998) measured the effect of *Aspergillus oryzae* fermentation extract (2 or 5mg/ml) on the growth rates of pure cultures of nineteen types of ruminal bacteria. The authors determined that ten species of bacteria were unaffected (neither increased nor decreased growth rate) by Aspergillus oryzae supplementation, suggesting that *Aspergillus oryzae* had no antibacterial effects. These authors reported the growth rates of the bacteria that digest fiber, including *Ruminococcus albus* and *Fibrobacter succinogenes* increased with the addition of *Aspergillus oryzae* extract. Increased growth rate of bacteria that utilize lactate, *Megasphaera elsdenii, Selenomonas ruminantium*, and *Selenomonas lactilytica* was also observed.

Aspergillus oryzae fermentation extract had no effect on degradation of cell walls, cellulose, or hemicellulose and did not affect ruminal anaerobic or cellulolytic species in cannulated beef cows (Varel and Kreikemeier, 1994a). Researchers also reported that ruminal ammonia concentration was not affected, but total VFA tended to be higher and pH tended to be lower when a high level (27g) of *A. oryzae* extract was fed. The recommended dosage for *A. oryzae* extract is 3g (Varel and Kreikemeier, 1994a).

In vitro results indicate that *A. oryzae* fermentation extract may stimulate the degradation of grass NDF by affecting the ruminal microorganisms (Waldrip and Martin, 1993). In vivo work by Varel and Kreikemeier (1994b), reported *A. oryzae* fermentation extract had little effect on the degradation of bromegrass or alfalfa NDF in non-lactating cows, yet the total ruminal population increased. Varel and Kreikemeier (1994b) also concluded that the effect of *A. oryzae* fermentation extract on ruminal metabolism was not dose dependant. In situ NDF digestion was not affected even at three times the recommended dose of *A. oryzae*.

Denigan et al. (1992) reported that 1.5 g/d but not 6 g/d, of *A. oryzae* fermentation extract increased DMI in lactating cows, but milk yield was not affected. Gomez-Alarcon et al. (1990) also observed increased DMI and milk yield in early lactation cows fed high concentrate diets with *A. oryzae* fermentation extract. Canton et al. (1993) reported that 2 g of extract altered the DMI of steers grazing cool season pastures, but the effect on DMI varied by season.

Monensin:

Monensin is a monocarboxylic acid ionophore which is a common dietary additive in ruminant diets and exerts positive effects on feed efficiency and nitrogen and energy utilization (Plaizier et al., 2000; Tedeschi et al., 2003).

Ionophores have been used in beef diets because of the improvement in the efficiency of nutrient utilization and reduction in the risk of ruminal acidosis and bloat (Chalupa et al., 1980; Bergen and Bates, 1984). Including monensin as a premix or a controlled release capsule has been extensively researched in dairy cattle rations and the results have been variable in terms of DMI. Inclusion of monensin did not influence (Ramanzin et al., 1997; Broderick, 2004) or decreased (Sauer et al., 1998) DMI for lactating dairy cows. Tedeschi et al. (2003) speculated the variation between studies may be due to differences in stage of lactation and the reduced DMI may be a consequence of the cows reaching positive energy balance and eating to their energy requirement. When cows are in a positive energy balance (late lactation/dry) dietary supplementation with monensin may increase the energy available per unit of feed consumed (Mcal/d), reducing the DMI required for cows in a negative energy balance during early lactation,

the additional energy available through monensin use could improve performance, reduce body reserve losses, or both (Tedeschi et al., 2003).

Arieli et al (2001) that with monensin and rbST supplementation from 4 wk prepartum through 9 wk postpartum monensin treatment may improve the energy status of transition cows. The authors used 27 multiparous cows divided into 4 treatment groups. Treatments were control, monensin, rbST, and monensin plus rbST. No differences were observed in plasma glucose concentrations among treatments. However, monensin was associated with a numerical increase in plasma glucose concentrations and rbST with a numerical decrease in plasma glucose. The NEFA concentrations were not altered by rbST, but monensin supplementation decreased blood NEFA concentrations immediately surrounding calving. The authors observed that rbST and monensin have different effects on energy partitioning in prepartum cows and that monensin treatment may improve the energy status of transition cows.

Dietary monensin had no effect on the proportion of individual VFA and the acetate to propionate ratio (Benchaar et al., 2006). Ali-Haimoud et al. (1995) observed no effect of monensin on the acetate to propionate ratio, in contrast with Ruitz et al. (2001) who reported that monensin decreased the acetate to propionate ratio in dairy cows. Conflicting results may be due to differences in inclusion levels of monensin and to interactions between dietary components and monensin. Ramanzin et al. (1997) reported that monensin decreased the acetate to propionate ratio to a greater extent when lactating cows were offered a low forage diet (50:50) than when offered a high forage diet (70:30).

Benchaar et al. (2006) used ruminally cannulated Holstein cows to compare the effect of supplementing essential oils and monensin independently and in combination on digestion, ruminal fermentation characteristics, and milk yield and composition. Intake of DM was not affected by treatment and apparent digestibility of DM, OM, NDF, and starch were similar among treatments. The apparent digestibility of ADF was increased for diets supplemented with essential oil versus the control. Apparent digestibility of CP increased for cows supplemented with monensin versus no monensin. Ruminal pH increased with the addition of essential oil (6.5 versus 6.39). Ruminal ammonia N level was lower with monensin supplemented diets compared with diets without monensin (12.7 versus 14.3 mg/100 mL). These authors noted no change in total VFA concentration or molar proportions of individual VFA. Milk yield was similar among treatments, but milk fat was lower for cows supplemented with monensin (3.8 versus 4.1%). They noted that the reduced milk fat concentration in cows supplemented with monensin was associated with a higher level of *trans* 10 18:1, a strong inhibitor of milk fat synthesis. It was concluded from this study that the addition of essential oils and monensin had limited effects on digestion, ruminal fermentation characteristics, and on milk yield and composition (Benchaar et al., 2006).

Heat Stress:

Heat stress is caused by a combination of environmental factors (temperature, relative humidity, solar radiation, air movement, and precipitation). Many indices combining different environmental factors to measure the level of heat stress have been proposed. A temperature–humidity index (THI) is a single value representing the

combined effects of air temperature and humidity associated with the level of thermal stress (Bohmanova et al., 2007). This index was developed as a weather safety index to monitor and reduce heat stress related losses. There are several temperature-humidity indices and they differ in their ability to predict heat stress. Some indices are better suited to certain areas. Indices with larger weights on humidity seem to be more suitable for humid climates. In contrast, in climates where humidity does not reach levels that could comprise evaporative cooling, indices with more emphasis on ambient temperature are more suitable (Bohmanova et al., 2007).

The challenge of heat stress spans the southern United States and in areas subject to extended periods of high ambient temperature and high relative humidity. Several methods of dealing with heat stress have been identified. Beede and Collier (1986) offered three management strategies to aid in minimizing the effects of heat stress: 1) physical modification of the environment (shade, cooling), 2) genetic development of heat tolerant breeds, and 3) improved nutritional management practices. It seems apparent that no single plan is capable of countering the problem heat stress presents, rather a combination of strategies is required (West, 2003).

The combined effects of high ambient temperature and relative humidity reduce milk yield in lactating dairy cows. The decline in milk yield is a result of reduced the DMI which occurs under heat stress conditions (NRC, 1981). In addition, greater maintenance costs associated with heat stress further reduce the efficiency of energy use by the cow (National Research Council, 1981). A typical practice in the dairy industry is to reduce the amount of fiber in diets during hot weather. Cows offered low-fiber diets during hot weather produced more milk than cows offered a high fiber ration (Tsai, et al.,

1967). In contrast, in a study by Cummins (1992) cows offered low fiber diets reduced DMI by a greater degree than cows offered a high fiber diet when ambient temperatures increased. This suggests that total energy intake and the resulting metabolic heat production may influence DMI more than fiber content of the diet.

In a study conducted using Tifton 85 bermudagrass to determine the effect of different NDF concentrations on cow performance and nutrient digestibility during hot weather, workers reported that increasing dietary NDF reduced DMI, but the decline was not greater in hot weather compared with cool weather, suggesting that high fiber diets do not contribute to a greater heat stress load (West et al., 1999). These authors suggested that optimizing fiber content to maintain intake during cool temperatures and the use of high quality, but not necessarily low levels of dietary fiber during hot weather may be the most advantageous.

Hyperthermia (either from pyrexia or environmentally induced) negatively effects dairy production in a variety of ways, thus heat stress is a costly issue to the global dairy industry (St-Pierre et al., 2003). The two most noticeable effects of heat stress are reduced DMI and milk yield. Unabated heat stress can cause a 50% or more reduction in feed intake (Huber, 1996) and even in well cooled dairies, milk production can decrease by >10% (Collier et al., 1982). In addition to reduced DMI, heat stressed cows are thought to have increased maintenance costs (≥ 30 %) because maintaining homeothermia presumably has a large energy cost (Morrison, 1983; Huber, 1996; Fox and Tylutki, 1998). Because of decreased energy availability and increased energy utilization, heat stressed cows enter into a calculated state of negative energy balance (Moore et al., 2005). The heat stress induced deficiency in energy and nutrient

availability is thought to restrict milk yield during a thermal load. Subsequently, the energy density of the diet is often increased during hot weather (Schwartz et al., 2009).

Methods of increasing energy density in the diet include feeding more grain or supplemental fat. Increasing the grain content of the diet must be done with caution because heat stressed cows are already prone to ruminal acidosis (Kadzere et al., 2002). Another strategy is the addition of fats to the ration to increase energy intake and subsequent milk yield under heat stress, but there are limits to this (Huber, 1996). The limits come because feeding fats, oils, and even bypass fats can decrease fiber digestion, cause milk fat depression, and reduce DMI (Bauman et al., 2008).

Transition Cows:

The transition period for dairy cows is typically defined as 3 wk prepartum through 3 wk postpartum. The transition period can be divided into two phases: 7 to 0 d prepartum which is characterized by a 30% decline in DMI (Bertics et al., 1992; Grummer, 1995) and 0 to 21 d postpartum when DMI increases rapidly. The transition period is marked by significant change in metabolism as the cow prepares for parturition and the subsequent lactation. Typically, DMI during the first 5 wk of lactation is insufficient to match the increasing energy demands of lactation. During this period a cow is in negative energy balance. The energy available from consumed feed is less than the energy output in the form of milk (Hippen et al., 2008). The transition period is a major source of concern to producers because of the economic costs incurred during this period. The costs come as lowered milk yield, reduced reproductive efficiency, treatment costs and cull loses (Burhans et al., 2003). Researchers from the University of Minnesota

reported on a large data set which indicated that almost 10% of cows that calve leave the herd as either market cows or dead animals within two months of calving (Overton, 2006). Field observations and research data show that about 50% of all cows on average calve with some type of transition metabolic health issues as well as mastitis and lameness (Ferguson, 2001; Overton, 2006). Several of the metabolic disorders (ketosis, retained placenta, displaced abomasum, dystocia, metritis) affecting dairy cows during the postpartum period are interrelated and have been linked to the prepartum diet (Curtis et al., 1985). There are a variety of risk factors for postpartum problems, but the major focus should be on three basic physiological functions: meeting the nutrient demands of lactation, maintaining normal blood Ca levels through the transition period, and reducing the degree of immunosuppression that occurs around calving (Goff,. 2003). These factors are interrelated and the degree of change in DMI that occurs during the initial preparturient period seems to be highly correlated with both immune function and postparturient intake (Overton, 2006). The rapid increase in energy demand at parturition has resulted in recommendations for energy dense diets 2 to3 wk before and after calving (NRC, 2001). Researchers suggest that increasing the energy intake during the transition period may result in positive effects on the health, reproductive performance, and milk yield in high production dairy cows (Grummer 1995). Curtis et al. (1985) reported that increased energy content of the diet during the prepartum period was related to a reduced occurrence of displaced abomasum and increased protein content in the diet was associated with a decrease in ketosis and retained placenta.

Several methods to increase energy availability have been evaluated. One method is to increase ruminal carbohydrate availability through grain processing (Hale, 1973;

Huntington, 1997; Lykos and Varga, 1995; Owens et al., 1997; Theurer, 1986; Zinn, 1990). Processed grains may be beneficial to transition cows during the prepartum period by adapting the ruminal microbial population to subsequent postpartum diets, promoting ruminal papillae development, increasing the absorptive capacity of the rumen epithelium, and reducing lipolysis by increasing glucogenic precursors (Grummer 1995). The protein value of processed grains, particularly steam-flaked corn may be improved by decreasing N solubility, increasing ruminal escape and increasing the post ruminal digestibility (Zinn 1990). Increasing starch degradability in the rumen leads to increased total tract digestibility and microbial yield. Therefore, increasing carbohydrate availability in the rumen should result in more nutrients being available to the cow for energy (Hale, 1973; Poore et al., 1993; Theurer 1986).

The DMI of late gestation cows can decrease about 30% just prior to calving, further complicating the problem of meeting the cow's energy demands (Bertics et al., 1992; Grummer 1995). Depending on the severity and duration of the decrease in DMI, cows may experience a negative energy balance prior to parturition in addition to the negative energy balance seen postpartum.

Dann et al., (1999) noted that feeding diets with higher levels of ruminally available carbohydrates to high producing transition dairy cows may be profitable. Researchers observed that cows offered a TMR with steam flaked corn for 4 wk prepartum consumed more energy than cows offered a TMR with cracked corn. The increase in energy intake resulted in reduced mobilization of adipose tissue as calving approached and lowered plasma NEFA concentrations. Also, they observed that postpartum cows offered a diet with steam flaked corn consumed more energy and

produced more milk with lower fat and MUN concentration, and had lower plasma NEFA concentrations then cows offered a diet with cracked corn. There were no effects on ruminal pH, volume, turnover rate, or turnover time by either prepartum or postpartum treatments. The authors reported trends for increased propionate and decreased acetate: propionate ratio with the steam flaked corn ration during the postpartum period.

Osbourne et al. (2009) determined the effect of supplemental glycerol and soybean oil in cow's drinking water on DM and water intake, calculated energy balance, and production performance of transition dairy cows. Ninety multiparous Holstein cows were used in the study and treatments were control (no glycerol or soybean oil added), 20g/L glycerol supplemented in the drinking water, and 10 g/L soybean oil supplemented in the drinking water. The trial lasted from 7 d prepartum to 7 d postpartum. They reported that the DMI for cows supplemented with either glycerol or soybean oil tended to be lower than controls but not statistically different. Water intake was also greater for the control cows than the average for the glycerol and soybean oil treatment cows' prepartum, and greater than the soybean oil group but similar to the glycerol group postpartum. Glycerol treatment cows consumed more water than soybean oil dosed cows both pre and postpartum. No differences were observed among the treatment groups for energy intake and energy balance pre or postpartum. Also, no differences were noted for serum NEFA and glucose concentrations throughout the experiment. There was no difference for serum BHB at parturition, but serum BHB concentration of the glycerol supplemented cows was greater than for the control and soybean oil supplemented cows during the prepartum period. During the postpartum period serum BHB concentration for control cows was greater than for the glycerol and soybean oil cows. No treatment effect

was noted for milk composition. Although water supplementation did not affect net energy balance or serum glucose and NEFA concentration, BHB concentration, in the serum was reduced. The reduction may have been unrelated to fat mobilization and due to the reduced DMI for the glycerol and soybean oil treatments, leading to lowered production and absorption of butyrate from rumen fermentation. The authors concluded that although the glucogenic properties of glycerol supplemented drinking water may not have been enough to cause a milk yield response, it did reduce the BHB concentration postpartum.

Ruminal Acidosis:

The importance of diagnosing and controlling subacute ruminal acidosis (SARA) was documented in a 500 cow dairy (Stone, 1999). Replacing high-moisture corn with corn meal increased ruminal pH, milk yield increased by 2.7 kg/d and milk fat and protein increased by 0.3 and 0.1 percentage points, respectively. Improved milk yield and component content resulted in an increased monthly income of $20,000 for the dairy, presumably in large part due to the reduction of SARA and an improvement in rumen microbial growth.

Acute acidosis and chronic acidosis are conditions that follow the ingestion of large amounts of readily fermentable carbohydrates. This condition is a serious concern for producers feeding ruminant diets high in concentrate because respiratory alkalosis can occur in animals exposed to heat stress conditions. Acidosis is defined as a decrease in the alkali (base excess) in body fluids relative to the acid (hydrogen ion) content (Stedman, 1982). Since the pH of body fluids is buffered by bicarbonate, the pH of body

fluids may or may not be depressed during acidosis, depending on the extent to which bicarbonate compensation is possible. Central nervous system function can be inhibited when bicarbonate concentrations are too low. Clinical diagnosis of acidosis requires that blood pH level fall below 7.35. Other clinical signs of acidosis are reduced ruminal pH (5.6 and 5.2 for chronic and acute acidosis, respectively), anorexia, reduced feed intake, diarrhea, and lethargic behavior (Owens et al., 1998; Elam, 1976; Elanco, 1993).

Acute acidosis typically occurs when an animal consumes a large excess of grain. Rumen pH drops to 5.2 or less as *Streptococcus bovis*, a lactic acid producing bacteria, produce large quantities of lactic acid (Nocek, 1997; Owens et al., 1998). Lactate levels have been low in studies involving dairy cattle with SARA (Mishra et al., 1970; Oetzel et al., 1999; Oba and Allen, 2000). Data suggests that ruminal pH in dairy cattle with SARA is typically closer to a pH range of 5.5 to 5.6 than 5.2 (Mishra et al., 1970; Oetzel et al., 1999; Keunen et al., 2002). *Streptococcus bovis* is generally regarded as the primary lactate producer when ruminal pH is above 5.0. The fermentation products of *Streptococcus bovis* depend on both pH and growth rate. Acetate and ethanol are produced above a pH of 5.7, and lactate concentrations do not increase notably until the pH drops below 5.2 (Russell and Allen, 1984). The condition of subacute ruminal acidosis appears to be more related to elevated total VFA as compared with lactate (Burrin and Britten, 1986; Britton and Stock, 1989; Oetzel et al., 1999). The rapid accumulation of lactate may occur in animals post-calving if the shift in fermentable carbohydrates between the prepartum and postpartum ration is too dramatic (Stone, 2004).

This condition is often the result of excessive intake of rapidly fermentable carbohydrates that leads to the accumulation of organic acids in the rumen (Britton and Stock, 1989; Oetzel et al., 1999). There are probably four groups of cattle that are high risk of SARA: transition cows, high DMI cows, and those exposed to highly variable rations and meal patterns or to poorly formulated diets. Oetzel et al. (1999) reported that 20% of commercial dairy cows in early to mid-lactation have rumen pH of < 5.5, which leads to increased incidence of DMI depression, loose feces/ diarrhea, decreased milk yield, liver abscesses, and lameness/ laminitis (Underwood, 1992; Nocek, 1997). The economic cost of SARA was estimated at $1.12 per cow per day (Stone, 1999) making SARA a serious concern to the dairy industry.

Transition cows are more prone to develop SARA if their rumen bacterial population and papillae have not been gradually adjusted to a higher starch ration prior to calving (Dirksen et al. 1985). Rumen papillae significantly increased in size and absorption capacity when cows were switched from a diet composed mainly of hay and straw to a higher energy diet containing a mixture of grass hay and grain two weeks prior to calving. Starch was gradually increased and fiber reduced during the postpartum period. Rumen papillae reached maximum length 4 to 5 wk post-calving. *In vivo* VFA absorption rates measured at 14 wk post-calving were significantly greater compared with cows fed the hay-straw diet (Mertens, 1992; Dirksen, 1989).

