EFFECT OF SITE OF INFUSION OF *LACTOBACILLUS ACIDOPHILUS* AND *PROPIONIBACTERIUM FREUDENREICHII* ON PRODUCTION AND NUTRIENT DIGESTIBILITY IN LACTATING DAIRY COWS

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IN LACTATING DAIRY COWS

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CHAPTER I

INTRODUCTION

Research has been conducted to determine the effects of feeding direct-fed microbials (**DFM**) to dairy calves (Cruywagen et al., 1996), cows (Stein et al., 2006), and feedlot cattle (Ghorbani et al., 2002). Each production system has its own reasons for feeding DFM, such as in a feedlot to aid in preventing subacute rumen acidosis (Beauchemin et al., 2003) or to help decrease the occurrence of *Escherichia coli* 0157-H7 shedding (Krehbiel et al., 2003). In dairy production the primary goal is to be profitable. Direct fed microbials may have the potential to help meet dairy production's goal. However, previous research has been inconsistent (McGilliard and Stallings, 1998; Nocek et al., 2003). Some research indicates that DFM increases milk production, whereas other research indicates no affect on milk production (Reath-Knight et al., 2007; Oetzel et al., 2003). Oetzel et al. (2003) showed a benefit to dairy cow health. No previous research could be found which determined the effects of site of delivery of DFM on apparent total tract digestibility or milk production. More research is needed to determine if DFM influence milk production, and if so, there mechanism of action.

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CHAPTER II

REVIEW OF LITERATURE

Dairy production is a challenging business. It is commonly known that the main goal of dairying is producing milk, but a lot of factors can influence a cow's milk production (**MP**). These factors include: stressors from environment (hot, cold, mud, dust), housing environment, management practices, diet, cow genetics, and the overall health of the cow. Dairy managers use tools and management practices to help minimize and/or prevent obstacles that may negatively affect milk production. One such tool is bacteria and/or yeast cultures in the diet. In human diets, bacteria and yeast cultures are referred to as probiotics, whereas in food animal production they are commonly referred to as direct fed microbials (**DFM**). Fuller (1997) defined probiotics or DFM as "preparations consisting of live micro-organisms or microbial stimulants which affect the endogenous microflora of the recipient." Microorganisms that can be used as DFM are considered normal microflora of the gut of a specific species are non-pathogenic and may include viruses, bacteriophages, fungi, yeast, and bacteria (Fuller, 1997). The direct mode of action for bacterial DFM in ruminants has not been fully determined, but has been suggested to include: changes in rumen fermentation; changes in the microbial

population in the rumen or lower gut; improvement of dry matter digestibility; increase in nutrient flow to the intestines; and changes in the immune system (Yoon and Stern, 1995; Krehbiel et al., 2003; Raeth-Knight el al., 2007). The purpose of this research is to determine the effects of bacterial based DFM on dry matter intake, rumen digestibility, rumen kinetics, milk production, milk components, and milk fatty acids.

Hypothesis

We hypothesized that administering a consistent dose of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* via ingestion, direct infusion into the rumen, or abomasum would result in an increase in milk production.

Types of Direct-Fed Microbials

There are many different types of DFM being used in livestock production. The two most common types of DFM being used are bacterial and fungal, or a combination of both. It is generally believed that the species or mixture of species being fed should be considered normal microflora of the gastrointestinal tract of the species of animal in that production system (Fuller, 1997). This can be narrowed further to life stages of the animal, such as neonate (preruminant) versus adult (ruminant) and/or production system, such as pasture based (high forage) or feedlot (high concentrate; Krehbiel et al., 2003).

Life stages in dairy production includes neonatal, juvenile (prebreeding), sub adult (breeding/pregnant), transition (changing from nonlactating to lactating), lactation

(early, mid and late), and nonlactating (dry). When feeding a DFM to dairy cattle the stages can be simplified as preruminating and ruminating. Within the ruminating life stages, transition through midlacation is the most common time when cows are fed a DFM (Francisco et al., 2002; Nocek et al., 2002; Nocek and Kautz, 2006; Oetzel et al., 2007).

Neonatal calves may experience several stressors and physiological changes within the digestive system. These calves are prone to many diseases causing enteritis and respiratory illness. Of these the main cause of morbidity and mortality is enteritis (Timmerman et al., 2005). Feeding *Lactobacillus* spp. and *Streptococcus* spp. may reduce the occurrence of enteritis (Khuntia and Chaudhary, 2002; Krehbiel et al., 2003). A study by Timmerman et al. (2005) demonstrated a combination of five strains of *Lactobacillus*: *L. acidophilus* W55, *L. salivarius* W57, *L. paracasei* spp. *paracasei* W56, *L. plantarum* W59, *L. lactis* W58, and *Enterococcus faecium* W54 reduced the occurrence of enteritis and coliform shedding in the feces of 1-wk old veal calves. Direct-fed microbials have also been shown to be a benefit when fed during rumen development. Feeding a yogurt containing *L. acidophilus* in a starter diet tended to increase ruminal function by increased rumination of Holstein calves at 30 d of age (Nakanishi et al., 1993).

Adult dairy cattle are primarily fed DFM from transition to midlacation due to the stressors involved at those stages in production. Many different types of DFM are fed to dairy cattle during this time. Some examples of these commonly fed bacterial species include: *Bacillus subtilis* (McGilliard and Stallings, 1998), *Dietzia* spp. (Click and Van Kampen, 2009), *L. acidophilus* (Krehbiel et al., 2003), *L. Casei* (Yasuda et al., 2006), *L.*

plantarum (Nocek et al., 2002), E. *faecium* (Oetzel et al., 2007), *P. freudenreichii* (Reath-Knight et al., 2007), and *P.* P169 (Stein et al., 2006). Yeast is also a commonly used fungal type DFM. Some commonly fed yeast species are: *Aspergillus oryzae* (McGilliard and Stallings, 1998), *Saccharomyces cerevisiae* (Oetzel et al., 2007), and *Trichosporon sericeum* (Mwenya et al., 2005). For every published paper showing beneficial results of one or a combination of these DFM species, there appears to be another showing no differences in observations. A summary of the available literature is provided in this review.

Theories: How Direct-Fed Microbials Work

Many theories have been developed on the mode of action of bacterial DFM. Lactobacillus bacteria thrive at pH levels around 5. They convert lactic acid to glucose to help stabilize ruminal pH. Propionic bacteria convert substrate to the volatile fatty acid (**VFA**) propionate. Propionic acid concentrations increase in the rumen and are absorbed into the portal blood. The propionic acid in portal blood is transported to the liver. The increased levels of propionate in the blood should lead to increased concentrations of key enzymes in the gluconeogenesis pathway such as pyruvate carboxylase (**PC**) and phosphenolpyruvate carboxykinase (**PEPCK**) in the liver. These enzymes are involved in the gluconeogenesis pathway that converts propionate into glucose. This leads to an increase in available glucose, which may be used by the mammary gland. Within the mammary gland, alveoli secretory epithelial cells increase lactose in the lumen of the

alveoli. Lactose is an osmotic regulator of milk production and causes an increase of water in the lumen and thus, an increase in milk production.

Transitioning Dairy Cattle and Health

The transition period (3 weeks prior to 3 weeks post calving) can be the most important stage of a cow's lactation cycle (Grummer, 1995; Drackley, 1999). To fulfill her genetic potential for milk production, the cow needs to go through the transition stage with as little stress and as few health problems as possible. A transition period with no ill effects will allow the cow to milk to her genetic potential during her lactation provided her nutritional needs are met. A successful transition period will optimize the profitability during that lactation (Drackley, 1999).

During the early lactation phase cows naturally go into a negative energy balance. After parturition, the cow has a sudden increase in demand for nutrients for milk production, while dry matter intake (**DMI)** of nutrients is below the requirement to supply lactation causing the negative energy balance (Drackley, 1999). For example, Bell (1995) reported that net energy for lactation ($\mathbf{NE_L}$) and metabolizable protein requirements could exceed diet consumption by 26% and 25%, respectively, in healthy cows less than one week after parturition. The mammary glands account for 97% of NE_L and 83% of metabolizable protein consumed when needs of milk production are calculated (Bell, 1995; Drackley, 1999). During early lactation little energy is available to supply maintenance. Body reserves are utilized to supply energy for maintenance and to further support milk production.

Most infectious diseases and metabolic disorders affecting dairy cattle occur during the transition period and early lactation phase (Drackley, 1999). Some common health disorders during the transition period include: parturient paresis (post-parturient hypocalcemia; i.e., milk fever), displaced abomasum, ketosis, fatty liver, retained placenta, metritis, and mastitis. The increased occurrence of these health disorders during the transition period is due to a combination of decreased DMI, parturition stressors and adjusting to lactation (Drackley, 1999). Wallace et al. (1996) reported that any health disorder during the transition period would cause a decrease in total 305-d adjusted milk production. A cow that had a displaced abomasum and ketosis produced 853 kg less 305-d mature equivalent milk yield than a cow that had no health disorders during the transition period. Dairy managers utilize management practices and products to prevent and treat health disorders during the transition period.

The suggestion has been made of using DFM to prevent illness and/or to improve the health of dairy cattle. Fuller (1997) summarized studies from 1977 to 1995 dosing different DFM to neonatal calves and obtaining beneficial results to health including decreased fecal coliforms, reduced occurrences of diarrhea, and less occurrence fever and antibiotic usage. Timmerman et al. (2005) showed the same trend with a tendency to decrease mortality, reduced occurrence of diarrhea with lower diarrheic days, reduced fecal coliform count, and reduction in required therapy and treatments for respiratory or digestive diseases. The use of a DFM as an alternative therapy for treating diseases has also been suggested. In a recent study by Click and Van Kampen (2009) feeding *Dietzia* ssp. C79793-74 for the treatment of adult paratuberculosis (Johne's disease) in dairy cows had a therapeutic effect by increasing survival rate and had a cure rate of 37.5%

with the early-stages of the disease. In terms of health, the transitioning or early phase dairy cow will benefit the most from DFM.

