DOSE RESPONSE OF A DIRECT-FED MICROBIAL ON MILK YIELD, MILK COMPONENTS, BODY WEIGHT, AND DAYS TO FIRST OVULATION IN PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

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Chapter I

Introduction

Over the last ten years, the average annual milk production per cow in the U.S. has increased 15.3%, from 7131 kg to 8423 kg, while the total number of dairy farms and cows in production have decreased 41.7% and 4.3%, respectfully (USDA, 2004). Fortunately, improved genetics and management of dairy cattle has accompanied this period in which fewer dairymen with fewer cows are trying to meet the continually growing demand for milk and dairy products (Lucy, 2001). There has also been a dramatic change in the structure of the dairy industry with over 40.7% of today's dairy operations milking over 500 plus cows compared to 33.4% of the operations milking over 200 plus cows just ten years ago (USDA, 2004). The incentive for increasing milk production comes from the desire to maximize farm income. The genetic potential for milk production sets the upper limit at which an individual cow can produce milk (Silvia, 2003). How close a cow comes to attaining her production limit is determined by not only management practices during lactation, but also by far-off and close-up dry period management practices under which she is subjected. The success of the transition period ultimately determines the profitability of the current lactation. Dairy cows with greater DMI, produced more milk, lost less BW, and ovulated earlier postpartum than those with lower intakes (Staples and Thatcher, 1990), thus, one of the primary challenges faced by

the dairy cow is the sudden increase in nutrient requirements for lactation at a time when DMI is limited (Drackley, 1999).

The rumen can be considered a self-contained ecosystem (Weimer, 1998) in which the microbe population, in a true symbiotic relationship with the host ruminant animal, aids in the digestion of the diet. The diet consumed by the animal supplies a constant substrate for the microbe population, while the rumen provides an anaerobic environment, a relatively constant temperature and pH, and the necessary volume and motility needed for fermentation by the microbes. The end products of microbial fermentation, volatile fatty acids (VFA) (primarily acetic, propionic, and butyric) and microbial protein, are utilized by the host animal (Mackie and White, 1990). Dairy cattle fed a 60:40 concentrate/roughage diet produced volatile fatty acids near the following proportions: 71% acetate; 21% propionate; and 8% butyrate while dairy cattle fed a 90:10 concentrate/roughage diet produced a volatile fatty acid proportions near 55% acetate; 40% propionate; and 5% butyrate (Oshio et al., 1987; Sutton et al., 2003). The efficiency of utilization for maintenance of acetic acid, propionic acid, and butyric acid is 0.59, 0.86, and 0.76, respectively (McDonald et al., 2002). The volatile fatty acids: acetate, propionate, and butyrate can supply up to 70 to 80% of the ruminants energy requirements. The theoretically efficiency of propionate as a source of energy for ATP/100 g nutrient is 108% compared with glucose (McDonald et al., 2002). The microbial protein leaving the rumen can supply up to 90% of the protein entering the small intestine (Aldrich et al., 1993; Russell and Rychlik, 2001; Verbic, 2002).

Propionate and amino acids from ruminal fermentation are major precursors for glucose production by the liver through gluconeogenesis (Drackley et al., 2001). Glucose

is required for the synthesis of lactose, the primary osmotic determinant of milk volume (Bell and Bauman, 1997), which is present in milk at a relatively constant percentage. The most commonly accepted theory of changes in ruminal propionate altering metabolism in lactating cows is that increases in ruminal propionate production results in a greater supply of this glucogenic precursor to the liver, thereby increasing glucose production (Sauer et al., 1989). Propionate spares glucogenic amino acids in gluconeogenesis and consequently reduces the maintenance cost of metabolizable protein and possible heat increment (Soest, 1994). Propionate and amino acids have an estimated maximal contribution to gluconeogenesis of 32% to 73% and 10% to 30%, respectively, ; as amino acids are utilized for protein synthesis, the extent the glucose precursor is supplied by propionate is of critical importance in the ruminant metabolism (Steinhour and Bauman, 1986). This critical importance arises from the fact that in late pregnancy glucose requirements increase four- to seven-fold in lactating dairy cows vs. nonlactating dairy cows (Bell and Bauman, 1997), with most of this increase met by gluconeogenesis.

With the onset of lactation, the energy requirements for milk production and maintenance of the dairy cow exceed available energy from the diet due to limited feed intake. Lactating dairy cows experience a postpartum negative energy balance that reaches its nadir during the first or second week postpartum and recovers at a variable rate (DeVries et al., 1999; Stevenson, 2001; Butler, 2003; Jorritsma et al., 2003). Lucy (2001) points out that high milk production should not be confused with negative energy balance; the highest producing cows in the herd may not necessarily be those with the greatest negative energy balance. Greater DMI in high-producing cows compensated for

greater milk production, so that energy balance was similar in high- and low-producing cattle.

Nutritional deficiency due to lactation has major implications on reproduction. Postpartum dairy cows first partition metabolizable energy toward milk production, then body condition gain, and finally to reproductive functions (Silvia, 2003). The partitioning of energy among the various destination points in the body is highly regulated and appears to be influenced, to some extent, by the ability of the individual cow (Veerkamp, 1998) and the nature of the products of rumen fermentation, particularly the relative proportions of VFA (McDonald et al., 2002). Lucy (2003) suggested the first phase of postpartum reproduction is the recovery of the hypothalamus and pituitary from the effects of pregnancy and the resumption of FSH and LH production leading to establishment of the LH surge mechanism; the latter of which is affected by energy balance. Low producing cows with poor dry matter intake (DMI) were shown to be at greater risk for anestrous and infertility than were high producing cows (Staples et al., 1990) while cows with higher DMI were more likely to show signs of estrus at first ovulation and to become pregnant by day 150 of lactation (Westwood et al., 2002).

In the ruminant animal, feed digestion involves a number of complex interactions between different microbial populations. In production animals, the rumen microbes are typically comprised of 10^{10}-10^{11} bacteria, 10^{5}-10^{6} protozoa, and 10^{3}-10^{5} fungi, 10^{5}-10^{7} anaerobic mycoplasma, and $5x10^{7}$ bacteriophages per ml of ruminal content (Church, 1988; Weimer, 1998; Russell and Rychlik, 2001; McDonald et al., 2002), as well as other transient microorganisms. The microbial population of the rumen is not fixed; the diverse populations of microbes present at a specific time are those that have adapted to the

specific conditions of the rumen ecosystem and anything that affects this ecosystem will ultimately affect what and how nutrients are available to the cow for productive and reproductive purposes. A comparison of rumen microbes in a group of animals under the same dietary and environmental conditions will show a high level of variation (both quantitatively and qualitatively) from animal to animal (Church, 1988; Weimer et al., 1999). A number of factors influence this variation including: type of diet (Weimer et al., 1999), frequency of feeding (Dehority and Tirabasso, 2001; Obispo and Dehority, 2002), time and location of sampling (Church, 1988; van Gylswyk et al., 1992; Davidson, 1998), ruminal pH (Therion et al., 1982; Russell and Wilson, 1996; Dehority and Tirabasso, 2001; Russell and Rychlik, 2001) feed transition (Tajima et al., 2001), stress (Fluharty et al., 1994; Loerch and Fluharty, 1999; Verbic, 2002), substrate adherence (Mackie and White, 1990; Russell and Wilson, 1996), and microbial type-substrate interactions (Mackie and White, 1990; Weimer, 1996; Weimer et al., 1999; Chen and Weimer, 2001).

The desire to increase the production and reproductive efficiency of dairy cows along with growing societal concern over the use of antibiotics and growth promoters in the dairy industry have ruminant nutritionists, along with microbiologists, interested in ways to manipulate the rumen microbe community to improve the economics and safety of ruminant animal agriculture. Therefore, products that can successfully manipulate or control ruminal microbe populations and fermentation should be of interest to dairy producers. The purpose of the following literature review is to describe alterations that occur in the rumen when manipulated with direct-fed microbials, glucogenic precursors, ionophores or other mediators of metabolism and their effect on VFA and glucose

metabolism, which affects milk production, milk components, and reproductive efficiency in the dairy cow.

Chapter II

Review of Literature

Alteration of Rumen Fermentation

Direct Fed Microbials

Growing concern over the possible risk of residues appearing in milk from the use of antibiotics and/or growth stimulants, not only in feed, but also in the producing animal, has the dairy industry searching for new and innovative feed additives to meet the increased nutrient demand of today's high producing dairy cow. Direct-Fed Microbials (DFM) are naturally occurring products that have been incorporated into the animal diet in an attempt to enhance production performance, alter ruminal fermentation and improve nutrient utilization.

The U.S. Food and Drug Administration (FDA) defines Direct-Fed Microbial (DFM) as "products that are purported to contain live (viable) microorganisms (bacteria and/ or yeast)." Since 1989, the FDA has required the use of the expression "Direct-Fed Microbial" instead of "Probiotics." Microorganisms known as a Direct-Fed Microbial are listed in the Official Publication of the Association of American Feed Control Officials (AAFCO) and have been reviewed by the FDA. Section 36, Fermentation

Products, defines animal feed ingredients derived from spent fermentation processes, and Section 96, Yeast, defines yeast products. For regulatory purposes, DFM products are considered a subclass of fermentation or yeast products because they are similarly produced. The AAFCO statement reads, "…contains a source of live (viable) naturally occurring microorganisms…," followed by a listing of each of the microorganisms and the content guarantee, as cfu/gram (FDA, 2004).

DFM categories are divided into the following categories; 1) Bacillus, 2) Lactic Acid Bacteria, and 3) Yeasts (Fungal). Yeasts are further divided into six different types; 1) Primary Dried Yeast, 2) Active Dried Yeast, 3) Irradiated Dried Yeast, 4) Brewers Dried Yeast, 5) Torula or Candida Yeast, and 6) Yeast Culture. Active Dried Yeast is defined as yeast dried to preserve a large portion of its fermenting power, and it must contain not less than 15×10^9 live yeast cells/g. Yeast Culture is a dry product composed of yeast and the media on which it was grown, dried in such a manner as to preserve the fermenting capacity of the yeast, with the media stated on the label. Some yeast culture products contain live cells, whereas other yeast culture products do not guarantee any live yeast cells in their product.

A DFM can elicit responses in several ways. In theory, DFM preparations are meant to augment normal ruminal microbe functions making the rumen more nutritionally efficient or more resistant to stress induced diseases (Davidson, 1998). Yoon and Stern (1995) categorized DFM modes of actions into the following categories: 1) stimulation of desirable microbial growth in the rumen, 2) stabilization of rumen pH, 3) altered ruminal fermentation pattern and end product production, 4) increased nutrient flow postruminally, 5) increased nutrient digestibility, and 6) alleviation of stress through

enhanced immune response. A DFM usually stimulates growth of ruminal bacteria, which contrasts ionophores, which are usually fatal to their targeted bacteria. Weimer (1998) puts forth that rumen bacteria can be successfully manipulated if consistent with the principles of microbial ecology:

a) Adaptation for utilization of a given compound requires that the compound be present at a concentration at which utilization will provide a selective advantage to the organism.
b) Increasing the concentration of catalyst will not enhance the rate of a substrate-limited process.
c) In an established ecosystem, invading (allochthonous) microbes will generally not compete effectively with native (autochthonous) microbial species.
d) End products of metabolism from one group of microbes can often be utilized as energy sources or growth substrate for other groups of microbes.
e) For a given metabolic task, specialists will usually outcompete or outperform generalists.

Although DFM have been recognized for several years with positive responses reported in the literature, the exact microbial mode(s) of action is still not clearly understood.

Bacterial Direct-Fed Microbials

Genus *Propionibacterium*

Propionibacterium are classified as a Gram positive, non-spore forming, non-motile, usually pleomorphic bacteria ranging in size from .3 to 1.3 μm in diameter and 1 to 10 μm in length. The cells arrange themselves in various patterns revealing "Chinese letters," "birds- in flight" and "picket fence" structures. Fermentation products from glucose include combinations of propionic and acetic acids and frequently lesser amounts of isovaleric, formic, succinic or lactic acids and carbon dioxide (Moore and Holdeman, 1974). *Propionibacterium spp.* are slow growing, acid intolerant (Kung, 2001), anaerobic to aerotolerant organisms which grow best at 30-37^{0}C at a pH near 7.0 (Moore and Holdeman, 1974).

The genus *Propionibacterium* consist of two principal groups, cutaneous and classical (dairy), differing mainly in their natural habitats. Cutaneous species are considered opportunistic organisms and members of the microbial population of human skin and intestinal tract. They have been associated with infections in humans and have been isolated from the feces of humans and other vertebrate animals (Zarate et al., 2004). Included in this group are four species: *P. acnes, P. avidum, P. granulosum*, and *P. lymphophilium.* Classical or dairy *Propionibacterium spp.* are extensively used by the dairy-food industry as starter cultures for Swiss-type cheeses where they are responsible for the "eyes" or "holes" and flavor formation (Rossi et al., 1999; Frohlich-Wyder et al., 2002). Included in this group are four species: *P freudenreichii, P jensenii, P. acidipropionici, and P. thoenii. Propionibacterium* is among the main species used in the

commercial biosynthetic fermentation production of Vitamin B_{12}, which is required in animals and humans for two enzymes, (R) - methyl-malonyl CoA and (R)-methionine synthase. All *Propionibacterium* strains employed for vitamin B_{12} fermentation production are efficient and produce very high yields (Martens et al., 2002) and have a huge advantage as they are food-grade allowing vitamin B_{12} to be produced without elaborate processing (Hugenholtz et al., 2002). Other beneficial applications include the production of antimicrobial agents such as propionic acid, propionins (antiviral peptides) (Grinstead and Barefoot, 1992), and bacteriocins, used as preservatives in the food industry which can inhibit bacteria, yeasts, and molds (Jack et al., 1995; Holo et al., 2002; Hugenholtz et al., 2002). *Propionibacterium* strains are also employed as inoculants in silage and grain (Merry and Davies, 1999; McDonald et al., 2002; Filya et al., 2004).

Propionibacterium are natural inhabitants of the rumen that have been shown to comprise up to 1.4% of the total microbe population on a high concentrate diet (Oshio et al., 1987). *Propionibacterium spp.* were shown to constitute 4.3% of the bacteria population isolated from the rumen epithelium surface of sheep fed a high roughage diet (Mead and Jones, 1981). Davidson (1998) showed naturally occurring rumen populations of propionibacteria ranging from 10^3-10^4 cfu/ml with two strains predominant; *P. acidipropionici* (96%) and *P. jensenii* (4%). Population varied among cows at different sampling times. Three of the five cows used in the study had detectable propionibacteria populations at two of the three sampling times, one cow had detectable populations at all three sampling times and one cow had no detectable population at any sampling time.

Several studies report the use of propionibacteria as a DFM in the feedlot segment of the cattle industry; however, few studies report the use of propionibacteria as a DFM in dairy cattle. Although *Propionibacterium* are lactate utilizers, due to their slow growth and acid intolerance, the primary focus has been on using propionibacteria for propionate production as a DFM. For example, cannulated steers fed a high concentrate diet supplemented with of *P. acidipropionici* DH42 (DH42) or DH42 plus *Lactobacillus plantarum* MC2 (LAB) (a lactate producing bacteria) at increasing dosage levels (0, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$ cfu/hd/d), with each dose level fed for 7 d, did not affect total VFA concentrations among dose levels or treatments (Kim et al., 2001a). Only $1x10^7$ DH42 had significantly greater levels of acetate, during the post-test period than the pre-test period. For all dosage levels of DH42 + LAB, acetate levels were greater in the post-test period vs. the other treatment periods. DH42 propionate levels were greater at all dosage levels, numerically increasing as the dosage increased, with a tendency to decrease in the post-test period. DH42 decreased the acetate/propionate ratio at all dosage levels except $1x10^8$, and did not return to pre-test levels in the post-test period, while DH42 + LAB increased the acetate/propionate ratio as the dosage increased. The DH42 butyrate concentration decreased as the dosage levels increased and returned to near pre-test levels in the post-test period, while DH42 + LAB butyrate levels were greater after the pre-test period except at the $1x10^9$ level. Ruminal pH was not affected by DH42 or DH42 + (LAB) supplementation (Kim et al., 2001a). In support of the in vivo study, in vitro studies using *P. acidipropionici* at $4.7x10^{10}$ cfu, the acetate/propionate ratio was significantly reduced after 6 h of fermentation (trail 1) and after 24 h (trial 2) (Kim et al., 2001b).

In contrast, Ghorbani et al.(2002) fed cannulated steers a high concentrate diet supplemented at $1x10^9$ cfu/g at 10 g/hd/d with either *Propionibacterium* P15 (P15) or P15 plus *Enterococcus faecium* EF212 (LAB) (a lactate producing bacteria) for a 3 wk period (2 wk adaptation with 1 wk measurement). No effect on ruminal concentrations of total VFA, propionate, ruminal pH or acetate/propionate ratio was reported. Acetate concentration was greater for steers fed P15 + LAB than for steers fed P15 or control. Butyrate concentration was greater in steers fed P15 than steers fed P15 + LAB or control. Total protozoa count, identified as mainly *Entodinium*, for steers fed P15 was significantly greater than steers fed P15 + LAB or control. *Entodinium*, which tend to increase butyrate concentrations, may have contributed to higher butyrate levels.

A four-unit dual effluent continuous culture system was used to evaluate the effects of P15 as a DFM on fermentation (Yang et al., 2004). Treatments were 1) control (no DFM); 2) P15 (PB) ($1x10^9$ cfu/g fed at 10 g/hd/d); 3) *Enterococcus faecium* EF212 (EF) ($6x10^8$ cfu/g/d at 10 g/d); or 4) EF plus *Saccharomyces cervevisiae* (yeast) (EFY) ($6x10^8$ cfu/g/d at 10 g/d). Innoculant for the fermenters was obtained 1 h after feeding ruminally fistulated steers the control diet. The control diet was dried, ground, and manually provided to each fermenter in two equal portions involving a 5 d stabilization period followed by 3 d sample collection. The DFM supplements were provided twice daily just prior to the diet. No significant difference between treatments for pH, acetate, propionate, butyrate or total VFA concentration, or acetate/propionate ratio was reported. The lack of effect on VFA numbers follows the lack of effect of treatments on digestibility in continuous culture system fermenter fluid (Yang et al., 2004). Counts of total bacteria in fermenter fluid taken directly from filtrate lines at 3 h after feed

provision tended to be higher for Control and PB than for EF or EFY. Surprisingly, lactate-utilizing bacterial numbers were higher for Control and EF than for PB or EFY. In contrast to Ghorbani et al.(2002), no protozoa were detected in the continuous culture system fermenter fluid (Yang et al., 2004), indicating its limited potential for extrapolation of results to in vivo conditions (Ziemer et al., 2000). However, Akay and Dado (2001), using in vitro fermentations with *Propionibacterium* P15 (PB) at (0, $1x10^3$, $1x10^6$, and $1x10^9$ cfu/g) showed a quadratic increase in total VFA, acetate and propionate concentrations with a tendency for butyrate to increase at a quadratic rate. When PB was autoclaved and compared to PB, a significant decrease in total VFA, acetate and propionate was observed suggesting that PB was the likely cause for the changes in VFA concentrations.

Propionibacterium jensenii strain 169 (P169) fed daily to multiparous dairy cows from 2 wk prepartum to 12 wk postpartum had no effect on milk production or milk fat and lactose percentage (Francisco et al., 2002). At wk 1 of lactation, cows fed P169 had a greater percentage of milk protein and solids-not-fat and plasma NEFA concentrations than did control cows. Body weight and plasma leptin concentrations tended to be greater in treatment cows while plasma glucose, insulin, and cholesterol concentrations were not affected by feeding P169. Average days to first and second ovulation as well as postpartum follicular growth did not differ between control and P169 treated cows (Francisco et al., 2002). This study provided the first evaluation of the use of Propionibacteria in dairy cattle and indicates supplemental feeding of P169 may alter some aspects of metabolism during lactation. Clearly further studies regarding the use of P169 in dairy cattle are warranted.

Nonbacterial Direct-Fed Microbials

Genus *Saccharomyces*

Nonbacterial DFM added to the diets of ruminants predominately consist of two main supplements: *Aspergillus oryzae*, a mold classified as a fungus, and *Saccharomyces cerevisiae*, a fungus, classified as yeast. Yeasts are nonfilamentous, unicellular fungi that are typically spherical or oval, ranging in size from 5 μm to 10 μm. Yeasts are Eukaryotic organisms and their properties differ from those of bacteria, which are Prokaryotic organisms. The baking and brewing yeast, *S. cerevisiae,* is the most well known species and is usually supplemented as either an "active dry yeast" or "yeast culture" product. Yeast cells have survived in the rumen from 12 h (Arambel and Kent, 1990) up to 45 h (Kung et al., 1997). Though yeasts have been shown not to multiply substantially in rumen fluid (Kung et al., 1997; Auclair, 2000), the increase in ruminal microbial numbers seems to be central to the action of the yeast in ruminants (Auclair, 2000).

Various studies have reported the ability of yeast supplementation to stimulate the population of specific groups of bacteria in the ruminant. Dairy cows supplemented with yeast culture (114 g/day) had decreased molar proportions of acetate, ruminal pH, and acetate/propionate ratio while molar proportions of propionate and cellulolytic bacteria numbers increased (Harrison et al., 1988). Yeast culture supplement fed to buffalo calves increased total bacteria, total viable bacteria and cellulolytic bacteria, 41.0%, 33.5%, and 57.4%, respectively. Total concentration of VFA, acetate, and acetate/propionate ratio

were also increased, while propionate concentrations were not affected (Kumar et al., 1997). Dawson et al. (1990) evaluating the effects of two feed supplements containing defined levels of live yeast and live yeast plus bacteria (lactobacilli) on the concentration and activity of ruminal bacteria in vitro and in vivo, found the feed supplement containing yeast and bacteria increased concentration of lactobacilli in vitro but not in vivo. In the same study, live yeast culture was compared to yeast culture supplement that had been autoclaved. Concentrations of cellulolytic and anaerobic bacteria were greater in live yeast culture along with a greater propionate concentration and decreased acetate/propionate ratio (Dawson et al., 1990).

Yeast culture was shown to provide soluble growth factors (organic acids, B vitamins, and amino acids) that stimulate in vitro growth of ruminal bacteria, that utilize lactate (*Selenomonas ruminantium* and *Megasphaera elsdenii)*, and digest cellulose (*Fibrobacter succinogenes* and *Ruminococcus albus)* (Callaway and Martin, 1997). Addition of 5% yeast culture filtrate to *S. ruminantium* increased acetate, proipionate and total VFA concentrations while no changes in acetate or propionate concentrations were seen in *M elsdenni*, while no changes in butyrate were seen in either culture. When *F. succinogenes* and *S. ruminantium* were incubated with cellulose and 5% yeast culture filtrate, cellulose disappearance increased as much as 11% in both cultures after 24h. An earlier study (Nisbet and Martin, 1991), showed a decrease in the acetate/propionate ratio due to a greater increase in propionate than acetate when *S. ruminantium* was treated with a yeast culture filtrate. Soto-Cruz et al. (2001) reported an increase in butyrate with no change in acetate or propionate with *M. elsdenni* in a continuous culture supplemented with yeast culture filtrate. Peptide fragments purified from *S. cerevisiae* were recently shown to

stimulate growth and lactate utilization and butyrate production in *M. elsdenni* (Rossi et al., 2004). These stimulatory peptides seem to be very unstable in the rumen environment, but seem to be continually supplied by metabolically active yeast to provide a low level of stimulation for beneficial rumen bacteria (Dawson, 2002). In a comparison of growth rates of ruminal bacteria from cattle and sheep, van Gylswyk et al. (1992) acknowledged that besides energy and nitrogen, constituents present in yeast extract were primarily responsible for the slow growth of specific bacteria species normally present in lower numbers in the rumen. Thus, the proportion of different bacterial species in the rumen may be directly dependent on the concentrations of specific growth factors or peptides.