Keunen et al. (2002) conducted two experiments to study the effect of an induced SARA on diet choice by dairy cows. The first experiment replaced 25% of the ad libitum DMI of the TMR with wheat barley pellets (WBP, 50% ground wheat, 50% ground barley). Researchers monitored rumen pH continuously via in-dwelling probes in mid to

late lactation cows. This diet resulted in a reduction in ruminal pH and extended the period of time pH was below 6.0, showing that this nutritional model successfully induced SARA. The second experiment was to determine whether SARA induced a change in feed preference or selection behavior using long alfalfa hay compared with alfalfa pellets. When given the choice between alfalfa hay and alfalfa pellets, cows chose the alfalfa hay more strongly when in a state of SARA (Keunen et al., 2002). The SARA model created a sufficiently large change in the dairy cow's rumen environment to invoke a dietary change in selection. Therefore, they surmised that dairy cows increased their dietary preference for a feed of longer particle size when given the appropriate choice during a bout of SARA (Keunen et al., 2002).

Laminitis is associated with nutrition, specifically with acute and subacute ruminal acidosis (Nocek, 1997; Vermunt, 2000). The specific relationship between laminitis and SARA have not been determined, but one of the theories associates SARA-induced damage to the ruminal epithelium, allowing for absorption of histamine and endotoxins. These and possibly other compounds disrupt normal circulation and cause inflammation within the hoof, leading to the occurrence of laminitis (Vermunt, 1992). Stone (2004) concluded that the nutritional program has a definitive effect on rumen health, which in turn affects hoof health and other effects of subacute ruminal acidosis. Ration formulation requires a balance between acid and buffer production. The occurrence of SARA can be reduced by considering the feed ingredients used in the ration, along with environmental conditions and management practices. The ration should be shifted toward additional physically effective NDF and less or slower fermenting non-

starch carbohydrate sources when the cow's environment (heat stress, comfort, stocking rates, ect.) or management is not as reliable as desired.

Hypocalcemia:

Hypocalcemia or milk fever is a life threatening condition observed in multiparous cows which typically affects about 8% of dairy cows, with reported herd frequencies ranging from less than 1% to greater than 20% (Kelton et al., 1998). Milk fever commonly occurs during calving or in the first 2 to 3 d postpartum, but can occur at anytime during lactation. Jersey cattle are more prone to milk fever then other breeds and older cows of any breed are more susceptible than primaparous animals. Subclinical hypocalcaemia is defined as plasma Ca concentration of approximately 5.5 to 8.0 mg/dl, which has been reported in up to 75% of periparturient cows (Reinhardt et al., 2005). The impact of the occurrence of milk fever extends beyond the consequences of clinical and subclinical hypocalcaemia alone. Parturient hypocalcaemia has been shown to increase the risk of dystocia, retained placentas, ketosis, and mastitis by 6.5, 3.2, 8.9, and 8.1% respectively (Curtis et al., 1983). Hypocalcemia has also been linked to decreased reproductive efficiency and increased culling rates (Erb et al., 1985). Systemic hypocalcaemia impairs intracellular Ca release in immune cells resulting in reduced immune cell activation following stimulation (Kimura et al., 2006).

Hypocalcemia occurs because of a sudden drop in blood Ca concentrations. During and near parturition, the onset of lactation results in a sudden loss in Ca through milk production. Serum Ca drops from a normal range of 10-12 mg/dL to 2-7 mg/dl. Typically serum Mg is increased and serum P less and cows are hyperglycemic. Milk

fever typically occurs within 72 h of calving and increases the occurrence of other complications like dystocia, uterine prolapse, and retained placenta. There are 3 stages of progression in hypocalcemia. In the 1st stage cows are able to stand but show signs of hypersensitivity and excitability. Cows may also be slightly ataxic and have fine tremors over the flank and loins and display ear twitching and head bobbing (Merck, 1998).

Cows will enter stage 2 if not treated. During stage 2 cows are unable to stand but can maintain strernal recumbency. Depression, dry muzzle, subnormal body temperature, cold extremities and smooth muscle paralysis are observed in this stage, and cows will often lay with their heads tucked against their flanks. Stage 3 follows where strernal recumbency can not be maintained and the cow becomes progressively more lethargic and with complete muscle flaccidity and can suffer from severe bloat. Cows that reach this stage will only survive a few hours without treatment (Merck, 1998).

Treatment of hypocalcaemia is directed at restoring the blood Ca content. Treatment can be an oral Ca paste in mild cases, but in the advanced stages of milk fever IV solutions of Ca solution is required. Phosphorus and Mg may also be included with the Ca solution. Hypocalcemic cows respond rapidly to treatment, heart rate and smooth muscle function recover and most cows are standing within 2 h of treatment (Merck, 1998). The best treatment however is prevention. The use of a negative DCAD and other diet and management practices help reduce the risk of milk fever, but high production mature cows, Jersey breed, and stressed cows will always have a risk developing hypocalcaemia.

Ketosis:

Ketosis has long been a challenge in the dairy industry and a problem that still needs resolution. Clinical ketosis (either primary or secondary) affects approximately 2-18% of cows during lactation, with the average being approximately 6% of cows (Kelton et al., 1998). Ketosis occurs when a cow is unable to metabolize the mobilized body fat in the form of NEFA. When the body utilizes body fat reserves as a source of energy, glycerol and fatty acids are released into the bloodstream. The glycerol is then converted to glucose by the liver (Krebs et al., 1966) and the kidneys (Krebs and Lund, 1966) to provide energy for cellular metabolism.

Subclinical ketosis is defined as blood concentration of BHB of 1200 umol/L or greater has been reported to affect 3-32% of lactating cows, depending upon DIM when sampled (Duffield et al., 1998; Duffield et al., 1997). Fat cows have a 1.6 fold greater risk for subclinical ketosis (Duffield et al., 1998) and clinical and subclinical ketosis is associated with increased risk for displaced abomasums and reduced reproductive efficiency (Overton, 2006). Most cows mobilize body fat reserves to meet energy demands during early lactation. Typically, cows will lose up to 0.75 units of body condition during the first 60 d of lactation. Cows that lose one or more units of body condition during this period are at increased risk of suffering from subclinical ketosis and have reduced conception rates at first service (Duffield et al., 1998; Domecq et al., 1997).

Ketosis is classified as type I or type II (Oetzel, 2003). Type I or insulin dependant ketosis is the commonly recognized form that occurs when an imbalance in energy consumed versus energy utilized. Cows suffering from this condition have a low insulin level due to being in a state of chronic hypoglycemia. These cows are unable to

meet the energy demands of early lactation, so excessive body fat is mobilized. The large amount of body fat mobilized stresses the capacity of the liver and results in the incomplete oxidation of fatty acids, the increase of ketones, and repackaged as VLDL's (Overton, 2006). Very low-density lipoprotein (VLDL) is a type of lipoprotein made by the liver. Once in circulation, VLDL comes in contact with lipoprotein lipase in the capillary beds in the body (adipose, cardiac, and skeletal muscle). Lipoprotein lipase (LPL) removes triglycerides from VLDL for storage or energy production. Cows with type I ketosis generally respond well to the normal treatments of drenching with glycerol or propylene glycol or IV glucose solutions.

Type II ketosis is commonly known as an insulin resistant ketosis or "fatty liver syndrome". Over conditioned cows are at greater risk for developing this type of ketosis but any cow that starts mobilizing body fat for energy prior to calving can be affected. The process of fat mobilization to meet energy needs is the same as seen in Type I ketosis, but it begins during the prepartum period instead of during the postpartum period. The excessive amount of fat traveling to the liver to be converted exceeds the liver's capacity to oxidize fat or form VLDL's. The result is an accumulation of fat in the liver. The accumulation of fat in the liver reduces the liver's ability to mobilize fat for energy and makes type II ketosis a more serious and difficult condition to treat compared with type I ketosis (Overton, 2006).

NEFA and BHB:

The concentration of NEFA tends to sharply increase just prior to calving. Grummer (1993) suggested that the rapid increase in serum NEFA concentration

immediately before calving may be hormonally regulated. The spike at calving may be associated with the sharp reduction in DMI and the elevation of lipolytic hormones occurring in conjunction with the onset of parturition (Vazquez-Anon et al., 1994). Pullen et al. (1989) reported that elevated concentrations of serum NEFA during the periparturient period is common in dairy cows and reflects increased reliance on adipose reserves to support energy requirements and milk fat synthesis. Another researcher observed that the degree of increase in serum NEFA after parturition was inversely related to DMI before parturition (Holstenius et al., 2003). Also, high serum NEFA concentration results in triacylglycerol accumulation in the muscle (Roberts et al., 1981) and liver (Roberts et al., 1981; Grummer, 1993) and has been associated with reduced DMI and greater incidence of metabolic disorders (Grummer, 1993).

Concentrations of NEFA and BHB are key metabolic factors used as herd based indicators of negative energy balance and subclinical ketosis in transition dairy cows, respectively (Duffield, 2000; Oetzel, 2004). An increase in NEFA secondary to lipolysis, stimulated by negative energy balance and value ≥ 0.40 mEq/L in ≥ 10% of an appropriate sample size of dairy cows tested between 2 and 14 d before calving is an indicator of excessive negative energy balance (Oetzel, 2004). Similarly, BHB values ≥ 14 mg/dL in ≥ 10% of an appropriate sample size of dairy cows tested within 5 to 50 days after calving indicates a problem with subclinical ketosis (Duffield, 2000; Oetzel, 2004).

Conclusion:

The need for alternative feed supplements and more information on how to best utilize recently available by-product feeds is an area of growing interest in the dairy

industry. Demand by some of the public for "natural" production and rising production costs are leaving producers in search of alternative ways to improve efficiency and cut costs. The use of bacterial inoculants and botanical supplements is an area with limited research and the need for further exploration. Glycerol has long been used in the treatment of ketosis but has not been an economically feasible addition to the ration at large. The expansion of biodiesel production has increased the availability of by-product glycerol and for many small refining operations the cost of refining glycerol to the purity level needed for other industries is cost prohibitive. Producers located near these biodiesel refineries may need to look at the inclusion of glycerol in the diet as an energy supplement or as a partial replacement for corn.

To date, there has been limited research with high yielding dairy cow diets using bacterial inoculants, botanical extracts, or glycerol individually or in combination. While, supplementing the transition dairy cow with glycerol has only been researched in short-term studies and the effects of supplementation past 3 wk postpartum has not been investigated. The objectives for this work were to investigate the effects of a combination of botanical extracts with glycerol and bacterial inoculants with glycerol in heat stressed dairy cows, and to evaluate the effects of various levels of dietary glycerol on ruminal fermentation in high yielding dairy cows and on the performance of the transition dairy cow.

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CHAPTER 3:

EFFECTS OF PLANT BOTANICAL EXTRACTS AND GLYCEROL ON EFFICIENCY AND PRODUCTION OF LACTATING DAIRY COWS DURING HOT WEATHER.[1, 2]

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ABSTRACT:

A trial was conducted to evaluate effects of a mixture of botanical extracts[1] with and without glycerol on milk yield, efficiency of yield, and nutrient digestibility in hot weather. The treatment compound was comprised of proprietary botanical additives and fermentation products selected for the potential ability to improve physiological response to heat stress. Forty-eight Holstein cows averaging 187 ± 16 days in milk (DIM) and 44.1 ± 0.46 kg/d of milk were used in a complete randomized block trial. The study was conducted June to August 2007. Cows were offered the control diet during a 2 wk standardization period, then blocked into groups of 4 based on parity, milk yield, and energy-corrected milk, and then randomly assigned within block to 1 of 4 treatments for 8 wk. Treatments were control (**C**), botanical extract[2] v1 (**T1**), botanical extract[3] v2 (**T2R**), and botanical extract[4] v1 with glycerol (**T1G**). Diets were corn silage based and balanced to be iso-caloric and iso-nitrogenous. No effects on DMI, milk yield, or energy corrected milk were observed except by lactation. Multiparous cows offered T2R and primiparous cows offered T1G treatments had similar milk yields which were greater than for cows fed C or T1 diets (P=0.02). An increase (P=0.01) in milk yield was observed for primiparous versus multiparous cows offered T1G. Decreased (P<0.02) milk fat percentage was observed for T1 versus C. An increase in NE balance by week was observed for T1G (42.2 Mcal/wk) compared with C, T1, and T2R (22.8, 20.6, and 36.1 Mcal/wk, respectively) possibly due because of improved ruminal efficiency. Cows offered T1G had numerically a higher weekly BW gain compared with other treatments.

[1] ThermalCare-D ® = mixture of botanical extracts and fermentation products
[2] ThermalCare-D ® v1
[3] ThermalCare-D ® v2 + RumeNext -D
[4] ThermalCare-D ® v1 + 454g/d glycerol

No effect on respiratory rate, skin temperature, body temperature or concentrations of serum glucose, urea N, or non-esterified fatty acid was noted. Cows offered botanical supplements tended to have increased DMI compared with C during the week during which digestibility measurements were recorded. Cows fed T1 and T2R exhibited improved ($P<0.05$) apparent digestion of DM, NDF and ADF compared with C or T1G. Results suggest ThermalCare® with RumeNext-D® may improve feed DMI and nutrient digestion of cows during hot weather. The addition of glycerol to ThermalCare® v1 was more beneficial to primiparous cows than multiparous cows.

Key Words: Botanical extracts, glycerol, heat stress, efficiency, digestibility

Abbreviation Key: **C** = Control; **T1**= ThermalCare® v1; **T2R** = ThermalCare® v2 plus RumeNext-D®; **T1G** = ThermalCare® v1 plus 454g/h/d glycerol.

INTRODUCTION:

Heat stress is a costly challenge for dairy producers leading to reductions in milk yield, growth rate, and reproductive performance, thereby costing producers millions of dollars a year. St. Pierre et al. (2003) estimated dairy producers lose approximately $900 million/yr due to heat stress. The current economic challenges facing the dairy industry accentuates the need to improve performance and efficiency under heat stress conditions. The dairy cow exhibits exquisite homeorhetic control in order to balance the metabolic demands of lactation. The increased metabolic energy demands that occur during heat stress result in reduced performance, which is compounded by a reduction in voluntary DMI. It has been estimated that DMI for a 600 kg cow producing about 27.2 kg of milk declines from 18.2 kg at 20°C to 16.7 kg (8.2%) at 35°C, and maintenance costs increase by 20% (NRC, 2006).

Dairy cows become heat stressed from the combined effects of high ambient temperature and the tremendous amount of metabolic body heat produced during lactation and the poor ability to dissipate the heat during hot weather. Metabolic heat accounts for about 31% of the energy consumed by a 600 kg cow producing 36.2 kg of 4% FCM (Coppock, 1985), and heat production increases with milk yield. The maintenance energy requirements increase during hot weather, which further reduces the energy available for milk synthesis. Heat increment of the diet, is defined as the increase in heat production following the consumption of a meal and heat associated with maintenance and with productive processes. Heat produced is substantial, and in moderate to high producing dairy cows the heat increment of feeds can be two-thirds of total heat production (Chandler, 1994).

The ability to enhance peripheral heat dissipation should help remove heat from the core of the body, effectively lowering body temperature and moderating heat stress. A reduction in body temperature should translate into greater DMI as well as improved efficiency of nutrient conversion to milk yield. In addition, less stress often results in fewer adverse health events with the potential for improved reproductive performance.

Glycerol is an odorless, colorless, hygroscopic, sweet tasting liquid by-product of the biodiesel industry. The increase in biodiesel production has lead to an increase in the availability of crude glycerol. This generates a potentially large source of crude glycerol for livestock producers. Glycerol has the potential to replace corn in the diet and has a projected feed value of 100-120% of corn. The energy value of glycerol makes it a viable alternative to corn and is a supplement that is basically "pure energy" (Hippen et al., 2008). Several studies reported that glycerol enhanced rumen fermentation and improved feed efficiency (Hippen, 2008; Garton et al., 1961; Remond et al., 1993; Schroder and Sudekum 1999; Dirksen et al., 1985; Linke et al., 2004).

ThermalCare® is a proprietary product developed by ADM Alliance Nutrition, designed to provide support to dairy cows during heat stress by supporting ruminal efficiency, digestive tract health and appropriate vasoactivity. This product is composed of selected fermentation and botanical extracts. The objective of this study was to determine the effects of two versions of botanical extract: v1[5], v2[6], and v1 + glycerol[7] on DMI, milk yield and composition, body temperature, and blood metabolites during heat stress.

[5] ThermalCare-D ® v1
[6] ThermalCare-D ® v2 + RumeNext -D
[7] ThermalCare-D ® v1 + 454g/d glycerol

MATERIALS AND METHODS:

The study was conducted during the summer of 2007 at the University of Georgia - Tifton Campus Dairy Research Center and all protocols for this study were approved by the University of Georgia Institute of Animal Care and Use Committee.

Cows and management:

Forty-eight lactating Holstein cows (twelve per treatment) were used in a 10 wk study. The study had a 2 wk standardization period followed by an 8 wk treatment period. Cows averaged 187 ± 16 DIM, 44.1 ± 0.46 kg/d of milk, 3.74 ± 0.24 % milk fat, and 2.84 ± 0.07 % of milk protein at the end of the standardization period. Cows were housed in a free stall barn with access to individual free stalls and fed behind Calan doors (American Calan, Inc., Northwood, NH). Training for Calan door use was initiated in mid-May, the standardization period occurred in early June and the treatment period started in late June and continued through early August. Supplemental cooling was provided by high speed fans and high pressure misters. All cows were administered rbST (Posilac, Monsanto, St. Louis, MO) and all diets contained Rumensin® (Elanco Animal Health, Greenfield, IN).

Diets were mixed and offered once daily behind electronic Calan doors, allowing individual intake to be determined. Diets were balanced to be iso-caloric and iso-nitrogenous. Amounts offered were adjusted to achieve 7-10% orts daily. Cows were milked twice daily at 0400 and 1500h. Prior to the treatment period a 2 wk standardization period was conducted during which all cows received the control ration. Baseline data was collected for all cows for use in covariate analysis of the data. In

addition, cows were blocked by average daily milk yield, stage of lactation and parity during the standardization period, into groups of 4, and randomly assigned to 1 of the 4 experimental diets within block. At this point, experimental diets were imposed for 8 wks.

Experimental treatments and design:

Dietary treatments consisted of a control (**C**), ThermalCare v1 (**T1**), ThermalCare v2 with RumeNext-D (**T2R**), and ThermalCare v1 plus 454g glycerol (**T1G**). Treatment premixes were added at manufacturer's recommended levels (2.27 kg/h/d) [Table 3.2].

The experimental design for this continuous study was a randomized complete block with repeated measures. Experimental model contained cow, treatment, week or sample, lactation number, covariate, and two and three way interactions. Data was analyzed using the Proc MIXED procedure of SAS version 9.1 (SAS, 2004). Significance of the treatments was determined using Tukey's (SAS, 2004).

Data collection:

The amount of feed offered and refused was recorded daily and adjustments were made as needed to achieve ad libitum intake. Milk yield was recorded twice daily by electronic meters (Alfa Laval Agric. Inc., Kansas City, MO). Milk samples were collected from 2 consecutive milkings each week and analyzed for milk fat and protein percentage, and somatic cell count by the Florida Dairy Farmers Laboratory (Bell, FL). Daily milk weights were recorded for 6 wk after the treatment period ended to track any

post treatment effects. Energy-corrected milk yield (ECM) was calculated using the following equation, which is defined as: ECM = (0.327 x kg milk) + (12.86 x kg fat) + (7.04 x kg protein) [Tyrell and Reid, 1964].