A study by Nocek et al. (2003) showed that during the transition period cows up to 70 days in milk (**DIM**) receiving DFM containing two strains of *E. faecium* at 5×10^9 colony forming units (**cfu**) and a yeast (*S. cervisiae*) at 5×10^9 cfu had an increase in DMI post-partum, and an increase in milk production and percentage of milk protein. It was observed that fewer cows were treated for various medical conditions in the DFM treatment group than the control. The cows receiving the DFM pre and post-parturition had a higher concentration of blood glucose (d 1 – 7, 59.3 vs. 51.1; d 8 – 21, 55.7 vs. 49.7; and d 22 – 70, 58.8 vs. 52.1) and insulin (d 1 – 7, 12.2 vs. 8.9 μM/L; and d 8 – 21, 16.8 vs. 10.6 μM/L) and a lower concentration of both β-hydoxybutyrate (**BHBA**; Nocek et al., 2003; Nocek and Kautz, 2006) and nonesterified fatty acids (**NEFA**; Nocek et al., 2003) when compared with the control cows. The decrease in BHBA levels may indicate a more efficient use of nutrients for production (Nocek and Kautz, 2006) and a decrease in negative energy balance (Drackley, 1999). Nocek et al. (2003) and Nocek and Kautz (2006) suggested that fatty acid mobilization from body reserves might be decreased causing the oxidation of fatty acids to be more precise and increasing the usage of carbohydrate energy from the diet. The DFM fed cows had a higher ruminal pH of 6.57 vs. 6.46 on d 1, 6.69 vs. 6.60 on d 2, and 6.72 vs. 6.59 on days 3, 4, and 5 than did the control cows (Nocek et al., 2003; Nocek and Kautz, 2006). The higher ruminal pH value is most likely caused by a balance of microorganisms that can utilize lactic acid and increasing the microorganism's ability to digest forages. This may cause the efficiency of microbial digestion in the rumen to be greater and increase total digestive health

(Nocek et al., 2002; Nocek et al., 2003), resulting in increased milk production and thus increased dairy efficiency.

As previously reported (Stein et al., 2006; Lehloenya et al., 2008) feeding *Propionibacterium* spp. increases the molar percentage of ruminal propionate. Propionate is a direct precursor to glucose through gluconeogenesis and increases portal glucose concentrations. The increase in glucose supplies more energy for metabolism and milk production. Propionate also increases energy in the form of acetyl CoA, a direct precursor to the Krebs cycle and increasing ATP. The increase in dairy efficiency may result from the increase in propionate allowing more energy to be utilized for maintenance. This increase in propionate may also allow more energy to be supplied to the immune system. The boosting of the immune system allows the cow to be in better health by fighting off invasive pathogens during the transition period.

Direct fed microbials may also help to achieve and maintain a healthy microenvironment within the intestines. The naturally occurring intestinal microflora have definite components that have protective attributes against infectious diseases. It is theorized that DFM affect the intestinal mucosa and systemic immune responses (Fuller, 1997; Yoon and Stern, 1995). Early studies on the effect of lactic acid bacteria on health has been summarized by Yoon and Stern (1995). It was suggested that feeding strains of lactic acid bacteria may have beneficial effects including antibacterial, anticarcinogenic, positive immune response, anticholesterolemic, and competitive attachment to the intestinal wall.

The use of DFM's as a management tool to decrease the occurrence of health disorders has the greatest impact during the transition phase of lactation and even more

during early lactation that results from the cow being in a state of negative energy balance. The increase in dairy efficiency, increase in propionate and increase in total gut health in theory makes DFM a good tool to reduce health disorders during the transition period. However, more research needs to be conducted with DFM and their effects on animal health.

Milk Production

Milk production is affected either positively or negatively by many factors. To help minimize the negative influences dairy farmers will do what they can to reduce stressors and feed high quality-feeds and feed additives. Direct fed-microbial's are one of these additives which have been shown to increase milk production and dairy efficiency. The precise mode of action for bacterial DFM has not been determined (Raeth-Knight et al., 2007), although many theories have been suggested. Effects of DFM may include the modification of microbial populations in the rumen and/or the lower gastrointestinal tract, change in rumen fermentation (VFA), increase in immune response, increase in feed digestibility, and increase in the amount of nutrients reaching the small intestine (Krehbiel et al., 2003; Yoon and Stern, 1995; Stein et al., 2006). Propionibacteria species are naturally occurring occupants of the rumen and produce propionate. Propionate is a major glucose precursor through hepatic gluconeogenesis (Sauer et al., 1989; Stein et al., 2006; Aleman et al., 2007). It has been theorized that propionate is 108% more efficient than glucose as a source of energy (McDonald et al., 2002; Stein et al., 2006). Therefore, feeding propionate producing bacteria directly may

positively stimulate metabolism by increasing the production of hepatic glucose (Francisco et al., 2002; Stein et al., 2006).

The first priority of metabolism for postpartum dairy cattle after maintance is to direct energy to support lactation (Lucy, 2001; Stein et al., 2006). In theory, the increase of peripheral glucose allows for a greater uptake of glucose by the mammary secretory epithelial cells encased by myoepithelial cells making up the alveoli. Mammary secretory epithelial cells synthesize and secrete a higher amount of lactose into the lumen of the alveoli. Water is transported in the lumen due to the osmotic imbalance of the high concentration of lactose. This leads to an increase in milk volume.

Stein et al. (2006) fed *Propionibacterium* strain 169 in a low dose 6×10^{10} cfu/cow/day and a high dose of 6×10^{11} cfu/cow/day. Stein et al. (2006) showed an average of 7.8% increase over the control in daily 4% fat corrected milk production. Similar results have been reported by Yasuda et al. (2006) when *Lactobacillus casei* was fed alone. Feeding a bacteria yeast mixture consisting of *Bacillus subtilis, L. acidophilus*, and a yeasts *Aspergillus oryzae* and *S. cervisiae* increased milk production by 33% in first lactation cows and muliparity before 180 days in milk (McGilliard and Stallings, 1998). Lehloenya et al. (2008) reported an increase of 13.5% for late lactation cows and a 16.6% increase for mid lactation cows being fed *Propionibacterium* strain P169 (Agtech Products Inc., Waukesha, WI) and Diamond V-XP yeast culture (Diamond V Mills Inc., Cedar Rapids, IA) during 30 weeks of lactation. No difference ($P > 0.10$) between treatment groups was detected for uncorrected milk production during early or mid lactation. Nocek et al. (2003) and Nocek and Kautz (2006) fed two strains of *E. faecium* at 5×10^9 cfu and *S. cervisiae* at 5×10^9 cfu and reported a statistical increase in

milk production up to 21 DIM and a tendency for increase up to 10 weeks postpartum. There seems to be inconsistency between studies for stage of lactation and the effect of DFM on milk production.

The inconsistency observed when feeding bacterial DFM can be further seen in the lack of response in milk production. Reath-Knight et al. (2007) reported no change in milk production when feeding two treatment combinations including *L. acidophilus* strain LA747 at 1×10^9 cfu/d and *P. freudenreichii* strain PF24 at 2×10^9 cfu/d (Treatment 1); *L. acidophilus* strain LA747 at 1×10^9 cfu/d, *P. freudenreichii* strain PF24 at 2×10^9 cfu/d, and *L. acidophilus* strain LA45 at 5×10^8 (Treatment 2); or the positive control (receiving the carrier lactose) to mid lactation cows. Similar results were observed by Francisco et al. (2002) when a species of *Propionibacterium* spp. was fed two weeks prepartum to 12 weeks postpartum. These results (or lack thereof) were also observed by Oetzel et al. (2007) when feeding a bacteria and yeast combination of *E. faecium* and *S. cervisiae*. Therefore, lack of response has been reported in studies that fed at least one DFM in contrast to the research where a response was observed. Whether the lack of response is from the type of DFM fed or other variables is not known. It is widely believed that bacterial DFM increase milk production; however, results are highly inconsistent (Krehbiel et al., 2003).

Milk lactose concentration is also highly inconsistent when DFM are fed. Some studies (Nocek and Kautz, 2006; Stein et al., 2006; Lehloenya et al., 2007) have reported an increase in milk lactose. Others have reported no change in lactose concentration (Francisco et al., 2002; Yasuda et al., 2006). An increase in lactose could be caused by an increase in propionate or a decrease in the acetate to propionate ratio. As previously

discussed, propionate is a precursor to gluconeogenesis, and thus increasing gluconeogenesis in the liver would increase glucose uptake by the mammary gland to secrete more lactose in the lumen (Stein et al., 2006). Following the idea that lactose is an osmotic regulator of milk production, with an increase of lactose there is an increase in fluid milk, which may or may not increase total lactose percentage.

Milk fat has been reported to be lower in cows fed DFM when compared with controls (McGilliard and Stallings, 1998; Francisco et al., 2002; Nocek and Kautz, 2006). The lower percentage of milk fat could be explained as a dilution effect from the increase of water into the lumen of the mammary system. Another possibility is that DFM causes a lower percentage of butyrate, which could result in lower milk fat (Stein et al., 2006). Some authors have reported an increase in milk fat (Nocek et al., 2003; Stein et al., 2006; Yasuda et al., 2006), suggesting a possible increase in molar proportion of butyrate to support an increase in milk fat. Nocek and Kautz (2006) and Raeth-Knight et al. (2007) reported no significant differences in milk fat yield or 4% fat-corrected milk when DFM were fed. The inconsistent results may be explained by the use of different bacteria, yeast, bacteria yeast combinations, dosing amount and or the varying diets.

Milk protein was increased 3 to 4% (milk protein average = 3.11%) over the control when lactating dairy cows were fed *Propionibacterium* strain 169 in a low dose of 6×10^{10} cfu/cow/day or a high dose of 6×10^{11} cfu/cow/day DFM for the first 25 wk of lactation compared to the control cows (milk protein = 3.00%). Milk protein was increased by 25% over the control in the first week of lactation when cattle were fed *Propionibacterium* strain 169 (Francisco et al., 2002). Possible ways that *Propionibacterium* strain 169 may increase levels of milk protein may be due to

improved rumen digestibility that spares amino acids and/or increases DMI that increases ruminal digesta and microbial protein flow to the small intestines (Stein et al., 2006). Milk solid non-fat (**SNF**) is calculated using the milk components protein and lactose. Because milk protein and lactose increased so did SNF by 9.15 percent for the high-dose (6×10^{11} cfu/cow of *Propionibacterium* strain 169) and 8.91 percent for the low-dose (6×10^{10} cfu/cow of *Propionibacterium* strain 169) compared to the control of 8.85 percent in the experiment by Stein et al. (2006). Similar results have been reported by Nocek et al. (2006), Yasuda et al. (2006), Lehloenya et al. (2008), and McGilliard et al. (1998). Most studies reported an increase in total milk production with a decrease of milk fat when compared to the groups not receiving a DFM.