Metabolic activity and not active reproduction of yeast was shown to be a vital part of the basic process leading to the beneficial response to yeast supplementation (Dawson et al., 1990). Koul et.al. (1998) fed a diet containing yeast culture cells ($5x10^9$ cfu/g) treated by autoclaving and γ-irradiation to buffalo calves (5 g/hd/d). The γ-irradiation yeast cells could no longer reproduce, but were metabolically active and increased ruminal pH, concentrations of total viable and cellulolytic bacteria, and total VFA concentrations. In contrast, feeding autoclaved yeast had no significant effect on these variables.

To evaluate *S. cerevisiae* as a live yeast (114 g/d, 60b live cfu) product or yeast culture product (114 g/d): milk yield, milk components, and ruminal parameters were compared on lactating cows (100 DIM) supplemented with *S. cerevisiae* (Higginbotham et al., 2001). Milk yield, 3.5% FCM, percentage of milk fat, protein, lactose, and SNF, rumen pH, and total VFA concentrations were unaffected by feeding *S. cerevisiae* ;

suggesting that similar responses can be realized by feeding either live yeast or yeast culture products. Lynch and Martin (2002) examined in vitro effects of *S. cerevisiae* as a live yeast or yeast culture product at two concentrations (0.35 and 0.73 g/L) at 24 h and 48 h on mixed ruminal microorganism fermentation in the presence of ground corn, soluble starch, alfalfa hay and Coastal bermudagrass hay. The live yeast contained 1000-fold greater yeast cell population than the culture supplement. Both concentrations of yeast culture lowered ruminal pH at 48 h, while both concentrations of live yeast increased ruminal pH. At 48 h, both concentrations of yeast culture and live yeast, increased acetate, propionate, butyrate, and decreased the acetate/propionate ratio. Thus, in vitro, live yeast or yeast culture products have similar effects on mixed ruminal microorganism fermentation.

The potential for different strains of yeast to stimulate growth of ruminal bacteria may be related to their ability to remove oxygen from the rumen fluid, as oxygen is detrimental to anaerobic bacteria in the rumen. Low concentrations of oxygen have been detected in the rumen, possibly entering via feed and saliva as the animal eats (Auclair, 2000). Bacterial growth is inhibited as is the adhesion of cellulolytic bacteria to substrate (Rode, 2002). Oxygen uptake of *S. cerevisiae* is several orders of magnitude greater than of rumen fluid. Respiration-deficient mutants of *S. cerevisiae*, failed to stimulate bacterial numbers, while the corresponding parent strains were beneficial for total and cellulolytic bacterial growth (Newbold et al., 1996). It is conceivable that stimulation of rumen bacteria is partly dependent on the respiratory activity of *S. cerevisiae*.

Dawson and Hopkins (1991) tested over 50 strains of *S. cerevisiae* and found only seven with the ability to stimulate growth of fiber-digesting bacteria. In a continuous

culture study using inocula from cannulated cows fed a corn silage diet comparing two different commercial yeast culture products (YC1 and YC2); Miller-Webster et al. (2002) found YC1 increased molar percentages of propionic acid, reduced acetic acid and lowered acetate/propionate ratio and mean nadir pH compared with YC2. Thus, indicating that specific yeast cultures may have different modes of action. Both YC1 and YC2 increased DM digestion, propionic acid production and protein digestion, and decreased acetic acid; indicating an influence of yeast on microbial metabolism. Newbold et al. (1995) suggested that different strains of *S. cerevisiae* alter rumen dynamics. Ruminal fermentation of four different strains of *S. cerevisiae*, NCYC 240, NCYC 694, NCYC 1088, and NCYC 1026, at 500 mg/d were evaluated in vitro and in vivo (in sheep). Ruminal pH, concentrations of acetate, propionate, or butyrate was not affected by treatment either in vitro or in vivo. However, in vivo, NCYC 1026 (a commercial product) and NCYC 240 significantly increased total and cellulolytic bacterial numbers; while in vivo, only NCYC 1026 significantly increased total bacterial numbers and NCYC 240 significantly increased cellulolytic bacterial numbers.

Studies have shown that yeast culture supplementation affects ruminal fermentation resulting in possible beneficial changes in digestion. Increases in dry matter intake were shown by yeast supplementation (Williams et al., 1990; Wohlt et al., 1991; 1998; Erasmus et al., 1992; Putman et al., 1997; Robinson and Garret, 1999; Dann et al., 2000). In contrast, Doreau and Jouany (1998) found no change in total and ruminal digestibility, duodenal flows of nonmicrobial and microbial N, pH, and VFA concentrations in cows supplemented with *S. cerevisiae* ($6x10^8$ x cfu/g at 50 g/hd/d) .

Improvements in milk production or FCM, after feeding live yeast or yeast culture, have been reported in several studies (Williams et al., 1990; Wohlt et al., 1991; 1998; Erasmus et al., 1992; Piva et al., 1993; Putman et al., 1997; Zhou, 2002; Kujawa, 2003). However, other studies have had no significant response of milk production to yeast supplementation (Erdman and Sharma, 1989;Arambel and Kent, 1990; Swartz et al., 1994; Kung et al., 1997; Dann et al., 2000; Kujawa, 2003;). Robinson and Garret (1999) found a tendency for increased milk production in primiparous cows but not multiparous cows. Most of the studies that have shown increases in milk production have also shown increases in DMI of the animals.

Milk component response to *S. cerevisiae* supplementation has also varied. Several studies have reported no significant changes in milk fat, lactose, protein, and SNF percentages (Erdman and Sharma, 1989; Arambel and Kent, 1990; Swartz et al., 1994; Kung et al., 1997; Dann et al., 2000) and SCC (Dann et al., 2000). Studies by Piva et al. (1993) and Putman et al. (1997) have reported increases in milk fat percentages whereas others, (Williams et al., 1990) have reported a decrease. In studies where increases (Williams et al., 1990; Wohlt et al., 1991) or decreases (Zhou, 2002) in milk protein percentages have been reported, no other milk components are affected.

The variability between findings of yeast supplementation in the diet of dairy cows in all probability is not due to one specific mode of action. A combination of factors which may include differences in experimental conditions including strain of yeast, age of the animal, stage of lactation, diet composition, amount and duration of yeast fed, environmental conditions (i.e., stress), or other dietary or animal factors may influence mode(s) of action.

Ionophores

Monensin and Lasalocid

Ionophores can be described as highly lipophillic compounds that facilitate the channeling of ions across a biological barrier (i.e., bacterial cell membrane composed of lipid bilayers) by combining with the ion (mobile carriers) or by increasing the permeability of the barrier (channel or pore forming). The exterior of the ionophore molecule is hydrophobic while the interior, which is able to bind to the ion, is hydrophilic. Ionophores shield the charge of the ion being transported, which aids in movement across the cell membrane. The backbone of the ionophore, which contains oxygen-containing heterocyclic rings, forms a torus-shape (Chow et al., 1994) into which the ion (monovalent or divalent) may fit. This "conformational cavity" contains ligand oxygens consisting of various functional groups (i.e., ether, alcohol, carboxyl, and amide) which bind the ion through ion-dipole interactions (Pressman, 1976). Once in the membrane, the ionophore-ion complex is driven by ion gradients and ionophore affinities. As the bound ion dissociates and is released from the ionophore, a proton is allowed to be picked up by the ionophore and the system proceeds in the reverse direction (Russell and Strobel, 1989). This extracellular/intracellular transport of ions can occur at a rate of over 1000 reactions per second (Pressman, 1976).

Ionophores used for feed additives perform as mobile carriers (Russell and Strobel, 1989) and are classified as carboxylic polyether ionophore antibiotics (Bergen and Bates, 1984). Within this group, some ionophores are classified as antiporters, which are able to

combine more than one cation, (i.e., exchange H^+ cations for monovalent cations); where as others are classified as uniporters, which bind only a single cation, (i.e., transport cations into the cell with no exchange for H^+) (Russell and Strobel, 1989; Ipharraguerre and Clark, 2003).

Ionophores do not exhibit the same affinity for all cations. Monensin, produced from *Streptomyces cinnamonensis* (Duffield and Bagg, 2000) and classified as a monovalent antiporter, has a 10 times higher affinity for Na^+ than K^+ (Pressman, 1976). Lasalocid, more lipophilic than monensin (Chow and Russell, 1992), produced from *Streptomyces lasaliensis* and classified as divalent antiporter, possesses a 3 to 10 time higher affinity for K^+ than Na^+ (McGuffey et al., 2001); with Ca^{++} affinity comparable to Na^+ (Bergen and Bates, 1984).

The prevalent intracellular cation of an organism is K^+, with the concentration of K^+ in the rumen being four to five-fold lower than that of Na^+ (Russell and Strobel, 1989). Ionophores transport ions across the cell membranes of susceptible bacteria resulting in reduced intracellular K^+, increased intracellular Na^+ and H^+, and lower cell pH. The implication of these actions depends on the sensitivity of the bacteria to ionophores. Gram-negative bacteria have a complex impermeable outer cell membrane and are usually more resistant to ionophores than gram-positive bacteria (Duffield, 2001; McGuffey et al., 2001; Ipharraguerre and Clark, 2003). However, bacteria that stain gram-negative can still show susceptibility to ionophores (Russell and Strobel, 1989). Ionophore sensitive bacteria will strive to maintain inner cell balance and use energy to expel the intracellular H^+ (i.e., ATP-ase pumps use 1 ATP per proton). This reduction in ATP (energy reserves) results in reduced cell division for growth and reproduction or cell

death (Duffield, 2001; McGuffey et al., 2001; Ipharraguerre and Clark, 2003). Reduction in susceptible gram-positive bacteria involved in fermentation processes that produce end products including, but not limited to, acetate, butyrate, lactate, hydrogen, and ammonia allows for the proliferation of non-susceptible gram-negative bacteria in the rumen associated with the production of propionate and succinate (Duffield and Bagg, 2000; Ipharraguerre and Clark, 2003). Such shifts in fermentation patterns would be expected to provide more metabolizable energy to the animal to be used for production purposes (Dawson, 2002).

Bergen and Bates (1984) list three major areas of metabolism that influence the improved efficiency of production with the use of ionophores. These include 1) increased efficiency of energy metabolism, 2) improved nitrogen metabolism in the rumen and 3) retardation of feedlot disorders, especially lactic acidosis and bloat. Probable modes of action of monensin have been summarized by Schelling (1984) and include modification of acid production and protein utilization, altered feed intake and digestibility, change in gas production, modified rumen fill and rate of passage, and other ruminal modes of action.

Although monensin and lasalocid are not presently licensed in the United States for use in lactating dairy cows, several studies have evaluated the effects of ionophores in lactating dairy animals. Results of treatment with monensin in dairy cows have not been consistent in the literature as to milk yield or milk component response and may be diet related. Ipharraguerre and Clark (2003) reviewed the use of ionophores, (sodium monensin), at doses of 80 to 350 mg/day, and found monensin did not affect (18 studies) or increased (14 studies) milk production by 2.6% (0.4 kg/d) to 11.2% (2.8 kg/d) with an

average of 7% (1.5 kg/d) above controls. The fat content of milk was either significantly (10 studies) or numerically decreased (20 studies) an average of 4.5% (1.9 g/kg) where cows were treated with 350 mg of monensin. Milk protein content of monensin-treated cows varied with a tendency to be lower than control; (ranging from a 0.7% increase to a 1.6% decrease) however, a significant decrease was reported in only three studies. In several of the experiments reviewed, milk fat and protein coincided, for the most part, with increased milk yield and suggests a dilution effect for the mechanism involved in decreasing milk fat and protein percentages (Ipharraguerre and Clark, 2003). McGuffey et al., (2001) reviewing the use of monensin (300mg/d from 12 to 44 wk) reported similar results as Ipharraguerre and Clark, (2003): milk yield increased an average of 1.3 kg/d, while milk fat and protein tended to decrease 0.05% and 0.01%, respectively.

Monensin supplemented in an alfalfa silage diet at 248 mg/d to multiparous lactating dairy cows (53 DIM and 39 kg/d of milk) reduced 3.5% FCM production, milk fat and protein percentage, SNF (tendency), acetate and butyrate concentrations and acetate/propionate ratio while increasing blood glucose levels and propionate concentrations (Broderick, 2004). In multiparous cows (35 DIM), milk yields were significantly increased when fed monensin at 150 mg/d and 300 mg/d but not 450 mg/d (Phipps et al., 2000). Milk fat and protein percentages were reduced at all treatment levels with no significant change in DMI. When monensin was fed at 300 mg/d for two consecutive lactation periods, milk yields were not affected by monensin whereas both milk fat and protein percentages were reduced (Phipps et al., 2000). In an earlier study by Sauer (1989), the addition of monensin at 15 and 30 g/ton from 2 wk prepartum to 3 wk postpartum had no effect on milk yield, milk protein or lactose concentrations, but

lowered milk fat at 15 g/ton level. In addition, molar proportions of acetate and butyrate in rumen fluid were decreased while molar percentages of propionate were increased thus, decreasing the acetate/propionate ratio.

Van Der Werf et al., (1998) evaluated the effect of 300 mg/d of monensin over two lactation periods in Jersey and Holstein cows and found that Jersey cows showed significantly lower milk production responses to monensin than Holstein cows, while Holstein cows with high breeding values for protein and fat showed greater milk production responses than those with lower breeding values. Milk fat percentages tended to decrease in Jersey and high-genetic Holstein cows with a tendency to increase in low genetic Holstein cows, while milk protein was not affected by monensin. The response to monensin on milk production for the first lactation showed a significant increase, (7% over control) while the second lactation increase was not significant, (4% over control).

McGuffey et al. (2001) reviewed the use of monensin controlled release capsule (MCRC), a device that delivers 335 mg of monensin per day for a 95 d period, reporting an average increase in milk yield of 1.1 L/d (6.1%) and protein yield of .03 kg/d (0.05%), with no change in milk fat yield. In a Canadian study (Duffield et al., 1999), involving over 1000 cows from 25 farms, MCRC boluses were administered to 503 cows (3 wk prior to calving) and 507 cows received placebos. A significant interaction between monensin and body condition score (BCS) of the cows (3 wk prior to calving) affected the first 90 d milk production. Monensin had no effect on milk production in cows classified as thin (BCS of ≤ 3) and increased milk production in cows classified in good body condition (BCS of ≤ 3.25-3.75) and as fat (BCS of ≥ 4). Monensin had no significant affect on milk fat or milk protein percentages (Duffield et al., 1999).

An Australian study (Beckett et al., 1998) involving over 1100 cows in 12 herds, a MCRC bolus administered 40 d prior to calving and at 50 postpartum reported that monensin increased milk production, although effects were different among the 12 herds. No significant differences were observed in milk fat or protein percentages or reproductive performance (i.e., days to first observed estrus, service and conception) (Beckett et al., 1998).

McGuffey et al. (2001) reviewed the use of lasalocid at 300 mg/d lasting from 10 to 17 wk and found no effect on milk yield or percentage of milk fat or protein compared to control. Similar results were reported by Erasmus et al. (1999) when lasalocid was supplemented at 0, 10, or 20 mg/kg in an alfalfa-based diet fed to multiparous and primiparous cows (2 wk prepartum to wk 17 postpartum) where DMI decreased linearly without affecting milk production or percentages of milk fat or milk protein. However, increasing lasalocid supplementation caused a significant linear decrease in MUN levels. Primiparous cows had increased BCS and increased feed efficiency (Erasmus et al., 1999). Weiss and Amiet (1990) also reported similar affects on milk production, where primiparous and multiparous cows (170 DIM) were fed a high roughage diet supplemented with 340 mg/d of lasalocid for 14 wk. Lasalocid did not affect milk yield or milk components however, DMI was slightly lower in treatment animals. Rumen samples taken on d 0, 7, 28, and 98 of the trial revealed on d 7 of treatment, lasalocid treated cows had lower ruminal acetate percentage and greater ruminal propionate percentage than control; no differences in VFA were observed on d 0, 28, and 98 of the study.

A two compartment glucose kinetics study (28 to 14d prepartum) to evaluate the singular and combined effects of somatotropin and monensin using U-^{13}C-labeled glucose, with treatments of control, bovine somatotropin (bST), 300 mg monensin, or bST plus monensin showed that treatment with 300 mg of monensin, increased glucose mass, distribution space and residence time in compartment one. Treatment with bST decreased those parameters, suggesting monensin may increase propionate supply and bST facilitates glucose flow into organs consuming glucose (Arieli et al., 2001). However, a study with the previously described treatments to d 63 of lactation, showed milk yield and milk components were not affected by any treatment (Vallimont et al., 2001).

VFA and Glucose Metabolism

The end products of microbial fermentation, volatile fatty acids (VFA) (primarily acetic, propionic, and butyric) are utilized by the host animal (Mackie and White, 1990). The VFA produced in the rumen has two outcomes: absorption through the rumen epithelium or passage from the rumen with the liquid phase, where as much as 24% may escape to the omasum and abomasum (McDonald et al., 2002). Dairy cattle fed a 60:40 concentrate/roughage diet produced volatile fatty acids near the following proportions: 71% acetate, 21% propionate, and 8% butyrate while dairy cattle fed a 90:10 concentrate/roughage diet produced a volatile fatty acid proportions near 55% acetate, 40% propionate, and 5% butyrate (Oshio et al., 1987; Sutton et al., 2003). When the acetate/propionate ratio decreases, methane production declines, and energy retention by cattle increase. The efficiency of utilization for maintenance of acetic acid, propionic

acid, and butyric acid is 0.59, 0.86, and 0.76, respectively (McDonald et al., 2002). The volatile fatty acids: acetate, propionate, and butyrate can supply up to 70 to 80% of a ruminant's energy requirements. The theoretically efficiency of propionate as a source of energy for ATP/100 g nutrient is 108% compared with glucose (McDonald et al., 2002).

With the onset of lactation, most dairy cows are not able to meet the energy requirements for maintenance and milk production from the diet due to inadequate feed intake and experience a postpartum negative energy balance (DeVries et al., 1999; Stevenson, 2001; Butler, 2003; Jorritsma et al., 2003). Glucose is required for the synthesis of lactose, the primary osmotic determinant of milk volume. Glucose requirements of superior lactating dairy cows may increase four- to seven-fold relative to nonpregnant or nonlactating cows (Bell and Bauman, 1997), with the lactating mammary gland accounting for up to 85% of whole body glucose supply (Bauman and Elliot, 1983). Dry matter intake does not increase as fast as the cow's glucose demand with days postpartum and thus positive energy balance is not achieved until 71 d to 95 d postpartum (DeVries et al., 1999; Coffey et al., 2002).

Cows with similar milk production fed the same amount of dietary energy may face different levels of actual negative energy balance; as the partitioning of energy among various destination points (i.e., maintenance, fertility or immunity) in the body is highly variable and appears to be influenced, to some extent, by the ability of the individual cow (Veerkamp, 1998) and the nature of the products of rumen fermentation, particularly the relative proportions of VFA (McDonald et al., 2002). Partitioning may involve two levels of regulation, homeostasis and homeorhesis (Bell and Bauman, 1997). Homeostatic control involves maintenance of physiological equilibrium or constancy of

environmental conditions with the animal. Homeorhesis is the orchestrated or coordinated control in metabolism of body tissues necessary to support a physiological state (Bauman and Currie, 1980). The liver is a central metabolic junction that plays a major role in the moderation, coordination, and distribution of nutrient flow to peripheral tissue (Seal and Reynolds, 1993). The nutrients removed by the liver or supplied by the liver depends on several factors: the mass of the liver, the rate of metabolism per unit mass of liver tissue, rates of blood flow supplies (arterial and portal), and the rate of transfer across cell membranes. (Drackley et al., 2001).

Reynolds et al. (2000b) measured liver mass in cows slaughtered at late gestation (7 d prepartum / 8.8 kg) or early lactation (22 d postpartum / 9.6 kg), and reported a 38% increase in DMI only increased liver mass by 9%; suggesting that increased liver mass only accounted for a small percentage of increased liver metabolism. Reynolds et al. (2000a) also reported that when DMI increased 44%, between 11 d prepartum to 11 d postpartum, hepatic blood flow increased 84 % while oxygen utilization increased 95%. The daily metabolic activity per gram of liver tissue doubles from 11 d prepartum to 11 d postpartum suggesting that the increased blood flow is a response to, and not a cause of, greater metabolic activity of the liver (Drackley et al., 2001). This supports earlier work by Overton et al. (1998) showing increased hepatic metabolic activity, where propionate conversion to glucose by liver slices was 19 and 29% greater at d 1 and d 21 postpartum, respectively, than at d 21 prepartum. Thus, the capacity of the liver to convert propionate to glucose seems to be responsive to propionate supply. Propionate supplied over 43% of the carbon for gluconeogenesis in steers fed a control diet (basal treatment), however,

when sodium propionate was administered (increasing propionate supply) carbon supplied by propionate increased to over 67% (Knapp et al., 1992).

Propionate and amino acids from ruminal fermentation are major precursors for glucose production by the liver through gluconeogenesis (Drackley et al., 2001). In ruminants, the discrepancy between predicted glucose from DMI and estimated glucose demand for production must be met by gluconeogenesis in the liver (Drackley et al., 2001). Reynolds et al. (2003) gives evidence of the increased contribution of lactate and alanine to glucose synthesis in the first days of lactation with this contribution declining as lactation progresses. Propionate spares glucogenic amino acids in gluconeogenesis and consequently reduces the maintenance cost of metabolizable protein and possible heat increment (Soest, 1994). In support of the former statement, circulating plasma free amino acid concentrations are significantly increased by intraruminal propionic acid infusion (Seal and Parker, 1995).

The usual pathways of propionate production from pyruvate by rumen bacteria are the succinate and acrylate pathways (Baldwin and Allison, 1983), accounting for approximately 70% and 30% of propionate production, respectively. Propionate is absorbed through the ruminal epithelium into the portal venous blood where it is preferentially and almost quantitatively removed by the liver (Bergman, 1990). As mentioned earlier, not all of the VFA produced in the rumen passes through the ruminal epithelium. Peters et al. (1990a) reported that propionic acid absorption and passage in beef steers, remains proportional across increased levels of production at 66% and 34%, respectively, thus the digestive tract distal to the rumen is an important site of propionate absorption. Further studies by Peters et al. (1990b) suggest that of the propionate exiting

the rumen, 93% to 97% was removed prior to entry into the duodenum and proposed the abomasum may be the site responsible for this disappearance. Likewise, not all propionate taken up by the liver is used to produce glucose (Bergman, 1990; Gabel et al., 2002). Propionate and amino acids have an estimated maximal contribution to gluconeogenesis of 32% to 73% and 10% to 30%, respectively (Seal and Reynolds, 1993).

As stated earlier, glucose is critical for the synthesis of milk lactose. An increase of its production rate in situations where the dietary supply of glucose is limiting, can be increased by increasing gluconeogenesis by enhancing the production of propionate, the only VFA that can be a major source of glucose (Bergman, 1990), or by increasing the postruminal supply of glucose (Hurtaud et al., 2000). Treatments involving infusing glucose in the duodenum and propionic acid (C3) in the rumen of dairy cows for 14 d increased raw milk production, milk protein and lactose percentages, while substantially decreasing milk fat percentage (Rigout et al., 2003). Results from (C3) infusion in the literature also were shown to lead to an increase in milk production and percent milk protein and a decrease in percentage milk fat (Rigout et al., 2003). Propionate (C3) infusion was also shown to alter ruminal VFA composition, increasing propionic acid and decreasing acetic and butyric acid (Rigout et al., 2003). Infusion of starch abomasally or ruminally for 14 d tended to increase milk yields, while infusion of starch abomasally tended to increase milk lactose and milk protein (Knowlton et al., 1998). Jugular infusion of glucose at two levels (low and high) for 11 d had no effect on milk production or milk fat, but high levels of glucose increased milk protein percentage (Amaral et al., 1990). Treatments comparing responses of early and mid-lactation cows to intraruminal

infusion with sodium propionate at six different concentrations lowered concentrations of plasma glucose and plasma insulin in early vs. mid-lactation cows (Oba and Allen, 2003). Ruminal acetate, propionate, and butyrate percentages decreased, increased and decreased, respectively, at a linear rate as concentration of sodium propionate was increased (Oba and Allen, 2003). Multiparous Holstein cows fed an energy supplement containing 78.43% propionic acid from 3 wk prepartum to 3 wk postpartum, showed a decrease in milk fat percentage with no change in other milk components, somatic cell count, or milk yield (Mandebvu et al., 2003).