Body temperatures were recorded every 15 min for 4 d during standardization and at wk 5 and 8 of the treatment period using intra-vaginal probes (HOBO® Water Temperature Pro, Onset Corp, Contoocook, NH) attached to blank CIDRs to obtain individual cow temperatures. Environmental temperature data from the USDA weather station approximately one mile from the dairy unit provided the daily minimum and maximum ambient temperature and relative humidity throughout the study. Cows were weighed weekly using electronic scales, following the p. m. milking and prior to access to feed or water**.** Skin surface temperature was measured using a thermal temperature gun (InfraPro3, Oaklon, Arrow Scientific, New Zealand) at three points on the body: the hip, midpoint of the rear udder, and the center of the head just below the eyes. Skin temperatures were measured between 1400 and 1500 h (prior to milking) once during standardization and during wk 5 and 8 of the treatment period. High pressure misters were turned off 2h prior to measurement to ensure that cows were dry.

Blood samples from the coccygeal (tail) vein were collected once during standardization and during wk 5 and 8 of the treatment period for analysis of serum glucose, urea N (Tifton Veterinary Diagnostic Lab, Tifton, GA), and non-esterified fatty acids (Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI).

Feed and ingredient samples were collected twice per week. The DM content was determined by drying in a forced-air oven at 55ºC for 48 h. Samples were composited by

week and ground to pass through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) for analysis of DM (AOAC, 1990), NDF, ADF (Van Soest et al., 1991), CP (Kjeltec 2200, Seattle, WA) ether extract, and minerals (AOAC, 1990).

Digestibility Study:

Digestibility study was conducted concurrently with the production study. The study occurred during a 14 d period composed of 10 d of marker equilibration followed by a 4 d collection period. Forty-eight cows were dosed with 24 g of Cr_2O_3 /d in the TMR beginning wk 5 of the experimental period. Daily feed and ort samples were collected during the 4 d collection period. Fecal grab samples were taken during the collection period at 12h intervals. The collection time was advanced by 3h each day (3/3, 6/6, 9/9, 12/12 am/pm). Fecal samples were frozen at -5ºC until composited for the complete collection period by cow. Samples of feed, orts, and feces were dried at 55ºC and ground through a Wiley mill (1-mm screen) and stored for later chemical analysis.

Diets, orts, fecal samples, and ingredient samples were analyzed for DM, NDF, ADF, crude protein, and chromium. Chromium content of feed, orts, and feces was determined using an atomic absorption spectrophotometer (AAnalyst 100/300, Perkin Elmer) after wet ashing (Ferrett, et al, 1999).

RESULTS AND DISCUSSION:

The DMI was not different among the treatments (P = 0.31) [Table 3.3]. Benchaar et al. (2006b) reported increase DMI when plant extracts were fed in the presence of monensin. Cardozo et al. (2006) reported increased DMI and water intake compared with

the control with the addition of 15% capsicum extract to the diet. Researchers on the current study observed cows fed the treatment T2R diet had a tendency to maintain or recover to earlier intake levels during heat stress conditions compared with cows offered the other treatments. No significant treatment by parity interactions was observed (Table 3.3).

Milk yield (Table 3.3) was not affected by treatment ($P = 0.53$), but a significant interaction of treatment and parity ($P=0.02$) was observed. A treatment x parity interaction was observed ($P= 0.01$). Multiparous cows offered treatment T2R produced more milk (40.9 kg/d) compared with multiparous cows offered treatment T1G (36.6 kg/d).

A reduction in milk fat percentage was observed for T1 (3.60 %) versus C (4.06 %) [$P = 0.03$] (Table 3.3). This is in contrast to work by Benchaar et al. (2006a), who reported no effect on milk composition with the inclusion of plant extracts in the diet. Also, T1 milk fat percentages were numerically lower compared with T2R (3.74%) and T1G (3.9 %). Milk protein was less for T1 (2.76 %) compared with T1G (2.93 %) [$P = 0.02$] and numerically lower compared with the other treatments. No differences were observed for ECM among treatment groups ($P = 0.56$) [Table 3.4]. An effect was noted for BW change among treatments by lactation ($P = 0.06$) when the difference in weekly average weight change was calculated (Table 3.4).

A numerical increase was observed in the NE balance by week for cows offered T1G compared with other treatments (Table 3.4). The NE balance can be attributed to the tendency of cows on the T1G and T2R diets to gain BW without increasing DMI and still maintain production. This response likely resulted from improved ruminal efficiency and

increased energy availability. Researchers also observed (P = 0.08) intake of NE (Mcal/d) tended to be highest for T2R and T1G compared with C and T1 (Table 3.4). The increase in NE may be attributed to the inclusion of dietary glycerol in the diet. Donkin and Doane (2007) reported that cows offered a diet containing 15% glycerol gained more BW compared with other treatments. Fisher et al. (1973) reported that cows offered a 6 % glycerol supplement lost less BW and remained in a more positive energy balance compared with the control.

No differences in respiratory rate, skin temperature, or body temperature was observed among the treatments (Table 3.5). A significant effect was seen by period, but this can be credited to differences in the ambient temperatures and relative humidity levels. Environmental temperatures ranged from 21.8-33 ºC and relative humidity levels for the treatment period ranged between 67.8-92% (Graph 3.1 and 3.2).

No differences were observed among treatments for blood glucose, BUN, or NEFA (Table 3.5), although a significant difference by period was observed. Greater BUN concentrations were observed for standardization samples compared with the wk 5 treatment period samples. The cause of this increase is likely due to higher than expected dietary crude protein concentration in the diet.

Table 3.6 showed a statistically significant increase in apparent DM digestibility for T1 compared with T1G (P = 0.04) and T2R compared with T1G (P = 0.01). Also, an increase in apparent NDF digestibility was seen with T1 compared with T1G (P = 0.02) and a numerical increase with T2R versus T1G (P = 0.08). Apparent ADF digestibility was lower for C versus T2R and T1 compared with T1G (P = 0.01) and T2R compared with T1G at (P<0.001). Results suggest that the addition of RumeNext-D® to Thermal

Care® improved total tract digestibility. Several studies have reported the potential of some botanicals and secondary plant metabolites including saponins, anise oil, capsicum extract, eugenol, and cinnamaldehyde to modify ruminal fermentation with in vitro studies (Cardoza et al., 2004, 2005; Busquet et al., 2005a,b, 2006; Klita et al., 1996; Hristov et al., 1999).

Post-trial milk yield data was collected for 6 wk. A trend was noted for T2R to remain above the control and other treatments but no statistically significant effects where observed.

CONCLUSION:

Results indicate that while there were no significant effects on DMI there was an increase in milk yield for multiparous cows offered the enhanced version of ThermalCare® v2 with RumeNext-D® (T2R). This occurred because of improved ruminal efficiency, which was supported by the total tract digestibility of DM, ADF, and NDF. More research is needed to determine the ruminal fermentation effects of ThermalCare v1 and ThermalCare® v2 with RumeNext-D®. Also, primiparous mid-lactation cows demonstrated an increased milk yield with the addition of glycerol to the ThermalCare® v1 (T1G) with no effect on weight gain. This suggests that the extra energy provided by the glycerol improved production in these animals by increasing the availability of energy in the diet.

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Table 3.1: Chemical composition of experimental diets and ingredients.

	%DM	%NDF	%ADF	%CP	%EE
C[1] TMR	50.1 ± 2.30	39.5 ± 0.73	24.2 ± 0.96	19.5 ± 0.55	5.7 ± 0.83
T1 TMR	50.1 ± 2.30	38.1 ± 0.07	24.5 ± 0.10	19.2 ± 0.25	5.7 ± 0.72
T2R TMR	50.5 ± 2.81	37.9 ± 0.32	25.2 ± 0.43	19.4 ± 0.63	5.5 ± 2.73
T1G TMR	51.8 ± 2.71	38.0 ± 0.55	24.7 ± 0.23	19.5 ± 0.23	5.9 ± 1.00
Corn Silage	40.8 ± 2.45	40.9 ± 0.67	27.5 ± 0.46	9.1 ± 1.09	N/A
Ground Corn	91.9 ± 1.56	13.2 ± 1.20	4.4 ± 0.61	9.9 ±0.92	N/A
Alfalfa Hay	91.2 ± 1.97	56.0 ± 2.42	47.3 ± 2.38	14.5 ± 0.37	N/A
Brewers Grain	26.4 ± 2.12	64.8 ± 1.17	34.4 ± 0.01	28.6 ± 0.27	N/A
WCS	93.2 ± 2.26	55.1 ± 0.23	41.2 ± 0.05	26.5 ± 0.79	N/A
SBM	93.2 ± 0.89	11.3 ± 0.69	6.4 ± 0.33	52.1 ± 1.03	N/A
Concentrate Mix[2]	94.1 ± 1.51	18.1 ± 1.30	12.2 ± 0.85	30.3 ± 2.85	N/A

[1]Control = (C), ThermalCare® v1 = (T1), ThermalCare® plus RumeNext-D® = (T2R), and ThermalCare® v1 plus 454 g/h/d glycerol l= (T1G).

[2]Concentrate Mix: 2.04% ProLak (H.J. Baker and Brother, Inc.) ; 6.06% soybean meal 48; 0.151% Ca 17%: P 21%; 0.182% pot-mag-sulfate; 0.454% K-minus; 0.151% limestone; 0.151% urea 45%N; 0.027% Availa-4; 0.154% MgO; 0.227% salt; 0.530% Na bicarbonate; 0.188% yeast; 0.121% trace mineral premix; 0.01% vitamin premix; 0.229% Rumensin 3.

Table 3.2: Experimental diet ingredients (DM basis).

Item	C[1]	T1	T2R	T1G
Corn silage	42.81	42.81	42.81	42.42
Alfalfa hay	4.49	4.49	4.49	4.46
Whole cottonseed	7.46	7.46	7.46	7.39
Ground corn	7.78	7.78	7.78	7.28
Brewers' grain	8.65	8.65	8.65	8.58
Soybean meal	0.0	0.0	0.0	1.33
Concentrate Mix[1]	21.60	21.60	21.60	21.40
Thermal Care premixes	7.21	7.21	7.21	7.15
Glycerin	0.0	0.0	0.0	0.79

[1]Control = (C), ThermalCare® v1 = (T1), ThermalCare® plus RumeNext-D® = (T2R), and ThermalCare® v1 plus 454 g/h/d glycerol l= (T1G)

Table 3.3: Performance of lactating Holstein cows fed diets supplemented with botanicals with and without glycerol.

Item	C[1]	T1	T2R	T1G	SE	*P* < Trt	*P* < Lactation[2]	*P* < Trt x Lactation
DMI (kg/d)	22.6	23.3	24.2	23.5	0.57	0.31	<0.0001	NS
Milk (kg/d)	38.3	39.0	39.7	38.5	0.69	0.53	0.33	0.02
Primiparous	39.0	39.3	38.4	40.4	1.09	_	_	-
Multiparous	37.6^{ab}	38.6^{ab}	40.9^{a}	36.6^{b}	0.91	_	_	-
Milk fat (%)	4.06^{a}	3.60^{b}	3.74^{ab}	3.90^{ab}	0.11	0.03	0.22	NS
Milk fat (kg/d)	1.57	1.41	1.47	1.50	-	-	-	-
Milk protein (%)	2.89^{ab}	2.76^{a}	2.84^{ab}	2.93^{b}	0.05	0.02	0.18	NS
Milk protein (kg/d)	1.11	1.09	1.11	1.12	-	-	-	-
ECM (kg/d)	38. 5	37.3	40.2	39.4	1.60	0.56	0.06	NS

[1]Control = (C), ThermalCare® v1 = (T1), ThermalCare® plus RumeNext-D® = (T2R), and ThermalCare® v1 plus 454 g/h/d glycerol = (T1G)

[21] Lactation= primiparous vs. multiparous

NS=No Significance (P< 0.35)

*Means with unlike subscripts in the same row are significantly different (P< 0.05)

Table 3.4: Efficiency of lactating Holstein cows fed diets supplemented with botanicals with and without glycerol.

						P <	
Item	**C[1]**	**T1**	**T2R**	**T1G**	**SE**	**Trt**	**Lactation[7]**	**Trt x Lactation**
Efficiency (ECM/DMI)	1.72	1.56	1.67	1.70	0.06	0.16	NS	NS
Efficiency (MY/DMI)	1.69	1.66	1.66	1.64	0.05	0.93	0.31	NS
NEBwk[2]	22.76	20.64	36.08	42.18	8.4	0.23	0.16	NS
NEBdy[3]	3.25	2.83	5.16	6.15	1.26	0.20	0.14	NS
NE intake (Mcal/d)	36.43	37.91	39.86	38.74	0.94	0.08	<0.0001	NS
NE milk[4] (Mcal/d)	27.89	25.37	27.82	27.07	1.15	0.38	0.01	NS
BW change[5] (kg/wk)	0.27	0.12	0.51	0.68	0.19	0.19	0.06	NS

[1]Control = (C), ThermalCare® v1 = (T1), ThermalCare® + RumeNext-D® = (T2R), & ThermalCare® v1 +s 454 g/h/d glycerol=(T1G)

[2] NE Balance per wk= NE intake (Mcal/d)-NE maintenance (Mcal/d) ± NE of tissue change (Mcal/d)-NE of milk (Mcal/d) [NRC, 2006]

[3] NE Balance by day= NE Balance by week/7

[4] NE milk= Milk (kg/d) x [(0.0929 x milk fat %) + (0.0563 x milk protein %) + 0.192)] (NRC, 2006)

[5] Difference in BW by week using a weekly rolling average

[6] Lactation= primiparous vs. multiparous

Table 3.5: Respiratory rate, skin temperature and blood metabolites of lactating Holstein cows fed diets supplemented with botanical supplements with and without glycerol.

						P <		
Item	**C**[1]	**T1**	**T2R**	**T1G**	**SE**	**Trt**	**Period**[2]	**Trt x Period**
Respiratory rate (breaths/min)	42.58	42.67	41.16	44.63	1.20	0.25	<0.0001	0.23
				Skin temperature(˚C)				
Head	35.8	35.9	35.9	35.7	0. 18	0.61	<0.0001	0.54
Hip	36.9	36.9	37.2	36.9	0.28	0.52	<0.0001	0.88
Udder	37.3	37.3	37.5	37.2	0.24	0.34	<0.0001	0.22
				Body temperature (˚C)				
	39.2	39.3	39.2	39.3	0.16	0.68	<0.0001	0.02
				Serum metabolite				
Glucose, mg/dl	65.10	64.25	65.37	64.07	0.89	0.69	<0.0001	0.25
Urea N, mg/dl	18.41	17.80	18.47	18.55	0.55	0.76	<0.0001	0.02
NEFA, mEq/L	0.23	0.23	0.22	0.20	1.51	0.76	0.01	0.90

[1]Control= (C), ThermalCare v1= (T1), ThermalCare® plus RumeNext-D®= (T2R), ThermalCare® v1 plus 454 g/h/d glycerol = (T1G)

[2]Period= 3 periods (standardization, wk 5 and wk 8 of the study).

Table 3.6: Apparent digestibility for lactating Holstein cows fed diets supplemented with botanicals with and without glycerol.

						P <	
	C[1]	**T1**	**T2R**	**T1G**	**SE**	**Trt**	**Lactation[2]**
Intake, kg/d							
DM	22.7	24.7	25.7	24.6	0.88	0.13	0.001
CP	4.5	4.6	5.0	5.0	0.17	0.09	0.001
ADF	5.2[a]	5.8	6.2[b]	5.7	0.24	0.04	0.004
NDF	8.5	9.1	9.3	8.9	0.37	0.49	0.004
	----------Digestibility %----------						
DM	60.3[abc]	62.0[a]	62.7[b]	58.5[ac]	0.90	0.009	NS
CP	61.3	63.0	63.8	62.0	0.84	0.18	NS
ADF	29.3[abd]	35.2[bc]	37.4[c]	27.5[d]	1.70	0.0004	NS
NDF	37.5	39.7[a]	38.5	33.2[b]	1.50	0.03	NS

[1]Control = (C), ThermalCare-D® v1 = (T1), ThermalCare-D® plus RumeNext-D® = (T2R), and ThermalCare-D® v1 plus 454 g/h/d glycerol = (T1G)

[2] Lactation= primaparous versus multiparous

* Means with unlike subscripts in the same row are significantly different (P<0.05).

Figure 3.1: Environmental temperatures outside the freestall barn for summer 2007.

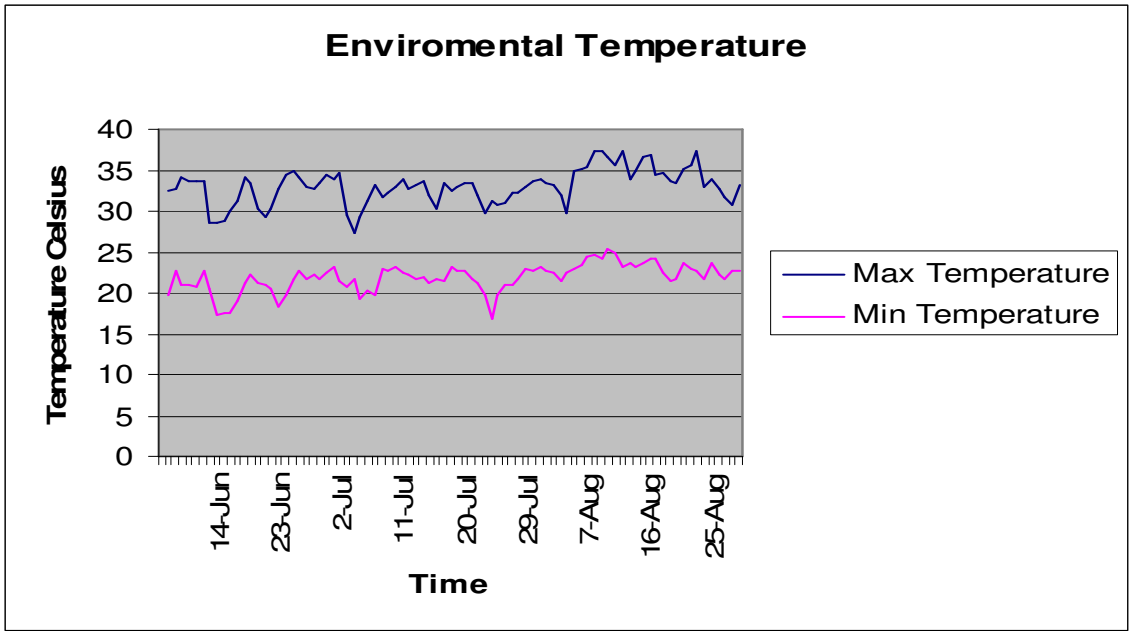
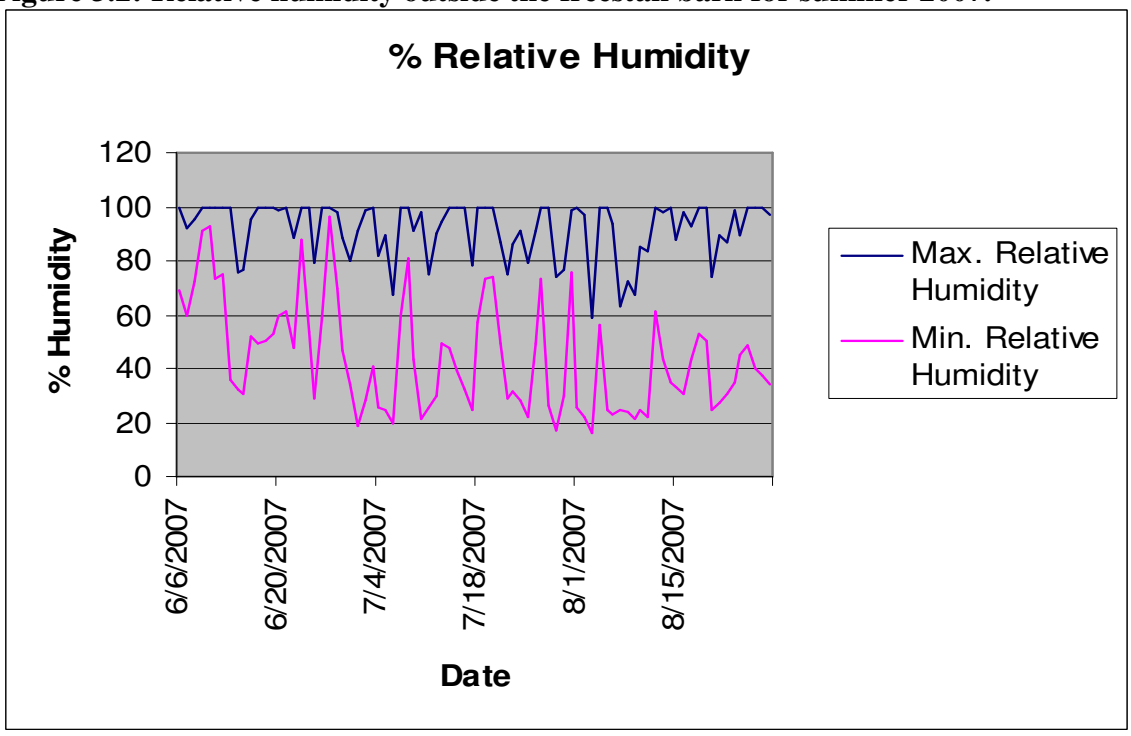


Figure 3.2: Relative humidity outside the freestall barn for summer 2007.