Digestibility

Supplementing DFM in dairy rations could improve ruminal digestion, increase dry matter intake and rate of dry matter digestibility of specific ingredients, stabilize rumen pH and increase molar proportions of propionate and butyrate (Nocek et al., 2003; Nocek and Kautz, 2006; Nocek et al., 2002; Oetzel et al., 2007; Stein et al., 2006). Propionate is perhaps the most important VFA in regards to being a precursor for glucose synthesis. Propionate can account for 61 to 67% of released glucose for lactating cows (Reynolds et al., 1994; Huntington, 2000; Krehbiel et al., 2003). From early to peak lactation, energy intake cannot keep up with energy demand, suggesting that propionate production may be insufficient (Overton et al., 1999; Krehbiel et al., 2003). *Propionibacteria* as a DFM is a known lactate fermenter and propionate producer

(Krehbiel et al., 2003). A DFM incorporating *Propionibacteria* spp. can reduce the lag in propionate production in the rumen during early to peak lactation. Stein et al. (2006) observed that feeding a high dose of propionibacteria strain 169 at 6×10^{11} cfu/cow/d had no effect on elevating the VFA acetate. It did however, increase the molar percentage of propionate over the low dose and control cattle by 17 to 18.5%, respectively, when 60, 120 and 175 days in milk were averaged. This increase affected the acetate to propionate ratio by lowering it by 13.3% to 15.4% compared with the low dose and control cattle, respectively. The high dose DFM also caused a lower pH value when compared to low dose and control cattle ranging from 6.94 to 6.65. Ruminal pH was not affected 60 d after the final treatment of DFM was given. Similar results were discussed by Yoon and Stern (1995) regarding an increase in molar propionate and butyrate while decreasing acetate and ruminal pH when *L. acidophilus* was dosed at 1.2 to 2.3×10^9 cfu/g. Nocek et al. (2002) observed similar results when dosing a combination of *E. faecium* at 1×10^5 cfu/mL of rumen fluid, *L. plantarum* at 1×10^6 cfu/mL of rumen fluid, and *S. cerevisiae* at 1×10^7 cfu/mL of rumen fluid. Ruminal pH increased with a level of DFM at 10^5 cfu compared to the control, while pH decreased at a higher DFM level of 10^6 and 10^7 cfu. Nocek el al. (2002) suggested that acid production could have overwhelmed the utilization of lactic acid with the higher doses. Some strains of bacteria used as DFM may maintain or prevent a decline in rumen pH by decreasing the production of lactic acid by an increase of lactic acid usage (Nocek et al., 2002). It has been suggested that propionate has a direct effect on DMI. Ruminal infusion of propionic acid into the rumen can cause a decrease in DMI. It has been proposed that one mechanism to regulate feed intake could be propionate receptors within the rumen activated by increased ruminal

proportion of propionic acid which decreases DMI (Baile, 1971). In addition, an indirect regulator of feed intake is the stimulation of insulin secretion by propionate (Lehloenya et al., 2008).

Dry matter intake has been shown to decrease when propionate was intraruminally infused (Oba and Allen, 2003). Similarly, Francisco et al. (2002) showed a decrease in DMI/kg of body weight in early lactation cows when P169 was fed. A study conducted by Raeth-Knight et al. (2007) demonstrated no statistical difference in dry matter intake (**DMI**), ruminal pH, or total VFA concentration when comparing two DFM treatments and a positive control. The treatments consisted of: *L. acidophilus* strain LA747 at 1×10^9 cfu/d and *P. freudenreichii* strain PF24 at 2×10^9 cfu/d (Treatment 1); *L. acidophilus* strain LA747 at 1×10^9 cfu/d, *P. freudenreichii* strain PF24 at 2×10^9 cfu/d, and *L. acidophilus* strain LA45 at 5×10^8 (Treatment 2); or the positive control receiving the carrier lactose. The lack of response could be explained by: the fact that the fermentation portion of the study was conducted using a 3 × 3 Latin square design with 28-d periods that consisted of no time gaps between treatment changes or rumen evacuation or wash. This could give rise to the possibility of treatment carry over to the following period and potentially washing out any significant differences between treatments; secondly, the treatment dosage of *P. freudenreichii* was lower than that dosed by Stein et al. (2006), suggesting that a higher DFM concentration of *P. freudenreichii* may be needed in order to achieve ruminal VFA concentration changes. Lastly, the study was conducted while the cows were in mid-lactation. Studies show when feeding a DFM that the greatest affect is observed during transition and early lactation (Francisco et al., 2002; Nocek et al., 2002; Nocek and Kautz, 2006; Oetzel, et al., 2007)

Rumen undegradable dry matter (**DM**) was lower ($P < 0.05$) and estimated ruminally available DM was greater ($P < 0.05$) on corn silage and haylage forage sources in cows supplemented with 2 strains of *E. faecium* at 5×10^9 cfu/d from 21 d prior to calving to 10 wk postpartum (Nocek and Kautz, 2006; Oetzel et al., 2007). At 72 h the rumen undegradable fraction was lower and the ruminally available DM was greater (Nocek and Kautz, 2006). The same cows fed DFM during postpartum had a greater DMI per day ($P < 0.01$) than the controls. Francisco et al. (2002) showed *Propionibacteria* fed cows (17 g/cow/d of a *Propionibacteria* culture [Agtech Products Inc., Waukesha, WI.]) had a lower DMI than the control cows when DMI is expressed as g/kg of body weight. In contrast Raeth-Knight et al. (2007) reported no significant difference in DMI, while feeding a different DFM. Reasons for the inconsistent results on DMI are hard to determine due to variables between the studies, including differences in bacterial species, dosing amount of DFM, diet ingredients, DIM of cows being studied, parity of cows, and possibly even facilities where cattle were being housed.

Raeth-Knight et al. (2007) reported no difference in apparent total tract digestibility of DM, NDF, CP and starch when compared across treatments or control cows. Cows on all treatments consumed similar DMI amounts. Lehloenya et al. (2008) showed similar results when feeding ruminally and duodenally cannulated Angus × Hereford steers on 4 different treatments: control, sorghum-silage based TMR; control plus P169 at 6×10^{11} cfu/steer/d; control plus Diamond V-XP yeast culture (**XPY**): 56g/steer/d; or control plus P169 at 6×10^{11} cfu/steer/d and XPY at 56 g/steer/d. There was no statistical difference ($P \geq 0.46$) between P169 × XPY treatments and control for organic matter (**OM**), NDF, and ADF intake, fecal output and apparent total tract

digestibility of OM, NDF, and ADF. *Propionibacterium* strain 169 × XPY tended to show an interaction for fecal output of OM, NDF and ADF and for apparent total tract digestibility of OM, NDF, and ADF. No differences were detected for ruminal digestibility of OM, NDF, and ADF or duodenal nutrient flow. Nocek et al. (2002) reported an increase in estimated ruminal digestible DM with the DFM *E. faecium, L. plantarum,* and *S. cerevisiae* fed 10^6 vs 10^5 or 10^7 cfu/mL of rumen fluid. Overall the digestion rate was greater for the two lower dosages, (10^5 and 10^6 cfu/ mL of rumen fluid) than for the 10^7 cfu/mL of rumen fluid of DFM, suggesting that rumen digestion of nutrients and fiber may be enhanced when pH is higher (Russell and Dombrowski, 1980; Russell and Wilson, 1996).

In summary, feeding DFM to lactating dairy cattle has shown differing results. It is suggested that DFM are dose dependent; too low results in no effect, and too high could have a dilution of effects as suggested by Nocek et al. (2002). Results can also be affected by the diet and the species or mixture of species of bacteria used as the DFM. The optimal time to achieve the greatest effect of utilizing a DFM may be during the transition period through early lactation. When utilized properly, feeding DFM is a tool that dairymen can use to help lessen transitioning health disorders, improve milk production and components, increase ruminal digestibility, improve dairy efficiency, and increase the overall health of the cow.

While DFM appear to have beneficial effects, no previous research could be found that determined the effects of bacterial DFM in lactating dairy cows via different routes of administration in the attempt to explain the site where DFM primarily function. In addition, previous research was lacking and limited on the effect of bacterial DFM on

rumen kinetics and rumen digestibility of nutrients in lactating dairy cows. Therefore, the following experiment was conducted to determine the effects of bacterial based DFM on dry matter intake, rumen digestibility, rumen kinetics, milk production, milk components, and milk fatty acids.

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CHAPTER III

MATERIALS AND METHODS

Experimental Design and Sample Collection

Four post-partum, multiparous (avg = 2.25 lactations) Holstein cows (DIM = 73.25 ± 20.11) fitted with type 9C rumen cannulas (Bar Diamond, Parma, ID) were used in a 4 × 4 Latin square design with 37 d periods to determine the effect of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* on milk production, digestibility, and metabolism. The experiment was in accordance with an approved OSU Animal Care and Use Protocol. A rumen content donor, dry Holstein cow was fed the same TMR as the cows on the treatments. Cannula surgeries were conducted at the OSU veterinary hospital 3 months prior to the start of the experiment to allow adequate healing time. Four treatments were allotted according to a Latin square design (**Table 2**) and consisted of (**Table 3**): 1) top dress 5 g of lactose and no DFM (negative control); 2) top dress 5 g of lactose, 10^9 CFU/g of *Lactobacillus acidophilus* and 10^9 CFU/g *Propionibacterium freudenreichii* (positive control); 3) ruminally infused DFM containing 5 g of lactose, 10^9 CFU/g of *Lactobacillus acidophilus* and 10^9 CFU/g *Propionibacterium freudenreichii* (RI); and 4) post-ruminally infused DFM containing 5 g of lactose, 10^9 CFU/g of

Lactobacillus acidophilus and 10^9 CFU/g *Propionibacterium freudenreichii* (PRI). Cows were housed in separate pens and fed a TMR balanced for mid-lactation Holstein cows two times daily (0630 h and 1830 h). Cows were milked two times daily (0530 h and 1730 h) in a double six herringbone milking parlor. There was a 14 d adjustment/wash out period (d 1 to d 14) followed by a 14 d infusion/treatment period (d 15 to d 27) and measurements were collected for 7 d (d 28 to d 35). Complete rumen evacuations were conducted on d 37 to prepare cows for the subsequent period. Rumen contents from the control donor were split between all 5 cows by weight. The DFM came in 5 g prepackaged foil pouches from Nutrition Physiology Corporation, Guymon, OK. Each pouch consisted of 5 g of lactose 1×10^9 CFU/g of *Lactobacillus acidophilus* and 1×10^9 CFU/g *Propionibacterium freudenreichii*. The pouches were stored in a -20ºC freezer for one week prior to usage when they were transferred to a -10°C freezer until feeding.

