DIRECT-FED MICROBIAL SUPPLEMENTATION TO CATTLE: EFFECTS ON MILK YIELD AND COMPONENTS, REPRODUCTION AND DIGESTION

By

KENEUOE VICTORIA LEHLOENYA

Bachelor of Science in Agriculture
The National University of Lesotho
Maseru, Lesotho
1995

Master of Science in Animal Science
Bunda College of Agriculture
Lilongwe, Malawi
1999

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 2007

DIRECT-FED MICROBIAL SUPPLEMENTATION TO CATTLE: EFFECTS ON MILK YIELD AND COMPONENTS, REPRODUCTION AND DIGESTION

Dissertation Approved:

Leon J. Spicer
Dissertation Adviser

Mark Payton

Clint R. Krehbiel

Gerald Horn

A. Gordon Emslie
Dean of the Graduate College

ACKNOWLEDGEMENTS

My first and ultimate thanks go to God, my Heavenly Father for His hand upon my life throughout the period that I spent studying at Oklahoma State University. My journey was not an easy one, but I know that God had guided me to this point in my life by His grace not by my might or my power.

My profound appreciation goes to Dr. Leon Spicer for serving as my major advisor and giving me an opportunity to work on my Ph.D. at Oklahoma State University. You have guided me right from the initial stage of my Ph.D. program until the end. Thank you so much for your patience, understanding and your scientific excellence.

Special thanks go to my committee members, Drs. Clint Krehbiel, Mark Payton, and Gerald Horn for their patience, support and their involvement in my PhD work.

I owe a great deal of thanks to David Jones and a crew at OSU dairy cattle center for their support and commitment to take care of our research cows. Without them, this work would have not been successful.

Joan, even though I met you towards the end of my program, you have filled the roles of mentor, assistant, and friend. You have patiently helped me along the way and my last part of research project would have not been possible if you had not been there for me.

My research would not have been possible without financial support from Agtech Inc. and Kellogg Foundation. I truly appreciate your valuable input.

To my very best friends, Cons and Tat (Dr. Morillo), I could not imagine my life without you here at OSU and even after you had graduated. I wish this relationship grows stronger and continues forever.

It will be approximately, three and half years after a sudden death of my beloved mother, M'athato Juliet Lehloenya, when this dissertation is completed. Because of her sincere love and dedication to take care of my son, Letlotlo Alexander Lehloenya, I dedicate this thesis with love to her and her only grandson who has been so supportive and understanding during the period we spent together here in the US. Thank you so much to both of you.

.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

Chapter III

Chapter IV

LIST OF FIGURES

Figure Page

Chapter III

Figure Page

Figure Page

CHAPTER I

INTRODUCTION

In general, the goal of any dairy industry is to maximize milk production without compromising animal health and reproductive performance. The way a cow is managed during the transition period determines her overall performance. During the transition period, 3 wk before parturition through 3 wk postpartum, many high producing dairy cows experience serious metabolic disorders (Goff and Horst, 1997). Occurrence of metabolic disorders during the transition period negatively affects the subsequent lactation, increase the risk of culling (Grohn et al., 1998), decrease milk production (Rajala and Grohn, 1998) and increase days to conception. Therefore, recently, improvements in dairy production have been focused on the transition period management, and much of the research is aimed at improving the understanding of cow physiology, as well as providing producers with tools to better manage cows during transition period. Despite these efforts, transition period continues to present a serious challenge because of the complexity caused by combination of physiological and metabolic activities that take place during this time.

One major metabolic challenge for cows once they calve is the tremendous nutrient demand to support milk production, at a time when dry matter intake (DMI) is reduced (Grummer, 1995; Drackley, 1999). This situation, combined with other stressors during

the periparturient period, can increase the risk of metabolic diseases and health disorders (Drackley, 1999).

In ruminants, there is little or no absorption of dietary carbohydrate as glucose (Bergman et al., 1974), unless large amounts of concentrates are fed making endogenous synthesis of glucose from non-hexose sources an essential requirement. This can be more significant during the transition period when the demand for glucose is substantial. The basal rate of gluconeogenesis is regulated by the availability of major precursors (Lindsay, 1978), which include propionate, amino acids, lactate and glycerol (Bergman, 1973; Lindsay, 1978), each contributing about 32 – 73, 10 – 30, 15, and 4 - 5%, respectively, of glucose production (Seal and Parker, 1994).

The lactating mammary gland utilizes most (i.e., 60 – 85%) of the glucose entering the circulation of ruminants (Knowlton et al., 1998). A substantial (i.e., 50 – 85%) part of the glucose taken up by the mammary gland is used for lactose synthesis (Amaral-Phillips et al., 1993; Knowlton et al., 1998). Despite the substantial contribution by gluconeogenesis, glucose continues to be limiting in high producing cows even in healthy cows, thus, supplementation becomes compulsory to ameliorate possible potential metabolic disorders leading to decreased milk production and prolonged days to first postpartum ovulation.

Researchers have struggled with finding possible solutions to alleviate the problems associated with this time period. Strategies to compensate for the decline in DMI have included increasing the caloric density of the diet. This may be achieved in two ways. The first method is to increase the readily fermentable carbohydrates in the diet by increasing the amount of grain feed and, hence reducing the amount of forage. This

however, may lead to metabolic disorders such as lactic acidosis and displaced abomasum. Another alternative to increasing energy intake in the diet is to add fat. Fat has approximately 2.25 times the calories of carbohydrates per gram and can be effectively added to the ration maintaining forage content. However, effects of fat on rumen microbial population have been noted by several authors. Sources of fat and its composition influence rumen microbial populations directly and indirectly through interaction with other feed ingredients.

Antibiotics have been used as one method of intervention. However, due to increasing public concerns over the residues in milk and producing animals resulting from the use of antibiotics and other anabolic hormones, there has been a shift to a use of natural feed supplements. Direct-fed microbials, including, bacteria, fungi and yeast have been used in place of antibiotics.

Propionibacteria have been used as a direct-fed microbial to prevent the risk of acidosis (Swinney-Floyd, 1997; Davidson, 1998; Ghobani et al., 2002, Yang et al., 2004) in feedlot cattle. Also, Propionibacteria have been demonstrated to reduce nitrate toxicity in cattle (Swatzlander, 1994). Propionibacteria have been used in dairy to enhance metabolism via increase in ruminate propionate production. Propionate is a major glucogenic substrate, spares glucogenic amino acids in gluconeogenesis and consequently reduces the maintenance cost of metabolizable protein (Sauer et al., 1989). The efficiency of utilization for maintenance of propionate is 0.86 compared to 0.59 and 0.76 for acetate and butyrate, respectively. The theoretical efficiency of propionate as a source of energy for ATP/100 g nutrient is 108% compared with glucose (McDonald et al., 2002). All

these benefits emphasize the importance of propionate, as a potential feed supplement during the transition period.

Recently, Propionibacteria, strain P169, has been shown to alter metabolism via increases in plasma leptin , glucose and insulin (Francisco et al., 2002; Aleman et al., 2007) in Holstein cows. Despite these positive effects of P169 on plasma hormones and metabolites, acute temporal changes of plasma insulin and glucose concentrations have not been evaluated. Moreover, P169 in the presence of yeast has been shown to increase propionate and consequently increase milk production and milk components in Holstein cows (Stein et al., 2006), however, systematic studies have not been conducted to compare the effect of P169 in conjunction with yeast to yeast alone. Therefore, a better understanding of how direct-fed microbials, specifically, yeast culture and P169 affect ruminal fermentation, nutrient intake and digestibility, and flow of microbial protein to the lower gut is necessary in order to increase production efficiency of dairy cows. The objectives of the research reported herein are as follows:

1) To evaluate the effect of feeding yeast alone or in the presence of Propionibacteria (P169) on milk yield, milk components, BW, BCS, and postpartum days to first and second ovulation.
2) To evaluate the acute temporal effect of feeding yeast with or without P169 on plasma glucose and insulin concentrations.
3) To evaluate the effect of feeding yeast, P169 and a combination of both on DMI, digestibility, rumen fermentation profile, duodenal flow and microbial protein synthesis and ruminal kinetics.

CHAPTER II

REVIEW OF LITERATURE

Physiologic and metabolic changes of dairy cows during the transition period

Dairy cows generally progress from positive energy balance before calving to negative energy balance early in lactation, to energy equilibrium for a variable period, and then to a positive energy balance during the last part of lactation (Amaral-Phillips et al., 1993: Knowlton et al., 1998). The transition period between the late pregnancy and early lactation certainly is the most interesting and challenging period of cow's lactation cycle. The length of time classified as the transition period has been defined differently by different authors. Drackley (1999) and Grummer (1995) defined transition period as the last 3 wk before parturition to 3 wk after parturition. It is a period marked by changes in endocrine status to accommodate parturition and lactogenesis (Grummer, 1995). This period is critically important to health, production, and profitability of dairy cows (Drackley, 1999). Most health disorders such as milk fever, ketosis, retained fetal membranes, metritis, displaced abomasum, and hepatic lipidosis occur during this time. Immunosuppression during the periparturient period leads to increased susceptibility to mastitis. Indeed, the incidence of environmental mastitis is greatest around parturition (Smith et al., 1985; Mallard et al., 1998). The primary challenge faced by cows is a sudden and marked increase of nutrient requirements for a rapidly growing fetus and for milk production, at a time when the DMI is limited, and thus nutrient supply lags behind.

During late pregnancy, glucose requirements increase four to seven fold in lactating compared to non-lactating dairy cows (Bell and Bauman, 1997). Moreover, studies with lactating goats, sheep and cows have shown that the glucose requirement of the mammary gland accounts for 60 to 85% of the glucose used by lactating ruminants and lactose synthesis accounts for 50 to 85% of the glucose extracted by the mammary gland (Amaral-Phillips et al., 1993). Therefore, maximizing energy intake is extremely important for nutritional management of cows in early lactation, not only for maximizing milk production, but also for preventing metabolic disorders (Oba and Allen, 2003). Maximal energy intake can be achieved by increasing the energy content of the diet or by manipulating the diet to maximize rumen fermentation.