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CHAPTER 4

EFFECT OF INCREASING CONCENTRATIONS OF DIETARY GLYCEROL ON RUMINAL ENVIROMENT AND DIGESTIBILITY IN LACTATING HOLSTEIN COWS. [1]

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ABSTRACT:

A study was conducted to evaluate effects of increasing concentrations of dietary glycerol on rumen environment, blood metabolites, and nutrient digestibility. Six ruminally cannulated Holstein cows averaging 56 days in milk and 37.9 kg/d of milk were used in the study. The study was conducted from May to July 2008. Experimental design was a 3x3 Latin square with a 3wk adjustment period followed by a 1wk collection period. Cows were blocked into groups of two by lactation, milk yield, and ECM. Cows progressed through three 4wk periods until exposed to all treatments. Diets were corn silage based and balanced to be iso-caloric and iso-nitrogenous. Treatments were control (**C**), 200g glycerol h/d (**G2**), and 400g glycerol h/d (**G4**). The DMI was greater for C than for G2 or G4 ($P < 0.01$). Milk yield was reduced ($P < 0.01$) for G4 compared with C or G2. Milk protein percentage was lower ($P < 0.001$) for C and G2 than for G4. Milk fat percentage was lower ($P < 0.01$) for G2 and G4 compared with C. Researchers observed an effect ($P < 0.04$) for ECM between C and G4, but G2 was not statistically different. A trend ($P < 0.18$) for improved efficiency (energy-corrected milk/DMI) was noted for G2 and G4 versus C. No effect by treatment on ruminal pH and ammonia (mean 6.06 and 11.19 mg/dl) was observed or for DM, NDF, ADF, or CP digestion. Also, no significant effect by treatment on blood glucose (mean 63 mg/dl) was observed. However, a trend for lower plasma urea N ($P < 0.09$) was observed for G4 (20.5 mg/dl) versus C and G2 (21.7 and 21.8 mg/dl). The acetate: propionate ratio was different ($P<0.03$) for C (2.65 ± 0.05) versus G2 (2.47 ± 0.05) and G2 versus G4 (2.3 ± 0.05) and C versus G4 ($P < 0.0001$). The decrease in milk fat percentage between G2 and G4 versus C agrees with the ruminal data because the drop in ruminal acetate level would

result in a reduction in milk fat percentage. The addition of glycerol to the diet alters the ruminal volatile fatty acid profile and may improve efficiency in dairy cows.

Key Words: glycerol, ruminal fermentation, VFA, digestibility, acetate, propionate

Abbreviation Key: **C**= Control; **G2**= Control plus 200 g/h/d glycerol; **G4**= Control plus 400 g/h/d glycerol.

INTRODUCTION:

Glycerol is a by-product of the biodiesel industry (Dasari et al., 2005) and the increase in biodiesel production has increased the availability of crude glycerol. By-product glycerol is generated from the transesterification of vegetable oils. Biodiesel production has increased in the United Sates from 0.5 million gal. in 1999 to 460 million gal in 2007 (National Biodiesel Board, 2008). Purified glycerol is used in the food, pharmaceuticals, cosmetics industries and other fields, but the cost of refining crude glycerol to a high purity level is prohibitive to many small refining operations (Pachauri and He, 2006). This generates a potentially large source of crude glycerol for livestock producers. Also, the increased demand for renewable energy has led to a record increase in corn and soybean prices leading dairy producers to search for alternative dietary energy sources that are more economical.

Glycerol is an odorless, colorless, hygroscopic, sweet tasting liquid that has the potential to replace corn in the diet and has a projected feed value of 100-120% of corn. The energy value of glycerol makes it a viable alternative to corn and is a supplement that is basically "pure energy" (Hippen et al., 2008). Linke et al., (2004) calculated the energy value of glycerol to be approximately 20 % greater than corn, yielding an NE_L of about 0.48 Mcal/kg. Schroder and Sudekum (1999) calculated the energy density of glycerol to range between 0.90 to 1.04 Mcal/kg NE_L. Several studies reported that glycerol enhanced rumen fermentation and improved feed efficiency (Hippen, 2008; Garton et al., 1961; Remond et al., 1993; Schroder and Sudekum 1999; Dirksen et al., 1985; Linke et al., 2004). The yield of glycerol from biodiesel is approximately 1 unit of glycerol for each 10 units of biodiesel produced. The U.S. biodiesel industry has a

projected national average production of over 2.2 billion gallons by the end of 2008, which would produce annually about 220 million gallons of by-product glycerol of 80% purity (Feedstuffs, 2007).

Previously, glycerol has been a cost prohibitive addition to the diet, but with growing biodiesel production more crude glycerol is being produced. The increased supply is reducing price and making the addition of glycerol to the ration a feasible choice for many producers. The objective of this study was to determine the effects of different levels of dietary glycerol on rumen function, blood metabolite, nutrient digestibility and passage rates in high production lactating Holstein cows.

MATERIALS AND METHODS:

The study was conducted during the summer of 2008 at the University of Georgia, Tifton Campus Dairy Research Center. Protocols for the trial were approved by the University of Georgia Institutional Animal Care and Use Committee.

Cows and management:

Six ruminally cannulated early to mid-lactation Holstein cows averaging 56 ± 18 DIM, 38.0 ± 8.20 kg/d milk, 3.77 % ± 0.97 milk fat, and 2.72 % ± 0.20 milk protein were used in a 3x3 Latin square design study. Cows were housed in a free stall barn with access to individual free stalls and fed behind Calan doors (American Calan, Inc., Northwood, NH). Cows were cooled using fans and high pressure misters. Training for Calan door use was initiated in early April; the study began in early May and continued until the end of July.

Diets were mixed and delivered once daily, ad libitum intakes were adjusted to achieve orts of 7-10% daily. Diets were formulated to be iso-caloric and iso-nitrogenous. (Table 4.1) Cows were milked twice daily at 0400 h and 1500 h.

Cows were blocked by average daily milk yield, stage of lactation, and parity during the standardization period, blocked into groups of 2 by rank, and assigned randomly to 1 of the 3 experimental diets within block and progressed through the three 4wk periods until exposed to all treatments.

Experimental treatments and design:

The experimental design for this study was a complete 3x3 Latin square design with a 3 wk adjustment period followed by a 1 wk collection period. Dietary treatments consisted of a control (**C**), control plus 200g glycerol per cow/day (**G2**), and control plus 400g glycerol per cow/day (**G4**). Glycerol was mixed with the TMR using a Super Data Ranger (American Calan, Inc., Northwood, NH) with whole cottonseed as a carrier. The glycerol used in this study was 80-85% glycerol, 14 % moisture, and 7 % sodium chloride. The methanol content was 18 ppm and was supplied by ADM Nutrition Alliance, Quincy, IL. The experimental model contained cow, treatment, hour, block, period, covariate, linear contrasts and two and three way interactions. Data was analyzed using the Proc MIXED procedure of SAS version 9.1 and significance of treatment effects was determined using Tukey's (SAS, 2004).

Data collection:

Feed intake was recorded daily and adjustments were made to maintain a 7-10% refusal rate. Milk yield was recorded twice daily by electronic weight meters (Alfa Laval Agric. Inc., Kansas City, MO) and summed daily. Milk samples were collected from four consecutive milkings each collection period and analyzed for milk fat and milk protein percentage, and somatic cell count by the Southeast Milk Incorporated laboratory (Bell, FL). Energy-corrected milk (ECM) yield was calculated using the following equation: ECM = (0.327 x kg milk) + (12.86 x kg fat) + (7.04 x kg protein) (Tyrell and Reid, 1964).

Blood samples from the coccygeal (tail) vein were collected once during each collection week for analysis of serum glucose and urea N, (Tifton Veterinary Diagnostic Lab, Tifton, GA). Rumen samples were collected on the third day of each collection week, at 0, 2, 4, 6, 8, and 10 h post feeding. Approximately 50 ml of ruminal fluid was collected and strained through three layers of cheesecloth and immediately analyzed for pH and ammonia levels. A 10 ml sub-sample was immediately mixed with 2 ml of metaphosphoric acid (25% w/v). The sample was then centrifuged at 10,000 x g for ten minutes, supernatant collected, and frozen for later analyses of VFA (Erwin et al.,1961) using a Thermo Scientific Trace GC Ultra (Thermo Fisher Scientific, Bellefonte, PA).

Feed and ingredient samples were collected 4 times during the collection week. The DM content was determined by drying in a forced-air oven at 55ºC for 48 h. Samples were composited by period and ground to pass through a 1mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) before analyses of DM (AOAC, 1990), NDF,

ADF, (Van Soest et al., 1991), crude protein, and ether extract (AOAC, 1990) [Table 4.2].

Nutrient Digestibility:

A digestion study was conducted concurrently with a 14 d period and consisted of a 10 d standardization period followed by a 4 d collection period, which was repeated for each block of the Latin square. All cows were dosed with 24 g of Cr_2O_3 / day in a top dress with ground corn. Daily feed and ort samples were collected during the 4 d collection period. Fecal grab samples were taken during the collection period at 12 hr intervals, with the collection time advancing by 3h each day (0300, 0600, 0900, 1200, 1500, 1800, 2100, and 2400 h). Fecal samples were frozen at -5ºC until being composited for the complete collection period by cow. Samples of feed, orts, and feces were dried at 55ºC and ground through a Wiley mill (1-mm screen) and stored for later chemical analysis.

Diets, orts, fecal samples, and ingredient samples were analyzed for DM (AOAC, 1990), NDF, ADF (Van Soest et al., 1991), crude protein (AOAC, 1990), and chromium. Chromium content of feed, orts, and feces was determined by atomic absorption spectrophotometer (AAnalyst 100/300, Perkin Elmer, Bellefontaine, PA) after wet ashing (Ferret, et al., 1999).

RESULTS AND DISCUSSION:

Dry matter intake was reduced (P= 0.004) was reduced with glycerol compared with the C versus G2 and G4 (Table 3). This is in contrast with work reported by Linke

(et al., 2004) and DeFrain (et al., 2004) who observed no difference in DMI when glycerol was included in the diet.

Milk yield ($P = 0.01$) was reduced for G4 and G2 compared to C (Table 4.3). The inclusion of glycerol resulted in reduced milk fat percentage ($P = 0.001$) with the glycerol included diets. In contrast, milk protein percentage increased ($P = 0.001$) 2.76%, 2.75%, and 2.8% for C, G2, and G4 respectively as glycerol concentration increased in the diet (Table 4.3). These results are in contrast with earlier work by Donkin and Doane (2007) who added 99.5% purified glycerol to the diet in place of corn and observed no affect on milk yield or composition. A transition cow study by Chung et al., (2007) using a dried glycerol product (food grade, 65% glycerol) also demonstrated no effect on milk yield or composition. Linke (et al., 2004) included glycerol at a rate of 498g and 998g in the diet of mid-lactation Holstein and Brown Swiss cows in a 3x 3 Latin square with no effect on DMI, milk yield, or FCM. These researchers did observe an improvement in feed efficiency with the inclusion of glycerol with milk to feed ratios of 1.46, 1.59, and 1.60 for the control, 498g/d of glycerol, and 998g/d treatments of glycerol respectively. There was no difference ($P = 0.18$) in feed efficiency for ECM/DMI, but a numerical trend was noted with increased efficiency of yield as glycerol levels increased (Table 4.3). ECM milk values declined ($P = 0.05$) between C and G4. The decrease in ECM values resulted from the reduction in milk fat percentage with the inclusion of glycerol in the diet. The decrease in ECM agrees with work by DeFrain et al., (2004) who reported greater ECM for controls compared with the glycerol treatments.

No statistical differences were observed among treatments for serum glucose or plasma urea N. Average serum glucose was 62.8 mg/dl and plasma urea N was 21.3 mg/dl for the treatments (Table 4.3).

Ruminal pH (average 6.06) and ammonia concentration were unchanged by treatments though a slight numerical trend for decreased ammonia concentration as glycerol level increased was observed (Table 4.4). No effect on total VFA concentration was observed ($P = 0.24$). Molar proportions of acetate decreased linearly ($P = 0.0001$) with increasing dietary glycerol content, C (61.1 %), G2 (59.1 %), and G4 (57.6 %). The reduction in milk fat percentage with increasing dietary glycerol was supported by the decreased ruminal acetate concentration observed with the addition of glycerol to the diet. Propionate increased linearly ($P = 0.0002$) with increasing dietary glycerol content. A notable change was observed for propionate between C and G4 and between G2 and G4. Butyrate concentrations increased with the addition of dietary glycerol (P= 0.0001). Less ruminal butyrate was noted for C compared with G2 and G4. Valerate concentrations also demonstrated a linear increase (P= 0.0001) with glycerol addition. No effect (P>0.35) was observed for iso-butyrate or iso-valerate concentrations. The acetate: propionate ratio decreased linearly with the increase in dietary glycerol ($P =$ 0.0001), C (2.65), (2.47), and G4 (2.3).

The effects on ruminal VFA concentrations agree with results reported in several studies. Schroder and Sudekum (1999) determined the ruminal effects of feeding glycerol to ruminally cannulated steers and reported that although glycerol addition did not alter diet digestibility, it did reduce the acetate: propionate ratio and linearly increased ruminal butyrate concentration, while stimulating greater water intake. They concluded that the

inclusion of dietary glycerol might benefit the dairy cow because of the potential to increase ruminal propionate concentration, increasing the supply of gluconeogenic substrate to the liver. Also, greater ruminal butyrate supports the growth of ruminal epithelial tissue and may increase nutrient absorption from the rumen (Dirksen et al., 1985). Greater water intake may be beneficial to cows under heat stress as an aid in cooling and yield.

DeFrain et al. (2004) conducted a transition cow study evaluating the inclusion of glycerol in the diet. They reported that ruminal fluid collected postpartum from cows offered glycerol had greater total VFA concentrations, increased molar proportions of propionate, and decreased acetate: propionate ratio compared with the control cows. Also, they observed molar proportions of butyrate tended to increase linearly in cows offered glycerol versus controls.

Linke et al., (2004) reported increased ruminal propionate and butyrate with inclusion of glycerol in the diet with no effect on DMI or yield of milk, or FCM. The authors reported on the effect of glycerol addition to feed versus drenching of glycerol on the ruminal environment. They observed that following glycerol administration concentrations of ruminal acetate decreased in all cows dosed with glycerol regardless of administration route. Propionate and butyrate increased with both methods of glycerol administration with a peak concentration reported at 4h post-feeding. The concentration of glucose increased in blood plasma for cows that were given glycerol via drenching or esophageal tube compared with the control or glycerol fed treatments. Also, concentrations of plasma insulin and *B*-hydroxybutyrate (BHB) were increased for drenching and esophageal tube treatments compared with the control and glycerol fed

diets. Researchers concluded from this study that in order for glycerol to be gluconeogenic it must be delivered in water to associate with the liquid fraction of the rumen contents or to be able to bypass the rumen in some form to be absorbed as glycerol and converted to glucose by the liver. Glycerol which is available to rumen microbes is highly fermentable and is converted to propionic and butyric acids. Garton et al., (1961) conducted in vitro incubations of glycerol and found that by 2 h nearly 25% of glycerol had disappeared and by 8 h nearly 90% of the glycerol was undetectable. The portion converted to butyrate is metabolized to BHB by the ruminal epithelium; therefore glycerol that is fed is actually ketogenic rather than gluconeogenic. However, if glycerol bypasses fermentation in the rumen it should be an efficient gluconeogenic substrate Garton et al., (1961).

The results of the digestibility study (Table 4.5) demonstrated no effects on nutrient intake or apparent digestibility (P=0.35), although a numerical trend for reduced intake was observed as the level of glycerol in the diet increased. These results agree with work that showed no effects on DMI or digestibility values with the addition of glycerol to the diet (Schroder and Sudekum, 1999; Donkin and Doane, 2007).

CONCLUSIONS:

Results indicate that the inclusion of dietary glycerol in the ration of high producing dairy cows has an effect on intake, production, ruminal fermentation pattern and feed efficiency. The effects on ruminal fermentation agree with several earlier studies. Thus it appears that the addition of rumen fermentable glycerol decreases the acetate: propionate ratio and increases butyrate which should increase the supply of

propionate for liver metabolism. Dietary glycerol provides a source of energy in the diet which may be an alternative for producers with the growth of the biodiesel industry. Glycerol improves rumen fermentation and feed efficiencies. Also, glycerol may be beneficial because of its ability to improve palatability in low doses, increase water consumption and as a viable substitute for corn in the diet.

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Table 4.1: Composition of experimental diets containing supplemental glycerol.

Ingredient	C[1]	G2	G4
	----------% of DM----------		
Corn silage	38.5	38.5	38.5
Alfalfa hay	10.5	10.6	10.6
Whole cottonseed	1.8	1.8	1.8
Ground corn	23.9	22.9	22.1
Wet brewers grain	12.1	12.1	12.1
Concentrate mix[2]	13.2	13.3	13.3
Glycerol	0.0	0.8	1.6

[1]Treatments were: Control =C; G2 = control plus 200 g/h/d glycerol; G4 = control plus 400 g/h/d glycerol.

[2]Concentrate mix the same for all treatment groups: 1.34% ProLak (H.J. Baker and Brother, Inc.) ; 6.47% soybean meal 48; 0.92% Megalac; 0.184% Pot-Mag-sulfate; 0.737% molasses black; 0.645% limestone; 0.369% DCAD plus (Arm & Hammer Animal Nutrition); 0.037% Availa-4 (Zinpro Corp); 0.184% MgO; 0.240% salt; 0.645% Na bicarbonate; 0.092% Vit. E "20,000"; 0.147% TM-Vit.; 0.276% Rumensin 3.

Table 4.2: Chemical composition of experimental diets and ingredients.

TMR	%DM	%NDF	%ADF	%Crude Protein	%Ether Extract
C[1] TMR	50.0 ± 0.51[2]	39.6 ± 1.81	26.1 ± 1.12	17.2 ± 0.53	4.4 ± 0.33
G2 TMR	49.3 ± 0.79	40.9 ± 1.94	27.1 ± 1.60	16.6 ± 0.29	4.7 ± 0.27
G4 TMR	49.9 ± 0.59	39.7 ± 1.53	26.3 ± 1.11	16.7 ± 0.62	4.7 ± 0.55
Ingredients:					
Corn silage	35.7 ± 5.60	49.3 ± 0.71	34.7 ± 0.49	8.7 ± 0.16	-
Ground corn	89.8 ± 2.19	18.2 ± 0.40	5.8 ± 0.08	9.1 ± 2.40	4.1 ± 0.62
Alfalfa hay	89.4 ± 3.13	55.3 ± 0.17	42.6 ± 0.52	16.8 ± 0.10	-
Wet brewers grain	24.1 ± 2.21	74.5 ± 1.20	43.5 ± 0.90	28.4 ± 1.02	-
WCS	91.5 ± 1.10	46.0 ± 0.23	35.3 ± 2.24	26.8 ± 1.91	-
Concentrate mix[1]	91.0 ± 1.54	27.3 ± 0.86	16.7 ± 0.38	36.9 ± 0.32	-

[1]Treatments were: Control =C; G2 = control plus 200 g/h/d glycerol; G4 = control plus 400 g/h/d glycerol.