Propylene glycol (PPG), used in the treatment of ketosis in dairy cows, is predominately metabolized in the rumen, increasing the proportion of ruminal propionate and decreasing acetate/propionate ratio (Nielsen and Ingvarsten, 2004). The remaining PPG is absorbed, without alteration, and enters gluconeogenesis via pyruvate (Nielsen and Ingvarsten, 2004). Administration of 500 mL PPG as an oral drench for the first 3 d postpartum, increased plasma glucose and liver glycogen concentrations the first 3 wk of lactation, but did not affect milk yield or milk components (Pickett et al., 2003). Nielsen and Ingvarsten (2004) reviewed the use of PPG from 2 wk prepartum to 8 wk postpartum with dosage levels ranging from 200 to 1000 g/d and reported no significant effects on milk yield, milk composition, and DMI. However, PPG did increase glucose and insulin levels, with physiological responses varying between trials. Juchem et al. (2004) also reported an increase in blood glucose and insulin concentrations, but only during the prepartum period when PPG was administered for 21 d prior to calving, with no affect on milk yield or milk components during the first 9 wk of lactation.

Because butyrate tends to inhibit hepatic propionate utilization and conversion to glucose (Bergman, 1990), milk production or its components could be altered with changes in ruminal butyrate. The infusion of butyric acid into the rumen had no affect on milk yield, but increased milk fat and protein while decreasing milk lactose and plasma glucose concentrations (Huhtanen et al., 1993). The decrease in plasma glucose was associated with a trend toward higher plasma urea concentrations suggesting that more amino acids were used for gluconeogenesis as the supply of propionate decreased and that of butyrate increased (Huhtanen et al., 1993). Similarly, Miettinen and Huhtanen (1996) reported the replacement of propionate with increased butyrate concentrations, decreased milk yield along with milk lactose and blood glucose levels, while increasing levels of milk fat.

Bovine Somatotropin

Bovine Somatotropin (bST) is a natural protein hormone found in cattle, synthesized and secreted from somatotroph cells in the anterior pituitary. Recombinant bST, first produced in 1982 and the first recombinant peptide approved for production use in domestic animals (Collier et al., 2001),varies from natural somatotropin only in the number of amino acid attached to the end of the bST molecule. Biological activity, however, is the same as natural somatotropin (Bauman, 1992). Since final approval by the FDA in 1993, recombinant bST has been commercially available to the U.S. dairy industry (Hartwig and Webber, 1993). A 21 state study by the National Animal Health Monitoring System (NAHMS) assessing the use of bST in dairy herds (USDA, 2003), found 15.2% of the participating dairy herds used bST in 2002 (compared to 9.4% in

1996). Over 54% of large herds (> 500 cows), 32% of medium herds (100-499 cows), while less than 9% of small herds (< 100 cows) used bST.

Milk production response to bST treatment are typically increases of 10 – 15%, but have been shown to vary due to stage of lactation, parity, genetic potential, plane of nutrition, and level of management (Thomas et al., 1991; Bauman, 1992; Hartwig and Webber, 1993; Etherton and Bauman, 1998; Tarazon-Herrera and Huber, 2000; Collier et al., 2001). Depending on the milk response and energy density of the diet, cows generally adjust their voluntary feed intake to support this increase in milk production (Bauman, 1992). Response to bST administered in early lactation (prior to peak yield) is minimal compared to administration in later lactation (Bauman, 1992). Milk production gradually increases after administration of bST, reaching maximum about day six (Peel and Bauman, 1987) and gradually returns to pretreatment levels if bST treatment ceases (Etherton and Bauman, 1998). When bST was administered to lactating dairy cows for four consecutive lactations, treatment with bST in previous lactations did not affect milk yields or milk yield responses to bST in subsequent lactations (Huber et al., 1997). Body condition score at the time of bST treatment influenced milk yield response (Santos et al., 2004): cows with BCS (≤ 2.75) had a 1 kg/d increase in milk yield over 110 d period, while cows with BCS (≥ 3.0) had a 3.5 kg/d increase over the same period. Treatment with bST shifts the lactation curve to the right (i.e., increasing persistence of lactation) (Bauman, 1992; Luna-Dominguez et al., 2000). With the increase in lactational persistency comes the opportunity for the producer to extend the calving interval, a possible management tool to maximize productivity and profitability of the dairy herd (Marsh et al., 1988; Galton, 1997). Moreira et al. (2000a) reported that when estrus

detection is eliminated by use of a timed AI (TAI) program, bST-treated cows had increased pregnancy rates to first service TAI. Moreira et al. (2002b) also reported administration of bST, at the time of insemination of superovulated cows, enhanced embryonic development and increased pregnancy rates of lactating recipient dairy cows following embryo transfer. Similar findings of bST reducing embryonic mortality were reported by Santos et al., (2004). Thus, there seems to be no inherent negative (or positive) effects of bST treatment on reproduction parameters (i.e., days open, fetal loss, cystic ovaries, gestation length) other than those associated with increased milk production and negative energy balance (Lucy, 2000; Collier et al., 2001). However, Santos et al. (2004) reported bST tended to reduce the percentage of multiparous cows detected in estrus, with no apparent effect on primiparous cows.

Milk fat, protein, lactose, and SNF did not seem to be significantly altered by bST treatment, however, slight variations, (i.e., increases in milk fat and increases or decrease in milk protein), may occur during the first few weeks after initiation of bST treatment, this variation seems to be transient and generally a normal balance is shortly re-established (Bauman, 1992; Etherton and Bauman, 1998; Huber et al., 1997; Tarazon-Herrera and Huber, 2000; Thomas et al., 1991; Velez and Donkin, 2004). Treatment with bST was shown to increase somatic cell count (SSC) in primiparous cows after 81 DIM, with no observed effect on multiparous cows (Santos et al., 2004). However, SCC do not seem to be altered by bST treatment over four consecutive lactations (Huber et al., 1997)

Somatotropin is a homeorhetic controller that orchestrates the portioning of nutrients (Peel and Bauman, 1987) and the metabolism of various body organs and tissues (Bauman, 1992) for increased milk synthesis. Some of the metabolic effects of bST are

mediated by insulin-like growth factor-I (IGF-I), but the main effect of bST is to inhibit insulin-induced lipogenesis which results in repartitioning of nutrients, thus increasing available nutrients for mammary gland milk synthesis (Etherton, 2004). Depending on the energy balance of the animal, lipogenesis (positive energy balance) and lipolysis (negative energy balance) are altered by bST treatment (Bauman, 1992; Etherton and Bauman, 1998). With bST administration, glucose oxidation by body tissue decreases and glucose production by the liver increases, due to the decreased ability of insulin to inhibit gluconeogenesis (Bauman, 1992; Etherton and Bauman, 1998). Knapp et al. (1992) reported that glucose synthesized from [1-C^{14}] propionate was 2.3 times greater in liver slices for bST treated cows than for control cows. Treatment with bST did not affect the conversion of [1-C^{14}] lactate or amino acid tracers to glucose (Knapp et al., 1992). Blood flow through the mammary gland is also increased during bST administration (Bell and Bauman, 1997), possibly as a coordinated response to increased mammary metabolic activity involving IGF-I, as there are abundant type I and II IGF receptors in bovine mammary tissue. After peak milk yield, the decline in mammary cell numbers or secretory activity accounts for the decline in the lactation curve. The effect of bST on persistency is due, in part, to IGF-I-induced maintenance of the mammary cell population rather than maintenance of cellular secretory rate (Capuco et al., 2003).

In summary, bST increases milk production by 1) inhibiting insulin induced lipogenesis and thus, repartitioning of nutrients to the mammary gland 2) reducing glucose oxidation by body tissue, 3) increasing blood flow to the mammary gland, and 4) maintenance of mammary gland cell population via stimulatory hepatic IGF-I secretion.

Factors Influencing Estrous Behavior

Regulation of the estrous cycle is controlled by orchestrated hormonal changes resulting in ovulation and the expression of standing heat (estrus). Cows spend less than 1% of the estrous period expressing standing behavior (Senger, 1994), thus estrous detection is one of the most important factors limiting reproductive efficiency in dairy cattle (Lopez and Shipka, 2003). The intensity (number of mounts) and the duration of estrus depends on the behavior of individual cows as well as social interactions among cows (Lucy, 2001). Standing behavior can be influenced by environmental factors such as environmental temperature (Gwazdauskas et al., 1983), time of day (Hurnik et al., 1975; Pennington et al., 1985) or other external and management factors such as number of cows in estrus per pen (Hurnik et al., 1975; Helmar and Britt, 1985; Floyd, 2001), age of the animal (Gwazdauskas et al., 1983), production of the animal (Lopez et al., 2004), and type of housing and footing (flooring surface) (Britt et al., 1986; Vailes and Britt, 1990; Rodtain et al. 1996; Lopez and Shipka, 2003). In addition, the percentage of cows detected in estrus increased from first to second ovulation postpartum in lactating dairy cows (Spicer et al., 1990; 1993).

Type of estrus, either prostaglandin induced or natural, did not affect estrus or its behavioral activities of multiparous Holstein cows (Pennington et al., 1985; Walker et al., 1996). Walton et al. (1987) however, recorded fewer standing mounts when Cloprostenol was used to induce estrus vs. a natural occurring estrus. No difference in breeding rate, conception rate, or pregnancy rate was observed when comparing Cloprostenol and Dinoprost use in dairy cows (Martineau, 2003). Gwazdauskas et al. (1983) found estrous activity of primiparous and multiparous cows randomly assigned to

barn, dry-lot or pasture areas was lowest for heifers and increased for older cows suggesting increased activity occurs with advancing lactation number and may be subject to conditioning or sexual experience. Walker et al. (1996) reported similar findings in that estruses for primiparous cows were nearly 50% shorter than for multiparous cows. Lopez et al. (2004) however, reported no differences between primiparous and multiparous cows in the duration of estrus (9.4 ± 0.6 h and 8.0 ± 0.6 h, respectively), number of standing events (8.1 ± 0.5 and 7.1 ± 0.4, respectively), and total standing time (26.3 ± 1.9 s and 23.9 ± 1.3 s, respectively). A significant difference was reported for low producing cows (< 39.5 kg/d) vs. high producing cows (≥ 39.5 kg/d), in the duration of estrus (10.9 ± 0.7 h and 6.2 ± 0.5 h, respectively), number of standing events (8.8 ± 0.6 and 6.3 ± 0.4, respectively), and total standing time (28.2 ± 1.9 s and 21.7 ± 1.3 s, respectively) (Lopez et al., 2004).

If the number of cycling, nonpregnant, animals in a group is low, regardless of the total group size, accurate detection of estrus may be difficult. Also, cows that are at the mid-stages of their cycles, (d 5 - d 16), are least likely to mount a cow that is in heat and consequently could be termed "poor heat detectors" (Diskin and Sreenan, 2000). There were 6.1 total attempted mounts and 2.3 stands recorded during estrus when one estrual cow and no preestrual cows were present in a pen compared to 31.8 total attempted mounts and 23.6 stands when at least two estrual and two preestrual cows were present (Helmar and Britt, 1985). In two different studies, 85% of all attempted mounts by heifers (Helmar and Britt, 1985) and 79% of mounting cows (Hurnik et al., 1975) were in preestrual or estrual stages of the estrous cycle. Attempted mounts by postesrtual heifers (96 attempts) was approximately half that of preestrual heifers (203 attempts) (Helmar

and Britt, 1985). As the number of animals in estrus simultaneously increased, there was a proportional increase in the number of mounts per hour and mounting activity increased at a decreasing rate for up to five active cows, then increased at an increasing rate up to nine active cows (Helmar and Britt, 1985). Dairy cows in a free stall area exhibited an average of 11.2 mounts per estrus during 7.5 h with one estrus cow and increased to 52.6 mounts during 10.1 h when three cows were in estrus at the same time (Hurnik et al., 1975). Similar findings were reported by Floyd (2001) in beef cows, where increasing the number of cows in estrus at the same time increased the number of mounts per estrus (when only one cow was 11.0 mounts were recorded, which increased to 30.9 mounts when 4 to 6 cows were in estrus) and decreased the interval between mounts.

In a study by Britt et al. (1986) lactating Holstein cows were observed for estrous behavior on either a dirt lot or a free-stall barn with a grooved concrete alley. Estrus was 4.4 h longer on dirt than on concrete and standing and mounting activity was 54% greater on dirt than on concrete. Britt concluded that the most important factor affecting sexual activity in lactating Holstein cows was the flooring surface(s) on which the cows were managed. Similar findings were reported by Vails and Britt (1990) where estrual cows were shown to prefer mounting tied estrual cows on dirt, nearly three times more often in a 30-minute period than those on concrete surfaces. Rodtian et al. (1996) also reported a significant reduction in estrus duration and number of mounting-mounted interactions on concrete vs. dirt flooring surfaces. Lopez and Shipka (2003) suggested that cows either felt uneasy trying to mount on concrete flooring or that they have learned from previous experiences to be fearful of mounting on concrete flooring.

At-Taras and Spahr (2001) compared the efficiency of the HeatWatch® system and the Heat Seeker® electronic Activity Tag system. Diagnosis of estrus was based on a combination of eight factors, including the two systems, visual factors, milk production, and rectal palpation. Blood or milk progesterone concentrations were not monitored. Cows were housed in free-stall barns with 30 to 50 other cows and had 24-hr access to a lot with a concrete floor. Efficiency was 86.8 % (trial 1) (6.7 average mounts/estrus averaging 3.2 s) when no conscious effort was made to synchronize cows and 71.7% (trial 2) (5.4 average mounts/estrus averaging 3.4 s) when cows were synchronized in groups of three or more cows. In contrast, Walker et al. (1996) using blood progesterone concentration to declare estrus on 51 head of cows housed in a free-stall barn, injected with prostaglandin after ultrasound confirmation of a corpus luteum, reported a 90% efficiency of the HeatWatch® system with an average of 10.1 mounts/estrus averaging 2.38 s. Xu et al. (1998) using milk progesterone concentration to declare estrus, also reported a 90% efficiency using HeatWatch® in a pasture situation involving spring and fall studies with Fresian and Jersey cows where the average duration of estrous for the two periods was 11.65 h involving 11.0 mounts /estrus averaging 2.45 s. At-Taras and Spahr (2001) mentioned at least two "false-negative" diagnoses occurring, where cows were visually observed standing to be mounted and no record of any mounts on the HeatWatch® system was recorded. Wilson et al. (2002) evaluating two groups of heifers synchronized with melengesterol acetate (MGA), reported 4 % and 9.5%, of the heifers standing to be mounted by visual observation were not recorded by the HeatWatch® system; efficiency of the HeatWatch® system was reported to be 80.9% and 77.7%, respectively. Dransfield et al. (1998) in a study involving 17 herds and over 3200 cows

in which herd size varied from 56 to 566 head with rolling herd averages ranging from 7330 to 10847 kg/yr, reported the number of standing events per estrus was 8.5 ± 6.6 with the duration of estrus ranging from 33 min to 35.8 h. Nearly one-fourth of the cows had estrus periods classified as low intensity (< 1.5 standing events per hour) and short duration (< 7 h). Standing events per estrus was shown to affect the probability of pregnancy with cows having fewer than three standing events per estrus having a 41% lower probability of pregnancy than cows having greater than three events per estrus (Dransfield et al., 1998). Lopez et al. (2004) evaluated estrous behavior using the HeatWatch® system on 131 primiparous and 136 multiparous cows, 50 DIM, housed in a free-stall barn with concrete flooring , where alleys were automatically scraped several times per day, and recorded the standing activity for 382 of 463 ovulations (82.5 % efficiency) with average d postpartum of 93 ± 6 d (range of 50 to 165 d). There were 15% of the recorded estruses consisting of only one standing event. Estrus was declared using plasma progesterone concentration and ultrasound conformation of a CL.

In summary, the efficiency of the HeatWatch® system ranged from 71% to over 90% with variation in the number of mounts and duration of mounts depending on the number of estrus cows in a pen, stage of the estrous cycle of nonestrus cows in a pen, age of cow (primiparous or multiparous), number of lactations, estrous behavior intensity, flooring surface, and milk production.

Postpartum Ovulation

The antagonistic relationship between high milk production and reproductive traits in dairy cows is not a modern day association. Eckles (1929) reported that dairymen

believed that breeding difficulties had increased over the same time interval as increased milk production, concluding a cause and effect relationship existed between the two. There seems to be contradictory evidence for an antagonistic relationship between increased milk production and reproduction traits in the modern dairy cow. In 1964, a test herd in Minnesota was divided into two lines of cows of equivalent genetic merit (control and select); pairing cows as closely as possible to age, sire, and production ability (Hansen, 2000). Differences in management practice between the lines were the selection of semen used for insemination. Matings for cows in the control line were sires selected to be near the mean for PTA for milk in 1964 or sons of these original sires. Matings for cows in the select line were from highest bulls for PTA for milk yield for the current year. Milk production increased over 69% in the select line vs. control (Hansen, 2000), with an increase in the average interval to first ovulation for the select line (43 ± 5 d) vs. control (29 ± 3 d) (Lucy, 2001). In a similar study of genetic merit comparisons of cows, Harrison et al. (1990) using "breed average" and "breed high" sires for milk production, reported no significant differences in days to first ovulation between "breed high" (31 ± 4 d) and "breed average" (29 ± 3 d) line of cows as milk production increased over 56% in the "breed high" vs. "breed average" line of cows. In a six-year study involving primiparous cows, evaluating 275 lactations (De Vries and Veerkamp, 2000), the first occurrence of luteal activity was reported to be 29.7 d with a range from 10 to 97 d, with the data being somewhat skewed, as the mode was 18 d. These questions arise: Is the decrease in reproductive traits caused by increased milk production? Or, are the effects of milk production on fertility noticed because every cow in the herd is potentially affected? Hansen (2000) points out that heritability for reproductive traits are low and

the majority of variation in reproductive traits is due to factors other than genetics while Darwash et al. (1997) gives evidence that a substantial proportion of variation to establish postpartum ovarian activity is additive genetically. Reports show that high milk yield, although contributory, may not be the major factor in the decline in reproduction in dairy cows, other disease and non-disease parameters may be involved (Drackley, 1999; Gröhn and Rajala-Schultz, 2000; Hansen, 2000; Lucy, 2001; Nebel, 2003). However, Fricke and Wiltbank (1999) conclude that milk production is the primary factor affecting the incidence of double ovulation in lactating dairy cows, as the incidence of double ovulation was three times higher in high producing cows (> 40 kg/d) than low producing cows (≤ 40 kg/d).

Although high milk yields have been associated with reduced reproductive performance, measures of postpartum ovarian activity have been more closely related to energy balance (EB) (Canfield and Butler, 1991; Beam and Butler, 1999). Energy balance is quantified using measures of milk production (quantity and composition), dietary intake (quantity and composition), and body weight (Spicer et al., 1990). Lucy (2001) points out that high milk production and negative energy balance (NEB) should not be confused. The highest producing cows in the herd may not be those in greatest NEB. Harrison et al. (1990) reported a greater DMI in "breed high" cows vs. "breed average" sired cows, which suggests the increased milk production was compensated for by increased nutrient intake. As stated earlier, this is true in bST-treated cows. Low producing cows with poor dry matter intake (DMI) were shown to be at greater risk for anestrous and infertility than were high producing cows (Staples and Thatcher, 1990),

while cows with higher DMI were more likely to show signs of estrus at first ovulation and to become pregnant by d 150 of lactation (Westwood, 2002).

Francisco et al. (2002) reported no significant difference in the average days to first ovulation in P169 treated vs. control cows, 44.8 ± 7.7 d and 30.1 ± 7.4 d, respectively. Francisco et al. (2003) reported that EB was the most influential contributor to the model predicting days to first ovulation, with DMI and FCM also making significant contributions. Zurek et al. (1995) reported that first postpartum ovulation occurred after the NEB nadir, in a range of 8 to 24 days, and at various NEB. In agreement with Spicer et al. (1990) reporting 24.4 ± 2.3 d to first ovulation, and Reist et al. (2003) reporting 23.9 ± 14.0 d to first ovulation, Zurek et al. (1995) found no relationship between mean EB and days to first ovulation, however the magnitude of the energy deficit seemed to be the important factor in the inhibition of first ovulation. De Vries and Veerkamp (2000) reported that a 2.38 Mcal. of NE_L/d lower nadir of EB delayed the start of luteal activity by 1.25 d. However, interval to first ovulation postpartum is positively associated with conception rate (Butler, 2000) and represents a relationship between energy status and reproductive performance.

The first phase of postpartum reproduction is the recovery of the hypothalamus and pituitary from the effects of pregnancy and the resumption of FSH and LH production, leading to establishment of the LH surge, a mechanism required for the ovulation of the dominant follicle; the latter of which is affected by energy balance (Beam and Butler, 1999; Hampton et al., 2003; Lucy, 2003). Currently, it is proposed that the day of EB nadir is more important than the degree of NEB (Beam and Butler, 1997) in suppressing LH pulse frequency. Results suggest that NEB does not affect follicle population or

follicular growth during early lactation (Beam and Butler, 1997; Butler, 2000; Diskin et al., 2003). Beam and Butler (1997) described the fate of follicular development: 1) ovulation of the first dominant follicle, 2) atresia of the first dominant follicle followed by a new follicular wave, or 3) development of a cystic ovary. Energy balance has been correlated to postpartum LH pulse frequency which usually begins to increase after NEB nadir (Canfield and Butler, 1991). Energy balance has also been associated with serum IGF-I concentrations (Spicer et al., 1990), glucose (Butler, 2000) and insulin levels (Canfield and Butler, 1991; Butler et al., 2004) which has been shown to increase levels of circulating estradiol in the absence of a change in LH pulsatility (Butler et al., 2004).

There appears to be both direct and indirect nutritional effects that orchestrate the alteration of ovarian function affecting the onset of estrus or anestrus and a major challenge facing the dairy industry is the development of ways to minimize the extent and/or duration of negative energy balance in postpartum cows.

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Chapter III

DOSE RESPONSE OF A DIRECT-FED MICROBIAL ON MILK YIELD, MILK COMPONENTS, BODY WEIGHT, AND DAYS TO FIRST OVULATION IN PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

Abstract

From two wk prior to parturition to 175 d postpartum, 38 primi- and multiparous Holstein cows were assigned to one of three treatment groups. Control (n = 13) received regular Total Mixed Ration (TMR). Low-dose (n = 14) received control TMR plus 6 x 10^{10} cfu/head Propionibacteria Strain P169 (P169). High-dose (n = 11) received control TMR plus 6 x 10^{11} cfu/head P169. Cows were housed in a free-stall barn divided into three separate free-stall and feeding areas. Cows were provided feed ad libitum. Supplemental P169 was fed daily (p.m.) as a top-dress on 4.5 kg of TMR. Cows were milked daily at 0400 and 1600. Weekly milk samples were collected at successive a.m./p.m. milkings and analyzed for percentage of milk fat, protein, lactose, and solids-not-fats (SNF), milk urea nitrogen (MUN) and somatic cell count (SCC). Rumen fluid was collected via intubation at 30 d prepartum and at d 60, 120, and 175 postpartum. Daily milk production expressed as 4% fat-corrected milk (FCM) was affected by treatment ($P < 0.003$) and week x parity ($P < 0.05$). High-dose and Low-dose P169 treated cows exhibited a 7.1 % and 8.5 % increase above controls in daily 4% FCM, respectively. Treatment x parity and week significantly influenced percentage of milk

fat, lactose and protein, while solids-not-fats (SNF) was influenced by treatment x parity and treatment x week. Propionate levels were significantly influenced by treatment ($P < 0.05$) such that High-dose P169 cows had 18.5% and 17.0% greater molar percentage of ruminal propionate than Low-dose P169 and Control cows, respectively. Acetate/propionate ratio was altered by treatment ($P < 0.06$) such that High-dose P169 cows had 15.4% and 13.3% lower acetate/ propionate ratios than Low-dose P169 and Control cows, respectively. Change in body weight postpartum was influenced by week x parity ($P < 0.02$) and treatment x parity ($P < 0.004$) such that High-dose P169 and Low-dose P169 multiparous cows exhibited a greater recovery of wk 1 body weight than Control multiparous cows. There was no treatment, parity, or interaction on days to first postpartum ovulation or on estrous behavior at 45 and 90 d after parturition. We conclude that P169 may hold potential as an effective direct-fed microbial to increase milk production in dairy cows.