For rumen infusion 0.60 meters of Tygon Fuel and Lubricant Tubing (Saint-Gobain, Akron, OH; 4.8 mm length, 0.48 cm ID x 0.79 cm OD) was inserted through the cannula into the ventral rumen making the infusion apparatus. A "quick-hose clamp" (Andwin Scientific, Addison, IL) was used to open and close the tube. The infusions were administered via a 60-mL syringe twice daily using sterile water and rinsed with 120 mL of sterile water followed by 60 mL of air to push remaining fluids through the tubing. Abomasal infusions were inserted using the technique described by Gressley et al. (2006). A similar apparatus was used with 2.74 meters of tubing and the addition of a rubber flange held in place by hose clamps cut and ground for smooth surfaces. The flange consisting of 4, 1.27 cm holes drilled on the outer corners to aid digesta flow was

placed through the omasal-abomasal ridge. The infusions were administered via 60-mL syringe 2 times daily with sterile water and flushed with 180 mL of sterile water followed by 60 mL of air after a physical check that the infusion tube was located in the correct position.

Prepackaged DFM was poured into a dry 60-mL syringe and sterile water was sucked out of a prepoured beaker, then lightly shaken until lactose was dissolved into solution. After administrating DFM via infusion lines sterile water was flushed through the syringe and infusion line 2 times for RI (120 mL) and 3 times for PRI (180 mL) to clean.

Feed Intake

TMR and orts were collected and weighed to evaluate daily dry matter intake (DMI; d 28 to d 35) and immediately placed in a -10°C freezer. Composition and ingredients of TMR are listed in Table 1. Animals were fed TMR for *ad libitium* intake 2 times daily with a target of 10% orts. Samples of orts from each treatment were collected before the morning feeding on d 28 to 35. Samples of TMR were collected and weighed before both feedings. Daily DMI was calculated by subtracting amount of TMR offered each day and subtracting the orts. At the end of each period, TMR and orts samples were thawed and composited by weight (100 g). The composited sample was subsampled (100 g) and dried. Samples were dried at 60ºC for 72 h for analyses of nutrient composition. Samples were then ground with a 2-mm screen in a Wiley Mill. All samples were evaluated for CP (LECO Truspec® CN, LECO Corporation, St. Joseph, MI), NDF and

ADF (ANKOM^{200}, ANKOM Technology Corporation, Fairport, NY), DM, OM, and ash (Galyean, 1997).

Milk Production and Composition

Milk weights were recorded at each milking (d 28 to 35) by using Heart of America DHIA (Manhattan, KS) milk meters and sampling equipment. All meters were calibrated by Heart of America DHIA to DHIA standards prior to the start of the experiment and checked monthly at the herd's routine DHIA testing program. The average milk production was calculated daily. Milk samples were taken at the PM milk shift on d 32 and at the AM shift on d 33 of each period. Two samples were collected: one in a DHIA milk sample tube with Micro-Tabs (milk preservative) added for milk composition, and two in a 50-mL polypropylene conical tube for milk fatty acid analysis. AM and PM samples from each treatment were composited by AM and PM milk yield. After compositing milk samples the samples taken in DHIA milk sample tubes were shipped to Heart of America DHIA (Manhattan, KS) to evaluate butter fat, lactose, milk urea nitrogen, protein, solids non-fat and somatic cell count. The other sample was frozen (-20°C) for fatty acid analysis via gas chromatography using the procedure based after Bligh and Dyer (1959) for total lipid extraction. Fatty acid and CLA derivitization procedure was based on Nuernber et al. (2002). Gas chromatography analysis was performed on a Hewlett-Packard 5890 Series II Gas Chromatograph equipped with a Hewlett-Packard 7673A Auto-Sampler and a J&W BD23 column (30m x 25mm x 0.25 μm film thickness). The GC was set at an inlet temperature of 250°C, split 1:25; detector

temperature was set to 300°C; flow was set to 1.0 mL/min at a temperature of 170°C; and the carrier gas was helium. The oven program was set for a temperature of 120°C held for 2 min, then increasing temperature by ramp 1 (12°C/min to 190°C) and ramp 2 (2.0°C/min to 224°C).

Digestibility

Chromic oxide was used as an indigestible marker to measure fecal output. Marker was dosed (10 g) in the rumen via the rumen cannula in preweighed 14.79 mL torpac gelatin capsules twice daily, at 0700 h and 1900 h for 7 d (d 27 to 35) prior to collection. On d 32 to 35, fecal grab samples were collected twice at 0700 h and 1900 h. Samples were stored in rectal palpation gloves in a -10ºC freezer. At the end of each period the fecal samples were composited by treatment at 100 g each on a wet weight basis. The samples were brought to the OSU Nutrition Physiology barn to be dried at 60ºC for 72 h and then ground with a Wiley Mill to pass through a 2-mm screen. Samples were then prepared in the OSU Animal Science Ruminant Nutrition Laboratory using the procedure described by Williams et al. (1962) and analyzed in the OSU Soils and Forage Laboratory by ICP (Inductively Coupled Plasma-Atomic Emission Spectroscopy) for chromium. The chromic oxide was used as an estimator of fecal output to calculate the digestibility of CP (LECO Truspec® CN, LECO Corporation, St. Joseph, MI), NDF and ADF (ANKOM^{200}, ANKOM Technology Corporation, Fairport, NY), and DM, OM, and ash (Galyean, 1997).

Ruminal Fluid Analysis

Rumen fluid was sampled starting on d 34 at 3-h intervals for 24 h starting at 0700 h and ending at 0700 h for analysis of VFA, ammonia, and pH. Rumen fluid was also collected on d 35 at 3-h intervals for 24 h starting at 0700 h and ending at 0700 h for analysis of Co-EDTA dilution. A total of 4 collection devices were made and assigned to a treatment. The devices were made up of 1.27 cm PVC capped on the sampling end with 0.32 cm holes drilled randomly for the first 7.62 cm of sampling end, an Erlenmeyer flask for sample collection, another Erlenmeyer flask as a vacuum trap, and a portable vacuum pump. Rumen fluid was collected in 3 different locations of the ventral sac of the rumen via collection apparatus inserted through the cannula opening. Rumen fluid was thoroughly mixed after collection. Rumen pH was evaluated with a VWR SympHony SP70P pH meter (Radnor, PA). Meta-phosphoric acid was added to two 50-mL polypropylene conical tubes followed by adding the mixed rumen fluid, making a 4:1 ratio of rumen fluid to meta-phosphoric acid. The samples were then inverted 6 times then immediately stored in a -10ºC freezer until frozen. All samples were then transferred to and stored in a -20ºC freezer until analysis.

Co-EDTA was prepared in the as described by Uden et al. (1980) prior to the start of each sampling day. The 0 h samples were collected followed by dosing 300 mL of Co-EDTA. After dosing into the rumen, Co-EDTA was thoroughly mixed into rumen contents.

Rumen fluid was prepared as described by Erwin et al. (1961) and Goetsch and Galyean (1983) and analyzed by gas chromatography (Hewlett-Packard 5890 Series II Gas Chromatograph) equipped with a Hewlett-Packard 7673A Auto-Sampler with a Phenomenex ZB-FFAP column (30m x 0.53mm x 1μm). Inlet temperature was 250ºC

and FID was set at 280ºC. Oven parameters were set with the initial temperature at 80ºC (held 0.2 min) then ramped at 15ºC/min to 145ºC (held 0.5 min), then ramped 45ºC/min to 235ºC with a final hold of 2.0 min. The carrier gas was helium set at a flow rate of 8 mL/min.

Rumen ammonia was analyzed according to Broderick and Kang (1980) and adopted to 96-well microplates (Beckman Coulter, Fullerton, CA). The modified procedure was: 1) centrifuge rumen fluid at 20,000 × g for 10 min in 12 mL centrifuge tubes; 2) pipette 2 mL of supernatant into 2 mL micro centrifuge tubes and centrifuge tubes in a table top micro-centrifuge (Fisher Scientific [Model 235C], Pittsburg, PA) for 15 min at 24,000 × g; 3) add 3 μL of centrifuged rumen fluid or distilled water for blank or working standards to individual wells; 4) add 150 μL of phenol reagent, put plate cover on and mix on plate shaker (VWR Micro Plate Shaker model 980130, Radnor, PA) at 300 rpm for 30 sec. covered with foil; 5) add 120 uL of hypochlorite reagent, put plate cover on and mix on plate shaker at 300 rpm for 30 sec under foil; 6) place covered micro plate on prewarmed 95ºC plate warmer (VWR [model 980130, Radnor, PA] for 5 min; 7) allow plates to cool to room temperature. Absorbance was measured according to the procedure using a plate reader (Multiskan Spectrum; Thermo Scientific, Waltham, MA). Coefficients of variation for ammonia was kept below 5% for intra- and inter-assay.

Blood Samples and Analysis

On d 34 pre and post prandial (0530 h and 0730 h) blood samples were collected via coccygeal vessel into serum separating, sodium fluoride and sodium heparin tubes purchased from the OSU Veterinary Hospital. Serum samples were allowed to sit over

night in a 5ºC refrigerator. All blood samples were centrifuged at 3,000 x g for 20 min to separate plasma and serum and pipetted into 2 mL micro centrifuge tubes. Samples were frozen at -20ºC until analysis was conducted to evaluate total protein, ß-hydroxybutyrate, plasma urea nitrogen (BUN), glucose, insulin-like growth factor 1 (IGF-1), insulin, lactate and non-esterified fatty acids (NEFA). All blood samples were processed in the OSU Animal Science Ruminant Nutrition laboratory.