[2] (±) Standard deviation

Table 4.3: Performance and blood metabolites of lactating Holstein cows fed diets supplemented with increasing levels of dietary glycerol.

Item	C[1]	G2	G4	SE	P< Trt
DMI (kg/d)	24.2[a]	23.0[b]	23.0[b]	0.29	0.004
Milk (kg/d)	37.8[a]	37.2[a]	35.8[b]	0.47	0.01
Fat (%)	3.46[a]	3.31[b]	3.35[b]	0.03	0.001
Fat (kg/d)	1.31	1.23	1.20	-	-
Protein (%)	2.76[a]	2.75[a]	2.80[b]	0.005	0.001
Protein (kg/d)	1.04	1.02	1.00	-	-
ECM[2] (kg/d)	36.7[a]	35.2[ab]	34.1[b]	0.53	0.004
Efficiency 1 (ECM/DMI)	1.53	1.54	1.50	0.03	0.66
Efficiency 2 (MY/DMI)	1.58	1.63	1.58	0.03	0.35
		Blood Serum Metabolites			
Glucose, mg/dl	62.7	62.5	63.3	1.31	0.89
Urea N, mg/dl	21.7	21.8	20.5	0.41	0.10

[1]Treatments were: Control =C; G2 = control plus 200 g/h/d glycerol; G4 = control plus 400 g/h/d glycerol.

[2]Energy corrected milk = (0.3246 x kg milk) + (12.86 x kg fat) + (7.04 x kg protein) (Tyrell and Reid, 1964).

* Means with unlike subscripts in the same row are different (P<0.05)

Table 4.4 Comparison of ruminal pH and VFA concentrations of lactating Holstein cows fed diets supplemented with increasing levels of dietary glycerol.

Item	C[1]	G2	G4	SE	P< Trt
pH	6.07	6.03	6.08	0.04	0.62
Ammonia	12.07	11.72	9.77	0.86	0.14
----------% ----------					
Acetate	61.1[a]	59.1[b]	57.6[c]	0.39	0.0001
Propionate	23.3[a]	24.1[a]	25.4[b]	0.35	0.0002
Butyrate	11.4[a]	12.3[bc]	12.4[c]	0.15	0.0001
Iso-butyrate	1.25	1.22	1.23	0.06	0.93
Iso-valerate	1.47	1.53	1.5	0.04	0.64
Valerate	1.51[a]	1.71[bc]	1.84[c]	0.05	0.0001
Acetate: Propionate	2.65[a]	2.47[bc]	2.3[c]	0.05	0.0001

[1]Treatments were: Control =C; G2 = control plus 200 g/h/d glycerol; G4 = control plus 400 g/h/d glycerol.

* No interaction observed between collection time and treatment

* Means with unlike subscripts in the same row are different (P<0.05)

Table 4.5: Nutrient intake and apparent digestibility for lactating Holstein cows fed diets supplemented with increasing levels of dietary glycerol.

Item	C[1]	G2	G4	SE	P< Trt
Intake (kg/d)					
DM	21.1	19.8	19.9	0.83	0.47
CP	3.4	3.0	3.0	0.11	0.09
NDF	7.6	7.2	7.2	0.34	0.75
ADF	5.0	4.7	4.6	0.21	0.53
----------Apparent digestibility, %----------					
DM	67.44	66.1	65.31	2.26	0.80
CP	67.66	69.88	69.1	0.82	0.27
NDF	44.7	47.46	46.93	1.6	0.48
ADF	39.66	42.73	41.85	2.06	0.60

[1]Treatments were Control =C: G2 = control plus 200 g/h/d glycerol; G4 = control plus 400 g/h/d glycerol.

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CHAPTER 5

EFFECTS OF THE ADDITION OF LIVE BACTERIAL INOCULANTS AND GLYCEROL TO THE DIET OF LACTATING DAIRY COWS ON APPARENT EFFICIENCY AND MILK YIELD.[1,2]

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ABSTRACT:

A study was conducted to evaluate the effects of a live bacterial inoculant and dietary glycerol on milk yield, efficiency of yield, and nutrient digestibility during hot weather. Sixty Holstein cows averaging 120 days in milk (DIM) and 36.2 kg/d of milk were used. The study was conducted from June through September 2008. Cows were fed a common diet during the 2 wk standardization period and were then divided into 4 groups of 15 by parity, milk yield, energy-corrected milk, and stage of lactation and assigned randomly to 1 of 4 treatments for 10 wks. Experimental design was a randomized complete block with a 2x2 factorial treatment arrangement. Treatments included control (**C**), bacterial inoculant[1] (**B**), bacterial inoculant with 400g h/d glycerol (**BG**), and control with 400g h/d glycerol (**G**). Bacterial inoculant is composed of $4x10^9$ CFU/h/d of a combination of *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24*. Diets were based on corn and ryegrass silages and balanced to be iso-caloric and iso-nitrogenous. DMI was 23.0, 22.2, 23.3, and 22.8 kg/d for C, B, BG, and G, respectively. Milk yield was 32.5, 32.8, 34.5, and 32.4 kg/d for C, B, BG, and G, respectively. No effect was seen for milk fat percentage or ECM among diets. Milk protein percentage decreased ($P < 0.08$) for cows offered G (2.72 ± 0.02) compared with C (2.8 ± 0.02). No effect on respiratory rate, skin temperature, body temperature or concentration of serum glucose or urea N was observed. An increase in efficiency ($P<0.01$) defined as milk yield/DMI was noted with G (1.51 ± 0.02) and BG (1.54 ± 0.02) compared with C (1.42 ± 0.02). Also, improved efficiency ($P<0.06$) for B (1.5 ± 0.02) versus C (1.42 ± 0.02) was observed. The addition of bacterial inoculants alone and with

[1] Bovamine ® containing $4x10^9$ CFU/h/d of a combination of *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24*.

glycerol had a positive effect on apparent efficiency compared with C. Results suggest that the addition of bacterial inoculants, glycerol or a combination of both may improve yield efficiency for cows subject to heat stress.

Key Words: glycerol, bacterial inoculant, efficiency, *Lactobacillus acidophilus NP51, Propionibacterium freudenreichii NP24*

Abbreviation Key: **C**= Control; **B**= Bovamine ®; **G**= Control plus 400g glycerol g/h/d; **BG**= Bovamine® plus 400 g/h/d glycerol

INTRODUCTION:

Direct fed microbials (DFM) are defined as a source of live, naturally occurring microorganisms (Krehbiel et al., 2003). DFM are used in the dairy industry to improve cow performance, feed efficiency, and health (Yoon and Stern, 1995). The increasing demands by some of the public for antibiotic and hormone "free" production has increased the interest in direct fed microbials. These products can be marketed as a "natural" feed additive and according to Nocek and Kautz (2006), the inclusion of DFM in dairy cow diets has become a generally accepted practice.

A review of the literature (Krehbiel et al., 2003) indicated that supplementing with DFM generally resulted in a 2.5 to 5% increase in ADG and a 2% improvement in feed efficiency in feedlot cattle. Other researchers (Nocek et al., 2006; Weiss et al., 2008; West, 2009) conducting studies with lactating dairy cows reported improved milk yields and efficiency with the addition of bacterial inoculants to the diet.

Researchers have shown that various strains of *Propionibacterium* can increase the molar proportion of ruminal propionate (Kim et al., 2000; Stein et al., 2006). The effect of feeding *Propionibacterium* alone, or in combination with other bacteria to dairy cows has been evaluated but the results have been inconsistent. The primary bacterial organisms fed to ruminants are *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*. Feeding these organisms together may be beneficial, because *L. acidophilus* is a lactate producing bacteria and *P. freudenreichii* is a lactate utilizing bacteria that produces propionate, a glucose precursor as a product of fermentation. (Raeth-Knight, et al., 2007)

The mode of action for DFM is still being debated among researchers, though a variety of methods have been suggested. These include the modification of the rumen or lower gut microbial population, alteration of ruminal fermentation patterns, increased intestinal nutrient flow, improved diet digestibility, and improved immune function (Yoon and Stern, 1995; Krehbiel et al., 2003).

Another area of growing interest is the use of by-product glycerol in the diet. Glycerol is an odorless, colorless, hygroscopic, sweet tasting liquid that has the potential to replace corn in the diet and has a projected feed value of 100-120% of corn. The energy value of glycerol makes it an alternative to corn and glycerol is a supplement that is basically "pure energy" (Hippen et al., 2008). Several studies have reported that glycerol enhances rumen fermentation and improves feed efficiency (Hippen, 2008; Garton et al., 1961; Remond et al., 1993; Schroder and Sudekum 1999; Dirksen et al., 1985; Linke et al., 2004). Previously, glycerol has been a cost prohibitive addition to the diet because of its value in other fields, but with growing biodiesel production more crude glycerol is coming on the market. The increased supply is making the addition of glycerol to the ration a feasible choice for many producers. The objective of this study was to determine the effects of the addition of a live bacterial inoculant or dietary glycerol alone and in combination on DMI, milk yield, blood metabolites, and apparent efficiency of high yielding dairy cows.

MATERIALS AND METHODS:

The study was conducted during the summer of 2008 at the University of Georgia, Tifton Campus Dairy Research Center. Protocols for the trial were approved by the University of Georgia Institutional Animal Care and Use Committee.

Cows and Management:

Sixty early to mid-lactation primiparous and multiparous Holstein cows (15 per treatment) were used in the study. The study was 12 wk with a 2 wk standardization period followed by a 10 wk experimental period. Cows were housed in a free stall barn with access to individual free stalls and fed behind Calan doors (American Calan, Inc., Northwood, NH). Training for Calan door use was initiated in May, the 2 wk standardization period was at the end of June and the treatment period began in early July and continued through September. Cows averaged 120 ± 12 DIM, 35.8 ± 0.5 kg/d milk, 3.62 ± 0.23 % milk fat, 2.78 ± 0.08 % milk protein and 41.2 ± 1.2 ECM at the end of the standardization period. Cows were cooled using fans and high pressure misters.

Diets were balanced to be iso-caloric and iso-nitrogenous, and based on corn silage and ryegrass silage (Table 5.1). Diets were mixed and delivered once daily and ad libitum intakes were adjusted daily to achieve orts of 7-10%. Cows were milked twice daily at 0400 and 1500h. Prior to the treatment period a 2 wk standardization period was conducted during which all cows received the control ration. Baseline data was collected for all cows for use in covariate analysis of treatment period data, adjusting for individual animal variation. In addition, cows were ranked by average daily milk yield, stage of lactation, and ECM during the standardization period and blocked into groups of 4 by

rank, and assigned randomly to 1 of the 4 experimental diets within block. At this point, experimental diets were imposed for 10 wk.

Experimental Treatments and Design:

Dietary treatments consisted of a control **(C)**, control plus 400 g/d/h of glycerol **(G)**, control plus bacterial inoculant **(BG)**, and bacterial inoculant plus 400 g/d/h of glycerol (**BG**) [Table 5.2]. The bacterial inoculant was added according to manufacturer's instruction at a concentration of $4x10^9$ CFU/h/d of a combination of *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24* mixed with 1kg of ground corn daily and top dressed onto the TMR. All treatment groups received a top dressing of ground corn either containing the inoculant or as blank controls.

The experimental design for this continuous study was a randomized complete block with 4 dietary treatments. Experimental model contained cow, treatment, week, lactation, covariate, and two and three way interactions. Cow within treatment was considered a random variable and week was included as a repeated measure. Data was analyzed using the Proc MIXED procedure of SAS version 9.1 (SAS, 2004) including repeated measures. Significance of the treatments was determined using Tukey's when treatment means were different (SAS, 2004).

Data Collection:

Feed intake was recorded daily and adjustments were made as needed to maintain a 7-10% refusal rate. Milk yield was recorded twice daily by electronic meters (Alfa Laval Agric. Inc., Kansas City, MO) and summed daily. Milk samples were

collected from two consecutive milkings each week and analyzed for milk fat and protein percentage and somatic cell count by the Southeast Milk Incorporated Laboratory (Bell, FL). ECM was calculated using the following equation: ECM = (0.327 x kg milk) + (12.86 x kg fat) + (7.04 x kg protein) [Reid and Tyrell, 1964].

Body temperatures were recorded every 15 min for 4 d during standardization and during wk 6 and 10 of the treatment period using intra-vaginal probes (HOBO Water Temperature Pro, Onset Corp, Contoocook, NH) attached to blank CIDRs to obtain individual cow temperatures. Environmental temperature data collected by HOBO environmental monitors (HOBO Water Temperature Pro, Onset Corp, Contoocook, NH) mounted over the freestall area in the barn recorded the daily minimum and maximum ambient temperature throughout the study. Cows were weighed weekly using electronic scales, following the p. m. milking and prior to access to feed or water**.**

Blood samples from the coccygeal (tail) vein were collected once during the standardization and during wk 6 and 10 of the treatment period for analysis of serum glucose and urea N at the University of Georgia, Tifton Veterinary Diagnostic Lab (Tifton, GA).

Feed and ingredient samples were collected twice per wk. The DM content was determined by drying in a forced-air oven at 55ºC for 48 h. Samples were compiled by week and ground to pass through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 1mm screen for analysis of DM (AOAC, 1990), NDF, ADF, (Van Soest et al., 1991), crude protein, ether extract, and minerals (AOAC, 1990).

Nutrient Digestibility:

A digestion study was conducted concurrently with a 14 d period and consisted of a 10 d standardization period followed by a 4 d collection period. Forty-eight cows were dosed with 23 g (DM basis) of Cr_2O_3 / cow/d in the TMR. Daily feed and ort samples were collected during the 4 d collection period. Fecal grab samples were taken during the collection period at 12 hr intervals, with the collection time advancing by 3h each day (0300, 0600, 0900, 1200, 1500, 1800, 2100, and 2400 h). Fecal samples were frozen at -5ºC until being composited for the complete collection period by cow. Samples of feed, orts, and feces were dried at 55ºC and ground through a Wiley mill (1-mm screen) and stored for later chemical analysis.

Diets, orts, fecal samples, and ingredient samples were analyzed for DM (AOAC, 1990), NDF, ADF (Van Soest et al., 1991), crude protein (AOAC, 1990), and chromium. Chromium content of feed, orts, and feces was determined using an atomic absorption spectrophotometer (AAnalyst 100/300, Perkin Elmer, Bellefontaine, PA) after wet ashing (Ferret et al., 1999).

RESULTS AND DISCUSSION:

The chemical composition of the diets offered during the treatment period is presented in Table 5.2. The average environmental temperature was 25.6˚C with a low of 17.4˚C and a high of 35.1˚C for the study period. Average body temperature was 38.6˚C and was similar for all treatments for the weeks monitored. No differences were observed among treatments for DMI, milk yield, or milk composition (Table 5.3). This is consistent with previous studies that reported no effect on DMI with the inclusion of *P.*

freudenreichii and *L. acidophilus* in the diet (Raeth-Knight et al., 2007; Weiss et al., 2008; West et al., 2009).

A numerical reduction in milk fat percentage was observed with the inclusion of glycerol in the diet. This agrees with a previous study conducted at the University of Georgia that showed a reduction in milk fat as glycerol level increased in the diet (Boyd et al. 2009). Donkin and Doane (2007), added 99.5% purified glycerol to the diet in place of corn and observed no effect on milk yield or composition and in a transition cow study Chung et al., (2007) used a dried glycerol product (food grade, 65% glycerol) and reported no effect on subsequent milk yield or composition. Milk protein percentage (P = 0.08) decreased with the inclusion of glycerol but the supplementation of bacterial inoculants had no effect (Table 5.3). The combination of bacterial inoculant and glycerol (BG) yielded the numerically lowest milk protein percentage. ECM was not affected by treatment (Table 5.3).

Efficiency of yield defined as ECM/DMI was not different ($P = 0.37$) by treatment but a slight increase for the B, G, and BG diets compared to C was observed (Table 5.3). The lack of significant difference can be attributed to the relatively similar milk protein and fat percentages among treatments. However, efficiency defined as MY/DMI was different (Table 5.3). The efficiency for the C (1.38 ± 0.04) was lower (P = 0.04) than for G (1.48 ± 0.04). Also, C was lower (P = 0.06) than BG (1.55 ± 0.04) and numerically lower than B (1.44 ± 0.04). Improved milk yield in the absence of greater DMI suggests that rumen function was improved either through improved digestion, modified ruminal environmental conditions, or greater microbial protein yield.

Dietary glycerol can impact ruminal fermentation patterns. Linke et al., (2004) reported that including glycerol at 498g and 998g in the diet of mid-lactation Holstein and Brown Swiss cows had no effect on DMI, milk yield, or FCM. The authors did note an improvement in feed efficiency with the inclusion of glycerol, with milk: feed ratios of 1.46, 1.59, and 1.60 for the control, 498g of glycerol, and 998g of glycerol, respectively. This corresponds to the improvement in feed efficiency observed in the present study.

Previous work has reported that various strains of *Propionibacterium* affect ruminal fermentation by increasing the molar proportions of ruminal propionate (Kim et al., 2000; Stein et al., 2006). Francisco et al., (2002) reported that early lactation cows fed 17 g of *Propionibacterium* culture (strain identified as P169 and supplemented at approximately 6×10^{10} cfu/d in (Stein et al., 2006) consumed less DM and produced similar amounts of milk as the control group. A subsequent study by Stein et al. (2006) demonstrated that early lactation, multiparous cows offered 6×10^{10} or 6×10^{11} cfu/d of *Propionibacterium* strain P169 produced about 8% more FCM than the control cows, but no difference was observed among the primiparous cows.

Weiss et al. (2008) conducted a study to determine the effect of a direct-fed microbial agent, *Propionibacterium* strain P169 on rumen fermentation, milk yield, and health of periparturient and early lactation cows. *Propionibacterium* strain P169 was fed at a rate of 6×10^{11} cfu/d. The authors reported that dairy cows fed *Propionibacterium* strain 169 at 6×10^{11} cfu/d had similar milk yield and composition as the control cows. The calculated energy expenditures for maintenance, milk yield, and BW change were similar among treatments, but cows offered the microbial supplement had a lower DMI

resulting in a 4.4% increase in the efficiency of converting dietary DM to NE_L. They attributed this improvement to altered ruminal fermentation patterns.

No effects were observed for NE balance, BW change, or blood metabolites by treatment (Table 5.4). The results from the digestibility study demonstrated a significant effect on the apparent digestibility of DM, CP, ADF, and NDF (Table 5.5). Treatment G was higher compared to C for apparent DM digestibility ($P = 0.04$). The inclusion of Bovamine ® (B) in the diet increased ($P = 0.006$) apparent CP digestibility compared to C and a numerical improvement for BG compared with C. Apparent ADF digestibility was improved (P= 0.06) for BG versus B and for G versus to BG. Also, researchers observed apparent NDF digestibility was increased (P = 0.05) for B versus C and for BG versus B (P = 0.04).