Introduction

In the last ten years, annual milk production per cow in the U.S. has increased 15.8%, while the number of dairy farms and cows in production have decreased 41.7% and 4.3%, respectfully (USDA, 2004). Fortunately, improved genetics and management have accompanied this period in which fewer dairymen with fewer cows are trying to meet the continually growing demand for dairy products (Lucy, 2001). The genetic potential for milk production sets the upper limit at which a cow can produce milk (Silvia, 2003). How close a cow comes to attaining her production limit is determined by management practices. One of the primary challenges faced by the dairy cow is the sudden increase in

nutrient requirements for lactation when a cow cannot consume sufficient dry matter (Drackley, 1999). Propionate from ruminal fermentation is a major glucogenic precursor for glucose production through hepatic gluconeogenesis (Sauer et al., 1989). Theoretic efficiency of propionate as a source of energy for ATP is 108% compared to glucose (McDonald et al., 2002). Postpartum dairy cows first partition ME toward milk production and body condition gain before reproductive functions (Silvia, 2003). Thus, nutritional deficiency due to lactation has major implications on reproduction. The desire to increase production and reproductive efficiency along with growing societal concern over the use of antibiotics and growth promoters, such as bST, in the dairy industry have producers interested in products that can successfully manipulate rumen microbe populations and fermentation products. The present study was conducted to test the hypothesis that manipulating ruminal fermentation with a direct-fed microbial, P169, would result in increased milk production, milk components, and reproductive efficiency of dairy cows.

Methods and Materials

Experimental Design and Sample Collection

From approximately two wk prior to parturition to 175 d postpartum, 19 primi- and 19 multiparous Holstein cows were randomly assigned to one of three dietary treatment groups, based on age, expected calving date, and (for multiparous) the previous year's lactation averages or (for primiparous) current PTA's (Predicted Transmitting Ability), a term given to estimate the genetic merit for a number of traits in dairy cattle including milk, protein, fat, and type traits. The Control treatment group (n = 13) received a lactation total mixed ration (TMR; Table 4.1) throughout the study. The Low-dose treatment group (n = 14) received the control TMR plus 6 x 10^{10} cfu/head Propionibacteria Strain P169 (Low-dose P169). The High-dose treatment group (n = 11) received the control TMR plus 6 x 10^{11} cfu/head Propionibacteria Strain P169 (High-dose P169). The 2 wk prepartum feeding period consisted of the respective fore-mentioned treatments with the substitution of the transition TMR (Figure 4.2) fed from 2 wk prepatum to the time of parturition. The Propionibacteria Strain P169 (P169) was prepared by Agtech Products Inc., Waukesha, WI. The previous 305 ME milk production for Control, Low-dose, and High-dose multiparous cows were 10265 ± 716 kg, 10439 ± 905 kg, and 10174 ± 905 kg, respectively, and did not differ ($P > 0.97$) among treatment groups. The genetic merit for milk production (PTA) for Control, Low-dose, and High-dose primiparous cows were +120 ± 104, +236 ± 78, and +187 ± 95 kg,

respectively, and did not differ (P > 0.67) among treatment groups. The number of treatment days prior to parturition did not differ (P > 0.28) among Control, Low-dose, and High-dose treatment groups (13.1 ± 2.1, 14.5 ± 2.0, and 18.0 ± 2.3 d, respectively). One Low-dose primiparous cow was taken out of the study at wk 23 due to health reasons. Cows in each treatment group were housed in the same open-air free-stall barn divided into three separate free-stall and feeding areas to prevent contact between treatment groups. Cows had free access to water and were provided with feed ad libitum. Cows were fed the P169 via top-dress on a small amount (4.5 kg) of TMR once per day (p.m.) while isolated in a free stall. To assure complete consumption of the control and treatment TMR, all cows were confined to free stalls after the p.m. milking until the respective control and treatment TMR was consumed. The TMR was composed of sorghum/sudan silage, alfalfa hay, Bermuda grass hay, whole cottonseed, corn gluten feed, Diamond V-XP yeast culture (Diamond V-XP Yeast Culture; Diamond V Mills Inc., Cedar Rapids, Iowa), and mineral concentrate (Table 4.1). Cows were fed twice daily at 0900 and 1600. Energy concentration of the diet was formulated to support daily milk production of at least 45 kg. The TMR was sampled weekly and composited monthly throughout the study for analysis. Lactation TMR averaged 17.07 ± 0.33% CP, 1.67 ± 0.006 Mcal/kg NE_l, 68.67 ± 0.55% TDN, 25.06 ± 0.87% ADF, 38.73 ± 1.0 % NDF, 0.97 ± 0.03% Ca on a dry matter basis. The TMR analysis was completed by Dairy One Inc., DHI Forage Testing Laboratory, Ithaca, NY.

Cows calved between August 26, 2002 to October 25, 2002, with no difference (P >0.36) in date of birth among treatment groups: average day of birth for the Control, Low-dose, and High-dose treatment groups were Julian day 270 ± 4.9, 261 ± 4.8, 269 ±

5.1, respectively. Local climatological data for the 30 wk experiment, collected 1.6 km from the O.S.U. Dairy Center, were obtained from the Oklahoma Mesonet, Norman, Oklahoma. The greatest weekly average maximum temperature (24.9 ± 1.6 ^{o}C) occurred at wk 1 of lactation and lowest weekly average minimum temperature (-4.3 ± 0.7 ^{o}C) occurred at wk 19 of lactation, and no significant differences were observed among treatment groups in weekly average minimum and maximum temperature during wk 1-25 or wk 25-30 (time of bST administration).

The original 175 d study was extended for an additional 28 d period to assess the effects of the three treatment groups during concomitant bST treatment. Following the final measurements for the 25-wk study, bST (POSILAC®, steril sometribove zinc suspension; 500 mg; Monsanto, St Louis, MO) was administered in the ischiorectal fossa (s.c.) on d 0, d 14, and d 21; this was in addition to the continuation of the once per day dosage of P169 to the applicable treatment groups for 28 d.

For a 90 d period, October 1, 2002 through December 31, 2002, postpartum feed intake was monitored for each of the treatment groups. A minimum of three measurements of daily feed intake by each treatment group per wk during the 90 d period was recorded with the average number of days between measurements being 1.4 ± 0.3 d. The average number of days postpartum at the beginning of the monitoring period for Control (n=7), Low-dose (n=11), and High-dose (n=7) treatment groups averaged 18.1 ± 3.9, 18.4 ± 3.2 and 18.4 ± 3.5 d, respectively. The number of days postpartum at the end of the feed intake measurements for Control, Low-dose, and High-dose treatment groups averaged 94.1 ± 5.6, 103.1 ± 4.1 and 95.8 ± 5.3 d, respectively.

Cows were milked twice daily at 0400 and 1600, and a.m. and p.m. milk yields were recorded. The a.m. and p.m. daily milk weights were totaled for the weekly milk production per cow. Milk production was corrected for percentage milk fat to 4% fat corrected milk (FCM) for statistical analysis. Milk samples were collected twice weekly during successive a.m. and p.m. milkings (the p.m. prior to and the a.m. of blood sample collection) and analyzed for percentage of fat, protein, lactose, and solids-not-fats (SNF); milk urea nitrogen (MUN) and somatic cell count (SCC) were quantified. Milk component analysis was completed by Heart of America DHIA, Manhattan, KS. The average number of days postpartum at first milk collection for Control, Low-dose, and High-dose treatment groups was 4.3 ± 0.3, 4.3 ± 0.2, and 3.9 ± 0.3 d, respectively, and did not differ ($P > 0.37$) among treatment groups.

Blood samples were collected, via venipuncture of the coccygeal vein/artery in vacutainer tubes (7 mL) containing EDTA, once weekly between 0530 and 0730, after cows exited the milking parlor. Samples were collected from wk 1 through wk 30 postpartum. Blood was stored on ice, transported to the lab and centrifuged at 1200 x g for 15 min (5^{o}C) and plasma was decanted and stored at -20^{o}C until progesterone was quantified. Days postpartum at first blood collection for Control, Low-dose, and High-dose treatment groups were 3.4 ± 0.6, 4.2 ± 0.6, and 3.3 ± 0.7 d, respectively, and did not differ ($P > 0.50$) among treatment groups. Weekly body weights were also recorded during the time of blood sample collection.

The HeatWatch® system (DDX Inc., Denver, CO) was used to monitor estrous behavior. A pressure sensitive radio transmitter enclosed in a nylon pouch, was attached to the tail head of the each cow. A repeater was used to aid in the signal relay from the

transmitter to the receiver at a computer. Transmitters were activated by a continuous pressure with a duration of ≥1 s from the weight of a mounting female. The average number of days postpartum when the electronic estrus activity sensors were placed on cows was 16.0 ± 2.3, 18.0 ± 6.3 and 17.0 ± 6.0 d for the Control, Low-dose, and High-dose treatment groups, respectively. Cows were observed during the p.m. feeding period to monitor the stability of the patches and to evaluate if reattachment was needed. The HeatWatch® system contained a daily supervisory check feature that reported the status of all transmitters and was monitored daily. A computer recorded the date, time, and duration of all mounts. Cows were subjected to two separate synchronization periods involving two i.m. injections, 11 d apart, using cloprostenol sodium (Estrumate®; Schering-Plough, 2 mL). Period I was implemented at 45 ± 0.6 d postpartum and Period II was implemented at 90 ± 1.0 d postpartum. Depending on the response of the individual animal to the $PGF_{2\alpha}$ injection, data recorded for Period I and Period II were totals of either a single observation (response to either the first or second $PGF_{2\alpha}$ injection) or an average of two observations (response to first and second $PGF_{2\alpha}$ injection). A response to $PGF_{2\alpha}$ in cows defined as > 1 ng/ml P_4 at time of $PGF_{2\alpha}$ administration and subsequent < 1 ng/ml P_4 1 wk after $PGF_{2\alpha}$ administration were considered cyclic (i.e., exhibiting luteal activity). The beginning of estrous behavior was defined as the first mount of two mounts within 4 h and the end of estrus was defined as the last mount with at least one mount in the preceding 4 h and no mounts in the following 12 h (Floyd, 2001). Cows were subjected to the AI protocol of the OSU dairy after Period II.

Rumen fluid was collected from all animals via intubation at 30 d prepartum. Postpartum rumen samples were taken at d 60, 120, 175 (end of original study), 204 (end of wk 4 bST administration) and 264 (60 d after end of P169 treatment). At each sampling time, approximately 100 to 150 mL of rumen fluid was collected and transferred into conical vials, placed on ice and shipped to the Agtech Inc. Laboratories (Waukesha, WI) for volatile fatty acid (VFA) analyses. Average day prepartum of rumen collection for the Control, Low-dose, and High-dose treatment groups was 34.0 ± 3.3, 29.7 ± 3.2, and 33.0 ± 3.5 d, respectively, and did not differ ($P > 0.63$) among treatment groups.

Plasma P_4 concentrations were determined using a solid-phase ^{125}I radioimmunoassay (RIA) (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) designed for the direct, quantitative measurement of progesterone in plasma and validated for bovine plasma (Stewart et al., 1996; Maciel et al., 2001). Sensitivity of the assay, defined as 95% of total binding, averaged 0.028 ng/ml (12 determinations). Intraassay and interassay coefficients of variation averaged 6.8% and 11.7%, respectively.

Statistical Analysis

Milk production (corrected to 4% fat), milk components and body weight data were analyzed as repeated measures using PROC MIXED procedure of SAS, (SAS Institute, Cary, NC). Cow nested within treatment and parity was considered to be random and all other effects in the model were considered fixed. The model of the covariate structure for repeated measurements was an autoregressive with a lag equal to one. If main effects or

their interactions were significant, mean separation was accomplished using paired student's t-test.

VFA and pH data were analyzed as repeated measures using the MIXED procedure of SAS (SAS Institute, Cary, NC) with d -30 used as a co-variable. The model included treatment, parity, and day as main effects with all interactions included. If main effects or their interactions were significant, mean separation was accomplished using paired student's t-test.

Interval to first ovulation, mounting activity, and mounting behavior were analyzed using the GLM procedure of SAS (SAS Institute, Cary, NC). The model included treatment as the main effect. If main effect was significant, mean separation was accomplished using paired student's t-test.

Response to $PGF_{2\alpha}$ treatment and pregnancy was analyzed by the FREQ procedure of SAS (SAS Institute, Cary, NC) using a Chi-square statement.

Chapter IV

Results

Milk Production

Daily milk production, calculated as 4% fat-corrected milk (FCM) was affected by treatment ($P < 0.003$) and week x parity ($P < 0.05$) with no other main effect or interaction being significant ($P > 0.15$). Daily 4% FCM production, averaged across primiparous and multiparous cows, was greater in both the High-dose (32.2 ± 0.6 kg/d) and Low-dose (32.7 ± 0.6 kg/d) P169 treatment cows vs. the Control cows (29.9 ± 0.6 kg/d) during the 25-wk study. There was no significant difference between the High-dose and Low-dose P169 treatment cows (Figure 4.1). During the 25 wk study, High-dose P169 treatment cows exhibited a 7.1 % increase above the Control in daily 4% FCM production and the Low-dose P169 treatment cows exhibited an 8.5 % increase above the Control. Similarly, uncorrected daily milk production increased 8.1 % in the High-dose (34.2 ± 1.0 kg/d) and 7.7 % in the Low-dose (34.0 ± 0.9 kg/d) P169 treatment cows above the Control cows (31.4 ± 0.9 kg/d). Daily 4% FCM production in primiparous cows significantly increased from wk 1 through wk 3 and remained constant throughout the remainder of the 25 wk lactation period (Figure 4.2). Daily 4% FCM production in multiparous cows showed a significant increase from wk 1 through wk 5, after which, a significant decrease in milk production was not seen until after wk 17 of lactation (Figure

4.2). The percentage decrease in milk production from peak production at wk 6 (40.4 kg/d) for multiparous cows and wk 7 (27.6 kg/d) for primiparous cows, to wk 25 of lactation was 15.1 % and 0.6 %, respectively.

To compare data to a previous study (Francisco et al., 2001), data were analyzed separately for wk 1-12 and wk 13-25. Daily 4% FCM production for wk 1-12, displayed a tendency to be affected by treatment ($P < 0.07$) such that 4% FCM, averaged across primiparous and multiparous cows, was greater in Low-dose P169 cows (32.7± 0.7 kg/d) than either the High-dose P169 cows (31.9 ± 0.8 kg/d) or Control cows (30.3 ± 0.8 kg/d). Daily 4% FCM production for wks 13-25 was affected by treatment ($P < 0.01$) such that 4% FCM, averaged across primiparous and multiparous cows, was greater in both the High-dose (32.5 ± 0.8 kg/d) and Low-dose (32.8 ± 0.8 kg/d) P169 cows vs. the Control cows (29.6 ± 0.8 kg/d). Expressed as a percentage increase above the Control, High-dose P169 cows exhibited a 8.9 % increase and the Low-dose P169 cows exhibited a 9.7 % increase in daily 4% FCM production during wk 13-25.

When bST was administered for 5 wk at the end of the initial 25 wk study, 4% FCM production was significantly affected by treatment ($P < 0.03$) and parity ($P <0.001$) with no other main effects ($P > 0.40$) or interactions ($P > 0.30$). Daily 4% FCM production during the bST administration averaged 31.9 ± 1.2, 33.7 ± 1.1, and 29.6 ± 1.1 kg/d for the High-dose, Low-dose P169 treatment and Control cows, respectively. During bST administration, daily 4% FCM production averaged 28.8 ± 0.9 kg/d for primiparous cows and 34.7 ± 0.9 kg/d for multiparous cows. Average 4% FCM production after 5 wk of bST administration (i.e., wk 30) for both primiparous and multiparous cows did not significantly differ from wk 25 (wk 0 of bST treatment) milk production. Daily 4% FCM

production was greater in the Low-dose P169 treatment cows vs. Control cows exhibiting a 12.1 % increase ($P < 0.05$) in daily milk production over the Control cows during the 5 wk bST administration. High-dose P169 did not differ from either the Low-dose P169 or Control treatments.

Milk Components

Milk Fat

Milk fat percentage was altered by treatment x parity ($P < 0.02$) and week ($P < 0.001$) with no other main effects ($P > 0.30$) or interactions ($P > 0.45$). Percentage milk fat was significantly greater in the Low-dose P169 and Control multiparous cows vs. the High-dose P169 multiparous cows, but there was no difference among treatment groups in primiparous cows (Figure 4.3). Milk fat percentage decreased significantly from wk 1 to 4 and plateaued after wk 5 with no significant change (increase or decrease) between wk 5 and 25 (Figure 4.4).

When milk fat data were analyzed separately for wk 1-12 and wk 13-25, treatment had no significant effect. However, milk fat percentage was altered by week ($P < 0.001$) during wk 1-12, such that milk fat percentage decreased significantly from wk 1 to 4. During wk 13 – 25, parity ($P < 0.03$) affected milk fat percentage such that multiparous cows ($3.7 \pm 0.1\%$) had a greater percentage milk fat than primiparous cows ($3.5 \pm 0.1\%$).

During the 5 wk bST administration, there was no significant effect of treatment, parity, week, or their interactions on milk fat percentage ($P > 0.13$) (data not shown).

Milk Lactose

Milk lactose percentage was influenced by treatment x parity ($P < 0.03$) and week ($P < 0.001$) with no other main effects ($P > 0.45$) or interactions ($P > 0.35$). Milk lactose percentage was significantly greater in the High-dose P169 (5.12 ± 0.04 %) vs. Low-dose P169 (4.91 ± 0.04 %) multiparous cows; with the Low- and High-dose P169 multiparous cows significantly greater than Control (4.77 ± 0.03 %) multiparous cows (Figure 4.5). The lactose percentage in High-dose primiparous cows (5.04 ± 0.04 %) did not differ from Control primiparous (4.93 ± 0.04 %), but was greater ($P < 0.05$) than Low-dose primiparous cows (4.89 ± 0.03 %). Weekly milk lactose levels increased from wk 1 to wk 7 of lactation, after which milk lactose levels did not change.

When milk lactose data were analyzed separately for wk 1-12 and wk 13-25, milk lactose percentage was influenced by treatment ($P < 0.002$) and week ($P < 0.0001$) during wk 1 - 12, such that percentage of milk lactose was significantly greater in the High-dose P169 (5.04 ± 0.04 %) vs. Low-dose P169 (4.88 ± 0.04 %) and Control (4.82 ± 0.04 %) cows; weekly milk lactose levels increased from wk 1 through wk 7 of lactation. During wk 13-25, milk lactose percentage was influenced by treatment x parity ($P < 0.0001$) similar to that observed in wk 1 – 25.

The milk lactose levels during the 5 wk bST administration was influenced by treatment x parity ($P < 0.06$) and followed a pattern very similar to lactose levels during wk 1 – 25 (data not shown). No other main effects or interactions ($P > 0.30$) were significant.

Milk Protein

Milk protein percentage was influenced by week ($P < 0.001$) with a tendency to be influenced by treatment x parity ($P < 0.11$) with no other main effects ($P > 0.40$) or interactions ($P > 0.20$). Weekly milk protein percentage significantly decreased from wk 1 to 4, and then gradually increased through wk 15, with no change thereafter (Figure 4.6). High-dose and Low-dose P169 treatments tended to increase ($P < 0.11$) milk protein percentage in multiparous cows vs. Control multiparous cows (Figure 4.6); these differences were not evident in primiparous cows (data not shown).

When milk protein data were analyzed separately for wk 1-12 and wk 13-25, milk protein percentage was influenced by week ($P < 0.001$) during wk 1-12, such that weekly milk protein percentage exhibited a significant decrease from wk 1 to 4. There was no significant difference in milk protein percentage due to treatment, parity, week, or their interactions during wk 13 – 25.

Milk protein percentage during the 5 wk bST administration was influenced by week x parity ($P < 0.002$) and treatment x week ($P < 0.09$) with no other main effects ($P > 0.45$) or interactions ($P > 0.15$). Multiparous cows showed no significant change with week, while primiparous cow response varied with time of bST administration (every 2 wk) (Figure 4.7). The High-dose P169, Low-dose P169 and Control treatments also exhibited a weekly response that varied with the time of bST administration (data not shown).

Milk Solids-Not Fats

The percentage of milk solids-not-fats (SNF) was influenced by treatment x parity ($P < 0.02$), week ($P < 0.008$), and treatment x week ($P < 0.10$) with no other main effects (P

> 0.35) or interactions (P > 0.15). The percentage SNF was greater in both High-dose (9.16 ± 0.09%) and Low-dose (8.94 ± 0.09%) P169 multiparous cows vs. Control (8.67 ± 0.07%) multiparous cows (Figure 4.8). However, differences were not evident among High-dose P169, Low-dose P169 and Control primiparous cows (9.08 ± 0.08, 8.87 ± 0.06, and 9.01 ± 0.09%, respectively) during wk 1 – 25 (data not shown). Averaged across all treatment groups, percentage SNF significantly decreased from wk 1 to wk 4, then slowly increased through wk 12 and then remained constant through wk 25 (Figure 4.8).

When milk SNF data were analyzed for wk 1-12, percentage of milk SNF was influenced by treatment x parity (P < 0.05) and week (P < 0.0001); percentage SNF followed a similar profile of response as the wk 1 – 25 analysis. The wk 13 – 25 analysis revealed a significant treatment effect (P < 0.01), with High-dose P169 cows (9.15 ± 0.07%) having a greater percentage of SNF than Low-dose P169 (8.91 ± 0.06%) and Control cows (8.85 ± 0.07%).

Milk SNF percentage during the 5 wk bST administration was affected by week x parity (P <0.04) with no other main effects (P > 0.15) or interactions (P > 0.13). Primiparous cows exhibited a greater increase in percentage SNF over multiparous cows in response to the first bST injection, after which percentage SNF response to bST varied with the time of administration within parity groups (Figure 4.9).

Milk Urea Nitrogen

Milk urea nitrogen (MUN) levels were influenced by week (P < 0.001) and week x parity (P < 0.08) with no other main effects (P > 0.30) or interactions (P > 0.12). The

general trend was an increase in MUN levels over the first 15 wk, but specific effects (i.e., increases and decreases) varied depending on the week of lactation and parity of the animal (Figure 4.10).

When MUN data were analyzed separately for wk 1-12, MUN levels were influenced by week ($P < 0.001$) with changes similar to that observed in the wk 1–25 analysis. For wk 13-25, MUN levels were altered by treatment ($P < 0.05$) with a tendency to be influenced by parity ($P < 0.07$). MUN levels were greater in High-dose P169 (15.56 ± 0.28 mg/dL) and Low-dose P169 cows (15.76 ± 0.26 mg/dL) than Control cows (14.86 ± 0.26 mg/dL). MUN levels in multiparous cows tended to be greater than in primiparous cows ($P < 0.07$) during wk 13-25.

During the 5 wk bST administration MUN levels were influenced by week ($P < 0.04$) with no other main effects ($P > 0.90$) or interactions ($P > 0.15$). Levels of MUN were lowest at wk 29 (14.38 ± 0.37 mg/dL) (2 wk after the 2nd bST injection) and greatest at wk 28 (15.67 ±0.32 mg/dL) (1 wk after the 2nd bST injection at wk 27).

Somatic Cell Counts

Somatic cell counts (SCC) for wk 1-25 were influenced by week x parity ($P < 0.001$) with no other main effects ($P > 0.25$) or interactions ($P > 0.75$). Primiparous cows exhibited a greater SCC at wk 1 than multiparous cows, 1871.0 ± 193.1 and 370.0 ± 204.7 x10^3/ ml, respectively, and SCC in multiparous cows were greater than in primiparous cows at wk 3, 167.7 ± 189.8 and 504.3 ± 196.9 x10^3/ ml respectively. From wk 4 to 25, no significant difference in SCC was observed between parity groups (Figure 4.11).