Kits available commercially were used for the colorimetric determination of plasma urea nitrogen (Urea Nitrogen Reagent, Teco Diagnostic, Anaheim, CA), total protein (Total Protein [Biuret] Reagent Set, Pointe Scientific, Canton, MI), non-esterified fatty acids (HR Series NEFA-HR [2], Wako Pure Chemical Industries Ltd., Richmond, VA), ß-hydroxybutyrate (ß-hydroxybutyrate Reagent Set, Pointe Scientific, Canton, MI), lactate (Lactate [Liquid] Reagent Set, Pointe Scientific, Canton, MI), glucose (Liquid Glucose [Hexokinase] Reagent Set, Pointe Scientific, Canton, MI), insulin (Insulin ELISA, DSL-10-1600, Diagnostic Systems Laboratories, Webster, TX) and insulin-like growth factor 1 (Non-Extraction IGF-1 ELISA, DSL-10-2800, Diagnostic Systems Laboratories, Webster, TX) concentrations. Microplates (96-well; Beckman Coulter, Fullerton, CA) were used for all analyses. Absorbance was measured according to manufacturer recommendations for each metabolite using a plate reader (Multiskan Spectrum; Thermo Scientific, Waltham, MA). Intra- and inter-assay coefficients of variation for analysis of each metabolite were below 5 and 7.5%, respectively.

Ruminal Evacuation

To decrease the possibility of cross treatment contamination between periods, complete rumen evacuations were conducted on d 37. Contents were collected in 208.20 liter Rubbermaid trashcans (Rubbermaid, Fairlawn, OH) and weighed. After thoroughly mixing, four samples were taken and stored in a -20ºC freezer until analysis of DM could be obtained. Contents of any cow receiving a DFM was discarded and replaced with a mixture of fresh feed along with control cow and donor cow rumen contents. Extra feed was also offered on evacuation day to allow the cows to consume adequate amounts for fill.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS with animal within period as a random effect using LS means and orthogonal contrasts (control vs. TD, RI, PRI; TD vs. RI, PRI; RI vs. PRI) to separate significant treatment differences. Values were considered significant at ($P < 0.05$); if the value was between 0.05 and 0.10 it was considered a tendency towards significance. Treatment × time interactions were tested for DMI, ruminal pH, ruminal NH_3, VFA, and milk production. If no significant treatment × time interaction was detected then data was presented by treatment. Data presented are Least squares means.

RESULTS

No difference in DMI was detected among treatments (Table 4), but a trend (P = 0.09) was observed when comparing TD vs RI and PRI. Cows fed TD (25.6 kg DMI) consumed less DM than cows on RI and PRI (27.2 kg DMI); no treatment × time interaction was observed. Kilograms of fecal output did not differ (P = 0.70) among treatments. In addition, total tract digestibility did not differ among the four treatments for DM, CP, NDF or ADF (Table 4). Ruminal fluid dilution rate (**FDR**), ruminal fluid volume (**RFV**), ruminal turnover time (**TT**), and ruminal fluid flow rate (**FFR**) of ruminal digesta (Table 5) did not differ among control, TD, RI, and PRI treatments. Ruminal pH did not differ among treatments and there was no treatment × time interaction. Site of administration of DFM had an effect on volatile fatty acid (**VFA**) concentration. Specifically, a difference was detected (P < 0.05) for valerate (Table 6) with cows fed TD having greater (P < 0.05) valerate than RI and PRI cows, but not differing from control. A trend was also detected for butyrate (P = 0.10) with PRI being less than control, but the same as TD and RI. For isovalerate (P = 0.10), with control and TD cows were different from each other but not from RI or PRI cows. In addition, a trend (P = 0.09) was observed for molar proportion of butryate (Table 7) with TD (14.4%) differing from PRI (13.6%), but not differing from the control and RI treatments

(avg = 14.0%). No treatment × time interactions were detected for VFA.

No treatment × time interactions ($P > 0.10$) were observed for ammonia. The way DFM was administered affected mmol/L of ruminal NH_3 ($P = 0.04$, Table 6) with control cows having a greater concentration of ammonia (4.81) than that of TD (4.29), RI (3.51), and PRI (3.31) treatments. By orthogonal contrast control cows had greater ($P = 0.02$) ruminal NH_3 compared with TD, RI, and PRI treatments.

Route of DFM administration had no effect ($P > 0.10$) on milk production or components (Table 8). Post-ruminal infusion of DFM increased ($P < 0.01$) the milk fatty acid concentration and as a percent of total milk fatty acids (Table 9, 10) of C8:0 compared with control, TD, and RI cows. By comparison PRI was statistically higher in concentration ($P = 0.003$) verses RI. While as a percent of total composition, only control verses TD, RI, and PRI were different ($P = 0.01$) by comparison for C8:0. There tended to be a difference in the μg/100 g concentration of 17:0 (RI verses PRI, $P = 0.10$), 20:1 (RI verses PRI, $P = 0.08$), 20:2 (RI verses PRI, $P = 0.08$), and 20:3n-3 (TD differing from RI and PRI, $P = 0.07$; and TD verses RI and PRI, $P = 0.02$) milk fatty acids. There was no effect of DFM on total milk fatty acid classes (SFA, NSFA, MUFA, PUFA, n-6 PUFA, n-3 PUFA, and CLA) by concentration or percent of composition (Tables 11, 12).

Direct fed microbial treatments had no treatment × time interaction ($P > 0.10$) for blood metabolites. Differing applications of DFM had no effect ($P > 0.10$) on the blood metabolites (Table 13) glucose, total protein, blood urea nitrogen, β-hydroxybutyrate, non-esterified fatty acids, insulin-like growth factor 1, or lactate. A trend was observed ($P = 0.06$) for insulin when comparing RI (4.72 μIU/mL) verses PRI (3.19 μIU/mL)

DISCUSSION

Site of infusion or top dressing *L. acidophilus* and *P. freudenreichii* had no affect on DMI compared to the control, although top dressing the DFM tended to result in lower DMI compared with infusing it in the rumen or abomasum. Nocek et al. (2002) and Nocek et al. (2003) showed similar results with no difference in DMI when DFM was fed to postpartum dairy cows. In addition, a field study conducted by Oetzel et al. (2007) showed no difference in DMI when feeding a DFM compared to a placebo. Raeth-Knight et al. (2007) reported no difference in DMI between two DFM dosing amounts of *L. acidophilus* and *P. freudenreichii* compared to control when fed to mid-lactation Holsteins. The authors suggested that no difference in DMI may be due to cows receiving the same TMR and consuming comparable amounts of DM. In contrast, others have reported a decrease in DMI when DFM was fed pre and postpartum (Francisco et al., 2002; Nocek et al., 2003), or an increase in DMI (Nocek and Kautz, 2006). Variation in DMI in response to DFM is possibly due to different diets, different DFM, and/or the dosing amount of DFM, among other factors.

No significant difference was determined between the four treatments on apparent total tract digestibility of DM, CP, NDF, and ADF. Values for apparent total tract digestibility for DM, CP and NDF are within the range reported by previous research (Nennich et al., 2003). The effects of *L. acidophilus* and *P. freudenreichii* on nutrient digestibility has been previously determined by Raeth-Knight et al. (2007), where

apparent total tract digestibility of DM, NDF, CP, and starch did not differ, similar to the present results. Ruminal digestibility of DM from forage was increased in cows fed *Enterococcus faecium* with yeast for 21 d prepartum through 70 d postpartum (Nocek and Kautz, 2006). Raeth-Knight et al. (2007) explained that similar results could be observed when feeding *L. acidophilus* or *E. faecium* due to them both being homofermentative lactic acid bacteria. However, in the Nocek and Kautz (2006) study, the combination with yeast did not allow that comparison to be made. We conclude that feeding *L. acidophilus* and *P. freudenreichii* without yeast does not impact DMI or total tract apparent nutrient digestibility.

The DFM treatments had no affect on rumen digesta kinetics (FDR, RFV, TT, and FFR). No previous research could be found which had evaluated the effect of bacterial DFM on rumen digesta kinetics in lactating dairy cattle. As stated earlier no statistical difference was observed in DMI. It could be expected that cattle with similar DMI fed the same TMR may have similar rumen digesta kinetics. Lehloenya et al. (2008) reported no difference in ruminal kinetics when *Propionibacterium* strain 169 was fed to Angus × Hereford steers with similar DMI across treatments.

Ruminal pH did not differ among routes of DFM delivery. Raeth-Knight et al. (2007) showed similar results between all treatments when feeding *L. acidophilus* strain LA747 and *P. freudenreichii* strain PF24 at 2 different levels (1×10^9 CFU/d and 2×10^9 CFU/d, respectively; 1×10^9 CFU/d and 2×10^8 CFU/d respectively; or lactose control) with a high pH averaging 6.42 and a low averaging 5.98 across all treatments. Stein et al.

(2006) showed different results with a decrease in ruminal pH when *Propionibacterium* strain 169 was fed at a high dose of 6×10^{11} CFU/d compared to low dose of 6×10^{10} CFU/d and control. Therefore, dose of *Propionibacterium* may impact ruminal pH. Nocek et al. (2002) reported even lower ruminal pH values when a combination of bacterial and yeast DFM containing *Enterococcus faecium* at 1×10^5 cfu/mL of rumen fluid, *Lactobacillus plantarum* at 1×10^6 cfu/mL of rumen fluid, and *Sacchromyces cerevisiae* at 1×10^7 cfu/mL of rumen fluid were supplemented via a ruminal cannula. The ruminal pH was below 5.5 for 13.1 h for the 10^5 or 10^7 cfu/mL of ruminal fluid versus 16.1 h for the 10^6 cfu/mL of ruminal fluid dosed cows. Cattle receiving the 10^5 treatment had a higher daily average pH than that of 10^6 or 10^7 (pH 5.8 vs 5.6 and 5.5, respectfully).