Present results are in contrast to work by Raeth-Knight et al., (2007) who used mid-lactation cows to determine the effects on yield, nutrient digestibility, and rumen fermentation of *Lactobacillus acidophilus* and *Propionibacteria freudenreichii*. Supplementing midlactation dairy cows with these microbes had no effect on apparent nutrient digestibility or rumen fermentation. The P169 strain of *Propionibacterium* altered ruminal metabolism of the cannulated steers toward increased propionate production without having an effect on feed intake, duodenal flow, microbial nitrogen synthesis, or ruminal kinetics (Lehloenya et al., 2008).

However, Nocek et al. (2006) observed that when supplementing direct-fed microbial products, transition dairy cows produced more milk and consumed more DM during the pre and postpartum periods. However, treated cows experienced a lower, milk

fat percentage compared with non-supplemented cows. Ruminal digestion of forage DM was increased in cows supplemented with direct fed microbials.

CONCLUSIONS

Results of this study indicate that the inclusion of Bovamine®, a source of live bacterial inoculants *Lactobacillus acidophilus NP51* and *Propionibacterium freudenreichii NP24* in the ration of high production dairy cows does improve apparent efficiency defined as (MY/DMI) and apparent digestibility of dietary nutrients. Addition of dietary glycerol with Bovamine® had a positive impact on efficiency of (MY/DMI) and a numerical improvement in milk yield was observed compared with the other treatments. Dietary glycerol alone improved efficiency (MY/DMI) in comparison with the control, while maintaining a similar milk yield with reduced DMI. The effects of both glycerol and Bovamine® can be attributed to changes in ruminal fermentation and improved ruminal efficiency. In conclusion, the addition of live bacterial inoculants and dietary glycerol alone or in combination may be beneficial to improvement of performance and efficiency in high yielding dairy cows. Further research is needed to determine the ruminal fermentation effects of Bovamine® and dietary glycerol in the ruminant.

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Table 5.1: Experimental diets containing bacterial inoculant and glycerol expressed on a DM basis.

Item	C[1]	B	G	BG
	----------% of DM----------			
Corn silage	20.15	20.15	20.21	20.21
Alfalfa hay	15.52	15.52	15.57	15.57
Ryegrass silage	12.29	12.29	12.33	12.33
Ground corn	25.21	25.21	23.46	23.46
Wet brewers grain	13.07	13.07	13.12	13.12
Megalac	0.79	0.79	0.79	0.79
Concentrate mix[2]	12.97	12.97	13.01	13.01
Glycerol	0	0	1.51	1.51

[1]Control = C, Bacterial inoculant = B, Control plus 400 g/h/d glycerol =G, and Bacterial inoculant plus 400g/h/d glycerol =BG.

[2]Composition of concentrate mix (% of DM): 2.04% ProLak (H.J. Baker and Brother, Inc.) ; 6.06% soybean meal 48; 0.151% Ca 17%: P 21%; 0.182% Pot-Mag-sulfate; 0.454% K-minus; 0.151% limestone; 0.151% urea 45%N; 0.027% Availa-4; 0.154% MgO; 0.227% salt; 0.530% Na bicarbonate; 0.188% yeast; 0.121% trace mineral premix; 0.01% vitamin premix; 0.229% Rumensin 3.

Table 5.2: Average chemical composition of diets containing bacterial inoculant and glycerol.

	DM	NDF	ADF	CP	Ether extract
	----------(% of DM)----------				
C [1]	54.4 ± 1.48	45.7 ± 0.31	28.9 ± 0.37	17.5 ± 0.38	4.4 ± 1.41
B	53.0 ± 2.50	42.5 ± 0.68	26.4 ± 0.84	17.9 ± 1.24	4.7 ± 0.95
BG	53.4 ± 2.16	41.3 ± 0.44	27.4 ± 0.43	17.4 ± 1.95	5.1 ± 1.30
G	52.8 ± 1.20	41.8 ± 1.50	27.6 ± 0.31	18.2 ± 1.12	5.9 ± 1.00
Ingredients:					
Corn silage	35.7 ± 5.6	49.3 ± 0.71	34.7 ± 0.49	8.4 ± 0.13	N/A
Ryegrass silage	50.4 ± 1.93	54.9 ± 1.0	40.6 ± 0.64	16.8 ± 0.44	N/A
Ground corn	89.8 ± 2.19	16.6 ± 1.34	5.6 ± 0.37	9.2 ± 1.83	N/A
Alfalfa hay	89.4 ± 3.13	54.5 ± 0.16	42.6 ± 0.52	16.9 ± 0.02	N/A
Wet brewers grain	24.1 ± 2.21	73.7 ± 1.16	43.5 ± 0.90	28.2 ± 0.94	N/A
Concentrate mix	91.0 ± 1.54	26.6 ± 0.90	16.7 ± 0.38	37.5 ± 0.20	N/A

[1]Control = C, Bacterial inoculant = B, Control plus 400 g/h/d glycerol =G, and Bacterial inoculant ® plus 400g/h/d glycerol =BG.

Table 5.3: Performance of lactating Holstein cows fed diets supplemented with live bacterial inoculants and/ or dietary glycerol.

	Treatment					P<		
Item	**C[1]**	**B**	**G**	**BG**	**SE**	**Bacterial Inoculant**	**Glycerol**	**Interaction[2]**
DMI (kg/d)	24.3	21.8	22.9	23.4	0.93	0.41	0.94	0.19
Milk (kg/d)	32.8	32.3	31.1	35.0	2.51	0.64	0.85	0.67
Fat (%)	3.62	3.57	3.47	3.45	0.13	0.80	0.28	0.89
Fat (kg/d)	1.19	1.15	1.08	1.21	-	-	-	-
Protein (%)	2.80	2.78	2.77	2.68	0.04	0.18	0.08	0.43
Protein (kg/d)	0.92	0.90	0.86	0.94	-	-	-	-
ECM (kg/d)	31.5	31.6	30.6	32.8	1.21	0.37	0.92	0.37
Efficiency (ECM/DMI)	1.39	1.39	1.41	1.50	0.05	0.46	0.20	0.37
Efficiency (MY/DMI)	1.38^{b}	1.44^{ab}	1.48^{a}	1.55^{a}	0.03	0.10	0.01	0.86

[1]Control = C, Bacterial inoculant= B, Control plus 400 g/h/d glycerol =G, and Bacterial inoculant plus 400g/h/d glycerol =BG.

[2] Interactions between Bacterial inoculant and Glycerol

* Means with unlike subscripts in the same row are significantly different (P<0.05).

Table 5.4: Net energy balance of lactating Holstein cows fed diets supplemented with live bacterial inoculants and or dietary glycerol.

						P <		
Item	**C**	**B**	**G**	**BG**	**SE**	**Bacterial Inoculant**	**Glycerol**	**Interaction[4]**
NEBdy[1]	4.62	4.39	5.72	4.72	1.03	0.49	0.54	0.71
NE intake (Mcal/d)	38.3	38.6	40.3	38.8	1.22	0.38	0.60	0.46
NE milk[2] (Mcal/d)	11.2	10.7	11.4	11.2	0.57	0.59	0.62	0.76
BW change[3] (kg/wk)	0.92	1.05	0.80	1.03	0.14	0.61	0.20	0.74
Serum glucose (mg/dl)	58.4	61.0	60.9	57.8	1.70	0.90	0.82	0.10
Serum Urea N (mg/dl)	19.5	19.8	18.6	19.9	0.74	0.24	0.56	0.42

* Control = C, Bovamine ® = B, Control plus 400 g/h/d glycerol =G, and Bovamine ® plus 400g/h/d glycerol =BG.

[1] NE Balance per day= NE intake (Mcal/d)-NE maintenance (Mcal/d) ± NE of tissue change (Mcal/d)-NE of milk (Mcal/d)/7 (NRC, 2006)

[2] NE milk= Milk (kg/d) x [(0.0929 x milk fat %) + (0.0563 x milk protein %) + 0.192)] (NRC, 2006)

[3] BW change by week over the study period.

[4] Interaction between Bacterial Inoculant and Glycerol

Table 5.5: Apparent digestibility for lactating Holstein cows fed diets supplemented with live bacterial inoculants and or dietary glycerol.

	C[1]	B	G	BG	SE	Bacterial Inoculant	Glycerol	Interaction[2]
Intake, kg/d								
DM	20.5	22.8	22.7	23.8	1.04	0.11	0.14	0.57
CP	3.5	4.1	3.9	4.3	0.19	0.01	0.05	0.62
ADF	6.0	6.0	6.2	6.5	0.29	0.63	0.20	0.67
NDF	9.0	10.1	9.7	10.2	0.45	0.09	0.41	0.50
	---------- % ----------							
DM	67.3^{a}	70.3^{ab}	70.8^{b}	69.1^{ab}	0.91	0.47	0.19	0.01
CP	65.6^{a}	70.3^{b}	69.1^{b}	69.6^{b}	0.94	0.008	0.15	0.03
ADF	51.5^{ab}	53.5^{b}	56.6^{a}	49.2^{b}	1.4	0.06	0.78	0.002
NDF	56.8^{b}	61.4^{a}	61.2^{ab}	56.6^{b}	1.22	0.99	0.92	0.0005

[1]Control = C, Bovamine ® = B, Control plus 400 g/h/d glycerol =G, and Bovamine ® plus 400g/h/d glycerol =BG.

[2] Interaction between Bacterial inoculant and Glycerol

* Means with unlike subscripts in the same row are significantly different (P<0.05).

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CHAPTER 6

EFFECTS OF SUPPLEMENTING THE TRANSITION COW DIET WITH DIFFERENT LEVELS OF DIETARY GLYCEROL ON POSTPARTUM PERFORMANCE, EFFICIENCY, AND BLOOD METABOLITES.

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ABSTRACT

A study was conducted to determine the effects of dietary glycerol on dry matter intake (DMI), milk yield and components, blood metabolites, and apparent efficiency in the transition cow diet. Forty-eight transition cows (25 primiparous and 23 multiparous) were included. The study was conducted from February to October of 2008 at the University of Georgia, Tifton Campus, Dairy Research Center. The study was from 3 wks prepartum to 8 wk postpartum. The study design utilized a randomized complete block design with a 2x2 agreement of treatments. Treatments were: prepartum control and postpartum control (**CC**); prepartum control and postpartum 400g/h/d glycerol (**CG**); prepartum 200g/h/d glycerol and postpartum control (**GC**); prepartum 200g/h/d glycerol and postpartum 400g/h/d glycerol (**GG**). Cows were assigned to treatment by previous or predicted production and estimated calving date. Diets were corn silage based and balanced to be iso-caloric and iso-nitrogenous. Postpartum DMI ($P = 0.15$) was 16.1, 17.3, 18.7, and 16.7 kg/d (± 0.81) for CC, CG, GC, and GG respectively). Milk yield ($P = 0.59$) was 32.4, 34.4, 35.0, and 34.8(± 1.70 for CC, CG, GC, and GG respectively). Milk fat percentage ($P = 0.77$) was 3.5%, 3.4%, 3.5%, and 3.8% (± 0.27 for CC, CG, GC, and GG respectively). Milk protein percentage ($P = 0.08$) was 3.4%, 3.9%, 3.8%, and 4.0% (± 0.27 for CC, CG, GC, and GG respectively). No effect on serum glucose or blood urea N (average 66.1 and 15.2 mg/dl respectively) was observed. Non-esterified fatty acids and B-hydroxybutyrate concentrations were not affected by treatment (average 0.62 mEq/L and 8.0 mg/dl respectively). The use of glycerol in the diet resulted in a numerical improvement in milk yield, milk components, and energy-corrected milk compared with controls. The use of glycerol in the postpartum ration alone resulted in a positive effect

on efficiency. The use of dietary glycerol may be more beneficial to the transition cow when included in the postpartum diet than in the prepartum diet.

Key Words: glycerol, transition cow, apparent efficiency

Abbreviation Key: CC = Prepartum Control and Postpartum Control; **CG =** Prepartum Control and Postpartum 400g/h/d glycerol; **GC =**Prepartum 200g/h/d glycerol and Postpartum Control; **GG** = Prepartum 200g/h/d glycerol and Postpartum 400g/h/d glycerol

INTRODUCTION

The demand for glucose or other energy sources increases dramatically as the dairy cow transitions from gestation to lactation. If the energy needs are not met, cow health and milk yield may be comprised. During late gestation, nutritional demands from the fetus increase dramatically. Bertics et al., (1992) reported that DMI declines by 30% in the week before calving. Bell (1995) observed that increased nutrient requirements for milk synthesis postpartum are significantly higher than DMI can meet. Therefore minimizing the difference between DMI and nutrient demand during the transition period is essential because this is when the largest imbalance occurs (Grummer, 1995).

Increasing energy intake during the transition period can improve lactation performance, health, and reproductive efficiency in high producing dairy cows (Curtis et al., 1985; Grummer, 1995). One means of accomplishing this may be to include dietary glycerol in the ration. Glycerol is readily fermented in the rumen to propionate, a major precursor for gluconeogenesis in early lactation dairy cows (Reynolds et al., 2003), and increasing the ruminal synthesis of propionate may increase glucose supply, reduce ketosis, and provided increased energy for lactose synthesis. Increasing ruminal propionate can also increase energetic efficiency by reducing fermentation losses (Rogers and Davis, 1982) and by reduced heat increment (Orskov and Allen, 1966).

Glycerol is an odorless, colorless, hygroscopic, sweet tasting liquid that has the potential to replace corn in the diet and has a projected feed value of 100-120% of corn. The energy value of glycerol makes it an alternative to corn and is a supplement that is basically "pure energy" (Hippen et al., 2008). Linke et al., (2004) calculated the energy value of glycerol to be approximately 20 % greater than corn yielding an NE_L of about

0.47 Mcal/kg. Schroder and Sudekum (1999) calculated the energy density of glycerol to range between 0.41 to 0.47 Mcal/kg NE_L. Glycerol has been recognized for years as a way to alleviate the symptoms of ketosis when delivered as an oral drench (Leng, 1970; Johnson, 1955; Fisher, 1973). Several research studies reported that glycerol enhances rumen fermentation and improves feed efficiency (Hippen, 2008; Garton et al., 1961; Remond et al., 1993; Schroder and Sudekum 1999; Dirksen et al., 1985; Linke et al., 2004).

Glycerol has been a cost prohibitive addition to the diet because of its value in other fields, but with growing biodiesel production more crude glycerol is available. The yield of glycerol from biodiesel is approximately 1 unit of glycerol for each 10 units of biodiesel produced. The biodiesel industry has a projected national average production of over 2.2 billion gallons by the end of 2008, which would produce annually about 220 million gallons of by product glycerol of 80% purity. (Feedstuffs, 2007) Greater supply is making the addition of glycerol to the ration a feasible choice for many producers. The objective of this study was to determine the effects of prepartum and postpartum transition cow diets with different levels of glycerol on DMI, milk yield and components, blood metabolites, and efficiency.

MATERIALS AND METHODS

The study was conducted from January to October of 2008 at the University of Georgia, Tifton Campus Dairy Research Center. Protocols for the trial were approved by the University of Georgia Institutional Animal Care and Use Committee.

Cows and management:

Forty-eight transitional Holstein cows (12 per treatment) were used in a complete randomized complete block design. The study utilized 25 primiparous and 23 multiparous cows. Training for Calan doors (American Calan, Inc., Northwood, NH) was initiated at least 2 mo prior to calving. After cows were trained to use Calan doors, they were turned into a dry lot until 4 wk before their due date. At 4 wk prior to calving cows were brought back into the barn and reintroduced to Calan gates for the beginning of the study. Feed intake data was recorded from 3 wk prior to calving to 8 wk post calving. All calving cows had access to a dry lot, after calving cows were maintained in a freestall barn with access to individual free stalls. Dry cows and fresh cows were maintained in separate pens during the study. Cows were cooled using fans and high pressure misters.

Diets were mixed and delivered once daily and fed behind electronic Calan doors, allowing individual intake to be determined. Diets were balanced to be iso-caloric and iso-nitrogenous (Table 6.1 and 6.2). After calving cows were milked twice daily at 0400 and 1500h.

Multiparous cows were blocked by previous 305 d milk yield, due date and randomly assigned to a treatment. Primiparous cows were randomly assigned to treatments based on calving dates. Treatments were equally divided between primiparous and multiparous cows with the exclusion of the GC group, which had 7 primiparous animals.

Experimental treatments and design:

The experimental design for this study was a randomized complete block design with a 2x2 arrangement of treatments. Treatments consisted of a prepartum control and postpartum control (**CC**), prepartum control and postpartum 400 g/h/d of glycerol (**CG**), prepartum 200 g/h/d glycerol and postpartum control (**GC**), and prepartum 200 g/h/d glycerol and postpartum 400 g/h/d glycerol (**GG**) [Table 6.1 and Table 6.2]. Glycerol was mixed with whole cottonseed as a carrier in the TMR using a Super Data Ranger (American Calan, Inc., Northwood, NH). The glycerol used on in this study was contained 80-85% glycerol, 14 % moisture, 7 % sodium chloride, and 18 ppm methanol (ADM Nutrition Alliance, Quincy, IL). Experimental model for statistical analysis contained cow, treatment, lactation, week, contrasts, and two and three way interactions. Contrasts were: 1. CC versus GC + CG + GG; 2. CC + CG versus GC + GG; 3. CC + GC versus CG + GG. Data was analyzed using the Proc MIXED procedure of SAS version 9.1 (SAS, 2004). When treatment was significant difference of means was determined using Tukey's (SAS, 2004).

Data collection:

Amount of feed offered and refused were recorded daily and adjustments made as needed to maintain a 7-10% refusal rate. Milk yield was recorded twice daily by electronic meters (Alfa Laval Agric. Inc., Kansas City, MO) and summed daily. Milk samples were collected from two consecutive milkings each week and analyzed for milk fat and protein percentage, and somatic cell count by the Florida Dairy Farmers laboratory (Bell, FL). Energy-corrected milk yield (ECM) was calculated using the

following equation, which is defined as: ECM (kg/d)= (0.327 x kg milk) + (12.86 x kg fat) + (7.04 x kg protein) [Tyrell and Reid, 1964]. Cows were weighed weekly using electronic scales, following the p. m. milking and prior to access to feed or water**.**

Blood samples were collected from the coccygeal (tail) vein 1 wk prior to calving, calving, 2 wk, 4 wk, 6 wk, and 8 wk post calving for analysis of serum glucose, urea N, (University of Georgia, Tifton Veterinary Diagnostic Lab, Tifton, GA), serum calcium, non-esterified fatty acids and B-hydroxybutyrate (Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI).

Feed and ingredient samples were collected twice per week. The DM content was determined by drying in a forced-air oven at 55ºC for 48 h. Samples were compiled by week and ground to pass through a 1mm screen on a Wiley mill (Arthur H. Thomas, Philadelphia, PA) for analysis of DM, NDF, ADF, (Van Soest et al., 1991) CP (Kjeltec 2200, Seattle, WA), ether extract, and minerals (AOAC, 1990).

RESULTS AND DISCUSSION

The chemical composition of pre- and postpartum diets and ingredients is shown in Table 6.3. No significant effect on prepartum intake was observed (Table 6.4), but a numerical trend for lower intake with the addition of glycerol was observed. Postpartum DMI was numerically higher ($P = 0.15$) for CG and GC compared with CC and GG. An expected difference in intake was noted by lactation with multiparous cows consuming an average of 3.5 kg/d more DM than primiparous cows.

Results are similar to those of DeFrain et al. (2004) who offered diets top dressed with corn starch or glycerol to transition cows. The diets were offered from 21d

prepartum until 21d postpartum. Prepartum DMI was greater for control cows compared with cows offered the glycerol treatment but no significant treatment effects were observed for postpartum DMI.

Milk yield (Table 6.4) did not differ by treatment but was numerically higher for CG, GC, and GG diets compared with CC. Milk fat percentage was similar among treatments. Milk protein percentage increased ($P = 0.08$) with inclusion of dietary glycerol for GG, GC, and CG compared with CC. A positive contrast ($P = 0.01$) was observed for the inclusion of glycerol in diet. Energy-corrected milk showed a numerical but not significant effect by treatment for CC, CG, GC, and GG respectively.