When SCC data were analyzed separately for wk 1-12, week x parity ($P < 0.001$) influenced SCC with primiparous cows (256.3 ± 80.6 $x10^3$/ ml) having a greater SCC than multiparous cows (148.7 ± 82.9 $x10^3$/ ml) (Figure 4.12). For wk 13 - 25, SCC were significantly altered by treatment ($P < 0.007$), with both High-dose P169 (36.0 ± 29.8 $x10^3$/ ml) and Low-dose P169 (92.0 ± 27.5 $x10^3$/ml) cows exhibiting a significantly lower SCC vs. Control cows (168.5 ± 28.0 $x10^3$ /ml) (Figure 4.12).

During the 5 wk bST administration, SCC tended to be altered by treatment ($P < 0.09$) with no other main effects ($P > 0.20$) or their interactions ($P > 0.60$) being significant. The SCC of High-dose P169 cows (38.6 ± 28.0 $x10^3$) were lower than Control ($P < 0.03$; 123.4 ± 26.3 $x10^3$) cows. Low-dose P169 ($P < 0.14$; 95.6 ± 26.3 $x10^3$) cows did not differ from High-dose P169 or Control cows.

Volatile Fatty Acids (VFA)

Acetate

The molar percentage of ruminal acetate was affected by day such that molar percentage of acetate was greater at d 120 (67.2 ± 0.9%) and 175 (66.7 ± 0.9%) compared to day 60 (63.6 ± 0.9%). The molar percentage of ruminal acetate was not affected by treatment ($P > 0.30$) and averaged 64.4 ± 1.2%, 66.4 ± 1.1% and 66.7 ± 1.1% for High-dose P169, Low-dose P169, and Control cows, respectively, between d 60 to d 175. Molar percentage of ruminal acetate at d -30 was 71.5 ± 1.5%, 74.0 ± 1.3% and 72.8 ± 1.3% for High-dose P169, Low-dose P169, and Control cows, respectively.

At the end of the 5 wk bST administration (d 204), molar percentage of ruminal acetate was not affected by treatment, parity, or their interaction with molar percentages of 64.3 ± 1.7%, 66.2 ± 1.2% and 66.6 ± 1.2% for High-dose P169, Low-dose P169, and Control cows, respectively.

At d 265 (60 d after end of P169 treatment), the molar percentage of ruminal acetate was influenced by treatment ($P < 0.04$) such that High-dose P169 (67.0 ± 1.0%) cows exhibited a lower molar percentage of acetate than either the Low-dose P169 (70.0 ± 1.0%) and Control (70.7 ± 0.9%) cows.

Propionate

Analysis for changes in ruminal propionate levels from the (-30 d) pretreatment status revealed that propionate levels were influenced by treatment ($P < 0.05$) such that High-dose P169 cows, averaged across d 60, 120, and 175, had an 18.5% and 17.0% increase in the molar percentage of rumen propionate over the Low-dose P169 and Control cows, respectively (Figure 4.13). Molar percentage of ruminal propionate at d -30 was 15.7 ± 1.4%, 14.1 ± 1.2% and 13.5 ± 1.3% for High-dose P169, Low-dose P169, and Control cows, respectively.

At the end of the 5 wk bST administration (d 204), molar percentage of ruminal propionate was not affected by treatment, parity, or their interaction, and averaged 22.9 ± 1.0%, 23.8 ± 0.9%, and 22.6 ± 0.9% for High-dose P169, Low-dose P169, and Control cows, respectively.

At d 265 (60 d after end of P169 treatment), the molar percentage of ruminal propionate was not affected by treatment, parity, or treatment x parity interaction with

molar percentages of 21.7 ± 1.1%, 19.7 ± 1.0% and 19.6 ± 1.0% for High-dose P169, Low-dose P169, and Control cows, respectively.

Acetate/Propionate Ratio

Changes in the acetate/propionate ratio from the (-30 d) pretreatment status was affected by treatment ($P < 0.06$). The High-dose P169 (2.91 ± .17) cows, averaged across d 60 , 120, and 175, had a 15.41% and 13.33% lower acetate/ propionate ratio compared to the Low-dose P169 (3.44 ± .15) and Control (3.36 ± .11) cows, respectively (Figure 4.13). At the end of the 5 wk bST administration (d 204) and at d 265 (60 d after end of P169 treatment), acetate/propionate ratio was not affected by treatment, parity, or treatment x parity interaction.

Butyrate

The molar percentage of ruminal butyrate was affected by day ($P < 0.001$) such that molar percentage of butyrate was greater at d 60 (15.7 ± 0.6%) compared to d 120 (11.8 ± 0.6%) and d 175 (11.4 ± 0.6%). There was a tendency for the molar percentage of ruminal butyrate to be affected by treatment ($P < 0.11$) with levels of 12.3 ± 0.6%, 13.9 ± 0.5%, and 12.7 ± 0.5% for the High-dose P169, Low-dose P169, and Control cows, respectively. Molar percentage of ruminal butyrate at d -30 was 12.8 ± .9%, 11.9 ± .8% and 13.3 ± .9% for High-dose P169, Low-dose P169, and Control cows, respectively.

At the end of the 5 wk bST administration (d 204), the molar percentage of ruminal butyrate was not affected by treatment or treatment x parity interaction averaging 11.1 ± 0.3%, 11.0 ± 0.3%, and 10.6 ± 0.3% for High-dose P169, Low-dose P169, and Control

cows, respectively. The molar percent of ruminal butyrate was influenced by parity ($P < 0.07$) with multiparous cows (11.3 ± 0.3%) exhibiting a greater molar percentage of ruminal butyrate than primiparous cows (10.6 ± 0.2%).

At d 265, (60 d after end of P169 treatment), the molar percentage of ruminal butyrate was not affected by treatment, parity, or treatment x parity interaction, averaging 10.5 ± 0.6%, 10.7 ± 0.6%, and 9.8 ± 0.5% for High-dose P169, Low-dose P169, and Control cows, respectively.

Ruminal pH

Analysis for changes in rumen pH from the (-30 d) pretreatment day status revealed that pH was influenced by treatment ($P < 0.02$) such that High-dose P169 cows averaged across d 60, 120, and 175 had a lower pH than Low-dose P169 and Control cows with pH values of 6.65 ± 0.07, 6.94 ± 0.06, and 6.86 ± 0.06, respectively (Figure 4.14). At the end of the 5 wk bST administration (d 204) and d 265 (60 d after end of P169 treatment), ruminal pH was not affected by treatment, parity, or treatment x parity interaction.

Change in Weekly Body Weight (BW)

Body weight expressed as % of wk 1 BW was influenced by week x parity ($P < 0.02$) and treatment x parity ($P < 0.004$) such that High-dose P169 and Low-dose P169 multiparous cows exhibited a greater recovery of wk 1 BW vs. Control multiparous cows, recovering 98.4 ± 1.3%, 100.0 ± 1.1% and 95.3 ± 1.0%, respectively. Nadir BW occurred at wk 5 for the multiparous cows with percentage weight loss from wk 1 at 4.0 ± 2.4%, 2.9 ± 2.2% and 7.5 ± 1.7 %, respectively, for the High-dose P169, Low-dose P169

and Control multiparous cows. Control primiparous cows exhibited a greater recovery of wk 1 BW than High-dose P169 primiparous cows recovering 100.5 ± 1.2% and 97.2 ± 1.13%, respectively. Low-dose P169 primiparous cows (98.7 ± 1.0%) did not differ from either the Control or the High-dose primiparous cows (Figure 4.15). Nadir BW also occurred at wk 5 for the primiparous cows with percentage BW loss from wk 1 averaging 9.0 ± 2.0%, 7.2 ± 1.7% and 7.1 ± 2.1%, respectively, for the High-dose P169, Low-dose P169 and Control primiparous cows. Nadir percentage BW loss occurred for both primiparous and multiparous cows at wk 5, and by wk 25, primiparous cows had recovered 104.2 ± 1.1% of wk 1 BW vs. 100.0 ± 1.2% for multiparous cows of wk 1 BW.

During the 5 wk bST administration, change in BW was influenced by parity ($P < 0.006$) and week ($P < 0.05$) such that primiparous cows (averaged across treatment and wk) recovered 105.7 ± 1.30% of wk 1 BW vs. multiparous cows recovering 101.9 ± 1.30%. During the 5 wk bST administration, averaged across treatment and parity, cows continued to gain BW, with BW increasing from 102.0 ± 1.03% at wk 25 to 105.3 ± 1.1 % at wk 30 of wk 1 BW.

Postpartum Interval to First Ovulation and Estrous Behavior

Interval to First Ovulation

There were no treatment, parity, or treatment x parity interaction on days to first ovulation after parturition. Days to first ovulation averaged 32.4 ± 6.0, 30.8 ± 5.3 and 32.9 ± 5.6 d for the High-dose, Low-dose and Control treatment groups, respectively.

Period I and Period II Estrous Behavior and Pregnancy

No difference existed in the percentage of cows in each treatment group that were cyclic during estrous synchronization Period I (89.6%) or Period II (97.4%) or in the percentage of cows responding (overall mean = 87%) to the prostaglandin treatment (Table 4.3). In addition, there were no significant differences between High-dose P169, Low-dose P169, and Control cows for number of mounts (5.1 ± 2.8), mount duration (3.2 ± 1.2) or duration of estrus (13.3 ± 4.2) for Period I or Period II synchronization (Table 4.3). The percentage of cows demonstrating observable estrus (as detected by the HeatWatch® system) during Period I (45 d PP) and Period II (90 d PP) averaged 57.5% and 60.2%, respectively. No difference existed in the percentage of pregnancy rates at 30 wk postpartum for Control (23.0%), Low-dose P169 (21.4%), and High-dose P169 (36.3%), treatment groups.

Feed Intake and Efficiency

Feed efficiency [kg of feed (DMI) / kg of BW] for the High-dose P169, Low-dose P169, and Control treatment pens averaged 4.24, 3.91, and 4.05, respectively.

Milk (dairy) efficiency [kg of 4% FCM / kg of feed (DMI)] for the High-dose P169, Low-dose P169, and Control treatment pens averaged 1.32, 1.47, and 1.44, respectively.

Table 4.1: Ingredient and Nutrient Composition of the Control Lactation Diet (DM basis). Diet was fed from parturition to d 265 of lactation.

Table 4.1

Ingredient and Nutrient Composition of the Control Lactation Diet (DM basis)

Ingredient	%	Nutrient	
Sorghum Silage	16.44	DM, % (as fed)	55.9
Alfalfa (RFV 180)	16.94	CP, %	17.1
Bermuda Grass Hay	6.53	ADF, %	25.1
Whole Cottonseed	4.07	NDF, %	38.7
Corn Gluten Feed	6.60	NE_l, Mcal/kg	1.67
MEGALAC®-R*	0.91	Ca, %	0.97
Lactation Cow Grain Mix		P, %	0.41
Ground Corn	27.84	Mg, %	0.36
Wheat Midds	8.16		
Soybean Meal	6.79	K, %	1.44
Extruded/Expeller SB Meal	2.59		
Calcium Carbonate	0.94	Na, %	0.25
Sodium Bicarbonate	0.54		
Diamond V XP Yeast **	0.55	S, %	0.20
Magnesium Oxide	0.27		
Salt – White	0.27		
Zinpro 4-plex ***	0.07	Zn, ppm	58.00
Vit/Trace Mineral pak	0.27		
		Fe, ppm	281.25
		Cu, ppm	16.83
		Mn, ppm	75.42
		Mo, ppm	1.20

* Arm & Hammer® Animal Nutrition Group, Princeton, NJ; MEGALAC®-R contains:
Fat (as fatty acids) - 82.5%, Calcium - 8.5%, IOD (moisture) – 3 to 4%

** Diamond V-XP Yeast Culture; Diamond V Mills Inc., Cedar Rapids, IA

*** Zinpro Corp., Eden Prairie, MN contains:
Zinc – 2.58%, Mn – 1.43, Cu – 0.90%, Co – 0.18%, Methionine – 8.21%, Lysine – 3.8%

Table 4.2: Ingredient and Nutrient Composition of the Control Transition Diet (DM basis). Diet was fed from d -14 to parturition.

Table 4.2

Ingredient and Nutrient Composition of the Control Transition Diet (DM basis)

Ingredient	%	Nutrient	
Sorghum Silage	28.53	DM, % (as fed)	60.8
Alfalfa (RFV 180)	0.00	CP, %	12.8
Bermuda Grass Hay	13.95	ADF, %	33.8
Whole Cottonseed	0.00	NDF, %	53.5
Corn Gluten Feed	21.87	NE_l, Mcal/kg	1.43
MEGALAC®-R*	0.00	Ca, %	0.80
Dry Cow Grain Mix		P, %	0.31
Ground Corn	21.26	Mg, %	0.39
Wheat Midds	3.92		
Soybean Meal	3.94	K, %	1.08
Extruded/Expeller SB Meal	3.43		
Calcium Carbonate	1.28	Na, %	0.23
Sodium Bicarbonate	0.00		
Diamond V XP Yeast **	0.45	S, %	0.15
Magnesium Oxide	0.29		
Salt – White	0.20		
Zinpro 4-plex ***	0.20	Zn, ppm	72.75
Vit/Trace Mineral pak	0.38		
Calcium Chloride	0.20	Fe, ppm	247.75
		Cu, ppm	18.75
		Mn, ppm	103.5
		Mo, ppm	< 1.0

* Arm & Hammer® Animal Nutrition Group, Princeton, NJ; MEGALAC®-R contains:
Fat (as fatty acids) - 82.5%, Calcium - 8.5%, IOD (moisture) – 3 to 4%

** Diamond V-XP Yeast Culture; Diamond V Mills Inc., Cedar Rapids, IA

*** Zinpro Corp., Eden Prairie, MN contains:
Zinc – 2.58%, Mn – 1.43, Cu – 0.90%, Co – 0.18%, Methionine – 8.21%, Lysine – 3.8%

Figure 4.1. Effect of feeding Propionibacteria on milk production through 25 wk of lactation. Top Panel: 4% Fat corrected milk production in multiparous cows (n=19) fed High-dose (n=5) and Low-dose (n=6) Propionibacteria P169 and Control (n=8) rations for wk 1 to 25. Bottom Panel: 4% Fat corrected milk production in primiparous cows (n=19) fed High-dose (n=6) and Low-dose (n=8) Propionibacteria P169 and Control (n=5) rations for wk 1 to 25.

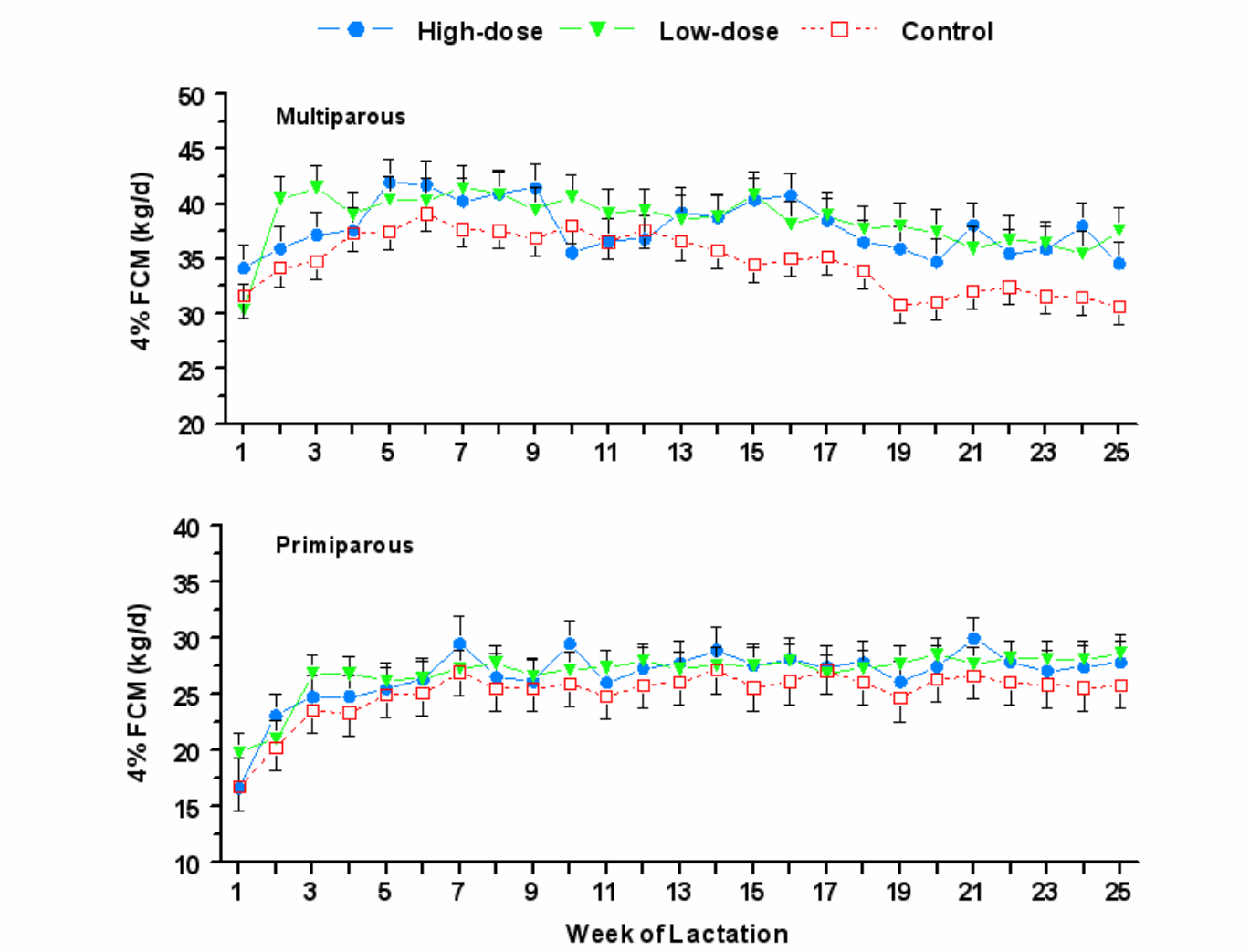

High-dose
Low-dose
Control
Multiparous
4% FCM (kg/d)
50
45
40
35
30
25
20
1
3
5
7
9
11
13
15
17
19
21
23
25
Primiparous
4% FCM (kg/d)
40
35
30
25
20
15
10
1
3
5
7
9
11
13
15
17
19
21
23
25
Week of Lactation

Figure 4.2. 4% Fat-corrected milk production for week x parity interaction in multiparous (n=19) and primiparous (n=19) cows during wk 1 to 25 of lactation. Data were pooled across treatments. * Means (± SEM) are significantly different from preceding week ($P < 0.05$). Arrow indicates numeric peak milk production for each parity group.

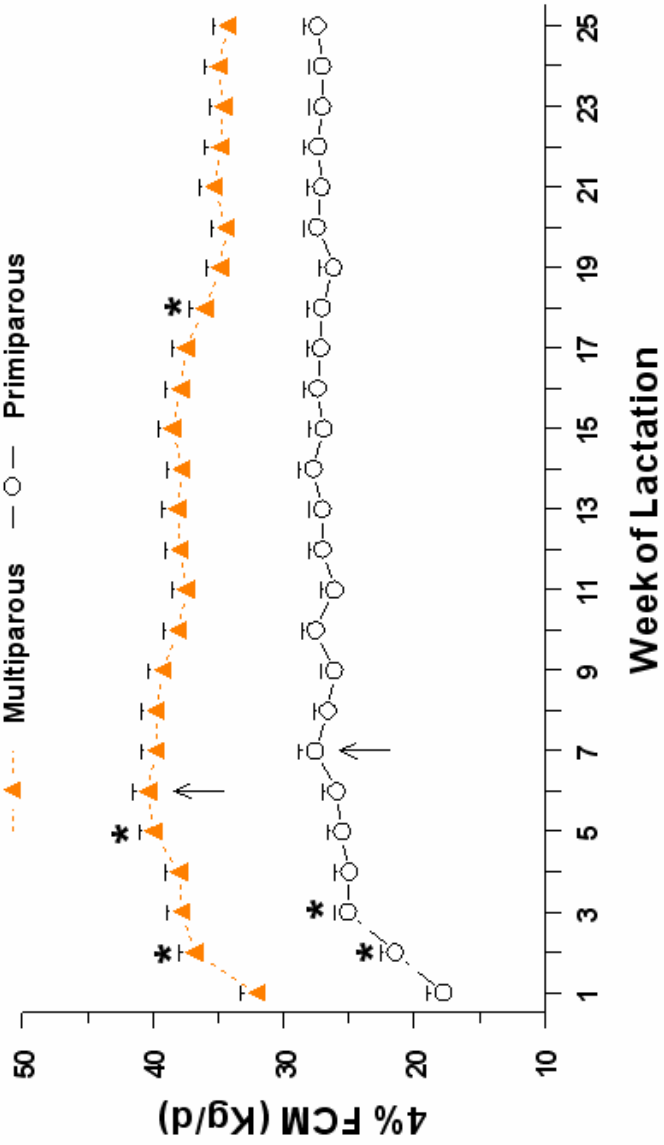

Multiparous
Primiparous
4% FCM (Kg/d)
Week of Lactation
10
20
30
40
50
1
3
5
7
9
11
13
15
17
19
21
23
25

Figure 4.3. Milk fat percentage for treatment x parity interaction in multiparous and primiparous cows fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations during wk 1 to 25 of lactation. [a,b] Means (± SEM) without a common superscript differ ($P < 0.02$).

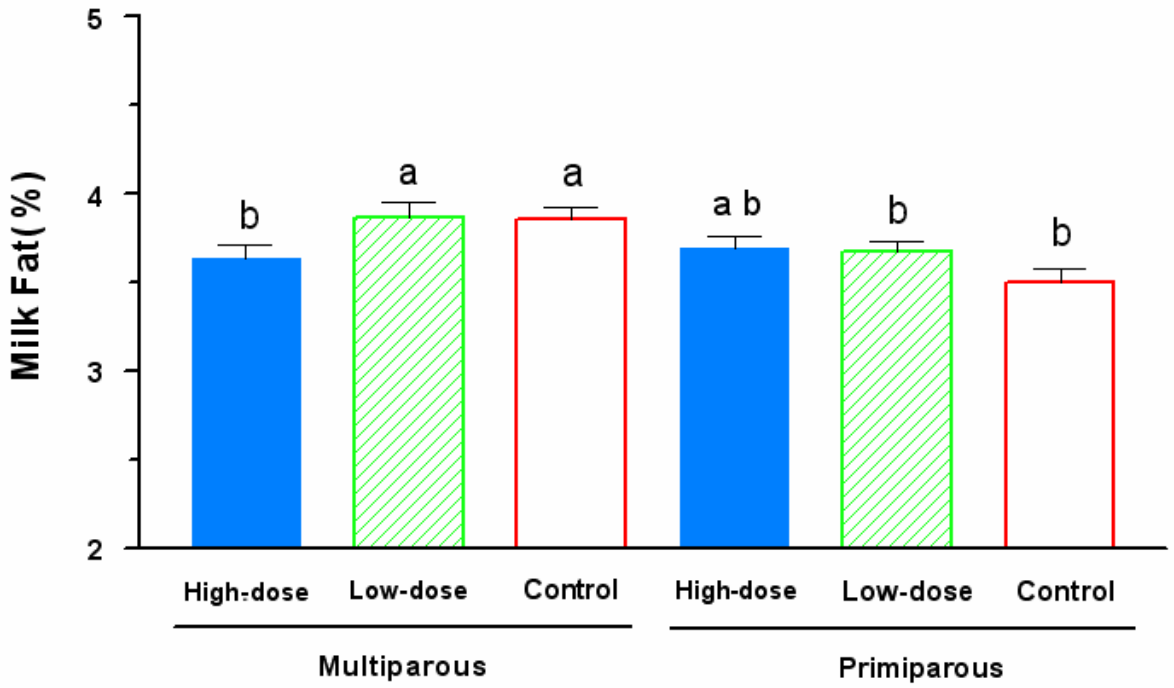

Milk Fat(%)
5
4
3
2
b
a
a
a b
b
b
High-dose
Low-dose
Control
High-dose
Low-dose
Control
Multiparous
Primiparous

Figure 4.4. Milk fat percentage during wk 1 to 25 of lactation in multiparous (n=19) and primiparous (n=19) cows fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations. Data from cows were pooled across treatment and parity. * Means (± SEM) are significantly different from preceding week ($P < 0.001$).