Mechanisms regulating voluntary feed intake are not well understood for cows in early lactation (Oba and Allen, 2003). Physical fill can be the most dominant mechanism limiting DMI for high yielding cows around peak lactation (Allen, 2000), but it may contribute less in early lactation (Ingvartsen and Anderson, 2000). Dry matter intake starts to decrease before parturition with the lowest level occurring at calving (Ingvartsen and Anderson, 2000). Typically, feed intake is reduced by 30 to 35% (Bertics et al., 1992; Grummer, 1995). Average values of DMI for the prefresh transition period have been reported to range between 1.7 and 2.0% of body weight (BW) (Hayirli et al., 1999). However, this is not a constant value and it can be influenced by the ration that is fed, the stage of transition period, body condition score (BCS) and parity (Hayirli et al., 2002).

Propionate and NEFA are the primary metabolic fuels extensively utilized by the ruminant liver (Emery et al., 1992). Glucose, acetate, and butyrate are the other major metabolic fuel for ruminants but are not extensively utilized in the liver. NEFA

concentrations are maximal at parturition (0.7 to1.2 mEq/L) with a slow decrease after three days postpartum (Melendez et al., 2002). These findings corroborate the elevated fat mobilization occurring around parturition in dairy cattle. Extreme rates of lipid mobilization lead to increased uptake of NEFA by liver and increased triglyceride (TG) accumulation (Drackley, 1999). When blood glucose concentrations increase, lipogenesis predominates over lypolysis and thus NEFA release from adipose tissue is suppressed (Herdt, 2000). When glucose concentration decreases as occurs just after calving, NEFA mobilization for adipose tissue is stimulated (Herdt, 2000; Melendez et al., 2002).

The primary end products of ruminal fermentation, VFAs, have been studied as possible signals that lead to the cessation of eating (Forbes et al., 1992). Infusion of 7.1 Mcal of net energy for lactation as acetate or propionate led to decrease in DMI when a high forage diet was fed alone; propionate infusion reduced intake to a greater extent than did acetate infusion (Shepherd and Combs, 1998). Propionate infusion decreased DMI by both decreasing meal size and increasing intermeal interval in mid lactation while increasing meal size only for early lactation (Oba and Allen, 2003). Propionate infusion might stimulate gluconeogenesis and oxidative metabolism in the liver simultaneously and thereby increasing plasma glucose concentration and decreasing DMI linearly. Allen (2000) proposed that hypophagic effects of propionate are from its oxidation in the liver.

In ruminants, glucose and amino acids serve as the major fuel supply for the developing fetus. Glucose and amino acids are both required by the mammary gland for lactose and protein synthesis, respectively (Herdt, 2000). Glucose demand in Holstein cows has been estimated at 1000 to 1100 g/d during the last 21 d of gestation, but increases sharply after calving to approximately 2500 g/d 21 d postpartum (Drackley et

al., 2001), however, these requirements are based on several factors such as, parity, body weight and milk production of individual animals. Ruminants are not entirely dependent on dietary glucose; as a result they are constantly dependent on gluconeogenesis (Herdt, 2002). The liver serves as a central organ in adaptation to the maintenance of body fuel supplies and consequently it is the key regulator of glucose supply to other tissues (Herdt, 2002). The major gluconeogenic precursor in ruminants is propionic acid produced in the rumen. Its contribution to gluconeogenesis has been estimated to be 32 to 73% (Seal and Reynolds, 1993). Liver uptake of propionate by portal circulation is almost 100% (Herdt, 2002); however, the capacity of the liver to convert propionate to glucose seems to be responsive to the amount of propionate supplied and the physiological stage of the animal (Drackley et al., 2001). Hepatic propionate metabolism is modulated during the transition period. For example, hepatic blood flow in cows increases by 84% from 11 d prepartum to 11 d postpartum (Reynolds et al., 2000). In addition, propionate conversion to glucose by the liver is 19 and 29% greater at day 1 and 21 postpartum, respectively, than at d 21 prepartum (Overton et al., 1998).

Amino acids, lactate and glycerol serve as secondary substrates for gluconeogenesis in ruminants (Herdt, 2002). Contribution to glucose production has been estimated to be 10 to 30% for amino acids, 15% for lactate and small amounts (about 3 – 4%) for glycerol (Seal and Reynolds, 1993). Alanine and glutamine account for 40 to 60% of the glucogenic potential of all the amino acids; therefore, they typically make the greatest contribution to glucose synthesis (Drackley et al., 2001).

Endocrine changes during transition period

Tremendous metabolic and endocrine adaptations must be made as dairy cows move from late gestation to early lactation in order to meet the overwhelming energy requirements (Drackley et al., 2001). Endocrine regulation of gluconeogenesis, ketogenesis and lipid mobilization includes insulin, glucagon, somatotropin, thyroid hormones, progesterone, estrogen, glucocorticoids, prolactin and leptin (Herdt, 2000, Drackley et al., 2001). Glucose levels in prepartum dairy cows are high until parturition (Grum et al., 1996). As a result, insulin concentrations are higher before calving than after calving and glucagon exhibits the opposite pattern (Herdt, 2000; Nelson and Cox, 2000; Drackley et al., 2001). In fact, basal concentrations of glucagon are elevated whereas the molar insulin/glucagon ratio decreases during early lactation (Sartin et al., 1985). Glucagon dominates hepatic metabolism and is more involved in short-term stimulation of gluconeogenesis and glycogenolysis in response to stress by direct effects on the liver (Weekes, 1991). Somatotropin concentrations are lower before calving than post calving with a peak at parturition (Grum et al., 1996). Collectively, these studies indicate an important role of glucagon as well as somatotropin in nutrient partitioning during early lactation.

Plasma thyroxine (T_4) concentrations gradually increase during late gestation, decrease approximately 50% at calving, and then begin to increase gradually during lactation (Kunz et al., 1985). Similar, but less pronounced, changes occur in 3,5,3'-triiodothyronine (T_3). Glucocorticoids concentrations increase dramatically on the day of calving and return to near prepartum concentrations the following day (Edgerton and Hafs, 1973). Whether these gradual and acute changes in thyroid and adrenal hormones

play a significant role in how the transition cow adapts to its lactational demands is not completely understood. What is known is that glucocorticoid creates an insulin resistant state thereby making glucose available to the mammary gland (Maciel et al., 2002).

Progesterone concentrations during the dry period are elevated for maintenance of pregnancy but decline rapidly approximately 2 d before calving (Chew, et al., 1979). Conversely, circulating estrogen concentrations remain high or increase during the transition period and are thought to participate in decreasing the DMI around calving (Grummer, 1993).

Leptin concentrations are high during pregnancy and decline to the lowest level at parturition due to loss in body fat (Liefers et al., 2003). Block et al. (2001) showed that leptin is negatively correlated with the amount of NEFA likely reflecting the reduction in adipocyte volume and increased lipolysis. However, the role leptin plays in metabolism is still unknown. All these physiological and metabolic adjustments occur to meet the challenging tremendous glucose needs that occur at this time.

Various strategies have been evaluated to ameliorate the dramatic changes in plasma hormones and metabolites during transition period to enable the liver to adapt quickly in order to meet the cow's increasing nutrient consumption and metabolic demand (Grummer, 1995). These strategies include increasing energy density of the diet (Schneider et al., 1987; Grum et al., 1996), and feeding numerous supplemental dietary components including fat (Spicer et al., 1993; Beam and Butler, 1998), ionophores (Phipps et al., 2000), *Aspergillus oryzae* extracts or *Saccharomyces cerevisiae* cultures, or both (Martin and Nisbet, 1992) and Propionibacteria (Francisco et al., 2002; Stein et al., 2006; Aleman et al., 2007). Also, drenching (Grummer et al., 1994) and feeding

propylene glycol (Christensen et al., 1997) have been used to decrease NEFA and increase plasma insulin and propionate in ruminal fluid. The majority of these strategies affect the rumen ecosystem, and therefore, it is important to briefly discuss the rumen ecosystem before evaluating how each of the above strategies have been used to manipulate the rumen microbial population in order to increase profitability and improve herd health.

The rumen

The rumen is a fermentation vat, open, complex and highly competitive microbial ecosystem. The rumen ecosystem enables a true symbiotic relationship between microbial population and the host ruminant (Church, 1993; Weimer, 1998). The feed that the animal consumes is fermented to VFAs and microbial cell protein to provide energy and protein, respectively (Weimer, 1998). Specifically, the rumen microbes hydrolyze the plant celluloses, hemicelluloses, pectins, fructosans, starches and other polysaccharides to monomeric or dimeric sugars which are further fermented to give various products such as acetic, propionic and butyric acids, methane and carbon dioxide (Hungate, 1988). Rumen microorganisms are predominantly strict anaerobes. Some oxygen can be tolerated as long as the fermentation is sufficiently efficient to facilitate the disposal of oxygen and the osmotic potential of the medium remains within normal limit (-250 to -450 mV). Facultative anaerobes consume oxygen and help to maintain the low oxygen potential (Van Soest, 1994). Population densities of 10^{10} to 10^{11} have been found for bacteria, 10^5 to 10^7 for protozoa and smaller amounts ($<10^4$) of anaerobic fungi and facultatively anaerobic bacteria (Leeddle, 1991; Mcallister and Cheng, 1996). The rumen has an internal temperature of 37°C and a pH of 6.8 to 7.0 under normal conditions.

Dilution rate varies and there is continual removal of fermentative end products which limits end product accumulation within the rumen (Hungate, 1966*;* Ogimoto and Imai, 1981).