Route of administration of DFM had an effect on m*M* concentration of valerate, with TD having a higher concentration than RI and PRI, but not different from control. Stein et al. (2006) reported an increase in the molar percentage of ruminal propionate, with cows fed the high dose of *Propionibacterium* strain 169 averaging 18.5% greater than the low dose and 17.0% greater than control. The higher propionate percentages affected the acetate/propionate ratio resulting in the high dose having a ratio that was 15.4% lower than the low dose and 13.3% lower than the control. Stein et al. (2006) also reported a treatment affect on the molar proportion of butyrate, with cows fed the low dose having a higher proportion (13.9%) than control cows (12.7%) and cows fed the high dose (12.3%). In the present experiment, a trend was detected for control cows to

have a higher m*M* concentration of butyrate than TD, RI, and PRI, suggesting a potential shift in fermentation pathways when DFM are fed or infused. In contrast, Raeth-Knight et al. (2007) observed no differences in total VFA concentration among previously described DFM treatments. Raeth-Knight et al. (2007) also reported no treatment affect on ruminal ammonia. Few studies could be found which had evaluated the effects of supplementing bacterial DFM to lactating dairy cows on ruminal VFA and ammonia concentrations. In the present experiment, it is unclear why control cows had greater ruminal ammonia concentration than TD, RI and PRI cows, or why TD cows tended to have greater ruminal ammonia than RI and PRI cows. However, it may suggest that feeding a DFM decreases ruminal protein degradation.

In the present study, milk production and milk components were not affected by treatment. Similar results were reported by Oetzel et al. (2007) when administrating 2 strains of *Enteroccus faecium* (5×10^9 cfu) and a yeast *Saccharomyces cerevisiae* (5×10^9 cfu) compared with control. Raeth-Knight et al. (2007) detected no difference in milk production and milk components when the previously described treatments were fed to mid lactation dairy cattle. Nocek et al. (2003) showed similar results with no difference in milk production between treatments when cows were fed the yeast *S. cerevisiae* (Biomate yeast plus, Milkwaukee, WI) and 2 strains of *Enteroccus* spp. (DFM fed at 90 g/cow/d), but milk protein was greater for DFM treated cows from wk 2 through 10 ($P < 0.05$). Nocek and Kautz (2006) reported different results with an increase in milk yield over the control group when 2 g of DFM/cow/d (Probios TC, Milkwaukee, WI),

Biomate yeast plus (1 g; 5×10^9 cfu), and 2 strains of *Enteroccus* spp. (5×10^9 cfu) were fed. They also reported that during the first 14 DIM the fat percentage was lower for DFM treated cows over the control. The DFM administered cows had no difference in milk protein, 3.5% FCM, MUN, and SCC. Stein et al. (2006) reported an increase in 4% FCM milk production and a greater amount of milk fat in low dose and control verses the high dose multiparous cows. It is possible that some bacterial strains of DFM have the greatest affect when fed through the transition phase to peak lactation as seen by the results by Stein et al. (2006), while others do not, such as the strains fed by Oetzel et al. (2007) and Nocek et al. (2003). The results reported by Raeth-Knight et al. (2007) suggest that propionic bacteria had no affect when fed during mid to late lactation, which is similar to the present study. A possible theory is that cows are not in a high negative energy balance during mid to late lactation and some metabolizable energy is going to body reserves rather than body reserves being utilized to help drive milk production. Raeth-Knight et al. (2007) suggested that a higher concentrate diet may be needed to provide more ruminal lactic acid concentration, which could be utilized by lactic acid utilizing bacteria to produce propionate. This does not follow the present study as the forage:concentrate ratio for Nocek et al. (2003) was 40:60 and in the present study forage:concentrate was 36:64. Nocek et al. (2003) suggested that if too much DFM is fed it can cause the level of ruminal acid to increase too high for the ruminant's ability to utilize the available acid.

In the present study, the dosed DFM affected milk fatty acid concentration (1.75 μg/100g) and percent of total composition (0.26) of 8:0. Treatment PRI was higher in concentration than the control, TD, and RIM, while there was no difference between control, TD, and RI. Slight increases in the fatty acid profile of the milk could possibly be the affect of dosing the bacteria post ruminally. No other studies could be found on the affect of bacterial DFM on milk fatty acid concentration or percent of composition in lactating dairy cattle when the DFM was administered post ruminally.

In the present study no statistical difference was observed on the blood metabolites glucose, total protein, blood urea nitrogen, β-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA), insulin-like growth factor 1 (IGF-1), and lactate. Similar results were reported by Francisco et al. (2002) when cows were fed 17 g/d of a *Propionibacteria* culture (Agtech Products Inc. Waukesha, WI) with no affect on plasma glucose (60.0 mg/dl) or IGF-1 (111.5 ng/ml). Francisco et al. (2002) reported that after wk 1 NEFA concentration decreased faster in DFM cattle than control cattle, suggesting that the cattle were not utilizing as much body reserves as the control cattle. Lehloenya et al. (2007), Nocek et al. (2003), and Oetzel et al. (2007) also reported no DFM treatment affect on glucose, insulin, BHBA, or NEFA. In the present experiment, there was a tendency for RI cows to have a higher insulin concentration (4.72 μlU/mL) than PRI cows (3.19 μlU/mL). This is similar to results reported by Lehloenya et at. (2007), where plasma insulin was greater for steers supplemented with bacterial DFM and yeast culture than control or yeast culture alone. Nocek et al. (2003) suggested that when

evaluating blood parameters in transitioning dairy cattle the best situation is to have a treatment increase blood glucose and insulin and decrease BHBA and NEFA concentration. By doing so the needs of the cows with high energy demand during early lactation would be better met by the diet suggesting the reduction of body reserve mobilization with more complete oxidation of fatty acids. In the present study no differences in DMI or nutrient digestibility among treatments may explain the lack of response on blood metabolites.

CONCLUSION

The affect of administration of the combination of *L. acidophilus* and *P. freudenreichii* to lactating dairy cows had no affect on DMI, milk production or milk components. Apparent total tract digestibility of DM, OM, CP, NDF, and ADF were similar across treatments. No differences were observed for rumen kinetics, rumen pH, rumen fermentation of acetate, propionate, butyrate, and acetate/propionate ratio, or NH_3. Lastly, the majority of milk fatty acids, and blood metabolites glucose, total protein, BUN, BHBA, NEFA, insulin, IGF-1, and lactate were similar across treatments. In conclusion, under the conditions of this study, the route of administration of *L. acidophilus* and *P. freudenreichii* had no affect on lactating dairy cow's performance, diet digestibility, rumen fermentation, milk fatty acids composition, or blood metabolites. We conclude based on previous literature that DFM might have their greatest effects during the transition period and early lactation. However, experiments are needed to determine if DFM could be fed with decreasing forage:concentrate in late lactation to sustain milk production.

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TABLES

Table 1. Ingredient and nutrient composition of the diet (DM basis).

Ingredient	
Bermuda grass, %	10.45
Alfalfa, %	25.25
Whole cottonseed, %	5.81
DDG with solubles, %	12.1
Corn gluten feed, %	9.97
Lactation cow grain mix	
Ground corn, %	21.56
Soybean meal, 48% CP, %	1.44
Soybean hulls, %	9.51
RUMOLAC *, %	1.17
Limestone, %	1.23
Sodium bicarbonate, %	0.82
Calcium diphosphate, %	0.18
Magnesium oxide, %	0.16
Salt white, %	0.18
Lactating premix, %	0.18
Components	
DM, % (as fed)	56.06
CP, %	15.15
ADF, %	21.14
NDF, %	40.14
NEl, Mcal/kg	1.70
Crude fat, %	6.50
Ash, %	3.81
Ca, %	0.96
P, %	0.41
Mg, %	0.36
K, %	1.05
Na, %	0.3
Zn, ppm	86
Cu, ppm	15
Mn, ppm	58
Mo, ppm	0.5

*Robt Morgan, Inc., Paris, IL; RUMOLAC contains: Fat (as fatty acids) – 84%, Calcium – 9.0%, Net energy of lactation – 1.34 Mcal/kg

Table 2. Treatment assignments and dosages.

Treatment	Dosage
Control	5 g of lactose top dressed on TMR
Top Dress	DFM mix[1] top dressed on TMR
Rumen Infusion	DFM mix[1] reconstituted in 60 mL of sterile water followed with 60 mL of sterile water
Post-Rumen Infusion	DFM mix[1] reconstituted in 60 mL of sterile water followed with 120 mL of sterile water

[1]Direct fed microbial (DFM) mix: 10^9 CFU/g of *Lactobacillus acidophilus*, and 10^9 CFU/g *Propionibacterium freudenreichii* with 5 g of lactose.

Table 3. Treatment assignments for 4 × 4 Latin Square.

	Cow			
Period	1	2	3	4
1	Control	Top Dress[1]	Rumen[2]	Post Rumen[3]
2	Top Dress	Post Rumen	Control	Rumen
3	Post Rumen	Rumen	Top Dress	Control
4	Rumen	Control	Post Rumen	Top Dress

[1]Top dress: direct fed microbials (DFM) applied to the top of the TMR.
[2]Rumen: DFM dosed to rumen via rumen infusion apparatus.
[3]Post rumen: DFM dosed post ruminally via infusion apparatus.

Table 4. Effects of type of administration of DFM on fecal output (kg), and apparent total tract digestibility of nutrients in lactating dairy cows.[1]

						P-Value[2]			
Item	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
Dry matter intake, kg	27.28	25.56	27.42	26.92	0.76	0.29	0.46	0.09	0.64
Fecal output[3], kg	8.35	7.89	9.54	8.78	1.01	0.70	0.75	0.32	0.60
Total tract digestibility									
Dry matter, kg	66.81	70.62	66.96	66.01	3.26	0.76	0.78	0.32	0.84
Organic matter, kg	68.98	72.05	68.51	67.94	2.82	0.74	0.87	0.29	0.89
Crude protein, kg	67.89	71.03	67.26	66.03	3.33	0.75	0.95	0.30	0.80
Neutral detergent fiber, kg	54.73	62.74	56.38	55.87	5.29	0.71	0.56	0.32	0.95
Acid detergent fiber, kg	54.69	62.16	56.17	55.90	4.91	0.71	0.56	0.32	0.97

[1]Data presented are Least squares means, treatment, *n*=4.
[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.
[3]Fecal output, kg: [Cr_2O_3 dosed g/d / Cr_2O_3 concentration in feces, (g/g of dry matter)] / 1000.

Table 5. Effects of type of administration of DFM on ruminal digesta kinetics in lactating dairy cows.[1]

						P-value[2]			
Item[3]	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
FDR %/hr	0.14	0.13	0.14	0.14	0.01	0.90	0.81	0.55	0.73
RFV, L	77.82	74.97	79.86	85.47	16.68	0.97	0.91	0.71	0.81
TT, hr	7.41	7.53	7.17	7.39	0.38	0.93	0.91	0.61	0.68
FFR, L/hr	10.55	9.73	11.01	11.35	2.11	0.95	0.95	0.58	0.91

[1]Data presented are Least squares means, treatment, *n*=4.