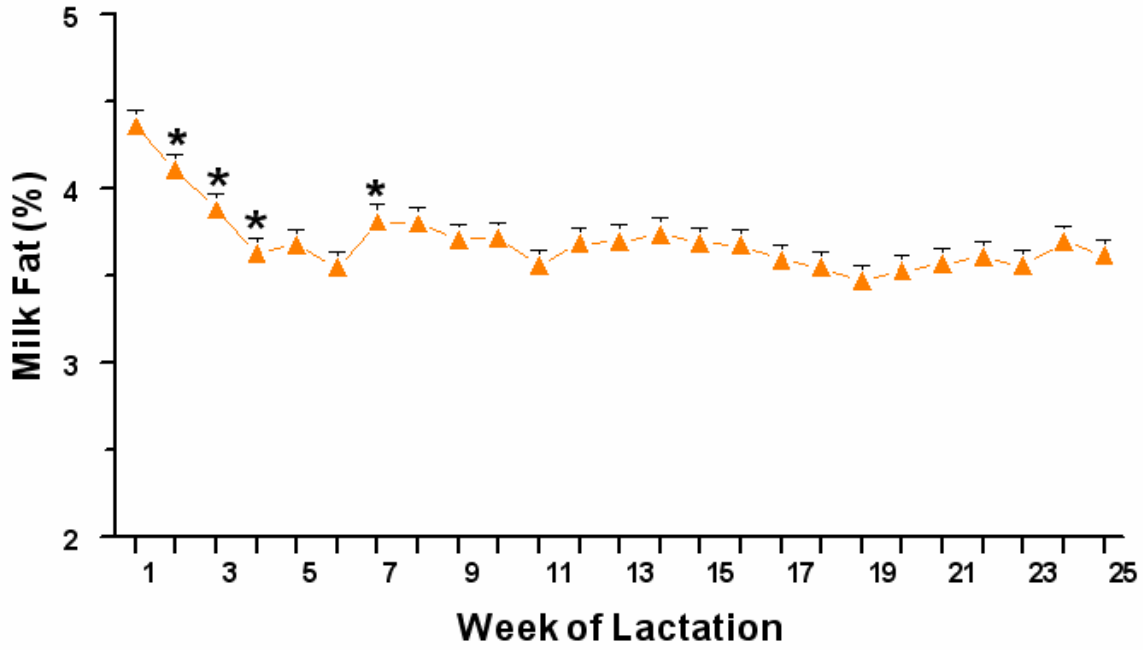

Milk Fat (%)
5
4
3
2
*
*
*
*
1
3
5
7
9
11
13
15
17
19
21
23
25
Week of Lactation

Figure 4.5. Effect of feeding Propionibacteria on milk lactose percentage during 25 wk of lactation. Top Panel: milk lactose percentage in multiparous cows (n=19) fed High-dose (n=5) and Low-dose (n=6) Propionibacteria P169 and Control (n=8) rations for wk 1 to 25. Bottom Panel: milk lactose percentage for primiparous cows (n=19) fed High-dose (n=6) and Low-dose (n=8) Propionibacteria P169 and Control (n=5) rations for wk 1 to 25.

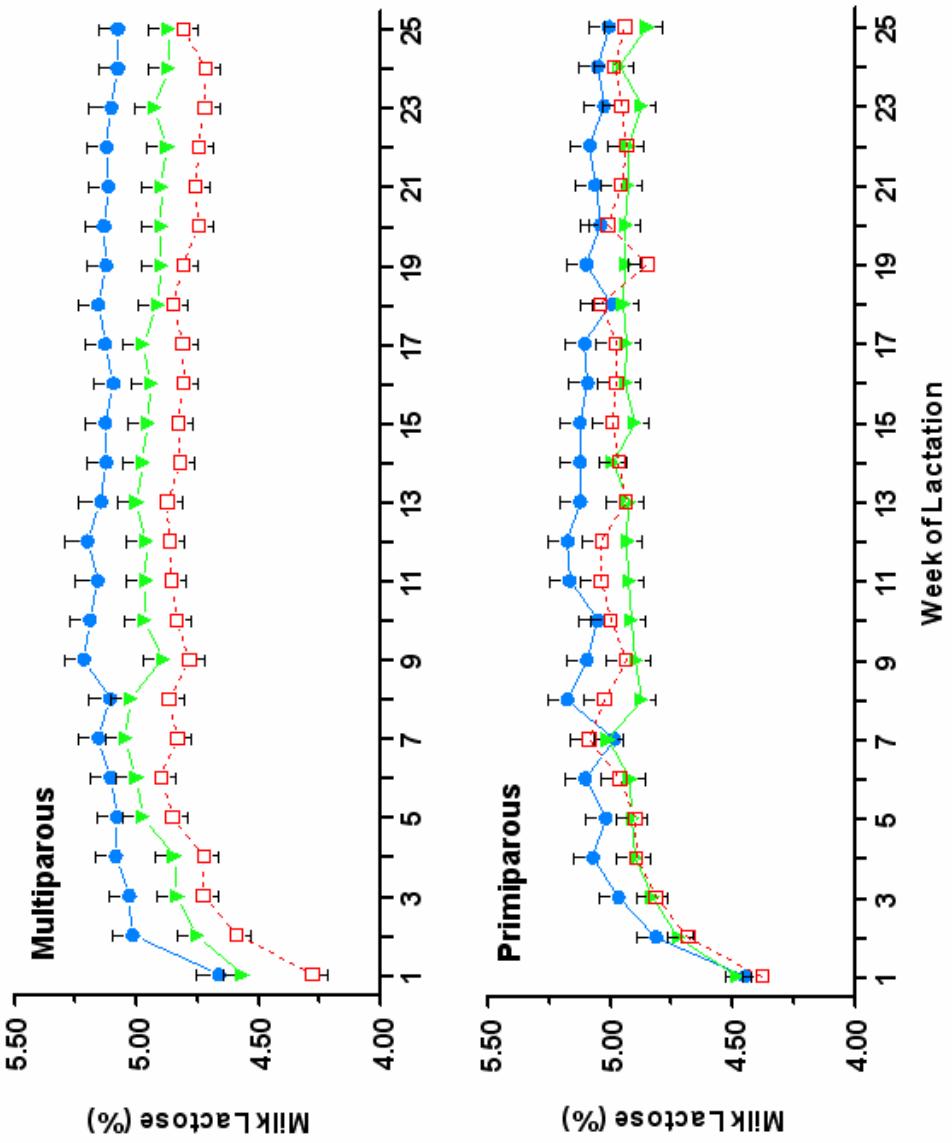

High-dose
Low-dose
Control
Multiparous
Primiparous
Milk Lactose (%)
Week of Lactation
5.50
5.00
4.50
4.00
1
3
5
7
9
11
13
15
17
19
21
23
25

Figure 4.6. Milk protein percentage during wk 1 to 25 of lactation in multiparous cows (n=19) fed High-dose (n=5) and Low-dose (n=6) Propionibacteria P169 and Control (n=8) rations.

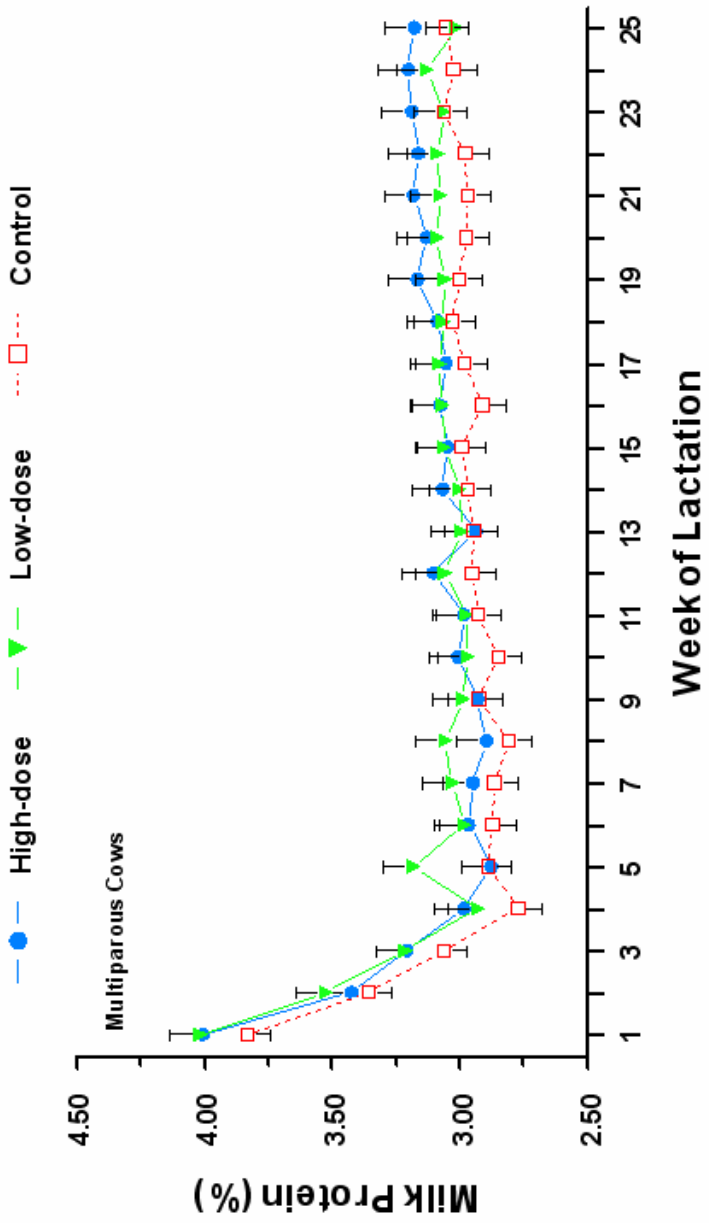

High-dose
Low-dose
Control
Multiparous Cows
Milk Protein (%)
4.50
4.00
3.50
3.00
2.50
1
3
5
7
9
11
13
15
17
19
21
23
25
Week of Lactation

Figure 4.7. Milk protein percentage for week x parity interaction in multiparous (n=19) and primiparous (n=19) cows fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations and treated with bST during wk 25 to 30 of lactation. Data from cows were pooled across treatments. * For primiparous cows, means (± SEM) are significantly different from preceding week ($P < 0.002$). Arrow indicates time of bST injection.

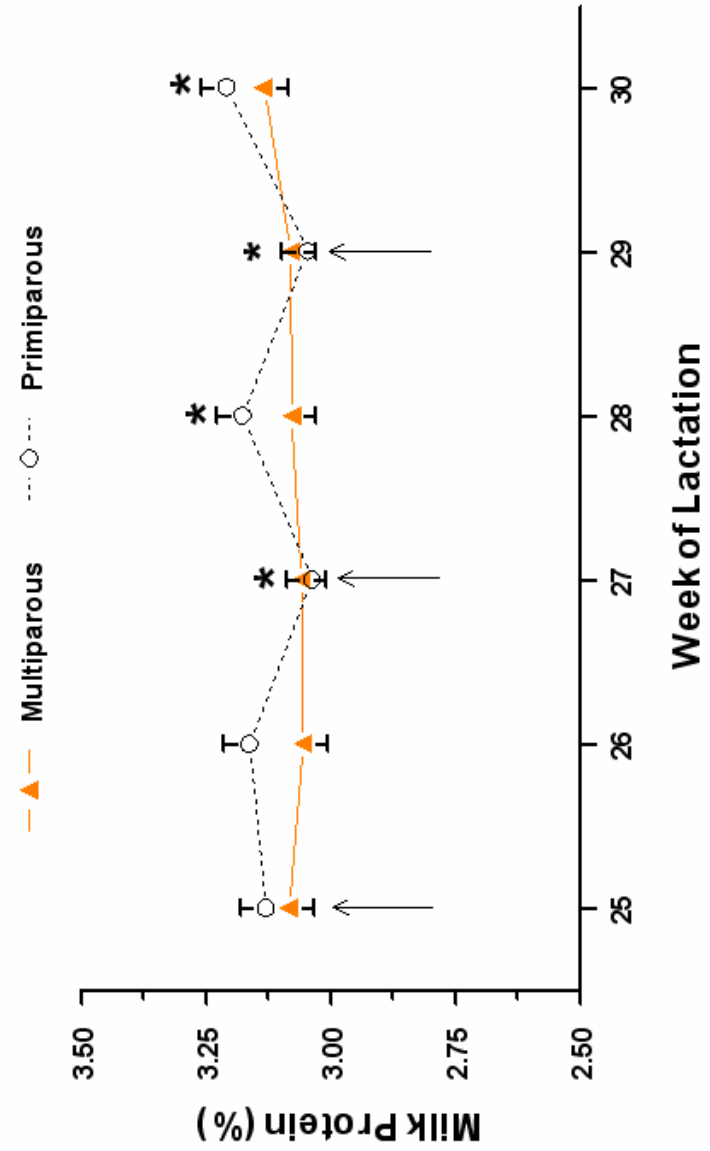

Multiparous
Primiparous
Milk Protein (%)
3.50
3.25
3.00
2.75
2.50
25
26
27
28
29
30
Week of Lactation

Figure 4.8. Milk solids-not-fat percentage during wk 1 to 25 of lactation in multiparous cows (n=19) fed High-dose (n=5) and Low-dose (n=6) Propionibacteria P169 and Control (n=8) rations.

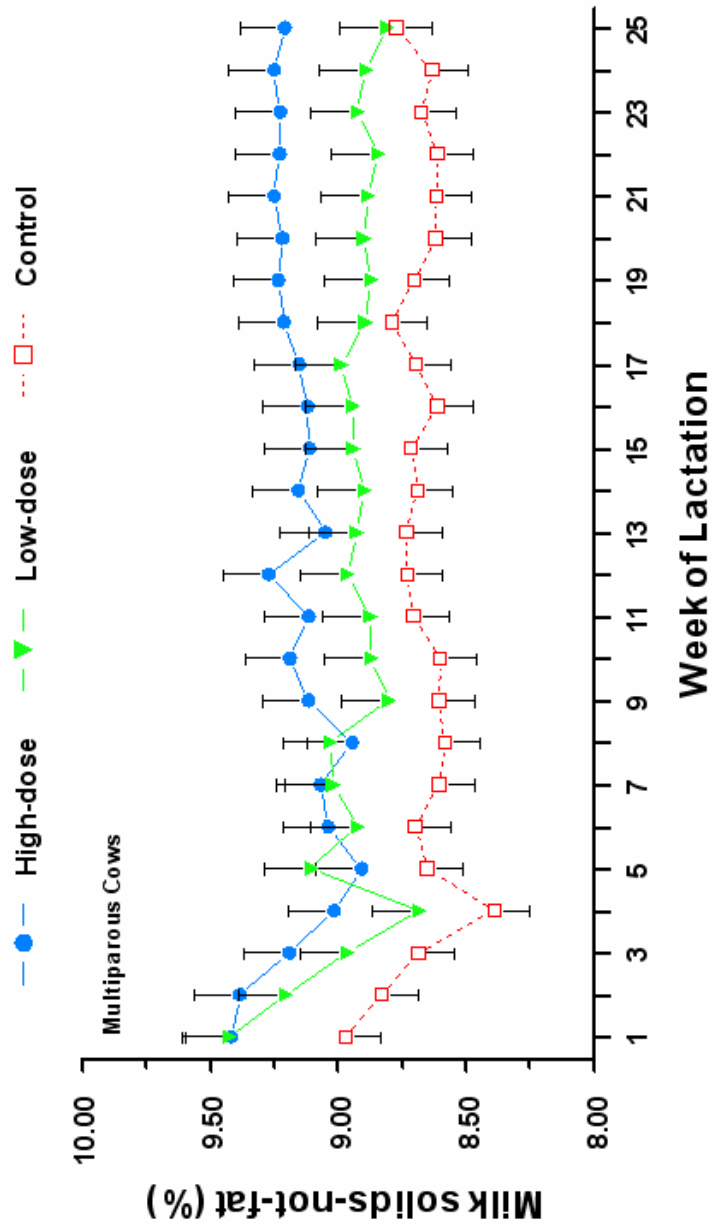

High-dose
Low-dose
Control
Multiparous Cows
Milk solids-not-fat (%)
10.00
9.50
9.00
8.50
8.00
1
3
5
7
9
11
13
15
17
19
21
23
25
Week of Lactation

Figure 4.9. Milk solids-not-fat percentage for week x parity interaction in multiparous (n=19) and primiparous (n=19) cows fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations treated with bST for wk 25 to 30 of lactation. Data from cows were pooled across treatments. * Means (± SEM) are significantly different from preceding week ($P < 0.04$); ^{a,b} Within week, means (± SEM) without a common superscript differ ($P < 0.04$). Arrow indicates time of bST injection.

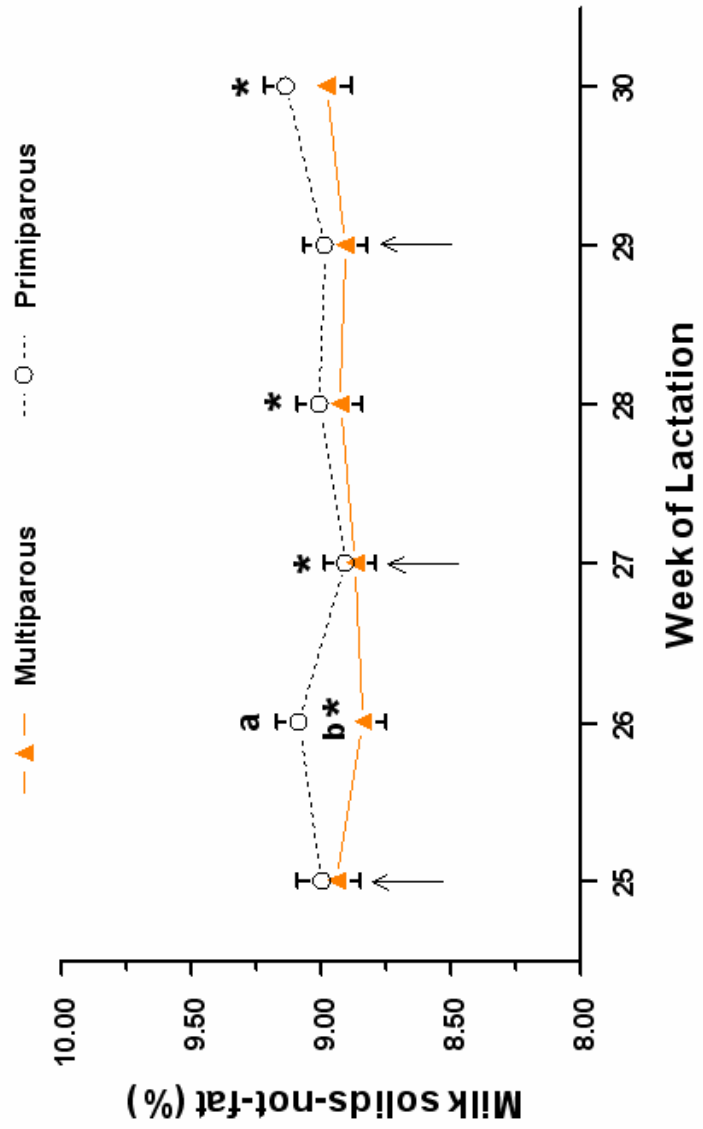

Multiparous
Primiparous
Milk solids-not-fat (%)
10.00
9.50
9.00
8.50
8.00
25
26
27
28
29
30
Week of Lactation
a
b*
*
*
*

Figure 4.10. Changes in milk urea nitrogen (MUN) during wk 1 to 25 of lactation in multiparous (n=19) and primiparous (n=19) cows fed a High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations. Data were pooled across treatments within multiparous and primiparous groups. $^{+}$ First mean to differ from wk 1 in multiparous cows. * First mean to differ from wk 1 in primiparous cows. $^{++}$ First mean to differ from wk 3 in multiparous cows. ** First mean to differ from wk 4 in primiparous cows.

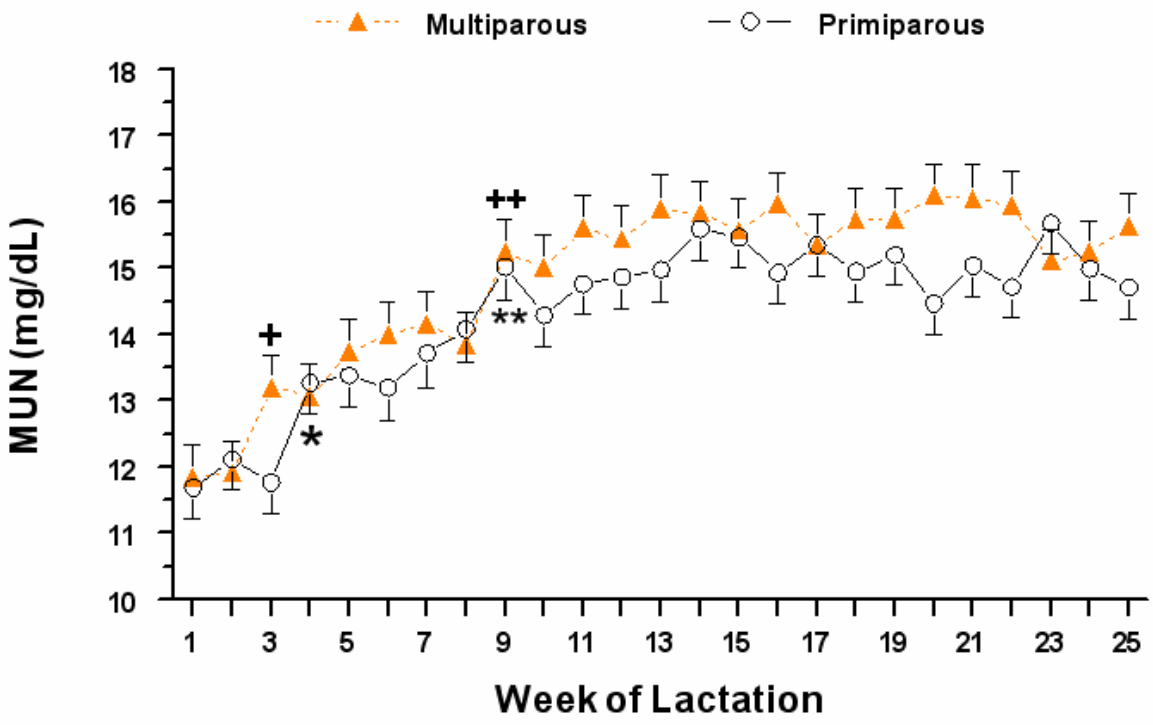

Multiparous
Primiparous
MUN (mg/dL)
Week of Lactation
18
17
16
15
14
13
12
11
10
1
3
5
7
9
11
13
15
17
19
21
23
25
+
*
**
++

Figure 4.11. Somatic cell counts (SCC; x 10^3/ml) for week x parity interaction in multiparous (n=19) and primiparous (n=19) cows during wk 1 to 25 of lactation. Data were pooled across treatment. * Means (± SEM) are significantly different from preceding week ($P < 0.001$). [a,b] Within week, means (± SEM) without a common superscript differ ($P < 0.001$).