Under a well balanced hay to concentrate ration, gram negative bacteria predominate (Dirksen, 1969). When the diet is abruptly changed to all concentrate, a large influx of carbohydrate is suddenly available in the rumen environment and gram positive bacteria such as *Streptococcus bovi*s and lactobacilli dominate (Dirksen, 1969, Slyter, 1976; Goad et al., 1998). The complete disappearance of protozoa and cellulolytic bacteria has been observed as pH decreases from 5.5 to 5.0 with an eventual cessation of bacterial population at pH of 4.0.

Gram negative bacteria including lactate utilizers and protozoa decrease in numbers and eventually die (Slyter, 1976) as the pH is affected by the heavy lactate production from *Streptococcus bovis* and the lactobacilli. Normally lactate utilizing bacteria find difficulty in multiplying to effective numbers if there is a sudden surge of lactic acid to the system (Mackie and Gilchrist, 1978, 1979). Megasphaera elsdenii is considered as one of the most important lactate utilizer (Slyter, 1976) and produces propionate. It utilizes 60 to 80% of the lactate fermented in the rumen (Counotte et al., 1981) and makes up 20% of the lactate utilizers in ruminants fed high concentrate diets (Mackie et al., 1984). Other lactate utilizers found in the rumen include *Selenomonas ruminantium*, *Veillionella alcalescens* and Propionibacteria species (Slyter, 1976).

Volatile fatty acid production

Volatile fatty acids are the major end products of carbohydrates fermentation by rumen microbes. Pyruvate is the primary intermediate of carbohydrates hydrolysis

leading to the production of the VFAs. End product formation primarily depends on the type of structural carbohydrate fermented and the type of bacterial species involved in the fermentation process. The primary VFAs produced in the rumen are acetic, propionic, and butyric acid (Gottschalk, 1986; Hungate, 1988). As the forage: concentrate ratio decreases, the acetate: propionate ratio also decreases. For example, forage: concentrate ratios of 100:0, will give VFA ratios of acetate, propionate, and butyrate of 71:16:7.9. When the forage to concentrate ratio is 50:50, the molar ratios of acetate, propionate and butyrate are 65.5:18.4:10.4. When there is more concentrate offered in the diet (80:20), the VFA ratio would be 53.6:30.6:10.6 (Annison and Armstrong, 1970). Rumen concentrations of VFAs are regulated by a balance between production and absorption whereby increased production rate induces higher VFA concentrations (Nagaraja et al., 1997). Volatile fatty acid production rates vary diurnally as a consequence of eating pattern, therefore rumen VFA concentration and pH vary as well. Fermentation peaks about 4 hours after feeding on a hay diet but occurs sooner if the diet contains more concentrate. The major factor affecting VFA absorption is their concentration (Van Soest, 1994). In general, acetate concentrations in the rumen ranges between 60 to 70 mmol/L, propionate between 18 to 25 mmol/L and butyrate between 10 to15 mmol/L (Nagaraja et al., 1997).

Acetate production is formed by the pyruvate formate lyase system. In this system, formate and acetyl-coenzyme A (CoA) are formed as intermediates. The formate is converted to carbon dioxide and hydrogen by other bacteria. This is the main pathway for acetate production in the rumen (Fahey and Berger, 1988). Another pathway involved in the production of acetate in the rumen utilizes pyruvate ferrodoxin oxidoreductase. It

produces ferrodoxin which is the acceptor for bacteria (Glass et al., 1977), carbon dioxide and acetyl-CoA. In either pathway, the resulting end product is acetate and bacteria such as *Megasphaera elsdenii* and *Veillonella alcalescens* have been observed utilizing the ferrodoxin-linked pyruvate oxidoreduction pathway (Fahey and Berger, 1988; Russell and Wallace, 1988).

Propionate production is primarily via the dicarboxylic acid pathways. The decarboxylation of succinate by *Selenomonas ruminantium* is the primary method of propionate production in the rumen of cattle (Wollin and Miller, 1988). However, other bacteria including *Veillonela* and Propionibacteria utilize the succinate pathway. Propionibacteria slowly converts succinate to propionate in the rumen and, therefore, contribute to propionate production. The more detailed account about propionate production by Propionibacteria will be discussed later. First the bacteria must produce succinate as an intermediate. A carboxyl group is then removed from the succinate by succinyl-CoA ultimately leading to the production of propionate. Nevertheless, there is an alternative pathway for the production of propionate. It has been named acrylate pathway and bacteria such as *Megasphaera elsdenii* have been implicated in utilizing it for the production of propionate (Paynter and Elsden, 1970). In this pathway, succinate or succinyl-CoA is not used as intermediates. Instead, pyruvate is converted to either form of lactate which is then converted to acrylyl-CoA and reduced to propinyl-CoA (Gottschalk, 1986). One third of the total propionate produced is via acrylate pathway (Fahey and Berger, 1988). The production of propionate is of vital importance to the ruminant because of its involvement in gluconeogenesis. Propionate is the only VFA which makes contribution to production of glucose in the ruminant animal.

Butyrate production is mainly via the reversal of the β-oxidation pathway (Fahey and Berger, 1988). In this pathway, acetacetyl-CoA, L(+)-β-hydroxybutyryl-CoA, and crotonyl-CoA are intermediates with butyryl-CoA being formed proceeded by butyryl phosphate; thus giving rise to butyrate (Gottschalk, 1986).

Microbial protein synthesis and milk production

Microbial protein is the most important source of essential amino acids for the lactating dairy cow for milk protein synthesis (Firkins et al., 2006) contributing about two thirds of the amino acids absorbed by ruminants (Cotta and Russell, 1997) or ranges from 50 to 100% (Clark et al., 1992). However, the errors associated with unrepresentative microbial sampling from the rumen and imprecise microbial markers as well as the differences between experiments (Firkins et al., 2006) contribute to variation in the quantification of the efficiency of microbial protein synthesis (E_{MPS}). The rumen degradable protein and carbohydrate availability determines the microbial protein synthesis in the rumen (Hoover and Stokes, 1991). Other than source and amount of nitrogen and carbohydrates, the amount and efficiency (defined as g of microbial crude protein (MCP)/ kg or 100 g of OM digested in the rumen) of microbial protein synthesis are affected by DMI, forage:concentrate ratio, rate of N and carbohydrate degradation, vitamins and minerals (such as sulfur and phosphorus) and rate of passage (Karsli and Russell, 2000). The average efficiency of microbial protein synthesis was predicted to be around 13 g MCP/100 g of total digestible nutrient (TDN) for beef cattle (NRC, 1997). The energy from the VFA needs to be further synchronized with metabolizable protein to improve the efficiency of conversion of dietary protein into milk protein (Firkins et al., 2006). Changes in the supply of individual VFA are related to microbial supply and

therefore milk yield and composition (Thomas and Martin, 1988). Increases in the supply of acetic acid are associated with increased milk yield and milk fat concentration, and butyric acid with milk fat concentration. However, propionic acid supply was inversely related with milk fat concentration and positively related with milk protein concentration (Firkins et al., 2006). These results are explained by the glucogenic or ketogenic nature of infused VFA and repartitioning effects of the VFA through hormonal changes (Dijkstra, 1994).

Methods utilized in manipulating ruminal fermentation

A goal of ruminant microbiologists and nutritionists is to manipulate the ruminal microbial ecosystem to improve efficiency of converting feed to products consumable by humans. Antibiotics have been used as feed additives for farm animals since the 2nd world war (Pusztai, et al., 1990). It has been the repeated evidence of their beneficial effects on the growth, milk production and overall health of livestock that has made them a popular choice in the industry. Nevertheless, the use of antibiotics is still widely accepted in the industry but carries the risk of bacterial strains becoming resistant and therefore affecting future therapeutic uses in both man and animal. The growing public concern over the use of antibiotics in the animal feed industry to control rumen fermentation, diseases, and improve animal efficiency has lead researchers to continue to develop natural feed supplements containing live microorganisms such as rumen bacteria, fungi and yeast.

In the next section, the various methods or feed additives used to modify rumen fermentation activities and its microbial population along with the use of antibiotics will be discussed.

Fat supplementation

Most approaches to alleviate the period of negative energy balance focus on mitigating the decrease in DMI and include partial substitution of forages with more energy-dense concentrates and fat supplements (Grummer, 1995). However, high amounts of concentrate may predispose transition cows to excessive decreases in DMI as they approach parturition and potentially increase incidence of displaced abomasum (Mashek and Grummer, 2003) and other metabolic disorders. Fat feeding increases the energy content of the diet without decreasing the forage to concentrate ratio and fiber content of the diet. Decreasing dietary forage to concentrate ratio has been shown to decrease the extent of biohydrogenation because the bacterial species capable of biohydrogenation are primarily cellulolytic (Harfoot and Hazlewood, 1988). The most commonly utilized fat sources for dairy cattle supplementation include oilseeds (whole soybeans, cottonseed, etc), vegetable oils, rendered fats (tallow, choice white grease, yellow grease, etc.) and speciality fats (calcium salts of long chain fatty acids, relatively saturated fatty acids, and hydrogenated tallow). Fat sources vary greatly in their structure and fatty acid composition (i.e., free or esterification form, chain length, and degree of unsaturation) which influence ruminal and post-ruminal metabolism of dietary fats, and ultimately, animal performance. The presence of unsaturated fatty acids from the diet and an altered rumen environment results in the formation of unique fatty acid intermediates that inhibit milk fat synthesis (Bauman and Griinari, 2003). One of these intermediates is trans-10, cis-12 CLA (Baumgard et al., 2000). Supplementation with conjugated linoleic acid (CLA) resulted in an 11 and 21% decrease in milk fat yield for CLA-1 and CLA-2,

respectively (Castaneda-Gutlerrez et al., 2005). However, in the same study, milk production and secretion of other milk components did not differ among treatments.