[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

[3]Ruminal fluid dilution rate (FDR): calculated from the slope of sample time by cobalt EDTA concentration at time of sampling; Ruminal fluid volume (RFV): calculated by dose of cobalt EDTA (mg) divided by the antilog of cobalt EDTA (mg/L) concentration at sampling time 0; Ruminal turnover time (TT): calculated 1/FDR; Ruminal fluid flow rate (FFR): outflow from the rumen in L/h = RFV × FDR.

Table 6. Effects of type of administration of DFM on ruminal pH, mM of volatile fatty acids (VFA), and mmol/L of ruminal NH_3 in lactating dairy cows.[1]

						P-value[2]			
Item	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
pH	6.07	6.09	6.13	6.13	0.05	0.77	0.37	0.56	0.92
Acetate, mmol/L	43.88	41.88	43.17	40.66	2.06	0.70	0.41	0.99	0.39
Propionate, mmol/L	17.89	18.35	18.42	16.96	0.91	0.66	0.99	0.56	0.26
Isobutyrate, mmol/L	3.49	3.44	3.45	3.42	0.04	0.57	0.21	0.92	0.51
Butyrate, mmol/L	11.85^{y}	11.63^{yz}	11.40^{yz}	10.61^{z}	0.37	0.10	0.14	0.17	0.13
Isovalerate, mmol/L	3.58^{y}	3.42^{z}	3.44^{yz}	3.49^{yz}	0.05	0.10	0.02	0.43	0.50
Valerate, mmol/L	4.13^{ab}	4.27^{a}	4.09^{b}	4.04^{b}	0.06	0.03	0.97	0.00	0.52
A:P Ratio	2.45	2.35	2.35	2.43	0.06	0.62	0.35	0.62	0.42
NH_3, mmol/L	4.81^{a}	4.29^{ab}	3.51^{b}	3.35^{b}	0.41	0.04	0.02	0.09	0.79

[a,b]Different superscripts within row indicate a *P*-value < 0.05.
[y,z]Different superscripts within row indicate a trend *P*-value < 0.10.
[1]Data presented are Least squares means, treatment, *n*=4.
[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

Table 7. Effects of type of administration of DFM on ruminal pH, molar proportion of volatile fatty acids (VFA), and mmol/L of ruminal NH_3 in lactating dairy cows.[1]

						P-value[2]			
Item	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
pH	6.07	6.09	6.13	6.13	0.05	0.77	0.37	0.56	0.92
Acetate, %	50.89	49.48	50.55	50.22	0.71	0.54	0.33	0.30	0.74
Propionate, %	20.97	21.74	21.84	21.15	0.41	0.37	0.21	0.63	0.25
Isobutyrate, %	4.37	4.48	4.33	4.68	0.17	0.45	0.53	0.90	0.14
Butyrate, %	14.15^{yz}	14.36^{y}	13.84^{yz}	13.64^{z}	0.22	0.09	0.42	0.02	0.52
Isovalerate, %	4.45	4.46	4.31	4.79	0.17	0.24	0.73	0.67	0.05
Valerate, %	5.16	5.48	5.13	5.51	0.19	0.34	0.32	0.50	0.16
A:P Ratio	2.45	2.35	2.35	2.43	0.06	0.62	0.35	0.62	0.42
NH_3, mmol/L	4.81^{a}	4.29^{ab}	3.51^{b}	3.35^{b}	0.41	0.04	0.02	0.09	0.79

^{a,b}Different superscripts within row indicate a *P*-value <0.05.
^{y,z}Different superscripts within row indicate a trend *P*-value < 0.10.
[1]Data presented are Least squares means, treatment, *n*=4.
[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

Table 8. Effects of type of administration of DFM on milk production and components in lactating dairy cows.[1]

						P-value[2]			
Item[3]	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
Milk production, kg	29.37	29.19	27.91	29.58	1.53	0.87	0.79	0.81	0.44
Butter fat, %	3.54	3.70	3.31	3.73	0.21	0.36	0.86	0.48	0.17
Protein, %	3.32	3.19	3.20	3.31	0.13	0.83	0.56	0.67	0.57
SCC	248.00	330.00	994.00	254.00	416.09	0.54	0.57	0.57	0.23
Lactose, %	4.61	4.64	4.64	4.73	0.13	0.91	0.66	0.80	0.62
SNF, %	8.79	8.71	8.71	8.93	0.22	0.88	0.99	0.69	0.49
MUN, %	13.85	13.49	14.77	12.99	0.82	0.49	0.92	0.71	0.14

[1]Data presented are Least squares means, treatment, *n*=4.
[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.
[3]Somatic cell count (SCC): number times 100,000; Percent solids non-fat (SNF); Milk urea nitrogen (MUN).

Table 9. Effects of type of administration of DFM on milk fatty acid contents (µg/100 g of fatty acids concentration) in lactating dairy cows.[1]

						P-value[2]			
Item	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
8:0	0.89[a]	0.99[a]	0.77[a]	1.75[b]	0.20	0.01	0.24	0.27	0.003
10:0	10.31	9.82	8.08	11.19	1.24	0.37	0.68	0.90	0.10
12:0	17.63	18.03	15.67	18.21	1.70	0.70	0.87	0.61	0.30
12:1	0.55	0.58	0.49	0.57	0.04	0.47	0.89	0.38	0.20
13:0	0.61	0.61	0.62	0.66	0.07	0.47	0.80	0.69	0.68
14:0	62.16	64.09	56.64	64.23	4.90	0.67	0.93	0.55	0.29
14:1	3.78	3.95	3.39	3.70	0.30	0.61	0.78	0.28	0.47
15:0	5.98	5.92	5.69	6.36	0.44	0.76	0.98	0.84	0.30
16:0	180.43	185.71	164.24	188.33	10.19	0.76	0.93	0.46	0.11
16:1	6.41	6.63	5.79	6.76	0.47	0.48	0.97	0.54	0.16
17:0	4.03	3.97	3.59	4.22	0.25	0.39	0.73	0.84	0.10
17:1	1.00	1.00	0.94	1.09	0.07	0.58	0.92	0.91	0.18
18:0	119.84	119.53	100.78	121.06	8.42	0.30	0.54	0.42	0.11
18:1 11*c*	6.91	6.77	6.65	7.16	0.41	0.84	0.92	0.79	0.39
18:1 11*t*	12.27	12.39	11.74	12.15	0.95	0.97	0.87	0.71	0.76
18:1 9*c*	185.22	180.55	162.78	187.08	11.40	0.44	0.53	0.69	0.15
18:1 9*t*	6.54	6.49	5.81	6.65	0.36	0.37	0.58	0.56	0.12
18:2 10*t*,12*c*	0.14	0.14	0.12	0.18	0.03	0.59	0.85	0.84	0.19
18:2 9*c*,11*t*	4.69	4.69	4.26	4.70	0.37	0.80	0.75	0.65	0.41
18:2n-6	33.96	34.84	31.11	35.30	2.27	0.57	0.94	0.56	0.21
18:2*tt*	6.82	6.57	6.23	6.88	0.36	0.59	0.55	0.98	0.22
18:3n-3	3.79	3.90	3.52	4.14	0.31	0.57	0.87	0.86	0.18
20:0	1.66	1.68	1.45	1.75	0.14	0.50	0.82	0.65	0.16
20:1	0.38	0.43	0.35	0.44	0.04	0.26	0.53	0.49	0.08
20:2	0.39	0.42	0.34	0.44	0.04	0.29	0.76	0.49	0.08
20:3n-3	0.37[yz]	0.41[y]	0.28[z]	0.28[z]	0.04	0.07	0.27	0.02	0.96
20:3n-6	1.99	1.97	1.78	2.08	0.28	0.89	0.89	0.90	0.46
20:4n-6	2.35	2.33	2.13	2.35	0.19	0.80	0.70	0.71	0.42
20:5n-3	0.52	0.55	0.61	0.52	0.08	0.85	0.70	0.92	0.44
22:0	0.81	0.82	0.70	0.83	0.08	0.62	0.77	0.56	0.25
22:1	0.24	0.40	0.26	0.32	0.07	0.41	0.31	0.22	0.57
22:6n-3	0.57	0.61	0.59	0.66	0.06	0.73	0.51	0.84	0.38
23:0	0.19	0.20	0.28	0.21	0.04	0.26	0.39	0.27	0.15
24:0	0.25	0.26	0.28	0.27	0.03	0.83	0.48	0.56	0.90

[a, b]Different superscripts within row indicate a *P*-value < 0.05.
[y,z]Different superscripts within row indicate a trend *P*-value < 0.10.
[1]Data presented are Least squares means, treatment, *n*=4.
[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

Table 10. Effects of type of administration of DFM on milk fatty acid contents (% of total composition of fatty acids) in lactating dairy cows[1]