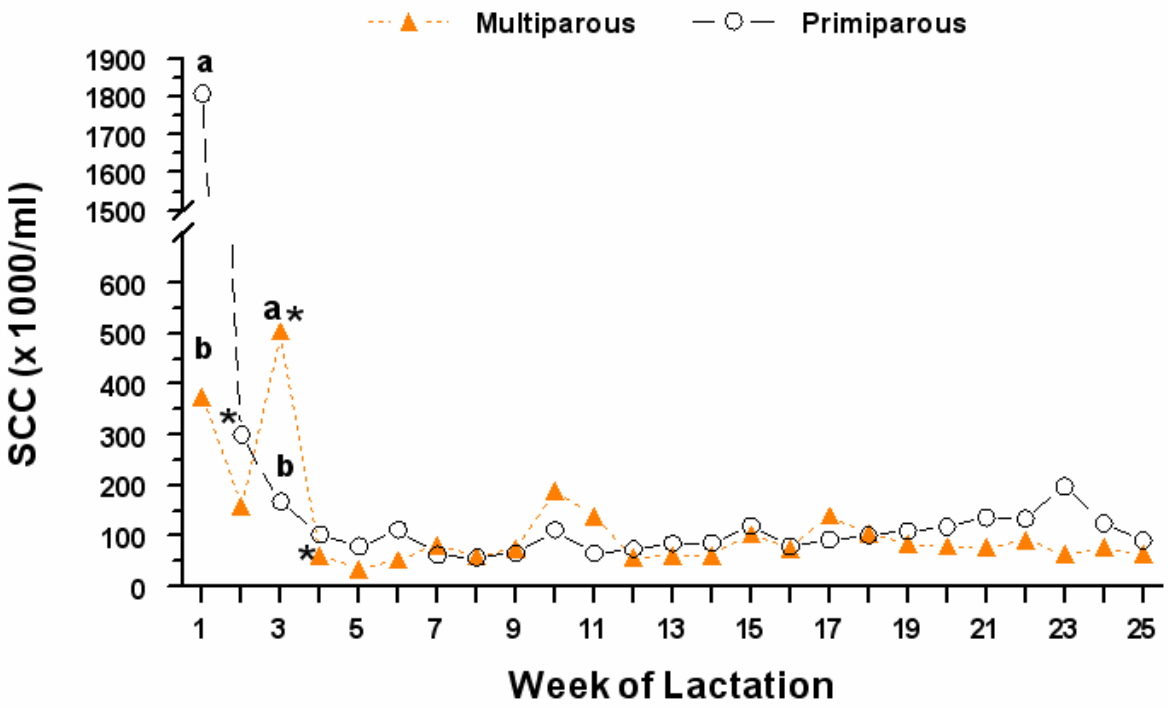

Multiparous
Primiparous
SCC (x1000/ml)
1900
1800
1700
1600
1500
600
500
400
300
200
100
0
a
b
a*
b
*
*
1
3
5
7
9
11
13
15
17
19
21
23
25
Week of Lactation

Figure 4.12. Average somatic cell counts (SCC; x 10^3/ml) in multiparous (n=19) and primiparous cows (n=19) fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations during wk 1 to 12 (Top Panel) and during wk 13 to 25 (Bottom Panel). Data were pooled across parity and week. [a,b] Means (± SEM) without a common superscript differ ($P < 0.003$).

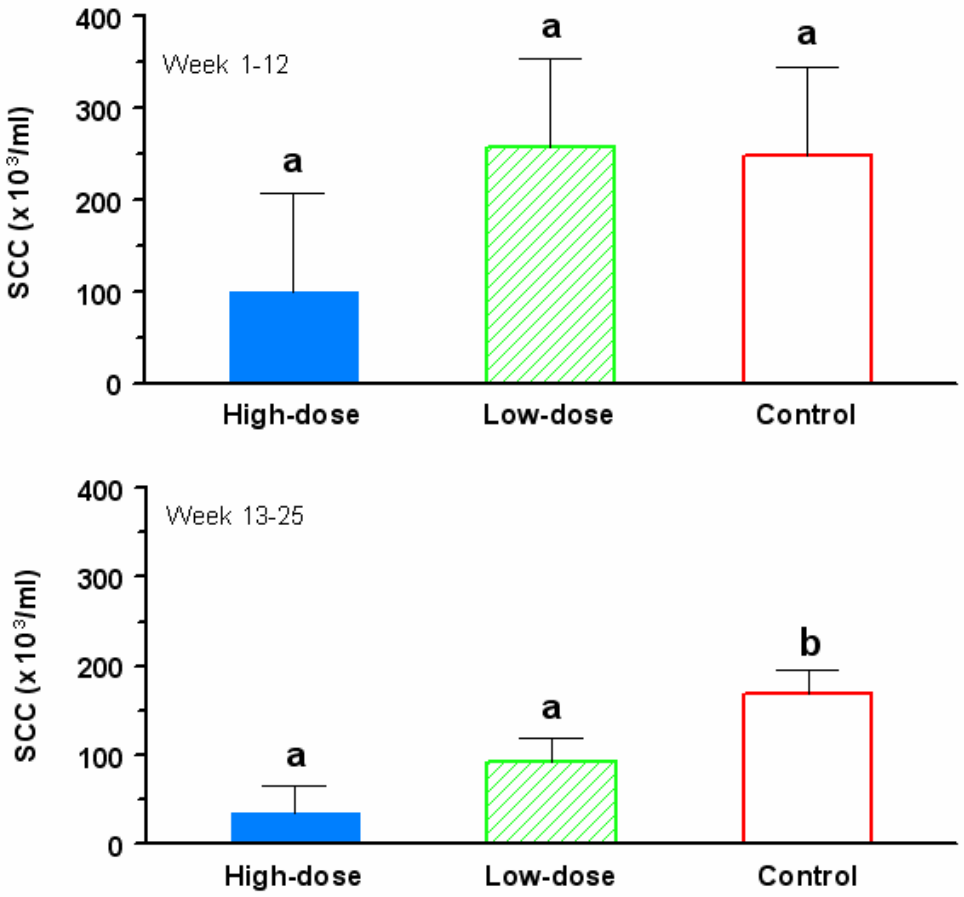

Week 1-12
SCC (x10³/ml)
400
300
200
100
0
a
a
a
High-dose
Low-dose
Control
Week 13-25
SCC (x10³/ml)
400
300
200
100
0
a
a
b
High-dose
Low-dose
Control

Figure 4.13. Effect of feeding Propionibacteria on ruminal volatile fatty acids (VFA). Left Panel: molar percent ruminal propionate (% of acetate, propionate, and butyrate) by treatment for multiparous (n=19) and primiparous (n=19) cows fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations for day +60, +120, and +175. [a,b] Means (± SEM) without a common superscript differ ($P < 0.05$). Right Panel: acetate/propionate ratio for multiparous (n=19) and primiparous cows (n=19) fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations for day +60, +120, and +175. [a,b] Means (± SEM) without a common superscript differ ($P < 0.06$).

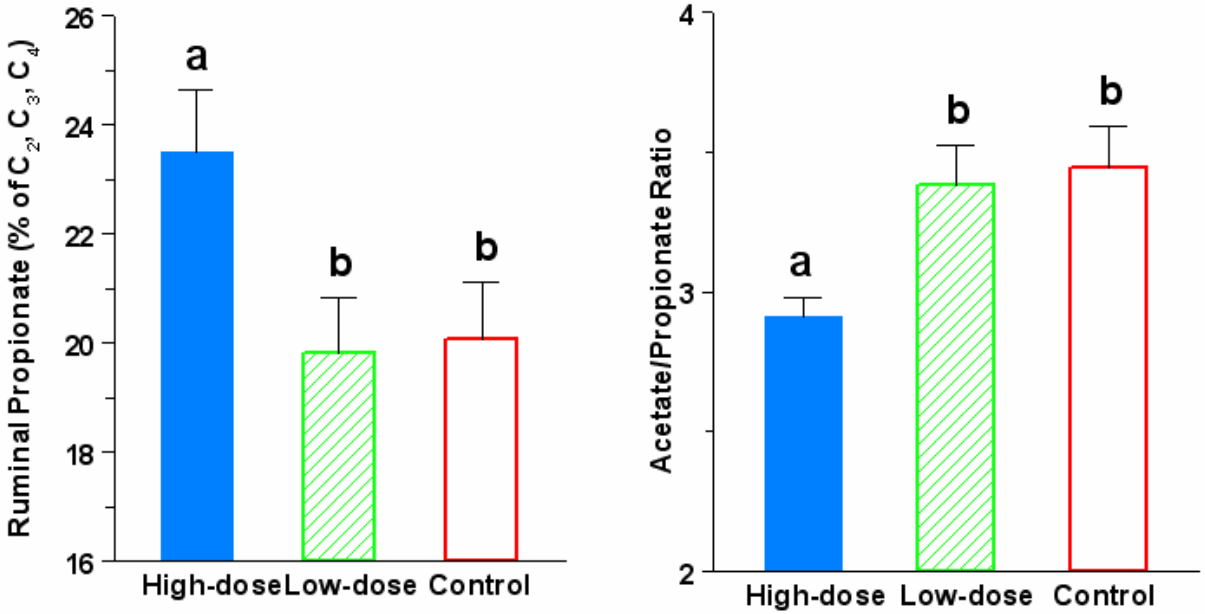

Ruminal Propionate (% of C2, C3, C4)
26
24
22
20
18
16
a
b
b
High-dose
Low-dose
Control
Acetate/Propionate Ratio
4
3
2
a
b
b
High-dose
Low-dose
Control

Table 4.3. Period I (d 45 of lactation) and Period II (d 90 of lactation) luteal activity and estrous behavior response of primiparous and multiparous dairy cows after synchronization with $PGF_{2\alpha}$.

Luteal Activity and Estrous Behavior Response of Dairy Cows after Treatment with $PGF_{2\alpha}$

Period	Treatment Group	Total, No.	Cyclic, No.	%	Response to $PGF_{2\alpha}$, No.	%	Number of Mounts, Total	Mount Duration (s)	Duration of Estrus (h)
(I)	High-dose	11	10	90.9	10	90.9	3.5 ± 0.6	5.3 ± 2.0	9.8 ± 6.2
	Low-dose	14	12	85.7	11	78.5	6.2 ± 3.4	2.5 ± 1.3	14.3 ± 4.0
	Control	13	12	92.3	12	92.3	7.1 ± 6.6	4.2 ± 1.8	18.7 ± 5.6
	avg.			89.6		87.2	5.6 ± 3.5	4.0 ± 1.7	14.3 ± 5.3
(II)	High-dose	11	11	100.0	11	100.0	3.6 ± 2.6	3.1 ± 0.6	16.5 ± 3.5
	Low-dose	14	14	100.0	13	92.8	5.2 ± 2.6	1.9 ± 0.5	8.3 ± 3.0
	Control	13	12	92.3	9	69.2	4.6 ± 2.7	2.1 ± 0.6	12.1 ± 3.8
	avg.			97.4		87.3	4.5 ± 2.6	2.3 ± 0.6	12.3 ± 3.2
	avg. I & II			93.5		87.3	5.1 ± 2.8	3.2 ± 1.2	13.3 ± 4.2

Figure 4.14. Ruminal pH for multiparous (n=19) and primiparous cows (n=19) fed High-dose (n=11) and Low-dose (n=14) Propionibacteria P169 and Control (n=13) rations for d -30, 60, 120, and 175 postpartum.

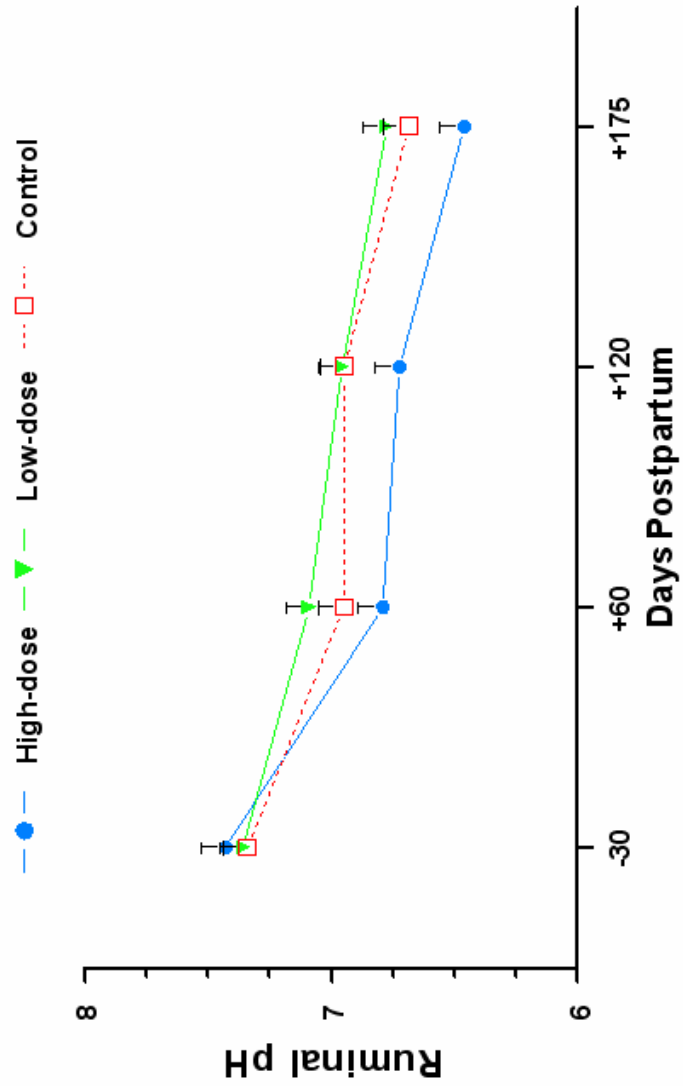
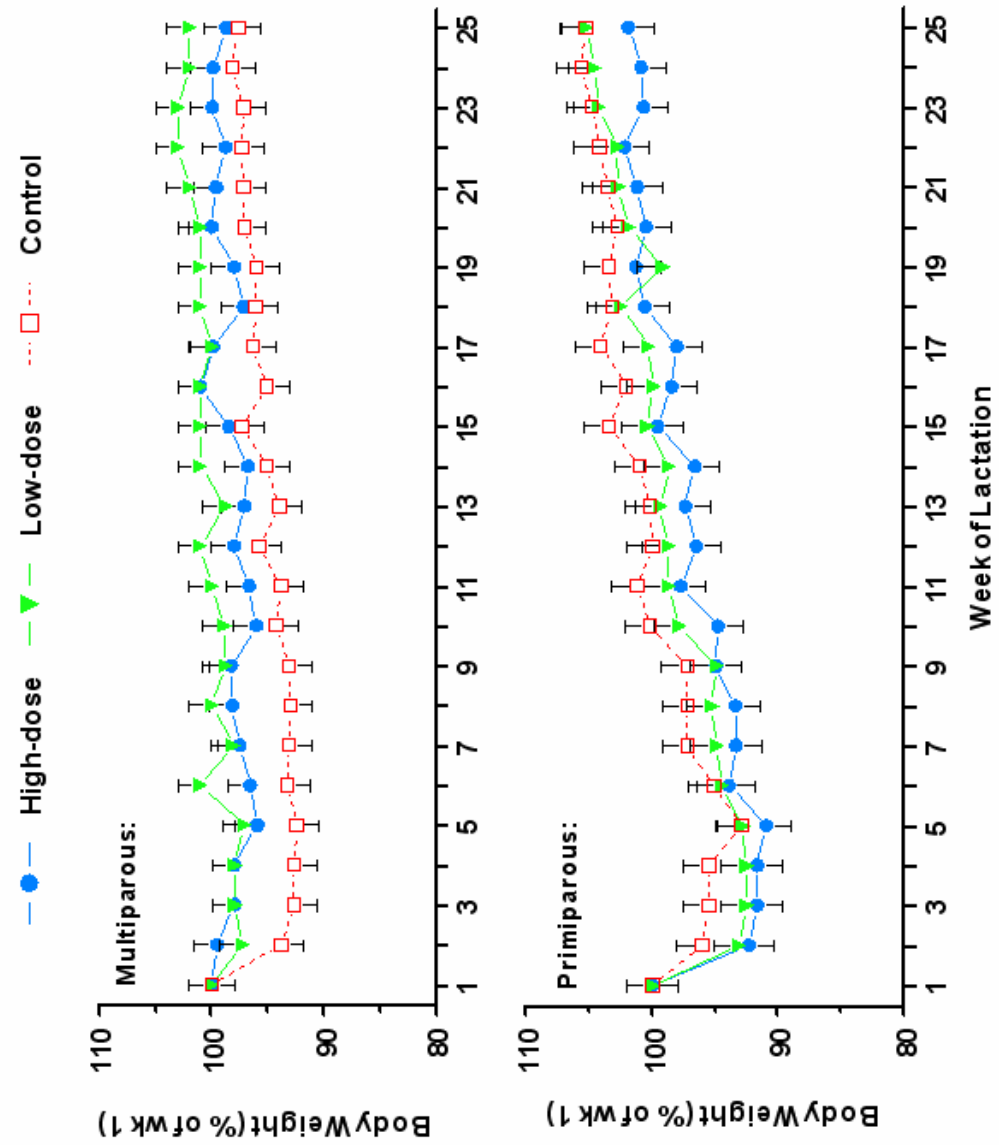

High-dose
Low-dose
Control
Ruminal pH
8
7
6
-30
+60
+120
+175
Days Postpartum

Figure 4.15. Effect of feeding Propionibacteria on body weight (BW) during 25 wk of lactation. Top Panel: changes in body weight between wk 1 and 25 of lactation in multiparous cows (n=19) fed High-dose (n=5) and Low-dose (n=6) Propionibacteria P169 and Control (n=8) rations. Data are expressed as percentage of wk 1 BW which averaged 739.4 ± 31.1 kg, 691.3 ± 28.2 kg, and 703.7 ± 24.0 kg for multiparous High-dose, Low-dose and Control groups, respectively. Bottom Panel: changes in body weight between wk 1 and 25 of lactation in primiparous cows (n=19) fed High-dose (n=6) and Low-dose (n=8) Propionibacteria P169 and Control (n=5) rations. Data are expressed as percentage of wk 1 BW which averaged 554.3 ± 28.2 kg, 522.0 ± 24.4 kg, and 528.9 ± 30.9 kg for primiparous High-dose, Low-dose and Control groups, respectively.


High-dose
Low-dose
Control
Multiparous:
Primiparous:
Body Weight (% of wk 1)
Week of Lactation
110
100
90
80
1
3
5
7
9
11
13
15
17
19
21
23
25

Chapter V

Discussion

In the present study, supplemental P169 feeding increased 4% FCM production by 8.5%. Previously, Francisco et al. (2002) reported that 4% FCM production was not affected by a 12 wk treatment with P169 supplementation at a dosage comparable to the Low-dose P169 level in the present study which extended over a 25 wk period and contained a yeast culture supplement. When milk production data of the current study was analyzed separately for wk 1-12 to relate to the data of Francisco et al. (2002) only a tendency ($P > 0.07$) for the High-dose and Low-dose P169 treatment to alter 4% FCM production was revealed. When analyzed separately for wks 13-25, 4% FCM production was significantly increased by P169 treatment with an 8.9% and 9.7% increase in the High-dose and Low-dose P169 treatment, respectively. These data suggest the possibility that the Francisco et al. (2002) study was not carried out over a period long enough to observe the persistency of lactation that occurred with P169 supplementation in the present study. Alternatively, because live yeast or yeast culture supplementation have been reported to increase milk production in several studies (Williams et al., 1990; Wohlt et al., 1991; 1998; Erasmus et al., 1992; Piva et al., 1993; Putman et al., 1997; Zhou, 2002; Kujawa, 2003), the presence of yeast culture in the TMR of the present study may have potentiated the effect of P169. Yeast supplementation has been shown to provide soluble growth factors (Harrison et al., 1988), stimulatory peptides (Rossi et al., 2004),

and other growth constituents (van Gylswyk et al., 1992) that stimulate the growth of specific groups of ruminal bacteria. However, other studies indicate no significant response of milk production to yeast supplementation (Erdman and Sharma, 1989; Arambel and Kent, 1990; Swartz et al., 1994; Kung et al., 1997; Dann et al., 2000; Kujawa, 2003). Thus, further research is warranted to investigate the potential interactions between supplemental yeast and P169 in regards to improving milk yield.

With the administration of bST, daily 4% FCM production was significantly greater in the Low-dose P169 treatment cows vs. the High-dose P169 treatment and Control cows, indicating that the positive effects of supplemental P169 feeding on milk production can be realized with a concomitant bST treatment. Average 4% FCM production after 5 wk of bST administration (i.e., wk 30) for all treatment groups did not significantly differ from wk 25 (wk 0 of bST treatment) milk production but, whether the persistence of lactation during this period of lactation when milk production declines was due to the positive effects of bST treatment could not be determined, because a "no bST" control for each respective treatment group was not included.

Increases in milk production are usually associated with increases in DMI (Williams et al., 1990; Erasmus et al., 1992; Putman et al., 1997; Robinson and Garret, 1999; Wohlt et al., 1991;1998), but individual feed intakes were not measured in the present study. Francisco et al. (2002) reported 9% lower DMI between wk 3 to 12 in P169 supplemented vs. control cows. Although individual DMI was not measured in the present study, pen averages for DMI from wk 3 to 14 for the High-dose P169, Low-dose P169 and Control treatment pens was 4.24, 3.91, and 4.05 kg feed intake/kg BW, respectively, and is similar to those reported by Francisco et al. (2002). Milk (Dairy)

efficiency [kg of 4% FCM / kg of feed (DMI)] is a measure of the ability of the cow to convert feed to milk, and can vary with DMI and season of year, from 1.1 to 1.8 kg/kg DMI for 3.5% FCM (Britt et al., 2003). Milk (Dairy) efficiency for the High-dose P169, Low-dose P169 and Control treatment pens was estimated at 1.32, 1.47 and 1.44, respectively. Additional research will be needed to further elucidate the effect of P169 feeding on DMI and milk efficiency.

Consistent with the results of the present study, the use of *Propionibacterium spp.* as a DFM in the feedlot segment has been reported to increase propionate levels, decrease acetate and butyrate levels, and decrease acetate/propionate ratios (Kim et al., 2001a). In support of the in vivo results of Kim et al. (2001a), Akay and Dado (2001) using autoclaved *Propionibacterium spp*. in vitro indicated that *Propionibacterium spp.* was responsible for the alteration in VFA concentrations. Other studies have found no effect of feeding *Propionibacterium spp*. on rumen fermentation patterns in vivo (Ghorbani et al., 2002) or in vitro (Yang et al., 2004). The strain of *Propionibacterium spp*. used does not explain the difference between studies. Kim et al. (2001a, 2001b), Akay and Dado (2001), and the current study used different strains of *Propionibacterium spp*., while Ghorbani et al. (2002), Yang et al. (2004), and Kim et al. (2001b) used the same strain of *Propionibacterium spp*. at similar doses in their respective studies. Kim et al. (2001b) reported non-significant increasing ruminal propionate levels as treatment levels increased, whereas in the present study ruminal propionate levels increased only in the High-dose P169 treatment which contained a sixty-fold greater level of propionibacteria than the highest treatment level of Kim et al. (2001b). The Low-dose P169 level in the present study was six-fold greater than the highest treatment level of Kim et al. (2001b)

and did not alter ruminal propionate levels. Ghorbani et al. (2002) fed a similar level of *Propionibacterium spp*. as the high treatment dosage of Kim et al. (2001b) and found no effect on ruminal propionate levels. Thus, differences in dose of propionibacteria alone cannot explain ruminal fermentation discrepancies among studies. In contrast to the present study, Ghorbani et al. (2002) and Kim et al. (2001b) did not feed a yeast supplement with the propionibacteria treatment. Like the effect of yeast on milk production, the effect of yeast supplementation on ruminal fermentation patterns is varied. Dairy cows supplemented with yeast culture had decreased molar proportions of acetate, and acetate/propionate ratio while molar proportions of propionate increased (Harrison et al., 1988). Miller-Webster et al. (2002) comparing two commercial yeast culture products (YC1 and YC2), reported both strains increased DM digestion, molar proportions of propionic acid and decreased acetic acid, indicating an influence of yeast on microbial metabolism. Kumar et al. (1997) reported increased concentration of total VFA, acetate, and acetate/propionate ratio, with propionate concentrations remaining unaffected when yeast culture supplement was fed to buffalo calves. Most studies however, report no changes in ruminal fermentation patterns with yeast supplementation (Erdman and Sharma 1989; Arambel and Kent, 1990; Williams et al., 1990; Erasmus et al., 1992; Piva et al., 1993; Swartz et al. 1994; Kung et al., 1997; Putman et al., 1997; Robinson and Garret, 1999; Dann et al., 2000). Francisco et al. (2002) did not measure ruminal VFA production in P169 supplemented cows. Rumen sampling methods, collection time, and frequency differed between the current study (intubation) and those of Kim et al. (2001b) and Ghorbani et al. (2002) (cannulation). Although intubation samples were possibly contaminated with saliva, the proportions of VFA should not

change for an individual sample (Molento et al., 1999). More likely, the time of rumen fluid collection, relative to feeding time may have a profound effect on rumen VFA, since ruminal propionate transiently increases after feeding.