Fats or lipids are the most concentrated source of energy containing 1.7 to 2.3 times more energy than protein and carbohydrates. Fat is the most common ingredient utilized to raise the caloric density. It has been proven that unprotected fat can only be supplemented at levels 3 – 5% of the concentrate in the ration without depressing fermentation in the rumen and thereby, reducing milk fat production (Palmquist and Conrad, 1978; Grummer, 1987). Protected oils pass through the rumen intact and are hydrolyzed in the abomasum.

Milk yield and composition have been variably influenced by added fat (Ferguson et al., 1990). Variable responses in production and composition with added fat may be related to the type and dietary concentration of fat used in different studies. Sharma and co-workers (1978) noted increased fat test and daily fat yields with 15% protected tallow added to dairy rations but indicated no difference in milk yields between treatments. Schneider et al. (1987) reported a significant milk production increase of 1.1 kg/cow/day with a trend toward fat test for early lactation cows receiving 0.45 kg of Megalac, by pass fat product daily. Borcherding and Wanner (1987) reported that feeding Megalac at a rate of 0.45 kg/cow/day produced a 3.4 kg/d increase in milk output of high producing cows and a 1.8 kg/cow/day increase for first lactation cows. They also noted that high producing cows receiving megalac supplementation maintained their body condition and remained on 12.5 month calving cycle. Similar studies that involved feeding inert fat or calcium of long chain fatty acids (Ca-FA, Megalac) at 2% DM reported no effect on DMI, milk yield, or composition but increased luteal phase progesterone concentrations

for cows fed inert fat than Control (Spicer et al., 1993). Beam and Butler (1998) supplemented postpartum cows with prilled fatty acids at 2.6% DM (Energy Booster-100, Milk specialists Co., Dundee, IL) during the first four wk of lactation and found lower DMI, higher 4%FCM yield and no improvement in follicular function or ovulation. A digestibility experiment with six mature sheep supplemented 20% fat coated with ruminal bypass protein showed no decrease in DMI and fiber digestibility, however, DM and nitrogen digestibilities were significantly higher relative to basal diet (chopped ryegrass hay) (Bayourthe et al., 1993). Conversely, Doreau et al. (1993) studied the effect of lactating dairy cows with a product made of calcium salts of rapeseed oil and found no treatment effect on OM digested, total N and microbial N flow at the duodenum or efficiency of N microbial synthesis. However, in sutu degradation of DM and NDF was higher for the diet supplemented with rapeseed oil fatty acids than for control diet. These studies show variable responses and, therefore, necessitate futher studies on supplemental effect of fats during early to mid-lactation.

Antimicrobials

Much of the research in the past 20 to 25 yr has focused on the effects of antimicrobial compounds on ruminal fermentation. Specifically, ionophore antibiotics are incorporated into ruminant diets to reduce energy losses associated with methanogenesis in the rumen (Russell and Strobel, 1989) and thereby improve efficiency of production. Ionophores inhibit Gram-positive bacteria to a much greater extent than Gram-negative bacteria because Gram-negative membranes are able to protect the cells from ionophores.

Ionophores are a group of carboxylic polyether antibiotics that facilitate the channeling of ions across a biological barrier such as bacterial membrane by combining

with the ion or increasing the permeability of the barrier. The ionophores used most commonly are monensin and lasalocid. Other ionophores include laidlomycin propionate, salinomycin and tetronasin. Ionophores alter bacteria flora of the rumen, leading to decreases in gram positive bacteria, protozoa, and fungi and increases the gram negative bacteria. The net effect of these changes in bacterial flora is increased propionate production; a decrease in acetate, butyrate, and methane production; increased DM digestibility and protein sparing effect (Hanson and Klopfenstein, 1979) with a resultant decrease in rumen ammonia concentration; and a decrease in ruminal lactate levels (Nagaraja et al., 1986). Monensin sodium aids in controlling lactic acidosis and pasture bloat, and is antiketogenic (by increasing serum glucose and reducing BHBA) (Nagaraja et al., 1981; Sauer et al., 1989). Through enhancing ruminal propionate production, lasalocid and monensin may decrease lactate production, decrease methane loss, and reduce proteolysis and deamination (Bergen and Bates, 1984; Schelling, 1984).

Monensin was discovered in an in vitro rumen batch fermentation screening program (Richardson et al., 1976). Monensin enhanced propionate production in a high roughage and high-grain diets by 49 and 79%, respectively (Van Maanen et al., 1978). Prenge et al. (1978) also demonstrated that monensin enhanced rumen propionic acid production by 44% in Holstein steers fed a 30% concentrate diet. These studies confirmed the early observations of monensin-mediated in vitro rumen propionate production and in vivo enhancements of rumen molar percentage of propionate are in fact indicative of elevated in vivo propionic acid production rates. The increase in rumen propionate is accompanied by a reduction in the amount of methane produced in the rumen (Nagaraja et al., 1997). These compounds seem to inhibit hydrogen-producing

bacteria such as *Streptococcus bovis* and *Lactobacillus spp*., *Ruminococcus*, and *Butyrivibrio* (Dennis et al., 1981; Russell, 1987; Van Soest, 1994) and thus to reduce precursors of methanogenesis (H_2, CO_2 and formate) (Russell and Martin, 1984). Monensin inhibits methanogenesis from formate, probably as a result of inhibition of nickel uptake by methanogenic bacteria, increased heat increment and the apparent digestibility of metabolizable energy (Wedegaetner and Johnson, 1983; Oscar and spears, 1990). Therefore, decreases in hydrogen production reduce ruminal methanogenesis and improve feed utilization by increasing the amount of metabolizable energy available to the animal as propionate (Bergen and Bates, 1984).

Monensin affects nitrogen metabolism by decreasing ammonia production in cattle fed a forage-based diet. This might be a result of reduced proteolysis, degradation of peptides and deamination of amino acids in the rumen (Yang and Russell, 1993; Nagaraja et al., 1997). Consequently, the feeding of ionophores constantly increase accumulation of non ammonia non protein nitrogen (i.e., peptides and amino acids) by inhibition of the hyperammonia producing bacteria in the rumen (Chen and Russell, 1991). Total tract nitrogen digestibility increases significantly about 8% due to higher flow of nitrogen of dietary origin to the duodenum (Machnuller et al., 2003). With feeding monensin, the specific activity of ammonia production by mixed ruminal bacteria is decreased by more than 30% and this decrease corresponds to about 10-fold decreases in the numbers of bacteria that ferment peptides and amino acids as an energy for growth (Yang and Russell, 1993). Ionophores have also been demonstrated to decrease ruminal urease activity (Starnes et al., 1984). Monensin prevents lactic acid build–up in the rumen by its selectivity towards gram-positive bacteria. The major lactic acid-producing bacteria

(*S. bovis* and *Lactobacillus spp.*) are inhibited while ruminal lactic acid-fermenting bacteria (gram-negative) are unaffected (Nagaraja et al., 1997). This effect was not repeated in a more recent study that used a monensin controlled-capsule or a premix in dairy cattle (Mutsvangwa et al., 2002). Monensin also decreases the incidence of frothy bloat as a result of reduction in microbial slime and gas production (Lowe et al., 1991; Nagaraja et al., 1997). Monensin reduces feed intake in both grain-fed and forage-fed cattle, probably due to low palatability or decrease in rumen turnover rate of liquids and solids, and consequently increase of ruminal fill (Stock et al., 1995; Nagaraja et al., 1997) or could be due to increase propionate concentration which has been implicated in decreasing feed intake.

Even though ionophores have been available for use in food animals for over 20 yr, there is still a limited information available about their use in lactating dairy cows (Duffield, 1997) because the U.S. FDA did not approve monensin sodium for use in lactating dairy cows for increased milk production efficiency until October 28, 2004 (FDA, 2004). In a well designed study conducted in Canada by Sauer et al. (1989), multiparous cows were gradually introduced to a monensin-containing concentrate one week prepartum and fed a complete diet containing 0, 15 or 30 g monensin/ton of dry matter for 3 wks postpartum (low and high monensin). Acetate molar proportion decreased, while propionate increased significantly between controls and high monensin-treated group. Milk production was similar but milk fat was lower in high monensin group than the control group (3.71% vs. 4.12%, respectively). Monensin primarily has been used as a powder mixed with concentrates. A capsule (containing 32 g of sodium monensin) of slow release of monensin has been developed (Abe et al., 1994; Lean et al.,

1994). Recently, in England, Phipps et al. (2000) reported that milk production was not different between cows fed either 0 or 300 mg/d of monensin over two lactations; however, percentage of milk fat and protein was lower in monensin supplemented cows than control cows in both lactations.

Animal health benefits have been demonstrated following monensin treatment in some, but not all the studies, and β-hydroxybutyrate concentrations are lower in monensin –treated vs. control cows (Sauer et al., 1989; Duffield et al., 1998b). Field trials with monensin have demonstrated a reduced prevalence of clinical ketosis and of multiple diseases as well as a decreased probability of being culled in the first 3 months of lactation (Duffield et al., 1999b).

Direct-fed microbials (DFM)

Societal concerns about antibiotic use and other growth stimulants in production agriculture and the need for producers to implement preventive measures against pathogen outbreaks in the food supply have highlighted the potential use of probiotic in feeding operations (Elam et al., 2003; Krehbiel et al., 2003). Kmet et al. (1993) defined ruminal probiotics as "live cultures of microorganisms that are deliberately introduced into the rumen with the aim of improving animal health or nutrition." The term probiotic is generic and all-encompassing, to include microbial cultures, extracts, and enzyme preparations (Elam et al., 2003). As such, the Office of Regulatory Affairs of the Food and Drug Administration (FDA, 2003), as well as the Association of American Feed Control Officials (AAFCO, 1999), have recommended the term direct-fed microbials to be used to describe feed products that contain a source of live, naturally occurring microorganisms and this includes bacteria, fungi and yeast. Of specific concern with

DFM are the specific organism(s) involved, dosage rate, site of establishment and duration of treatment.