						P-value[2]			
Item	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
8:0	0.13[a]	0.11[a]	0.09[a]	0.26[b]	0.03	0.01	0.01	0.56	0.16
10:0	1.40	1.42	1.32	1.67	0.14	0.34	0.34	0.68	0.68
12:0	2.60	2.59	2.59	2.63	0.19	1.00	1.00	0.98	0.93
12:1	0.08	0.08	0.08	0.08	0.00	0.97	0.97	1.00	0.76
13:0	0.09	0.09	0.10	0.09	0.01	0.66	0.66	0.47	0.39
14:0	9.04	9.23	9.43	9.13	0.55	0.96	0.96	0.74	0.94
14:1	0.56	0.58	0.56	0.52	0.05	0.87	0.87	0.89	0.55
15:0	0.87	0.86	0.93	0.90	0.04	0.58	0.58	0.49	0.59
16:0	26.37	27.05	27.00	26.71	0.64	0.87	0.87	0.47	0.81
16:1	0.94	0.97	0.94	0.95	0.04	0.97	0.97	0.73	0.74
17:0	0.59	0.58	0.59	0.59	0.01	0.85	0.85	0.85	0.40
17:1	0.15	0.15	0.16	0.15	0.01	0.52	0.52	0.50	0.43
18:0	17.49	17.38	16.81	16.92	0.74	0.89	0.89	0.60	0.58
18:1 11*c*	1.01	0.99	1.09	1.01	0.04	0.27	0.27	0.62	0.19
18:1 11*t*	1.84	1.88	2.03	1.95	0.13	0.72	0.72	0.45	0.48
18:1 9*c*	27.12	26.36	26.90	26.20	0.93	0.88	0.88	0.56	0.87
18:1 9*t*	0.96	0.95	0.98	0.94	0.02	0.73	0.73	0.86	0.67
18:2 10*t*,12*c*	0.03	0.02	0.02	0.02	0.01	0.84	0.84	0.49	0.96
18:2 9*c*,11*t*	0.69	0.68	0.70	0.66	0.04	0.94	0.94	0.86	0.94
18:2n-6	4.95	5.08	5.11	5.03	0.19	0.94	0.94	0.58	0.97
18:2*tt*	1.00	0.96	1.02	0.98	0.03	0.52	0.52	0.71	0.27
18:3n-3	0.55	0.57	0.58	0.59	0.03	0.84	0.84	0.47	0.63
20:0	0.24	0.24	0.24	0.25	0.01	0.81	0.81	0.86	0.96
20:1	0.05	0.06	0.06	0.06	0.00	0.21	0.21	0.09	0.35
20:2	0.06	0.06	0.06	0.06	0.00	0.86	0.86	0.46	0.71
20:3n-3	0.04	0.05	0.04	0.05	0.01	0.68	0.68	0.45	0.41
20:3n-6	0.29	0.29	0.30	0.30	0.03	0.97	0.97	0.83	0.67
20:4n-6	0.33	0.34	0.34	0.34	0.02	0.97	0.97	0.63	0.90
20:5n-3	0.07	0.09	0.08	0.07	0.01	0.51	0.51	0.30	0.30
22:0	0.11	0.13	0.11	0.12	0.01	0.48	0.48	0.52	0.34
22:1	0.03	0.04	0.04	0.04	0.01	0.81	0.81	0.41	0.67
22:6n-3	0.09	0.09	0.10	0.09	0.01	0.84	0.84	0.81	0.43
23:0	0.03	0.03	0.03	0.03	0.00	0.60	0.60	0.30	0.40
24:0	0.04	0.04	0.04	0.04	0.00	0.97	0.97	0.91	0.64

[a, b]Different superscripts within row indicate a *P*-value < 0.05.

[1]Data presented are Least squares means, treatment, *n*=4.

[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

Table 11. Effects of type of administration of DFM on total milk fatty acid classes (μg/100 g of fatty acids concentration) in lactating dairy cows[1]

						P-value[2]			
Item[3]	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
SFA	404.80	411.61	358.81	419.08	23.09	0.28	0.76	0.43	0.08
NSFA	278.91	275.60	249.16	283.45	15.65	0.43	0.88	0.62	0.20
MUFA	223.31	219.18	198.20	225.92	12.72	0.43	0.55	0.66	0.14
PUFA	55.60	56.43	50.96	57.53	3.51	0.58	0.88	0.62	0.20
n-6 PUFA	38.30	39.14	35.02	39.73	2.63	0.60	0.91	0.59	0.22
n-3 PUFA	5.26	5.46	4.99	5.59	0.38	0.70	0.84	0.72	0.28
CLA	4.83	4.83	4.38	4.88	0.37	0.75	0.77	0.67	0.35

[1]Data presented are Least squares means, treatment, *n*=4.

[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

[3]Total saturated fatty acids (SFA): all fatty acids without any double bond (8:0 to 24:0); Total unsaturated fatty acids (USFA): all fatty acids with double bond(s) (12:1 to 22:6n-3); Total monounsaturated fatty acids (MUFA): all fatty acids with a single double bond (12:1 to 22:1); Total polyunsaturated fatty acids (PUFA): all fatty acids with two or more double bonds (18:2 10*t*,12*c* to 22:6n-3); Total n-6 polyunsaturated fatty acids (PUFA): 18:2n-6, 20:3n-6, and 20:4n-6; Total n-3 polyunsaturated fatty acids (PUFA): 18:3n-3, 20:3n-3, 20:5n-3, and 22:6n-3; Total conjugated linoleic acid (CLA): 18:2 10*t*,12*c*, and 18:2 9*c*,11*t*.

Table 12. Effects of type of administration of DFM on milk fatty acid contents (% of total composition of fatty acids) in lactating dairy cows.[1]

						P-value[2]			
Item[3]	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
Total SFA	58.99	59.75	59.28	59.35	0.92	0.95	0.95	0.67	0.70
Total NSFA	40.82	40.27	41.20	40.11	1.00	0.58	0.58	0.88	0.62
Total MUFA	32.74	32.05	32.85	31.91	0.94	0.85	0.85	0.67	0.78
Total PUFA	8.08	8.22	8.35	8.19	0.25	0.90	0.90	0.56	0.88
Total n-6 PUFA	5.56	5.71	5.76	5.67	0.21	0.93	0.93	0.56	0.99
Total n-3 PUFA	0.75	0.79	0.79	0.80	0.04	0.77	0.77	0.31	0.99
Total CLA	0.71	0.70	0.71	0.69	0.04	0.95	0.95	0.78	0.93

[1]Data presented are Least squares means, treatment, *n*=4.

[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

[3]Total saturated fatty acids (SFA): all fatty acids without any double bond (8:0 to 24:0); Total unsaturated fatty acids (USFA): all fatty acids with double bond(s) (12:1 to 22:6n-3); Total monounsaturated fatty acids (MUFA): all fatty acids with a single double bond (12:1 to 22:1); Total polyunsaturated fatty acids (PUFA): all fatty acids with two or more double bonds (18:2 10*t*,12*c* to 22:6n-3); Total n-6 polyunsaturated fatty acids (PUFA): 18:2n-6, 20:3n-6, and 20:4n-6; Total n-3 polyunsaturated fatty acids (PUFA): 18:3n-3, 20:3n-3, 20:5n-3, and 22:6n-3; Total conjugated linoleic acid (CLA): 18:2 10*t*,12*c*, and 18:2 9*c*,11*t*.

Table 13. Effects of type of administration of DFM on pre and post prandial blood metabolites taken by coccygeal venipuncture in lactating dairy cattle.[1]

						P-value[2]			
Item[3]	Control	TD	RI	PRI	SEM	TRT	C_1	C_2	C_3
Glucose, mg/dL	48.76	48.29	50.55	47.52	1.66	0.62	0.99	0.72	0.20
Total protein, mg/dL	5.04	5.19	4.94	5.09	0.17	0.77	0.86	0.41	0.53
BUN, mg/dL	14.63	16.74	15.51	17.03	1.53	0.66	0.31	0.80	0.49
BHBA, mM	0.57	0.62	0.60	0.66	0.05	0.61	0.30	0.91	0.40
NEFA, mEq/L	175.99	202.09	150.25	174.69	35.36	0.78	0.99	0.37	0.63
Insulin, μIU/mL	3.15	3.05	4.72	3.19	0.55	0.12	0.43	0.19	0.06
IGF-1, ηg/mL	121.90	148.35	131.00	186.08	36.37	0.61	0.43	0.82	0.29
Lactate, mmol/L	2.17	1.76	1.90	1.67	0.23	0.44	0.14	0.95	0.48

[1]Data presented are Least squares means, treatment, *n*=4.

[2]Treatments analyzed by Least square means; Compared by orthogonal contrast, C_1 = control vs. top dress (TD), ruminal infusion (RIM), and post ruminal infusion (PRIM), C_2 = TD vs. RIM and PRIM, C_3 = RIM vs. PRIM.

[3]Blood urea nitrogen (BUN); β-Hydroxybutyrate (BHBA); Non-esterified fatty acids (NEFA); Insulin-like growth factor 1 (IGF-1).

VITA

Kyle Samuel Thompson

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF SITE OF INFUSION OF *LACTOBACILLUS ACIDOPHILUS* AND *PROPIONIBACTERIUM FREUDENREICHII* ON PRODUCTION AND NUTRIENT DIGESTIBILITY IN LACTATING DAIRY COWS

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EFFECT OF SITE OF INFUSION OF *LACTOBACILLUS ACIDOPHILUS* AND *PROPIONIBACTERIUM FREUDENREICHII* ON PRODUCTION AND NUTRIENT DIGESTIBILITY IN LACTATING DAIRY COWS

Pages in Study: 57 Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope and Method of Study:

The objective was to evaluate site of infusion of 10^9 CFU/g of *Lactobacillus acidophilus* and 10^9 CFU/g *Propionibacterium freudenreichii* (DFM) on DMI, rumen kinetics, ruminal VFA, digestibility, milk production, milk components, and blood metabolites in lactating dairy cows. A total of 4 Holstein cows equipped with ruminal cannulas were used in Latin square design experiment with 4 periods. Each period consisted of 14 d of no treatment to prevent crossover contamination, 14 d of adaptation to treatments, 8 d of sampling, and 1 d for ruminal evacuations for a total of 37 d. Within each period, cows were assigned to 1 of 4 treatments: 1) cows fed a TMR formulated to meet or exceed nutrient requirements plus 5 g of lactose twice daily without the addition of direct fed microbial (CONTROL); 2) cows fed the TMR with a daily dose of DFM top dressed on the feed twice daily 3) cows fed the TMR with ruminal infusion of the DFM administered twice daily (RI); and 4) cows fed the TMR plus abomasal infusion of the DFM twice daily (PRI). During the sampling period within each period, DMI and milk production were measured daily with set days for blood, and rumen fluid collection. Data were analyzed using the MIXED procedure of SAS with animal within period as a random effect.

Findings and Conclusions:

DMI was not different between treatments. No difference in rumen kinetics, pH, VFA (acetate, propionate, butyrate, and acetate/propionate ratio), ammonia, or digestibility. There was no difference in milk production ($P > 0.87$), butter fat ($P > 0.21$), milk protein ($P > 0.83$), lactose ($P > 0.91$), SNF ($P > 0.88$) , MUN ($P > 0.49$), and SCC ($P > 0.54$). No difference in most of the milk fatty acids except for 8:0 which had a greater concentration ($P > 0.01$) in PRI over control, TD, and RI. Route of administering DFM overall had no effects on DMI, rumen kinetics, ruminal VFA, digestibility, milk production or milk components in the present experiment.

ADVISER'S APPROVAL: Dr. Clint Krehbiel