The ionophores, monensin and lasalocid, have been shown to increase propionate concentrations and decrease the acetate/propionate ratio in the rumen in studies ranging from 2 wk prepartum (preP) to 17 wk postpartum (PP) (Brodercik, 2004); 3 wk preP to 3 wk PP (Sauer et al., 1989); and 24 wk to 38 wk PP (Weiss and Amiet, 1990). However, lasalocid was shown not to alter milk production in studies ranging from 2 wk preP to 17 wk PP (Erasumus et al., 1999); pre P to PP from 10 to 17 wk and for 24 wk to 38 wk PP DIM (Weiss and Amiet, 1990). Supplemental monensin feeding (typically 3 wk preP to 3-30 wk PP) has had variable effects on milk production as summarized by (Kennelly et al., 1998; McGuffey et al., 2001; Ipharraguerre and Clark, 2003; Brodercik, 2004). Overall, monensin increased milk production 2.6 to 11%, decreased milk fat 0.05 to 4.5% and decreased milk protein 0.01 to 1.6%. Sauer et al. (1989) and Green et al. (1999) reported no changes in percentage lactose with monensin treatment while Kennelly et al. (1998) reported variation of -6.0 to +11.0% in percentage lactose response to monensin and lasalocid treatments.

The use of glucogenic precursors to alter ruminal fermentation patterns and their subsequent effect on production parameters has varied. Infusion of propionic acid (C3) in the rumen of dairy cows for 14 d increased propionic acid, decreases acetic and butyric acid, and increased raw milk production, percentage milk protein and lactose, while substantially decreasing milk fat percentage (Rigout et al., 2003).

Multiparous Holstein cows fed an energy supplement containing 78% propionic acid from 3 wk preP to 3 wk PP, showed a decreased milk fat percentage with no changes in other milk components, SCC, or milk yield (Mandebvu et al., 2003). Nielsen and Ingvarsten (2004) reviewed the use of propylene glycol (PPG) (a treatment for ketosis in dairy cows), from 2 wk preP to 8 wk PP with dosage levels ranging from 200 to 1000 g/d and reported no significant effects on milk yield, milk composition, or DMI.

In the present study, milk fat percentage was lower in High-dose P169 vs. Low-dose P169 and Control multiparous cows, whereas no differences were seen in primiparous cows. Francisco et al. (2002) reported no changes in percentages of milk fat with P169 supplementation in multiparous cows. Because several studies have shown increases in milk fat percentage after live yeast or yeast culture supplementation (Erasmus et al., 1992; Piva et al., 1993; Putman et al., 1997), perhaps presence of yeast enhanced the effect of P169 in the present study. In a review of the literature, Bauman and Griinari (2003) discussed three theories of diet-induced milk fat depression: 1) alterations in rumen fermentation result in decreased production of acetate and butyrate to support milk fat synthesis, 2) increased rumen production of propionate and enhanced hepatic rates of gluconeogenesis cause an increase in circulating insulin, resulting in insulin–induced shortages of precursors for milk fat synthesis, and 3) synthesis of milk fat is inhibited by unique fatty acids produced as a result of alterations in rumen biohydrogenation. As ruminal percentages of acetate did not differ among treatments and the increase in percent ruminal propionate, with subsequent decrease of acetate/propionate ratio, was observed only in the High-dose P169 treatment, it is possible the milk fat depression observed in the present study is from insulin-induced shortages of glucogenic precursors.

Rigout et al. (2003) reported the mechanism for milk fat depression was different between infusion of duodenal glucose and ruminal propionic acid (C3). Propionic acid (C3) decreased milk fat production across all even number fatty acid chain lengths while odd number fatty acid short and medium-chain length increased milk fat. In contrast, glucose treatments decreased the production of even short- and long-chain fatty acids as production of all medium chain fatty acids and odd short chain fatty acids increased. Ipharraguerre and Clark (2003) indicated that the milk fat depression by ionophores may be due to reduced ruminal production of acetate and butyrate resulting in possible shortages of lipogenic precursors for fat synthesis. The molar percentage of ruminal butyrate tending to be lower in High-dose P169 vs. Low-dose P169 could help explain the differences in the reduction of milk fat percentage between the two treatment levels. Alternatively, because decreased milk fat percentages coincided with increased milk yield, a dilution effect could be part of the mechanism. The observed variability in the extent of milk fat depression associated with ionophore treatment may be due, at least in part, to the length of the observation period, as it appears that milk fat depression is more pronounced during the first few weeks of ionophore addition (Kennelly et al., 1998). A dilution effect does not seem to be the mechanism involved in the present study, as both High-dose and Low-dose P169 treatments increased milk production. Duffield et al. (2003) reported monensin significantly reduced milk fat percentage in TMR-fed but not component-fed cows. In addition, diets containing < 40.2 % in nonstructural carbohydrates reduced percentage milk fat, indicating interactions exist between certain dietary factors and monensin that may affect milk fat.

Both High-dose and Low-dose P169 treatment tended to increase milk protein percentage by 3.8% and 3.2 %, respectively, above Control multiparous cows during the 25 wk study. Francisco et al. (2002) reported an increase in percentage of milk protein only during wk 1 of lactation in P169 supplementation vs. Control. The effect of live yeast or yeast culture supplementation on milk protein has varied, with most studies reporting no changes in milk protein levels (Erdman and Sharma, 1989; Arambel and Kent, 1990; Erasmus et al., 1992; Piva et al., 1993; Wohlt et al., 1998; Dann et al., 2000) whereas, others report increases (Williams et al., 1990; Putman et al., 1997) or decreases (Zhou, 2002) in milk protein levels. McGuffey et al. (2001) reported an increase in milk protein percentage using controlled-release monensin. However these results differ from those reported in a review by Ipharraguerre and Clark (2003) where milk protein percentage in ionophore-treated cows tended to be lower than untreated cows, suggesting that a reduction in milk protein may be a dilution effect. Ruminal propionic acid (C3) infusion led to an increase in protein yield (Rigout et al., 2003). DePeters and Cart (1992) reported positive correlations between the amount and concentration of ME and either milk protein content or milk yield and suggested that increasing energy intake through the allocation of concentrates increased the protein content of milk. In a review by Emery (1978), the percentage of milk protein increased 0.15 units (% dev. from mean) for each megacalorie of increased daily intake of net energy when the increased intake came from grain or roughage. However, DePeters and Cart (1992) went on to report studies to the contrary where no change in protein content of milk was reported with increased concentrate feeding. Principal component analysis was performed to determine which variables explained the majority of the variation in the data from 40 publications

encompassing 59 trials and 217 treatments and revealed that total amino acid (AA) supply and total NE_l are the primary determinants of milk protein yield (Doepel et al., 2004). Energy availability to the animal affects the energy availability to the rumen microbes, which is a regulating factor of microbial protein yield. Estimations of bacterial metabolizable protein accounted for 55% of estimated total metabolizable protein supply (Doepel et al., 2004). Thus, changes in the animal's energy supply and the flow of microbial protein to the lower intestinal tract could result in increased milk protein levels with variations dependent on blood flow to the mammary gland and the efficiency within the mammary gland, as amino acids are absorbed by the mammary gland in quantities sufficient to account for protein synthesized within it (McDonald et al., 2002). Propionate spares glucogenic amino acids in gluconeogenesis and consequently reduces the maintenance cost of metabolizable protein and possible heat increment (Soest, 1994). In support of the former statement, circulating plasma free amino acid concentrations are significantly increased by intraruminal propionic acid infusion (Seal and Parker, 1995). Whether the P169-induced increases in milk protein levels in the present study was due to improved rumen function, increased DMI, or both will require further evaluation.

Francisco et al. (2002) reported no changes in percentages of milk lactose with 12 wk P169 supplementation. In the present study, milk lactose data analyzed separately for wk 1-12, showed no difference in the percentage of milk lactose between Low-dose P169 and Control cows and thus supports the findings of Francisco et al. (2002). However, the percentage of milk lactose was greater in the High-dose P169 vs. Low-dose P169 and Control cows for wk 1-12. During wk 13-25, milk lactose percentage was influenced similar to that observed in wk 1 – 25, where milk lactose percentage was significantly

greater in the High-dose P169 vs. Low-dose P169 multiparous cows , and the Low-dose P169 multiparous cows significantly greater than Control cows. Sauer, (1989) supplementing monensin from 2 wk preP to 3 wk PP showed no effect on milk lactose concentrations. Lactose is the only carbohydrate in milk and is composed of one molecule of glucose and one molecule of galactose, derived almost entirely from glucose, with virtually all the glucose derived from the blood (McDonald et al., 2002). Theoretically, supplementation with P169 should increase propionate and thus increase glucose concentration via gluconeogenesis. The lack of response in the study of Francisco et al. (2002) suggests that the dose of Propionibacteria was not large enough or measured over a period long enough to observe significant effects on milk lactose as seen in the present study.

Milk SNF consists mainly of lactose, protein, minerals, and to a lesser extent enzymes and vitamins. Francisco et al. (2002) reported the percentage of SNF, like milk protein levels, was greater in cows fed P169 vs. control on wk one of lactation. The present study indicated the percentage SNF was greater in both High-dose and Low-dose P169 multiparous cows vs. Control multiparous cows, but no differences in SNF existed among High-dose P169, Low-dose P169 and Control primiparous cows. These patterns follow closely the patterns of the individual milk components (lactose and protein) that are used in the calculation of milk SNF.

Milk fat, protein, lactose, and SNF do not seem to be significantly altered by bST treatment, however, slight variations, (i.e., increases in milk fat and increases or decrease in milk protein) may occur during the first few weeks after initiation of bST treatment, and this variation seems to be transient and generally a normal balance is shortly re-

established (Thomas et al., 1991; Bauman, 1992; Huber et al., 1997; Etherton and Bauman, 1998; Tarazon-Herrera and Huber, 2000; Velez and Donkin, 2004).

Changes in MUN levels paralleled milk yield: lower during the first 30 DIM, then increasing and peaking around 60-70 DIM, and then decreasing. Also in agreement with the present study, Francisco et al. (2002) reported no effect of P169 supplementation on MUN levels during wk 1 to 12 of lactation. Similarly, the tendency for MUN concentrations to be higher in multiparous cows vs. primiparous cows is likely due to production levels and intake differences (Godden et al., 2001). However, for wk 13-25, MUN levels were greater in High-dose P169 and Low-dose P169 cows vs. Control cows. Increased MUN could be due to a reflection of increased DMI in the P169 groups or due to increased ammonia incorporation into microbial protein in the rumen, which increases protein supply for milk protein synthesis and decreases N loss (Nousiainen et al., 2004). Additional work will be required to ascertain the role of supplemental P169 on nitrogen balance in lactating dairy cows.

Somatic cells in milk include leukocytes, with neutrophils the predominant cell type during early inflammation (> 90%), and sloughed epithelial cells, and the level of SCC are directly related to the degree of stress on the udder (Sordillo et al., 1997). Thus, somatic cells are a reflection of an inflammatory response to an intramammary infection or some other stimulus of the immune system. The largest reductions in SCC takes place in the first two weeks postpartum where SCC are reduced by over 80 % after the sixth subsequent milking postpartum (Barkema et al., 1999). In the present study, primiparous cows exhibited a greater SCC at wk 1 than multiparous cows, while multiparous cows were greater than primiparous cows at wk 3. From wk 4 to 25, no significant difference

in SCC was observed between parity groups. For wk 13 - 25, SCC levels were significantly altered by treatment with both High-dose P169 and Low-dose P169 cows exhibiting a significantly lower SCC vs. Control cows. When the data was analyzed from wk 4 -25 (after the time of major fluctuations associated with the onset of lactation), the reduction in SCC that was seen at wk 13-25, could be seen beginning from wk 4. This treatment effect was exhibited through the 5 wk bST administration. Dan et al. (2000) reported no changes in SCC in primiparous and multiparous cows supplemented with a yeast culture. Whether the P169-induced reduction in SCC is from a direct stimulation of the immune system by the Propionibacteria organism (i.e., a metabolic substrate or cell-wall component which can serve as a chemoattractant for leukocytes) or the enhancement of the animal's metabolic system will require further study.

Francisco et al. (2002) reported no significant difference in the average days to first ovulation in P169 treated (44.8 ± 7.7 d) vs. control cows (30.1 ± 7.4 d). The present study also showed no significant difference in the average days to first ovulation in High-dose and Low-dose P169 treated vs. control cows. Days to first ovulation averaged 32.4 ± 6.0, 30.8 ± 5.3 and 32.9 ± 5.6 d for the High-dose, Low-dose and Control treatment groups, respectively. It appears the increased milk production from P169 supplementation had no adverse effect on days to first ovulation indicating that the increased milk production was compensated for by altered metabolism, increased nutrient intake and (or) nutrient supply.

The estrous behavior response (i.e. number of mounts, mount duration, and duration of estrus) did not significantly differ among treatment groups, and the estrous behavior response data recorded by the HeatWatch® system was consistent with previously

published data (Walker et al., 1996; Xu et al., 1998; Dransfield et al., 1998; At-Taras and Spahr, 2001; Lopez et al., 2004). However, the efficiency of the HeatWatch® system for detecting estrus in the present study (57% and 62%) was lower than previously reported values of 72 – 95%.

This inconsistency could arise from several factors. The total number of animals for each treatment group was low (i.e., 11-14 head) and the cows remained on concrete throughout the study. In addition, only 1 to 3 cows per treatment group were synchronized at any given week during the study in order that the 45 and 90-d postpartum average could be attained. Britt et al., (1986) reported standing and mounting activity was 54% greater on dirt than on concrete. In addition, over 17% of the cows that stood on dirt declined to stand on concrete while over 10% of the cows that mounted on dirt declined to mount on concrete. Similar findings were reported by Vails and Britt, (1990) where estrual cows were shown to prefer mounting tied estrual cows on dirt, nearly three times more often in a 30-min period than those on concrete surfaces. Lopez and Shipka, (2003) suggested that cows either felt uneasy trying to mount on concrete flooring or that they have learned from previous experiences to be fearful of mounting on concrete flooring. If the number of cycling, nonpregnant, animals in a group is low, regardless of the total group size, accurate detection of estrus may be difficult. Also, cows that are at the mid-stages of their cycles, (d 5 - d 16), are least likely to mount a cow that is in heat and consequently could be termed "poor heat detectors" (Diskin and Sreenan, 2000). Hurnik et al. (1975), Helmar and Britt (1985) and Floyd (2001) all report similar findings: as the number of animals in estrus simultaneously increased, there was a proportional increase in the number of mounts per hour and increased mounting activity. The average total number

of mounts in the present study (i.e., 5-6) was similar to the total attempted mounts recorded (i.e., 6.1) by Helmar and Britt (1985) when only one estrual cow was present in a pen, but lower than 11 mounts recorded when one estrous cow was present in a free stall barn setting (Hurnick et al., 1975). Thus, the low number of cows synchronized each week per treatment group, the stage of the estrous cycle of the non-synchronized cows in each treatment group, and the concrete flooring surface likely affected the estrus detection efficiency of the HeatWatch® system in the present study. The increased number of detected estruses in Period I compared to Period II supports Spicer et al., (1990; 1993) where the percentage of cows detected in estrus increased from the first to second postpartum ovulations.

High-dose P169 treatment had a lower ruminal pH than Low-dose P169 and Control cows with pH values of 6.65, 6.94, and 6.86, respectively, and is consistent with increased propionate production. Even though the pH was statistically lower in the High-dose P169, in all probability, the pH level did not drop below a level that would cause a change in ruminal fermentation patterns due to shifts in microbial populations. Russell, (1998) reported no changes in acetate/propionate ratio or H_2 concentrations until the pH was below 5.5 to 5.7. At this point, there was a decrease in the acetate/propionate ratio and mirrored a reduction in CH_4 production supporting the idea that propionate production and methanogens are competing in alternative mechanisms of reducing equivalent disposal. At the end of the 5 wk bST administration (d 204) and d 265 (60 d after end of P169 treatment), ruminal pH was not affected by treatment, parity, or treatment x parity interaction.

With the onset of lactation, most dairy cows are not able to meet the energy requirements for maintenance and milk production from the diet due to insufficient feed intake and experience a postpartum negative energy balance (De Vries et al., 1999; Stevenson, 2001; Butler, 2003; Jorritsma et al., 2003) which results in body weight loss. In the present study, nadir BW occurred at wk 5 for both the multiparous and primiparous cows in all treatment groups. In contrast to Harris et al. (1990), control multiparous cows (lower producers) exhibited a 7.5% loss of wk 1 BW, at wk 5, than the Low-dose P169 group (2.9%) and High-dose P169 (4.0%). High-dose P169 and Low-dose P169 multiparous cows (higher producers) exhibited a significantly greater recovery of wk 1 body weight vs. Control multiparous cows (lower producers). In contrast to multiparous cows, control primiparous cows exhibited a greater recovery of wk 1 body weight than High-dose P169 primiparous cows, and Low-dose P169 primiparous cows not differing from either Control or High-dose primiparous cows. During the 5 wk bST administration, averaged across treatment and parity, cows continued to gain body weight. Postpartum dairy cows first partition metabolizable energy toward milk production, then body condition gain, and finally to reproductive functions (Silvia, 2003). It appears that the increased milk production from P169 supplementation had no adverse effect on changes in body weight, indicating that the increased milk production was compensated for by altered metabolism, increased nutrient intake and (or) nutrient supply.

The most commonly accepted theory of changes in ruminal propionate altering metabolism in lactating cows is that increases in ruminal propionate production results in a greater supply of this glucogenic precursor to the liver, thereby increasing glucose

production (Sauer et al., 1989). Although milk production, percentage milk protein, SNF, MUN and changes in percentage body weight were similar between the High-dose P169 the Low-dose P169 treatments, the presence of significant treatment differences in percentage ruminal propionate, acetate/propionate ratio, pH, and percentage milk fat and lactose lack clear explanation. Perhaps transient changes in rumen VFA were not detected with a single rumen fluid collection, particularly if the increases were short-lived. The increased milk production in the High-dose P169 treatment follows Sauer's theory of increased glucogenic precursors (i.e., propionate) increasing gluconeogenesis and is further supported by the increased percentage of milk lactose, increased ruminal propionate percentages, decreased acetate/propionate ratio, and depressed milk fat percentages. The Low-dose P169 treatment lacking the measurable effects of increased gluconeogenesis as observed in the High-dose P169 treatment may follow a different (or combination) mode of action(s) other than increased gluconeogenesis, such as those categorized by Yoon and Stern (1995): 1) stimulation of desirable microbial growth in the rumen, 2) stabilization of rumen pH, 3) altered ruminal fermentation pattern and end product production, 4) increased nutrient flow postruminally, 5) increased nutrient digestibility, or 6) alleviation of stress through enhanced immune response

Conclusions

Feeding High-dose P169 and Low-dose P169 to primiparous and multiparous cows increased daily milk yield and 4% FCM milk production during the 25 wk study suggesting that the Francisco et al. (2002) study was not carried out over a period long enough to observe the persistency of lactation that may occur with P169 supplementation. Milk fat percentage was lower in High-dose P169 multiparous cows while milk lactose and SNF percentage increased in High-dose P169 multiparous and primiparous cows. A tendency to increase milk protein was exhibited in both High-dose and Low-dose P169 multiparous cows. High-dose P169 treatment increased molar percentages of ruminal propionate, lowered ruminal pH, and somatic cell counts for wk 13 to 25, while decreasing acetate/propionate ratio and molar percentage of ruminal butyrate. Treatment or treatment x parity interaction did not affect MUN levels or molar percentage of ruminal acetate. Because molar percentage of ruminal acetate did not differ among treatments, and the increase in percent molar ruminal propionate, with subsequent decrease of acetate/propionate ratio, was observed only in the High-dose P169 treatment, it is possible that the milk fat depression observed in the present study is due to insulin-induced shortages of glucogenic precursors. Changes in an animal's energy supply and flow of microbial protein to the lower intestine could result in increased milk protein levels with variations dependent on mammary blood flow and the efficiency of amino acid use within the mammary gland.

High-dose and Low-dose P169 multiparous cows recovered a greater percentage of wk 1 body weight than Control multiparous cows at the end of the 25 wk, while no differences were seen among any reproductive measures. It appears that the increased

milk production from P169 supplementation had no adverse effect on changes in body weight or on reproductive measures indicating that the increased milk production was compensated for by altered metabolism, increased nutrient intake and (or) nutrient supply.

During bST administration, High-dose and Low-dose P169 had a tendency to increase milk yield over Control. High-dose P169 increased milk lactose levels and lowered SCC. Protein and SNF percentage, in primiparous cows, responded to time of bST injections, as did weekly MUN levels. Treatment or treatment x parity did not alter rumen acetate, propionate, or butyrate percentages, or pH. Primiparous and multiparous cows continued to recover body weight during bST administration, wk 25 to 30. These studies provide evidence for potential use of P169 as a direct-fed microbial for improving milk production in the dairy industry. Further studies should be conducted involving a larger number of cows in commercial settings to determine if P169 should be developed for commercial use.

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VITA

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Candidate for the Degree of

Master of Science

Thesis : DOSE RESPONSE OF A DIRECT-FED MICROBIAL ON MILK YIELD, MILK COMPONENTS, BODY WEIGHT, AND DAYS TO FIRST OVULATION IN PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

Major Field: Animal Science

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Title of Study: DOSE RESPONSE OF A DIRECT-FED MICROBIAL ON MILK YIELD, MILK COMPONENTS, BODY WEIGHT, AND DAYS TO FIRST OVULATION IN PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

Pages in Study: 157 Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope of Study: This study was conducted to investigate whether manipulating ruminal fermentation with propionibacteria would result in positive economic impact on milk production, milk components, and improved reproductive efficiency in dairy cows. From two wk prior to parturition to 175 d postpartum, 38 primi- and multiparous Holstein cows were assigned to three treatment groups: Control (n = 13) received Total Mixed Ration (TMR); Low-dose treatment (n = 14) received control TMR plus 6 x 10^{10} cfu/head Propionibacteria Strain P169 (P169); High-dose treatment (n = 11) received control TMR plus 6 x 10^{11} cfu/head P169. P169 was fed daily (p.m.) as a top-dress on 4.5 kg of TMR. Cows were milked twice daily. Twice weekly milk samples were analyzed for percentage milk fat, protein, lactose, and solids-not-fats (SNF), milk urea nitrogen (MUN) and somatic cell count (SCC). Rumen fluid was collected at 30 d prepartum, and at d 60, 120, and 175 postpartum.

Findings and Conclusions: Daily 4% fat-corrected milk (FCM) production was affected by treatment and week x parity. High-dose and Low-dose P169 treatments exhibited a 7.1 % and 8.5 % increase in daily 4% FCM production vs. Control, respectively. Treatment x parity and week influenced percentage milk fat, lactose, and protein, where High-dose P169 decreased milk fat percentage and increased milk lactose and protein percentage vs. Control. SNF was influenced by treatment x parity and treatment x week. Propionate levels and acetate/propionate ratio were influenced by treatment such that High-dose P169 had an 18.5% and 17.0% increase in molar percent ruminal propionate over the Low-dose P169 and Control, respectively, with High-dose P169 having a 15.41% and 13.33% decrease in the acetate/ propionate ratio from Low-dose P169 and Control, respectively. Change in body weight from wk 1 was influenced by week x parity and treatment x parity. High-dose and Low-dose P169 multiparous cows had a greater recovery of wk 1 body weight vs. Control. No treatment, parity, or interaction on days to first ovulation after parturition was observed. We conclude P169 may hold potential as an effective direct-fed microbial to increase milk production.

Advisor's Approval: Dr. Leon J. Spicer