In general, the mechanisms responsible for the beneficial effects of DFM have not been studied extensively (Krehbiel et al., 2003). However, the few that have been reported include, decrease in ruminal acidosis in feedlot cattle and dairy cows, improve immune response in stressed calves, a decrease in the area below subacute ruminal pH, increases in ruminal propionate concentrations, increases in protozoal numbers, and in viable bacterial counts. Effects on some blood variable (lower CO_2 and LDH) also suggest a reduced risk of acidosis (Krehbiel et al., 2003), increased plasma hormones (Francisco et al., 2002; Aleman et al., 2007) and increased milk production (Nocek and Kautz, 2006; Stein et al., 2006) in dairy cows. More research is needed to describe the mode of action of DFM. The following sub-sections report some of the DFM that have been evaluated in cattle industry.

Bacterial Direct-fed Microbials

Many microorganisms are used in DFM formulations. The most common bacterial organisms in DFM products for ruminants are lactobacilli (Kung, 1997) and Propionibacteria species. Limited research has evaluated the efficacy of bacterial DFM for lactating cows. In general, increased milk yield has been a consistent response, whereas changes in milk composition have been variable.

Lactobacillus

Jaquette et al. (1988) reported that milk yield was 1.8 kg/d greater for cows fed a diet containing 2.0 x 10^9 cfu of *L. acidophilus* per day compared with those fed a control diet. Dry matter intake, milk fat and milk protein were not affected by *L. acidophilus.* In a

more recent experiment, Gomez-Basauri et al. (2001) evaluated the effect of a supplement containing *L. acidophilus*, *L. casei*, *Enterococcus (Streptococcus) faecium* (total lactic bacteria = 10^9 cfu/d) and mannanoligosaccharide on DMI, milk yield, and milk component concentrations. Cows fed lactic acid bacteria and mannanoligosaccharide consumed 0.42 kg less DM and produced 0.73 kg/d more milk. Other experiments have been conducted with combinations of fungal cultures and lactic acid bacteria (Block et al., 2000). Milk yields were increased by 1.08 and 0.90 kg/d, respectively, when lactating cows were fed *Saccharomyces cerevisae* in combination with *L. acidophilus* or 5 x 10^9 cfu of yeast in combination with 5 x 10^9 cfu of *L. plantatum faecium*. In contrast to feeding bacterial DFM directly, Colenbrander et (1988) found that treatment of alfalfa silage with L. acidophilus did not improve DMI, milk yield, or milk composition in dairy cows, but efficiency (kg of fat-corrected milk/kg of feed) of milk production was improved by 7.1%. These studies suggest that bacterial DFM fed alone or in combination with fungal cultures might be efficacious for increasing milk production by lactating cows. However, the results might differ depending of the mode of application used.

Propionibacterium

Propionibacteria are mainly divided into two groups according to their habitats: the classical and the cutaneous strains. The classical strains include those bacteria found in cheese and dairy products (Grappin et al., 1999). However, they have also been found in silage fermentations and in fermenting vegetables (Babuchowski, et al., 1999; Merry and Davies, 1999). The cutaneous strains include those bacteria that are found on human skin. One acne bacillus, originally described as a Corynebacterium is an example of this

group and is referred to as the “acne group strain” or the cutaneous propionibacteria (Cummins and Johnson, 1986).

There are distinctive characteristics amongst the morphology of these two types of propionibacteria. The classical propionibacteria tend to exhibit a shorter and thicker rod while P. acnes tend to show a longer and slender irregular rod shape (Cummins and Johnson, 1986). In general, Propionibacteria are gram-positive, rod-shaped, non-spore forming, non-motile, facultative anaerobic bacteria. Although they can grow between the temperature of 5 °C and 40 °C, growth is most rapid at 37° C (Cummins and Johnson, 1986). Propionibacteria have an optimum pH for growth, between 6.5 and 7.0 although the optimum range for different species ranged from 4.6 to 8.5. Propionibacteria are used routinely for making Swiss cheese. After primary culture ferments lactose to lactate, Propionibacteria serve as the secondary culture to ferment lactate to propionate, acetate and CO_2 during cheese ripening (Hettinga and Reinbold, 1972) giving Swiss cheese its characteristic myopic eyes and flavor. The four recognized dairy-related genus Propionibacterium includes, *P. freudereichii*, *P. acidopropionici, P. theonii, and P. Jensenii*. Less favorable species include *P. acnes, P. avidum, P. granulosum and P. lymphophilum*.

Propionibacteria are also important as a DFM in beef cattle to prevent “nitrate” toxicity (Swartzlander, 1994). Because of their ability to produce Vitamin B_{12} and propionic acid, the genus Propionibacterium has been used for industrial production of these products (Perlman, 1978). Propionibacteria also has found use as an inoculant for grain and silage (Woolford, 1975; Flores-Galarza, 1985). Based on their ability to utilize lactate and grow at a low pH, species of propionibacteria recently have been studied as

DFM to aid in prevention of ruminal acidosis. Parrott (1997) determined that certain selected strains of propionibacteria increase the lag time before lactic acid accumulated and suppressed the rate at which H^+ concentration increased in ruminal fluid in-vitro.

Propionibacteria (classical type) are natural inhabitants of the rumen and their population varies according to species, sampling times, individual animals and the ration provided to the animals. In sheep, Propionibacteria were shown to constitute 4.4% of the bacterial population isolated from the rumen epithelium surface when a high roughage diet was fed (Mead and Jones, 1991). Propionibacteria comprised 1.4% of the ruminal microflora in the rumen of animals fed forage and medium concentrates (Oshio et al., 1987). However, in cattle, Davidson (1998) reported that the population of Propionibacteria ranges from 10^3 -10^4 cfu/mL of rumen fluid. Propionibacteria have the ability to convert lactic acid and glucose to acetic acid and propionic acid. Although propionibacteria can metabolize lactic acid, they are probably too slow growing and acid intolerant to prevent an acute acidosis challenge, therefore, their use has been focused on propionate production rather than lactate fermentation for use as a DFM.

In cattle industry, propionibacteria have been used as a DFM to prevent the risk of acidosis in feedlot cattle receiving high concentrate diets (Ghorbani et al., 2002; Yang et al., 2004). Ghorbani et al. (2002) reported that supplemental feeding of Propionibacterium P15 (P15) or P15 + *Enterococcus faecium* (EF212) to steers fed high concentrate diets had no effect on ruminal pH, blood pH, blood glucose, propionate, isobutyrate, or acetate/propionate ratio. However, acetate concentrations were greater in steers fed EF212 compared with Control steers or those fed P15 alone. In support of these results, Yang et al. (2004) found no effect of DFM (P15, EF212 or EF212 +

Saccharomyces cerevisiae) on the fermenter pH, acetate, butyrate or total VFA concentrations, or acetate/propionate ratio in feedlot cattle. The lack of response on VFA was supported by lack of treatments on digestibility in continuous culture system fermenters fluid (Yang, 2004). In contrast, Kim et al. (2001a) showed that propionate increased and acetate/propionate ratio decreased as *P. acidopropionici* dosage increased (0, 10^7, 10^8, 10^9 and 10^{10} cfu). Similarly, Kim et al. (2001b) reported that the acetate/propionate ratio decreased after in vitro fermentation for 6, 12 and 24 h when *P. acidopropionici* was used at a concentration of 4.7 x 10^{10} cfu. Total VFA, acetate, and propionate concentrations increased when Propionibacteria strain P5 was fed at 1 x 10^3, 1 x 10^6 and 1 x 10^9 cfu/g (Akay and Dado 2001). However, Propionibacteria (P5), decreased DM digestibility by 2% and fiber digestion by 14% but autoclaving Propionibacteria did not increase DM or fiber digestibility. A decrease in NDF digestion occurred because of increased VFA concentrations, particularly acetate, which might inhibit acetate producing bacteria, thus slowing the rate of fiber digestion. The data suggested that the resulting decrease in digestibility with the addition of Propionibacteria was primarily due to the carrier, which had high starch content and accounted for up to 2.8% of the DM in the fiber digestion assay (Akay and Dado, 2001).

An in vitro ruminal model suggest that *P. jensenii* strains have the greatest potential to utilize lactic acid in the rumen of beef cattle fed high concentrate diets due to increasing pH and the suppression of lactic acid accumulation (Parrot, 1997). Swinney-Floyd (1997) reported that during the first 9 d of a 21 d experiment for the evaluation of adaptation to high concentrate diets, acidosis never occurred and lactic acid

concentrations did not accumulate in ruminally-cannulated heifers inoculated with mixed culture of *P. acidipropionici* and *P. freudereichii*.

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 SNF 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. 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.

In a second study that evaluated a higher dose of P169 in the presence of yeast culture reported that, 4%FCM was greater in high-dose P169 (32 ± 0.5 kg/d) and low-dose P169 (33 ± 0.5 kg/d) vs. Control cows (Stein et al., 2006). Cows fed high-dose P169 had higher milk lactose % than low-dose P169 fed cows. Percentage milk fat was significantly greater in the low-dose P169 and Control vs. high-dose multiparous cows but there was no difference among treatment groups in primiparous cows. The percentage SNF was greater in both low- and high-dose P169 than Control multiparous cows. Between wk 13-25 of lactation, MUN levels were greater in high-dose and low-dose P169 vs. Control cows. Weekly body wt from wk 1 – 25 were greater in high-dose P169 than low-dose P169 and Control multiparous cows. Body weight in primiparous cows did not differ among groups (Stein et al., 2006).

Plasma glucose was 5.5% lower in high than low-dose P169 multiparous cows; and low-dose P169 primiparous cows had 6% greater plasma glucose levels than high-dose P169 and Control primiparous cows (Aleman et al., 2007). In addition, high-dose P169 cows had greater plasma insulin than Control cows during wk 1-12 (Aleman et al., 2007). Plasma IGF-1, NEFA and leptin concentrations did not differ among diet groups between wk 1 and 25 whereas low-dose cows had 25% greater plasma cholesterol levels than high-dose P169 and Control multiparous cows (Aleman et al., 2007). Collectively, these studies support the idea that feeding P169 may improve metabolism and suggest that supplemental feeding of P169 in conjunction with yeast culture may improve milk production if fed for a period longer than 12 wk postpartum.