Donkin and Doane (2007) reported similar results using glycerol to replace corn and corn gluten at up to 15% of DM in the diet. DMI decreased for the 15% glycerol during the first 7 d of the study but recovered thereafter. Donkin and Doane (2007) observed that milk yield and composition was not affected by glycerol. Cows offered the 15% glycerol gained more BW after wk 8 compared with other treatments. They concluded that glycerol could be included at up to 15% of diet DM in lactating cow diets without negative effects.

No effect on apparent efficiency (ECM/DMI) was observed for treatments (Table 6.4). However a trend ($P = 0.15$) for lower efficiency was for the GC cows compared to CC, CG, and GG, suggesting that glycerol in the prepartum ration alone may reduce efficiency. It may be advantageous to use glycerol in both pre and postpartum diets or in the postpartum diet only. A similar pattern was noted for efficiency defined as MY/DMI for GC compared with CC, CG, and GG.

Serum glucose (Figure 6.1) was not affected by treatment (Table 6.5) but a trend for higher serum glucose concentration prepartum with GC was observed. Figure 6.2 shows the serum glucose response for primiparous cows only, cows offered glycerol prepartum had a lower initial glucose level but peaked at calving similar to the control. At the 2 wk post sample the CG, GC, and GG cows maintained a higher level of serum glucose compared with CC cows. Figure 6.3 shows the serum glucose response for multiparous cows, which is similar to the primiparous cows (Figure 6.2). Treatment GC had a higher concentration ($P = 0.23$) of serum glucose at calving and trended above the other treatment postpartum. Multiparous GG cows had the lowest serum glucose levels both pre- and postpartum ($P = 0.06$). Serum urea N (Figure 6.4) was not affected by treatment, ($P = 0.17$) but a trend for higher levels was seen at wk 5 through wk 8 for the GC diet. *B*-hydroxybutyrate (BHB) levels ($P = 0.39$) were not altered by diet (Table 6.5; Figure 6.5). All treatment groups had BHB levels below the level associated with risk of subclinical ketosis. The CC and GC treatments maintained the lowest concentrations. Non-esterified fatty acids (NEFA) were not affected by treatment (Table 6.5; Figure 6.6; Figure 6.7; Figure 6.8). NEFA levels above 0.40 mEq/L were noted across all treatments. Figure 6.7 and Figure 6.8 show NEFA levels for primiparous and multiparous cows respectively, for both groups GG had the lowest NEFA levels at 1 wk pre calving. Multiparous cows on CC had a ($P < 0.0001$) higher spike at calving and maintained a higher NEFA concentration into wk 2 compared to the other treatments.

No effect by treatment on NEB or BW was observed (Table 6.6). Figure 6.9 shows change by week. There was a tendency for improved weight gain at wk 6 to 7 with the inclusion of glycerol in the postpartum diet. This increase in weight gain agrees with

work by Donkin and Doane (2007), who noted that cows supplemented with glycerol gained more weight after wk 8 compared with other treatment groups. Net energy intake for CC (28.6), CG (29.3), GC (32.0), and GG (28.8) were similar by treatment. Net energy content of milk was not different for CC (11.0), CG (10.8), GC (11.4), and GG (12.2), though a significant difference was recorded between primiparous and multiparous cows.

Chung et al. (2007) conducted a transition cow study using a dry glycerol product (food grade, 65% glycerol). The study was conducted from calving to 21 DIM using 39 multiparous Holstein cows. Feed intake, milk yield and composition, and serum insulin concentrations were not affected by treatment. The glycerol supplemented cows demonstrated an improved energy status during the wk 2 of lactation, indicated by greater concentrations of plasma glucose, lower concentrations of plasma BHB, and lower concentrations of urine ketones. No differences in DMI or milk yield during the first 3 wk of lactation occurred. Chung et al. (2007) observed a tendency toward greater milk yield for glycerol supplemented cows during wk 6 of lactation after the supplementation period had ended, suggesting a possible latent benefit of glycerol on energy status and subsequent milk yield.

DeFrain et al. (2004) proposed that the inclusion of glycerol in the diet would eliminate the need to restrain cows for drenching and still deliver a glucogenic substrate, reducing fatty liver complex and improving lactational performance. They used 30 Holstein cows from 14 d prepartum to 21 d postpartum. Treatments included a control, low glycerol (0.43 kg/d dry basis), and a high glycerol (0.86 kg/d dry basis). The prepartum DMI was higher for cows fed the control diet compared with the low glycerol

or high glycerol diets (13.3, 10.8, and 11.3 ± 0.5 kg/d respectively). The prepartum plasma glucose, insulin, BHB, NEFA, and ruminal profiles were not affected by treatment. However, rumen fluid collected postpartum from cows fed the high or low glycerol diets showed had total VFA, greater molar proportions of propionate and decreased acetate to propionate ratio. Also, molar proportions of butyrate were higher in the high and low glycerol treatments compared with the control diet. The postpartum plasma glucose concentration was greatest in cows offered the control diet compared with the low glycerol or high glycerol treatments. No affect was observed on DMI, BW, plasma NEFA, or liver lipids during the first 21 d postpartum among treatments. The yield of ECM during the first 70 d postpartum tended to be the greatest for cows offered the control diet. DeFrain et al. (2004) attributed the tendency for greater ECM yield for the control diet to be largely due to the lower milk fat yield for the low and high glycerol diets compared with control. The inclusion of glycerol in the diet tended to decrease milk fat percentage and MUN and decreased the ruminal acetate to propionate ratio. It is likely that glycerol underwent ruminal fermentation to propionate similar to a fermentable carbohydrate source. Schroder and Sudekum (1999) suggested that glycerol with different purity levels may be used to replace rapidly fermentable starches in ruminant diets at up to 10% of the diet dry matter. The results reported by DeFrain et al. (2004) are in agreement with work by Schroder and Sudekum (1999) and Khalili et al. (1997), where the ruminal acetate to propionate ratio decreased when feeding glycerol at 1.1 and 0.216 kg/d, respectively.

Schroder and Sudekum (1999) used ruminally cannulated steers to determine the ruminal effects of feeding glycerol. They observed that feeding glycerol did not affect

diet digestibility, but did decrease the acetate: propionate ratio, increase ruminal butyrate concentrations, and stimulated increased water intake. They concluded that these changes would benefit dairy cow by leading to an increase in ruminal propionate, increasing supply of gluconeogenic substrate to the liver. Also, increased ruminal butyrate may support improved growth of ruminal epithelial tissue and increase nutrient absorption from the rumen (Dirksen et al. 1985) and increased water intake would increase supply of water available to the mammary gland.

CONCLUSIONS:

Results of this study indicate that the inclusion of dietary glycerol may be of benefit to the transition cow by improving milk yield and components and ECM compared to the control treatment. The use of glycerol in the postpartum ration alone showed improved efficiency compared with the use of glycerol only in the prepartum diet. Further research is needed to determine the most beneficial time in the transition period and at what level to add glycerol to the ration.

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Table 6.1: Experimental prepartum dietary glycerol treatments shown on a DM basis.

Ingredient	C	G2
Corn silage	32.7	32.2
Alfalfa hay	11.6	11.4
Bermuda hay	15.4	15.2
Whole cottonseed	1.96	1.93
Ground corn	0	0
Brewers' grain	10.4	10.2
Pre-concentrate mix[1]	27.94	27.4
Glycerol	0	1.67

Control= **C**; Control + 200g/d/cow glycerol = **G2**.

[1]Pre-concentrate mix was composed of: 36.3 % soybean hulls; 26.9 % ground corn; 1.08 % limestone; 2.69 % Availa-4; 0.27 % Magnesium oxide; 0.67 % Rumensin 3; 0.54 % TM-Vit; 1.35 % Vit. E "20,000"; 30.2 % Bichlor.

Table 6.2: Experimental postpartum dietary glycerol treatments shown on a DM basis.

Ingredient	C	G4
Corn silage	38.39	38.52
Alfalfa hay	10.55	10.57
Whole cottonseed	1.81	1.82
Ground corn	23.94	22.12
Wet brewers grain	12.09	12.13
Post-concentrate mix[1]	13.22	13.27
Glycerol	0.0	1.57

Control = **C**; Control + 400g/d/cow glycerol = **G4**.

[1] Post-concentrate mix was composed of: 2.78% ProLak (H.J. Baker and Brother, Inc.) ; 6.47% soybean meal 48; 0.92% Megalac; 0.184% Pot-Mag-sulfate; 0.737% molasses black; 0.645% limestone; 0.369% DCAD plus (Arm & Hammer Animal Nutrition); 0.037% Availa-4; 0.184% MgO; 0.240% salt; 0.645% Na bicarbonate; 0.092% Vit.E "20,000"; 0.147% Zealand TM-Vit.; 0.276% Rumensin 3.

Table 6.3: The chemical composition of experimental diets and ingredients.

	DM	NDF	ADF	CP	EE
	---------- % of DM----------				
Experimental Diets					
[1]Pre-C	51.5 ± 5.2	47.1 ± 3.6	31.9 ± 3.1	16.6 ± 0.98	3.5 ± 0.45
G2	50.4 ± 5.2	45.7 ± 3.9	30.8 ± 3.4	16.4 ± 1.12	3.5 ± 0.57
Post-C	46.9 ± 4.3	38.9 ± 4.6	25.3 ± 3.7	17.1 ± 1.75	3.8 ± 0.69
G4	46.4 ± 3.9	37.7 ± 2.9	25.6 ± 4.8	17.1 ± 2.6	3.7 ± 1.06
Ingredients:					
Corn silage	35.7 ± 5.6	42.4 ± 3.6	28.1 ± 5.5	8.8 ± 2.8	N/A
Ground corn	89.8 ± 2.2	12.8 ± 2.6	4.6 ± 2.3	11.3 ± 6.6	4.1 ± 0.62
Alfalfa hay	89.4 ± 3.1	54.5 ± 6.5	43.3 ± 6.3	14.8 ± 6.6	N/A
Bermuda hay	88.7 ± 2.5	74.0 ± 9.4	47.9 ± 5.7	13.8 ± 4.1	N/A
Wet brewers grain	24.1 ± 2.2	64.0 ± 4.2	35.8 ± 5.8	25 ± 6.2	N/A
WCS	91.5 ± 1.6	38.6 ± 7.01	24.3 ± 5.3	16.4 ± 4.1	N/A
Pre-concentrate mix[2]	88.9 ± 3.4	34.2 ± 8.2	22.3 ± 7.2	22.2 ± 2.4	N/A
Post-concentrate mix[3]	91.0 ± 1.6	21.5 ± 4.04	12.5 ± 2.9	37.91 ± 2.5	N/A

[1] **Pre-C**= Prepartum Control; **G2=** Prepartum-Glycerol (200g/h/d); Postpartum **C**= Postpartum Control; **G4=** Postpartum Glycerol (400g/h/d)

[2] Pre-concentrate mix: See Table 6.1

[3] Post-concentrate mix: See Table 6.2

Table 6.4: Performance of transition Holstein cows fed diets supplemented with different levels of dietary glycerol.

	Treatment					P<		
Item	**CC**	**CG**	**GC**	**GG**	**SE**	**Trt**	**Lactation**	**Trt*Lactation**
Pre-DMI (kg/d)	7.96	7.83	7.61	6.47	0.73	0.45	0.81	0.84
Post-DMI (kg/d)	16.1	17.3	18.7	16.7	0.81	0.15	0.0004	0.57
Milk (kg/d)	32.4	34.4	35.0	34.8	1.70	0.59	0.002	0.60
Fat (%)	3.5	3.4	3.5	3.8	0.27	0.77	0.58	0.64
Fat (kg/d)	1.13	1.17	1.23	1.32	-	-	-	-
Protein (%)	3.4	3.9	3.8	4.0	0.18	0.08	0.09	0.20
Protein (kg/d)	1.10	1.34	1.33	1.39	-	-	-	-
ECM[1] (kg/d)	34.2	34.5	35.9	36.3	1.62	0.75	0.0001	0.87
Efficiency (ECM/DMI)	2.11	2.09	1.98	2.25	0.08	0.15	0.11	0.61
Efficiency (MY/DMI)	2.14	2.02	1.92	2.09	0.08	0.28	0.13	0.19

CC= Pre-calving-Control/ Post-calving-Control, **CG**= Pre-Control/Post-Glycerol (400g/h/d), **GC**= Pre-Glycerol (200g/h/d)/ Post-Control, and **GG**= Pre-Glycerol (200g/h/d)/Post-Glycerol (400g/h/d)

[1]Energy corrected milk = (0.3246 x kg milk) + (12.86 x kg fat) + (7.04 x kg protein) [Tyrell and Reid, 1965].

Table 6.5: Comparison of blood metabolite of transition Holstein cows fed diets supplemented with different levels of dietary glycerol.

	Treatment					P<		
	CC	**CG**	**GC**	**GG**	**SE**	**Trt**	**Lactation**	**Trt*Lact.**
Serum glucose (mg/dl)	68.5	63.9	70.4	61.5	3.4	0.23	0.06	0.50
Serum urea N (mg/dl)	14.3	15.6	16.2	14.7	0.65	0.17	0.80	0.51
Serum Ca (mg/dl)								
NEFA[1] (mEq/l)	0.61	0.66	0.63	0.57	0.05	0.67	<0.0001	0.84
Primaparous	0.48	0.59	0.52	0.43				
Multiparous	0.74	0.73	0.76	0.71				
B-hydroxybutyrate (mg/dl)	6.55	8.84	7.65	8.87	1.10	0.39	0.01	0.83
Primaparous	5.63	7.31	6.63	6.65				
Multiparous	7.46	10.37	8.66	11.1				

CC= Pre-Control/ Post-Control, **CG**= Pre-Control/Post-Glycerol (400g/h/d), **GC**= Pre-Glycerol (200g/h/d)/ Post- Control, and **GG**= Pre-Glycerol (200g/h/d)/Post-Glycerol (400g/h/d)

[1] NEFA= serum non-esterified fatty acids

Table 6.6: NE balance of transition Holstein cows fed diets supplemented with different levels of dietary glycerol.

	Treatment					*P* <		
Item	**CC**	**CG**	**GC**	**GG**	**SE**	**Trt**	**Lactation**	**Trt* Lactation**
NEBdy[1]	13.1	15.1	13.2	12.8	1.5	0.66	0.002	0.61
NE intake (Mcal/d)	28.6	29.3	32.0	28.8	1.22	0.22	0.001	0.82
NE milk[2] (Mcal/d)	11.0	10.8	11.4	12.2	0.99	0.75	0.09	0.89
BW change[3] (kg)	1.91	2.16	1.80	1.90	0.28	0.80	0.004	0.85

CC= Pre-Control/ Post-Control, **CG**= Pre-Control/Post-Glycerol (400g/h/d), **GC**= Pre-Glycerol (200g/h/d)/ Post-Control, and **GG**= Pre-Glycerol (200g/h/d)/Post-Glycerol (400g/h/d)

[1] NE Balance per wk/7= NE intake (Mcal/d)-NE maintenance (Mcal/d) ± NE of tissue change (Mcal/d)-NE of milk (Mcal/d)

[2] NE milk= Milk (kg/d) x [(0.0929 x milk fat %) + (0.0563 x milk protein %) + 0.192)] (NRC 2006)

[3] Weekly change in body weights over the study period

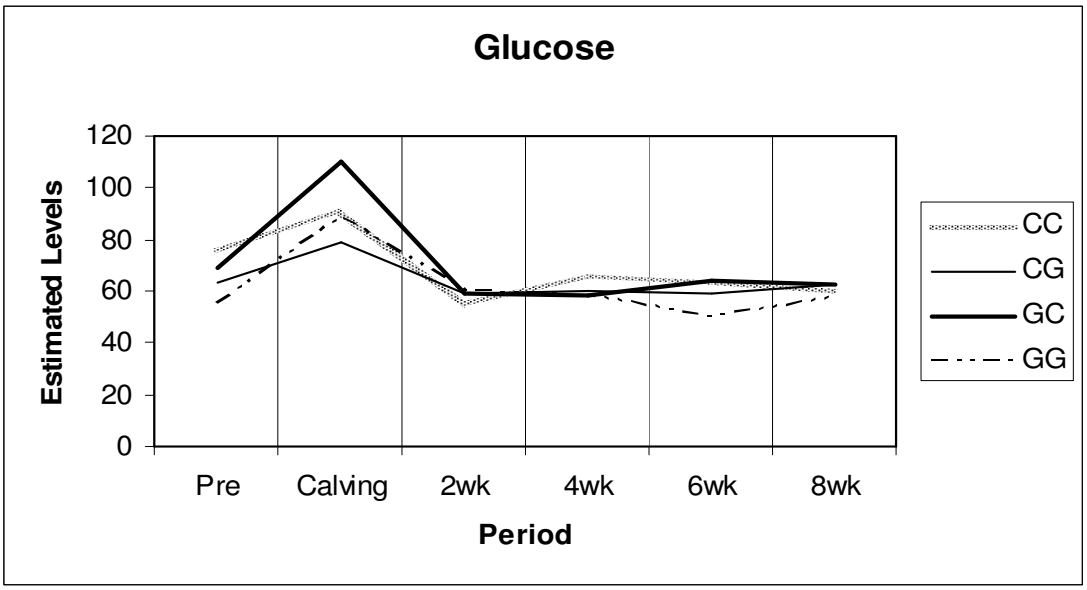

Figure 6.1: Serum glucose levels for transition cows supplemented with dietary glycerol.