Nonbacterial Direct-fed Microbials

Nonbacterial, direct-fed microbials added to ruminant diets generally consist of *Aspergillus oryzae* fermentation extract, or *Saccharomyces cerevisiae* cultures, or both (Martin and Nisbet, 1992). Results from in vivo and in vitro research have been variable regarding effects of non-bacterial direct-fed microbials on ruminant feedstuff utilization and performance. Some research has shown increased milk production, milk components and total tract digestibility of feed components, but others have shown little influence or no influence of non-bacterial direct-fed microbials on these parameters.

While some products contain and guarantee "live" yeast, most products based on *S. cerevesiae* and *A. oryzae* make no guarantee for supplying live organisms. Higginbotham et al. (2001) evaluated effect of feeding *S. cerevisiae* as a live yeast (114 g/d, 60b live cfu) product or yeast culture (114 g/d) on milk yield, milk components and ruminal parameters on lactating dairy cows. Milk yield, 3.5% FCM, milk fat percentage,

protein, lactose and SNF, rumen pH and total VFA concentrations were unaffected by feeding both live yeast product or yeast culture product suggesting that similar responses can be realized by feeding either of the products. Similarly, 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 10^3 fold greater yeast cell populations than the yeast culture supplement. Both concentrations of yeast culture lowered ruminal pH at 48 h, while both concentrations of live yeast increased pH. However, at 48 h, both concentrations of yeast culture and live yeast, increased acetate, propionate, butyrate, and decreased the acetate/propionate ratio. Therefore, these results further support the notion that live yeast or yeast culture product have some similar effects on mixed ruminal microorganism fermentation.

Yeast culture was shown to provide soluble growth factors such as organic acids, vitamin B and amino acids that stimulate growth of ruminal bacteria (*Selenomonas ruminantium* and *Megasphaera elsdenii*) that utilize lactic acid and digest cellulose (*Fibrobacter succinogenes* and *Rumincoccus albus*) (Callaway and Martin, 1997). Specifically, the presence of malate provided the stimulation of lactate utilization by *Selenomonas ruminantium* (Martin and Nisbet, 1992). However, it is likely that both DFM, *A. oryzae* and *S. cerevisiae*, are providing other stimulatory factors for rumen bacteria, such as B vitamins or branched chain VFA (Higginbothan et al., 1994), growth factors such as fumarate, and p-aminobenzoic acid, as well as amino acids, which are

required by *S. Ruminantium* for growth on lactate (Kanegasaki and Takahashi, 1967; Lenehan et al., 1978).

Harrison et al. (1988) fed *S. cerevisiae* to lactating cows. Ruminal acetate concentration, and molar acetate to propionate ratio all were lower but molar proportion of propionate and valerate were increased. However, ruminal liquid dilution rate and total tract apparent nutrient digestibilities were not altered by *S. cerevisiae* treatment. Newbold et al. (1991) suggested that *A. oryzae* stimulated the rate rather than extent of digestion. Evidence supporting this hypothesis came from the observation that *A. oryzae* stimulated DM digestion after 24 h, but not after 48 h, of incubation using the rumen stimulation technique (Rusitec) (Newbold et al., 1991). Supplementing diets of lactating dairy cows with 5% yeast culture filtrate to *S. ruminantium* increased acetate, propionate and total VFA concentrations while no change in acetate or propionate were seen in *M. elsdenii* and also butyrate concentrations did not change in any of the cultures. 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 24 h. Several studies demonstrated DFM treatment stimulates NH_3 production by mixed ruminal population (Arambel et al., 1987; Martin and Nisbet, 1990). Arambel et al. (1987) proposed that this increase in NH_3 production could be due to the DFM providing additional nutrients to the ruminal microorganisms or possibly by endogenous proteolytic activity of the DFM. Results were variable regarding pH and production of gas, VFA and NH_3 production. This variation between studies is likely due to differences in the type (or strain) and content of DFM, substrates, and culture conditions used. For example, 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 support of these previous results, Miller-Webster et al. (2002) had recently found that different yeast culture products (YC1 and YC2) have different modes of action. Only YC1 strain increased molar percentages of propionate, decreased acetic acid and lowered acetate to propionate ratio and mean nadir pH compared with YC2. The potential for different strains of yeast culture to stimulate growth may be related to their ability to remove oxygen from the rumen fluid as oxygen is detrimental to anaerobic bacteria in the rumen. Respiration-deficient mutants of *S. cerevisiae*, failed to stimulate bacterial numbers, while the corresponding parent strains were beneficial for cellulolytic bacterial growth (Newbold et al., 1996).

Other studies have also shown that inclusion of yeast culture (*S. cerevisiae*) in the diets of ruminants alter molar proportions of ruminal VFA (Newbold et al., 1990; Dawson, 1993; Ryan et al., 1993), increase nutrient digestibilities (Wiedmeier et al., 1987; Williams et al., 1991; Wohlt et al., 1991; Harris et al., 1992; Dawson, 1993), reduce ruminal NH_3 concentration (Harrison et al., 1988; Newbold et al., 1990; Erasmus et al., 1992), shift bacterial populations, and increase numbers of ruminal bacteria. (Wiedmeier et al., 1987; Harrison et al., 1988; Dawson et al., 1990; Dawson, 1993), increase numbers of ruminal protozoa (Edwards, 1991; Plata, et al., 1993), and alter the flow of N fractions to the duodenum (Williams et al., 1990; Edwards et al., 1991; Karr et al., 1991; Erasmus et al., 1992).

Few direct measurements of the effect of microbial protein flow to the duodenum have been made (Karr et al., 1991; Erasmus et al., 1992). Karr et al. (1991) reported lower flow of microbial N to the duodenum of sheep fed yeast culture. Erasmus et al.

(1992) fed 10 g/d of yeast culture (YEA-SACCR; Alltech Inc., Lexington, KY) to lactating dairy cows consuming a 35% forage diet that contained 25% wheat straw (% DM). Rate of passage of microbial N to the duodenum tended to increase (38 g/d above that of control cows), and the pattern of amino acids in duodenal digesta was altered. Specifically, supplemental feeding of yeast culture increased the flow of Methionine to the duodenum and tended to increase the flow of Lysine. Methionine and lysine are critically important in lactating dairy cows because they have been demonstrated to be the first two limiting amino acids in the diets of lactating dairy cows (Schwab et al., 1976; Schwab et al., 1993) and, therefore, required to be supplemented in the diets.

Contrary to Erasmus et al. (1992), flow of essential amino acids to the duodenum and the essential amino acid profiles of duodenal digesta and of mixed ruminal bacteria were not altered by yeast culture (Putman et al., 1997). Instead, the inclusion of yeast culture increased the ruminal concentration of isobutyrate in cows fed a low CP (16.1%) diet and decreased the concentration of isobutyrate in cows fed a high CP (18.8%) diet (Putman et al., 1997). Also, higher CP diets increased microbial N passage to the duodenum and had no effect on passage of nonmicrobial ammonia N, ruminal pH, concentrations of NH_3 and VFA in ruminal fluid and ruminal digestibility (Putman et al., 1997). Therefore, more research is needed to investigate the effect of yeast culture and its interaction with other DFM on N flow to the duodenum, microbial protein synthesis and passage rate of amino acids to the small intestine of dairy cows.

Dietary yeast culture for dairy cows has increased DMI and milk yield (Williams et al., 1991; Wohlt et al., 1991; Erasmus et al., 1992; Piva et al., 1993; Putman et al., 1997; Zhou, 2002; Kujawa, 2003) in some studies but not in others (Erdman and Sharma,

1989; Arambel and Kent, 1990; Kung et al., 1997; Dann et al., 2000). It is apparent that most of the studies that have shown increases in milk production have also shown increases in DMI possibly via increased fiber digestion. However, the precise mechanism by which yeast increases DMI will require futher studies.

Overall, data on studies using cows fed DFM have shown little change in milk composition and the results have been variable (Krehbiel et al., 2003). Several studies have reported no significant changes in milk components (Erdman and Sharma, 1989; Arambel and Kent; 1990; Wohlt et al., 1991; Swartz et al, 1994; Kung et al., 1997; and Dann et al., 2000) in response to *S. cerevesiae* supplementation. For example, Arambel and Kent (1990) fed TMR with or without yeast culture (*Saccharomyces cerevisiae* provided by Diamond V Mills, Inc., Cedar Rapids, IA) to Holstein dairy cows in early to midlactation and found no significant difference in milk composition (percentage of protein, fat, and lactose) and BW. Similarly, Dann et al. (2000) added yeast culture to diets of dry and lactating Jersey dairy cows (Primigravid and multigravid) and found no significant difference in the concentrations of fat, protein, lactose, total solids, and urea N in milk, as well as somatic cell count. However, in a study conducted by Harris and Webb (1998), increases in milk fat and milk protein percentages were significant in cows supplemented with yeast culture whereas Williams, et al. (1991) reported tendency for decreased milk fat percentage with feeding yeast culture and hay as a source of roughage.

Bacterial DFM fed in combination with fungal cultures might be efficacious for improving milk quality but the studies are minimal. Holstein cows fed a combination of DFM (2 specific *Enterococcus faecium* strains) and yeast showed higher lactose percentage and lower milk fat than control cows, but milk protein %, SCC and MUN

were not affected (Nocek and Kautz, 2006). Another study (McGilliard and Stallings, 1998) found that herds fed a supplement which contained dried fermentation products of *Aspergillus oryzae, Bacillus subtilis, Lactobacillus acidophilus*, and yeast cultures had decreased milk fat% with little effect on fat and protein yields and protein %.

Higginbotham et al. (1993) examined the effects of an *Aspergillus oryzae* extract on milk production and composition, rectal temperatures, and rumen metabolites in a commercial dairy herd in midlactation and detected no differences in milk protein and SNF%. In contrast to this, Higginbotham et al. (1994) examined the effect of a *Aspergillus oryzae* in combination with *S. cerevesiae* on milk composition in a commercial dairy herd in pluriparous Holstein cows in early lactation for 130 d and found no significant differences in milk fat or protein percentages. However, percentages of lactose and SNF were lower for the group fed *Aspergillus oryzae* plus yeast culture. The conditions under which non-bacterial DFM are fed alone or in conjunction with bacterial DFM to improve the overall performance of dairy cows vary and require further elucidation.

Propylene glycol

Propylene glycol is a glucogenic compound often used to treat ketosis postpartum in dairy cows (Nielsen and Ingvarsten, 2004) and a preventative for the development of fatty liver if administered to dairy cows during prepartum period (Studer et al., 1993). Propylene glycol has beneficial effects whether administered as a drench or as a part of concentrate fed once daily (Christensen et al., 1997). During feed restriction in heifers, propylene glycol administered by either method, increased plasma insulin and decreased NEFA concentrations. In support of this, oral drench of propylene glycol (500 ml)

increased plasma glucose and liver glycogen concentrations (Pickett et al., 2003). Although administration of propylene glycol has shown to increase blood glucose and insulin concentrations, this was not accompanied by increase in milk yield and improvement of milk components (Pickett et al., 2003). Administration of propylene glycol as part of a total mixed ration, however, failed to deliver desired effect.

Propylene is mainly metabolized in the rumen and shows decrease in the ruminal acetate to propionate ratio (Studer et al., 1993; Nielsen and Ingvartsen, 2004). The majority of propylene glycol is absorbed through the rumen epithelium and subsequently converted to glucose by sequential conversion to lactate, pyruvate and oxaloacetate (Schultz, 1971). Fisher et al. (1973), reported a depression in forage DMI when propylene glycol was fed at 9% of the concentrate mixture (460 ml/dl). However, when propylene glycol was delivered as an oral drench, the DMI was not depressed (Studer et al., 1993). The response of dairy cows to propylene glycol treatment varies mainly based on the mode of application, timing of treatment reference to parturition and duration of glycol. However, increase in glucose and insulin when propylene glycol is applied seems to be consistent in a lot of studies.

Role of glucose in milk synthesis

Milk synthesis takes place in mammary parenchymal tissue in specialized epithelial cells called alveoli. The uptake and utilization of substrates by the mammary gland is governed by several factors, including the availability of the substrates in the blood circulation, mass of tissue and blood flow to it, transport systems of the cells and organelles, activities of key enzymes and the hormonal milieu (Jones and Williamson, 1984). Glucose is a key energy source used for milk synthesis and the glucose demand by

the mammary gland increases during lactogenesis. In-fact, for the synthesis of milk, mammary gland uses on average, about 70% of the total amount of glucose available to lactating cows. Glucose uptake by the mammary gland the day after parturition is nine times greater than that removed by the mammary gland seven to nine days prepartum and five times that removed two days before parturition in dairy goats (Davis et al., 1979). With the mammary gland playing such a dominant role in the utilization of glucose, it is necessary to understand the role of glucose in milk synthesis.

Glucose is essential for milk synthesis. It is primarily utilized for lactose and glycerol synthesis in the mammary gland, with lactose synthesis being, fundamentally, the most important fate of glucose in the lactating ruminants. The availability of glucose to the mammary gland has an important impact on milk yield because lactose is the major osmoregulator in mammary uptake of water (Rook, 1979) and is a major determinant of milk yield (Davis and Collier, 1985). Danfaer (1994) showed that 0.4 mol of glucose is required per kg of milk synthesis. Increasing amounts of either post-ruminal glucose or ruminal propionic acid enhanced both milk and protein yield in lactating dairy cows when dietary supply of ruminal starch is low, such as with grass silage diets as reviewed by Rigout et al. (2003). Increased milk yield may result from changes in glucose supply to the mammary gland (Rigout et al., 2002). With duodenal infusions of glucose, an increased glucose appearance rate may be a key factor favoring increased glucose uptake by the mammary gland and increased milk yield (Rigout et al., 2002). Because propionate is the major precursor for hepatic glucose production (Danfaer et al., 1995), increased glucose production may also favor increased milk yield. Huhtanen et al. (1998) investigated the effects of intraruminal infusion of VFA and protein source on milk

production and blood metabolites and found that propionate infusion increased milk yield, milk protein yield (832 vs. 778 g/day) and milk lactose content (44.7 vs. 43.43.5 g/kg) and yield (1113 vs. 1023 g/d), whereas butyrate infusion was associated with a higher milk fat content (44.7 vs. 39.4 g/kg) and yield (1037 vs. 974 g/d).

There is growing evidence supporting that increase blood glucose concentrations increase milk protein synthesis, and therefore, increase milk protein. For example, Huhtanen et al. (1998) and Hurtaud et al. (2000) observed increased in milk protein yield with an increase in circulating glucose concentrations. The hypothesis that increase blood glucose spares glucogenic amino acid for being degraded to provide energy was not supported in this study because the increase in milk protein yield was not accompanied by an increase in plasma glucogenic amino acid concentration due to increasing glucose concentration in plasma of lactating dairy cows (Hurtaud et al,. 2000). Leonard and Block (1997) observed a decrease in milk protein yield due to intravenous glucose infusion. The difference in response could be due to the different methods used to administer glucose. More studies are needed to make conclusive remarks regarding the relationship that exist between milk protein and plasma concentration.

There are other studies that support Van Soest, (1994) hypothesis that propionate, spares glucogenic amino acids in gluconeogenesis and possible heat increment. This hypothesis was supported by significant increase in circulating free amino acid concentration by intraruminal propionic acid infusion (Seal and Parker, 1995) and significant increase in plasma concentration of glucogenic amino acids when lactating dairy cows fed grass based silage were infused with a propionate into the rumen (Lemosquet et al., 2004).

Much of the glucose extracted by the mammary gland not used for lactose synthesis is oxidized to synthesis ATP (Annison, 1983; Oddy et al., 1985). Glucose is also used to provide the α-glycerol phosphate as well as reducing equivalent in the form of NADPH (via pentose phosphate pathway) for milk fat synthesis (Forsberg et al., 1985a). However, only tracer quantities of fatty acids are derived from glucose (Forsberg et al., 1985a), indicating that glucose might not be a significant substrate in milk synthesis.

Propionate use by the liver

The blood flow and arteriovenous concentrations are major determinants of net appearance in portal blood and net hepatic utilization of the VFA by the liver (Bergman and Wolf, 1973). In general, the studies on VFA metabolism are complicated because some VFA do not reach the bloodstream because they are metabolized by the rumen epithelium during absorption. Like other VFAs , when propionate is administered into the rumen a part is metabolized into the rumen wall and only a part of rumen propionate appears in the portal vein. Portal appearance of lactate and alanine may increase (Seal and Parker, 1994) as a result of propionate infusion. However, many factors such as the duration of infusions, amounts infused and total energy intake will affect the results (Casse et al., 1994). Unlike other VFAs, the liver removes nearly all the propionate that reaches the peripheral circulation (Bergman and Wolff, 1971). Specifically, the mean portal recovery rates of propionate infused into the rumen are high and vary between 75 and 100% in steers (Seal and Parker, 1994) and in lambs (Majdoub et al., 2003).

Few studies have been done with lactating dairy cows to determine the contributions of propionate to gluconeogenesis during early lactation. Lomax and Baird

(1983) and Reynold et al. (1988) determined that propionate accounted for almost 46 to 55% of hepatic glucose production. The capacity of the liver to convert propionate to glucose seems to be responsive to propionate supply. To support this statement, Drackley et al. (2001) reported that daily metabolic activity of the liver doubles from 11 d prepartum to 11 d postpartum and this was accompanied by the increased blood flow. This observation agrees with an increased hepatic metabolic activity where propionate conversion to glucose by liver slices was 19 and 29 % greater at d 1 and d 21 postpartum (Overton et al., 1998). Propionate supplied over 43% of the carbon for gluconeogenesis in steer fed a basal treatment, however, when sodium propionate was administered, carbon from supplied by propionate increased over 67% (Knapp et al. 1992). Demigne et al. (1991) reported that propionate is a potent inhibitor of the other glucogenic pathways and this led Lemosquet et al. (2004) to speculate that glucose infused into both the duodenum and propionate into the rumen probably reduced gluconeogenesis from amino acids because plasma concentrations of glucogenic amino acids significantly increased with both glucogenic materials. To further support this point, Lemosquet et al. (2004) observed the tendency for increased plasma lactate concentration with the dose of ruminal propionate and led them to state that increased plasma lactate might indicate reduced lactate utilization for gluconeogenesis. Contrary to this, Majdoub et al. (2003) reported that in lambs infused with ruminal propionate at a similar dose to that used in Lemosquet et al. (2004), hepatic net flux of glucose remained unchanged despite an increase in portal availability of lactate and an unchanged fractional extraction by the liver. Whether the response varies depending on the species, it remains to be elucidated.

Increased gluconeogenesis is a requirement for high producing dairy cows to meet overwhelming glucose requirements for milk production during early lactation. Freetly and Ferrell (1998) studied the relationship between hepatic glucose release and milk production and the relationship between net hepatic uptake of gluconeogenesis precursors and milk production and found that net hepatic glucose release and net hepatic propionate uptake increased with increased milk production when multiparous were fed alfalfa based diet. Also, they observed net hepatic glucose release increases with increase hepatic propionate uptake suggesting an increase in metabolic fluxes through portal drain viscera and liver tissues increases milk production.