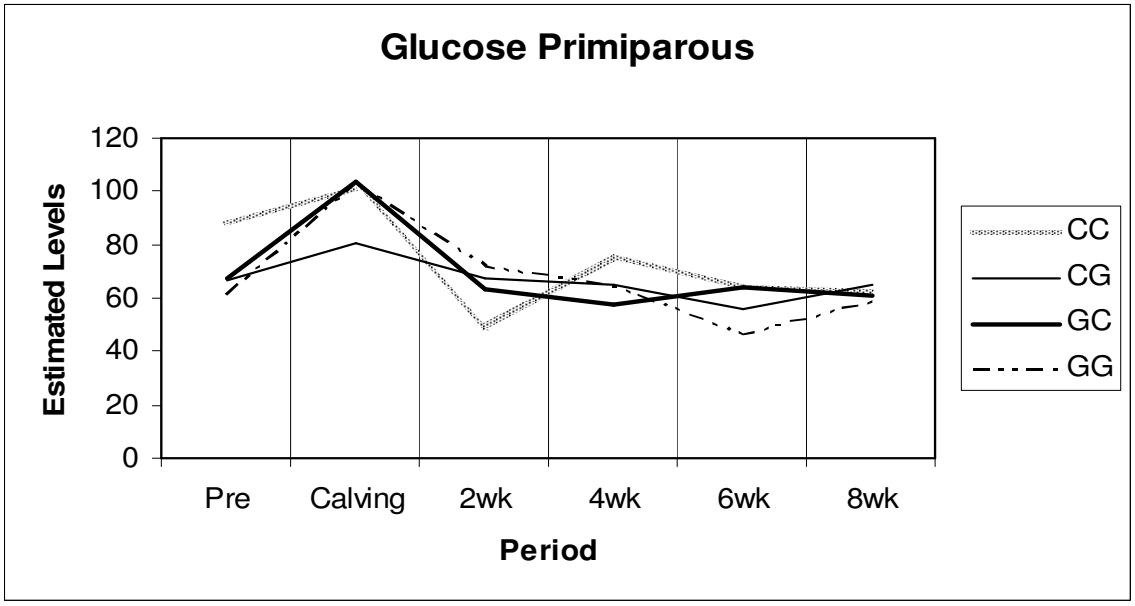

Figure 6.2: Serum glucose levels for primiparous transition cows supplemented with dietary glycerol

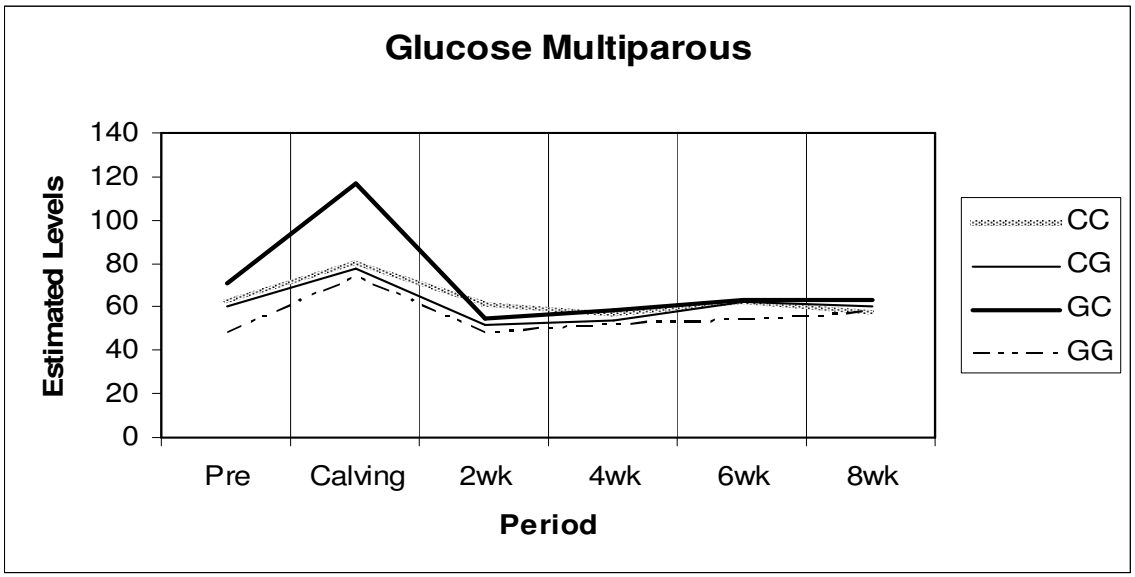

Figure 6.3: Serum glucose levels for multiparous transition cows supplemented with dietary glycerol

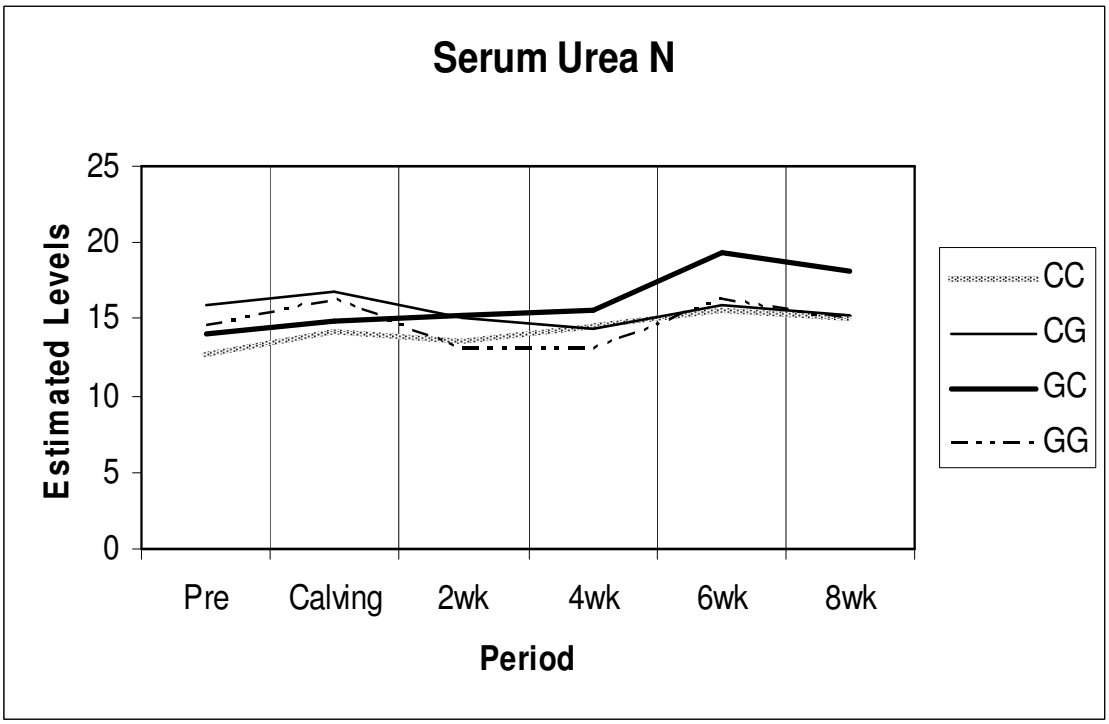

Figure 6.4: Serum urea N levels for transition cows supplemented with dietary glycerol

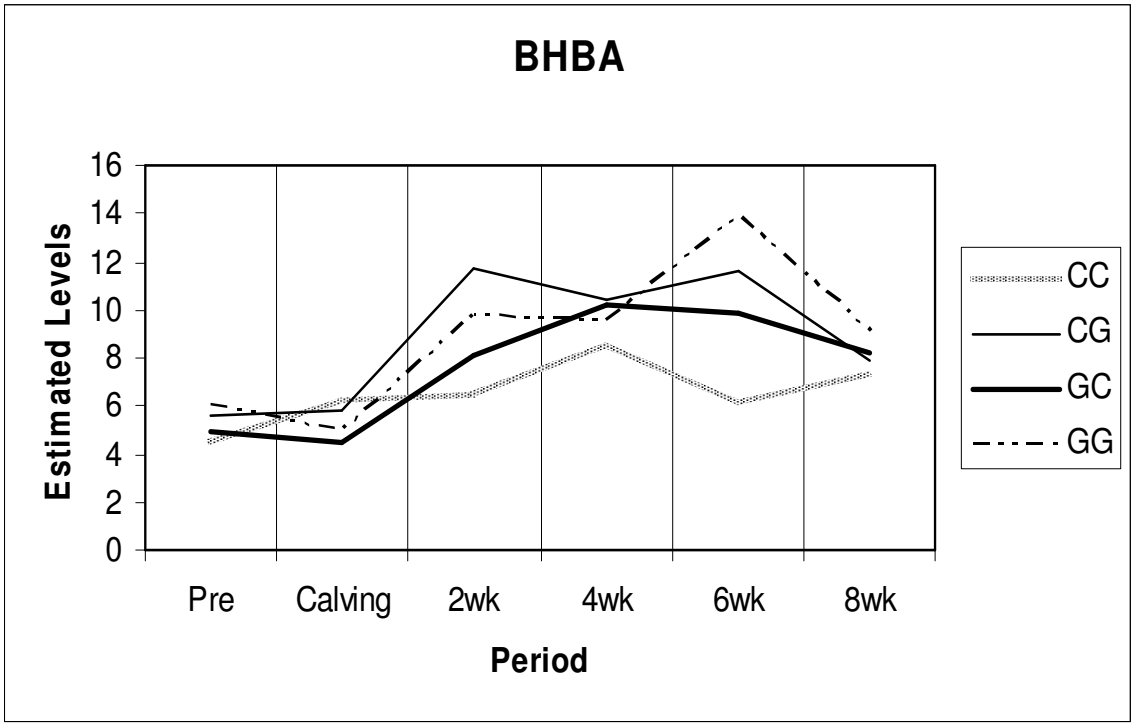

Figure 6.5: *B*-hydroxybutyrate levels for transition cows supplemented with dietary glycerol

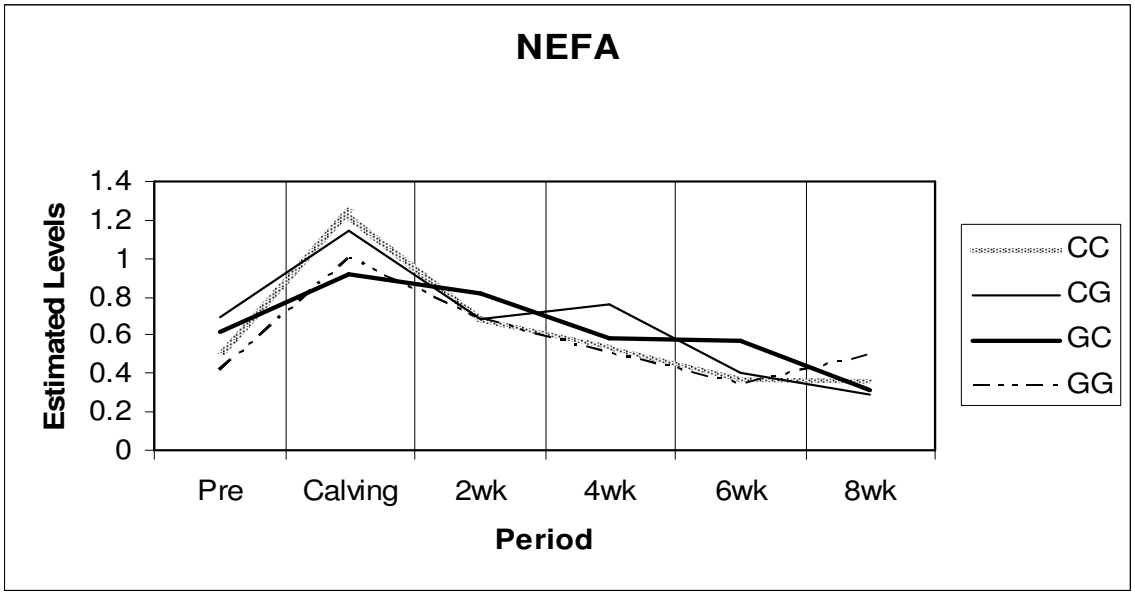

Figure 6.6: Non-esterified fatty acid levels for transition cows supplemented with dietary glycerol

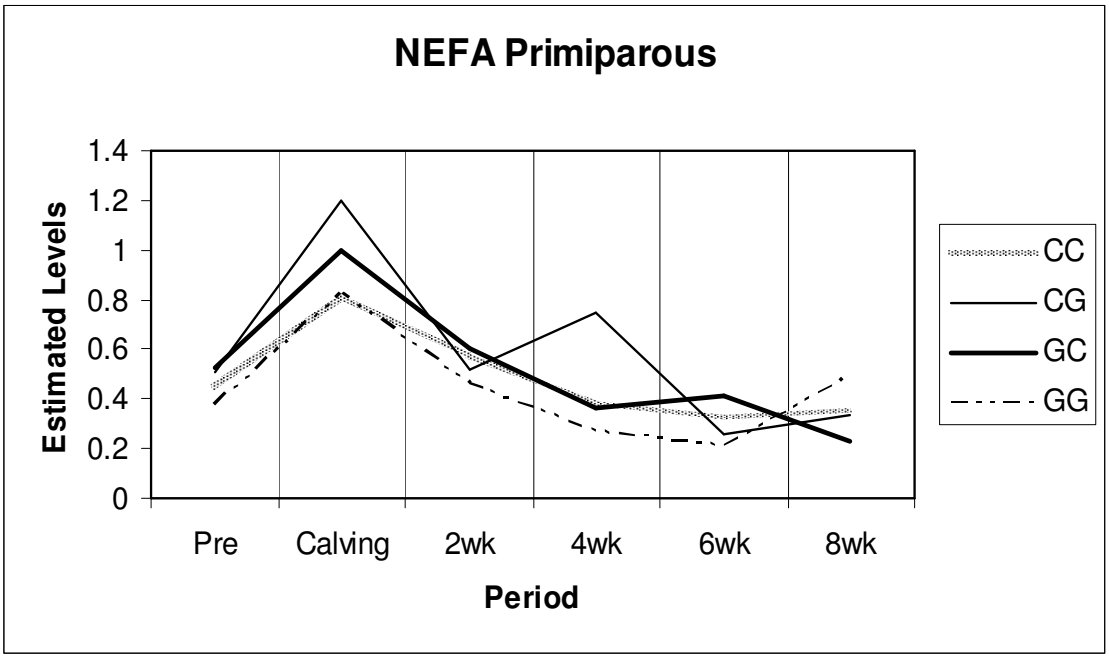

Figure 6.7: Non-esterified fatty acid levels for primiparous transition cows supplemented with dietary glycerol

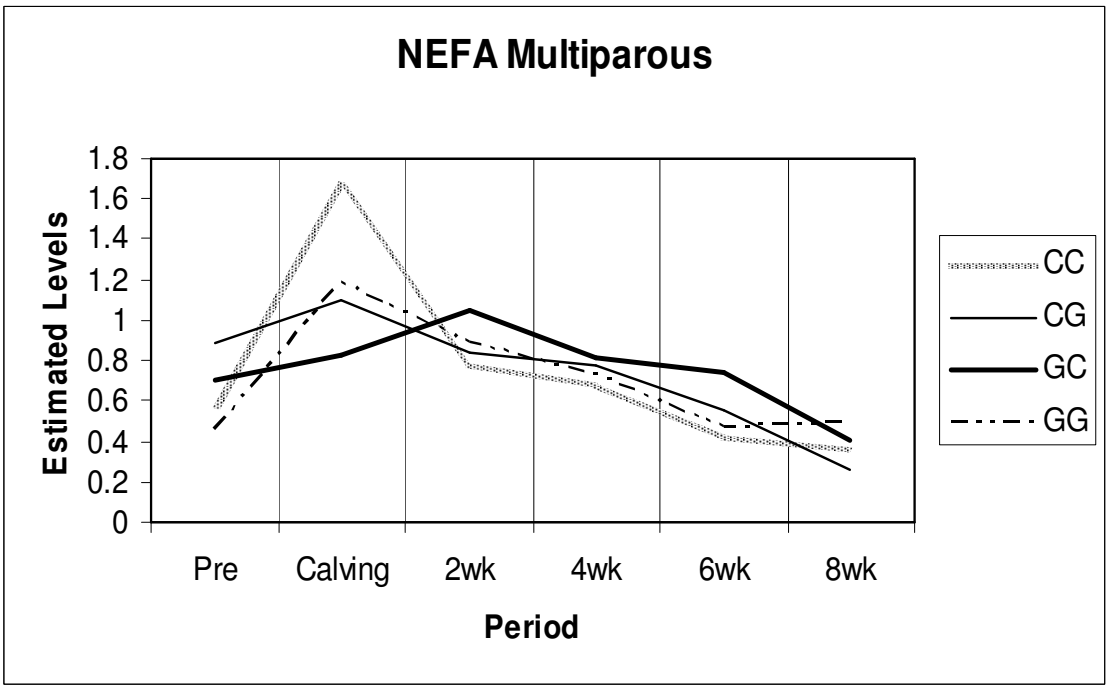

Figure 6.8: Non-esterified fatty acid levels for multiparous transition cows supplemented with dietary glycerol

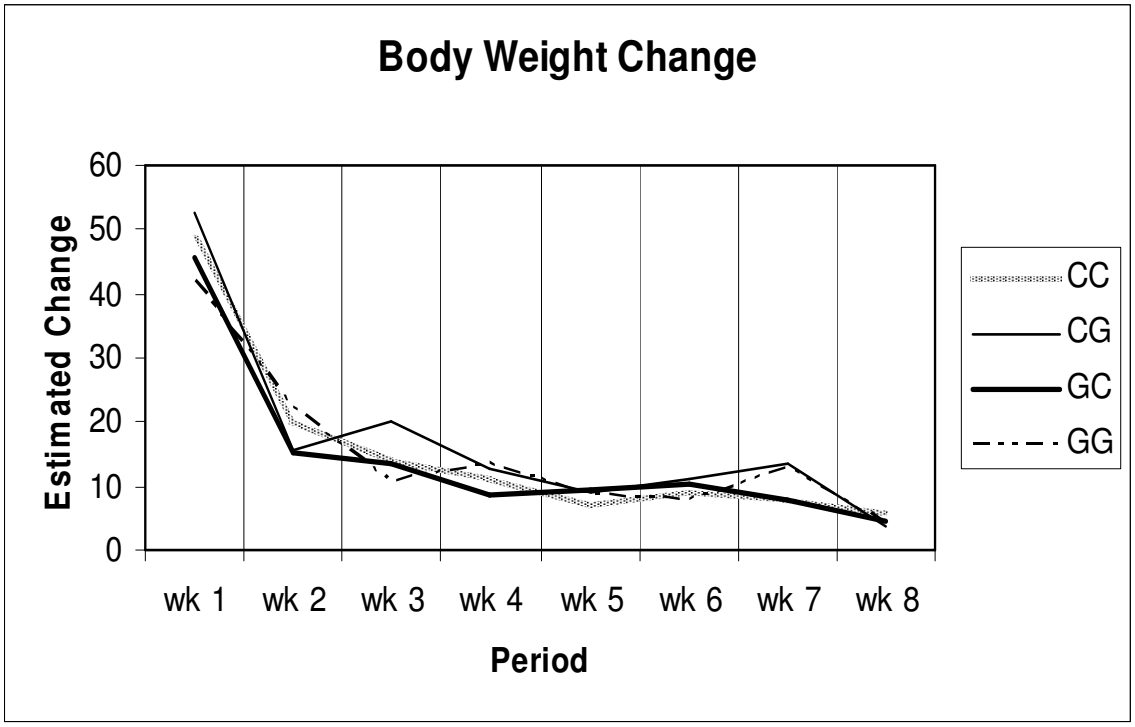

Figure 6.9: Body weight change by week with a rolling weekly average for transition cows supplemented with dietary glycerol.

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CHAPTER 7

CONCLUSIONS:

The changing economy and the shift in public opinion over the past decade has created the need for new and modified approaches to dairy production in the United States and internationally. The need to find the most efficient and economical way to meet the world's milk demand and to satisfy the public demand for "naturally" produced products is the challenge facing the dairy industry today. The search for natural supplements to replace products like rBST and maintain production is one faced by researchers, nutritionists, and feed companies alike. Another factor facing the dairy industry is rising feed costs, in particular corn and soybeans due to the growth in the ethanol industry and the rising cost of fertilizers required for crop production. Producers are looking for alternatives to expensive corn and energy ingredients in the diet, while still maintaining production levels.

Glycerol has commonly been used on the farm for years as a treatment for ketosis, but the high price tag has made the common use of glycerol in the diet unfeasible. The recent growth of the ethanol industry has altered the situation making by-product glycerol more affordable to the livestock producer. The challenge now lies in finding the best way to utilize glycerol in the ration. The results of the studies discussed earlier show the inclusion of glycerol can improve efficiency of production by improving the (MY/DMI) ratio in both early and mid-lactation cows. Also, glycerol was shown to work effectively with other supplements of interest like botanicals and bacterial inoculants.

The drive for natural or organic products by some segments of the public has left dairymen looking for natural supplements that improve or maintain performance and efficiency similar to synthetic supplements. The use of botanicals in the ruminant diet is still an area that requires much research before final conclusions can be made, but the results of the ThermalCare-D® study discussed earlier showed some promising results for improved efficiency, apparent digestibility, and milk yield with a blend of botanical supplements. The use of bacterial inoculants in the diet also shows a lot great promise for the future. The addition of live bacterial inoculants to the diet resulted in improved efficiency of production and apparent digestibility. The studies also showed a positive relationship between the inclusion of glycerol with either botanical supplements or live bacterial inoculants.

Further research needs to be conducted in all three of these areas in order to make the best possible feeding suggestions to producers. The rapidly changing market place and the economic challenges facing the dairy industry and all areas of agricultural production demand that the most efficient and economically sound practices must be found and utilized in order for the industry to continue to grow and prosper.