Effect of propionate on plasma hormones and metabolites

In ruminants, often less than 10% of the glucose utilized is absorbed from the lower gastrointestinal tract. Therefore, gluconeogenesis must supply more than 90% of glucose (Amaral et al, 1990). Since propionate is the major glucogenic precursor, it is important to understand its effect on insulin and glucagon, the major regulators of glucose production and disposal in ruminants (Bassett, 1978). Several studies have been conducted to evaluate the effect of propionate on plasma hormones and metabolites. In these studies, the responses differed depending on the method of administration and route of infusion, basal diet fed, dose given as well as the length of time that the propionate was administered. Few of these studies will be summarized in the next few paragraphs.

De Jong (1982) found that plasma insulin and glucagon concentrations increased initially in response to intraportal infusions of a mixture of propionate and n-butyrate at rate of 36.0 and 14.1 μmol.kg BW $^{-1}$ min^{-1} for 4 h in goats. Therefore, insulin and glucagon seem to be responsive to blood propionate. However, Bines and Hart (1984)

infused mixture of VFA into the rumen of cattle and observed that the specific omission of propionate from the mixture resulted in a major reduction in plasma insulin but not glucagon concentrations and concluded that only propionate was a major stimulant of insulin secretion. Sano et al. (1993) observed that plasma insulin and glucagon concentrations peaked at 2.5 to 15 min after the initiation of physiological propionate infusion and support the concept that propionate may be the major physiological regulator of pancreatic endocrine secretion in ruminants (Harmon, 1992). In the same study by Sano et al. (1993), the plasma glucose concentrations remained unchanged during propionate infusion at the rate of ≤ 16 μmol.kg BW^{-1} and, therefore, concluded that insulin and glucagons are not always mediated via changes in plasma glucose concentration as suggested by Istasse et al. (1987). Contrary to the studies above, the increase in plasma insulin concentration was not observed in peripheral blood with high dose of intraruminal infusion of propionate (Lemosquet et al., 2004). The absence of a significant increase in peripheral plasma insulin has already been observed in lactating cows receiving propionate infusions in the mesenteric vein (Casse et al., 1994) and in lambs (Majdoub et al., 2003).

While all the studies stated above involved infusion of propionate via different routes, studies that involve feeding of propionate have also been done. Aleman et al. (2007) reported that glucose concentrations in plasma samples collected weekly for 25 wk was 9% in PP cows fed low-dose (6×10^{10} cfu) P169 versus Control PP cows, but glucose concentration in MP cows did not differ among treatments. Consistent with this finding, Francisco et al. (2002) found no effect of feeding P169 (6×10^{10} cfu) on plasma glucose concentrations measured weekly during wk 1-12 of lactation in MP dairy cows.

Insulin concentrations were greater in PP cows fed high dose P169 vs. Controls and in MP cows fed high-dose P169 vs. low dose P169 during wk 1 – 25 (Aleman et al., 2007), but insulin did not differ between P169 low dose fed and Control MP cows during early lactation (Francisco et al., 2002). Feeding or infusion of propionate transiently increases both glucose and insulin concentrations in cattle (Subiyatno et al., 1996; Oba and Allen, 2003) and sheep (Sano et al., 1995) and these increases are short-lived (i.e. < 120 min).

Effect of feeding on plasma hormones and metabolites

Many of the daily patterns in the concentrations of hormones and metabolites in plasma of ruminants are related closely to feeding (Trenkle, 1978). Sano et al. (1990) studied insulin responsiveness to glucose and tissue responsiveness to insulin, using the hyperglycermic clamp and the hyperinsulinemic euglycermic clamp techniques. The measurements were taken before, during and after feeding sheep alfalfa hay and commercial concentrate diets. Their results suggested that insulin responsiveness to glucose tends to be enhanced during feeding but that tissue responsiveness to insulin is not changed over the feeding cycle in sheep.

Sutton et al. (1988) reported the effects of dietary modifications on the diurnal pattern of concentrations of certain metabolites and hormones in the peripheral blood of lactating dairy cows. In the cows fed twice daily, the concentrations of glucose tended to fall while that of insulin increased after meal. The maximum concentration and diurnal range of insulin concentrations were reduced by more frequent feeding of high concentrate (Sutton et el., 1988). Concentrations of glucose, BHBA and urea remained relatively constant in a herd that was fed by use of automatic distribution of concentrate (Eicher et al., 1999). However, significant diurnal patterns in glucose, BHBA and urea

concentrations were detected in a herd fed twice daily (Eicher et al., 1999; Borrebaek et al., 1990). Nielsen et al. (2003) studied the diurnal variation in metabolites in plasma and milk of dairy cows fed TMR with low energy or high energy and found that plasma glucose did not show any diurnal variation, however, plasma insulin was not measured in this study. This is contrary to earlier studies where concentrates have been fed twice daily. Collectively, these studies indicate that the daily pattern in the concentration of hormones and metabolites in ruminants varies and therefore, necessitates the frequent blood collection to detect transient increases in hormone and metabolite concentrations.

DFM and Reproductive performance

The impact of DFM on reproductive performance has not been studied extensively. Energy balance has been positively related to reproductive performance in dairy cattle and the topic has been reviewed (Butler and Smith, 1989). Most cows regardless of the ration fed are in a negative energy balance for 2 -3 months of lactation. DeVries and Veerkamp (2000) estimated that each decrease of 2.3 Mcal in energy balance nadir resulted in about a 1.5 day increase in time to resume luteal activity after calving. Villa-Goody et al. (1988) demonstrated that 75% of the variation in energy balance in early lactation Holsteins was associated with intake, while 25% was associated with milk yield. The transition program from the dry period and early lactation ration should be considered when minimizing the negative reproduction effects of negative balance.

Reproductive efficiency has major impact on profitability of dairy farms. Dairyman has utilized many methodologies in order to improve reproduction efficiency including the use of reproductive hormones to regulate and control the estrous cycle.

Nutritional strategies have also been utilized independently or in conjunction with hormonal programs to improve reproductive efficiency. For example, Jersey cows (14 primi gravid and 25 multigravid) were fed a TMR prepartum and postpartum with or without yeast culture, and day to first breeding was not affected by yeast (Dann et al., 2000). Also yeast supplement did not affect number of services per pregnancy, which averaged 2.1 (Dann et al., 2000). In another study, reproductive performance as measured by days to first service, days to conception and number of services per conception was not different between groups supplemented with *Aspergillus oryzae* fermentation extract (Amaferm) with or without yeast culture plus mineral-vitamin supplement (VitaFerm) in early lactation Holstein cows (Kellems et al., 1990).

Supplementation of Propylene glycol, a glucose precursor, has been examined as an alternative way to enhance reproduction. Miyoshi et al. (1995) drenched cows with 500 ml propylene glycol/day from day 7 to 42 postpartum and observed increased energy balance and blood glucose and insulin. Days to first ovulation was decreased (32 vs. 44), and duration of luteal phase during the first estrous was increased (6 vs. 13 days). Formigon et al. (1996) fed 300 ml propylene glycol/day for the final 20 days of the dry period and then drenched the same amount of propylene glycol on days 0, 3, 6, 9, 12 postpartum and found that percentage of cows remaining acyclic was reduced with propylene glycol as time postpartum increased. Because insulin and/or IGF-1 concentrations are greater in cows whose first dominant follicle ovulated (Beam and Butler, 1997), and are positively related to corpus luteum development and progesterone concentrations (Spicer et al., 1990; Thatcher et al., 1996), propylene glycol may be influencing reproduction through its effect on IGF-I secretion (Formigoni et al., 1996).

Francisco et al. (2002) and Stein et al. (2006) studies suggested that the inclusion of P169 in the diet of Holstein dairy cows might enhance metabolic performance, improve milk yield and milk components as well as body weight without compromising their reproductive performance via alterations in plasma metabolites and hormones. Unfortunately, there are no studies that have been conducted to date to evaluate the glucogenic precursor P169 in conjunction with yeast culture. Therefore, additional studies should be conducted to test the hypothesis that manipulation of microflora by feeding supplemental yeast culture in combination with P169 would offer an additional benefit on milk yield, milk components, body weight, and reproductive performance over the use of either feed additive alone.

Literature Cited

Abe, N., I. J. Lean, A. Rabiee, J. Porter, and C. Graham. 1994. Effects of sodium monensin on reproduction performance of dairy cattle. II. Effects on metabolites in plasma, resumption of ovarian cyclicity and estrus in lactating cows. Aust. Vet. J. 71:277-282.

Akay, V., and R. G. Dado. 2001. Effects of Propionibacterium strain P5 on in-vitro volatile fatty acids production and digestibility of fiber and starch. Turk. J. Vet. Anim. Sci. 25:635-642.

Aleman, M. M., D. R. Stein, D. T. Allen, E. Perry, K. V. Lehloenya, T. G. Rehberger, K. J. Mertz, D. A. Jones, and L. J. Spicer. 2006. Effect of feeding two levels of Propionibacteria to dairy cows on plasma hormones and metabolites. J. Dairy Res.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Amaral, D. M., J. J. Veenhuizen, J. K. Drackley, M. H. Cooley, A. D. McGilliard, and J. W. Young. 1990. Metabolism of propionate, glucose, and carbon dioxide as affected by exogenous glucose in dairy cows at energy equilibrium. J. Dairy Sci. 73:1244-1254.

Amaral-Phillips, D. M., A. D. McGilliard, G. L. Linderberg, J .J. Veenhuizen, and J. W. Young. 1993. Effects of decreased availability of glucose for dairy cows. J. Dairy Sci. 76:752-761.

Annison, E. F., and D. G. Armstrong. 1970. In: A. T. Phillpson (Ed.) Physiology of Digestion and Metabolism in the ruminant. p 422. Oriel Press, Ltd., Newcastle-upon-Tyne, England.

Annison, E. F. (1983). Metabolite utilization by the ruminant mammary gland. In 'Biochemistry of lactation'. (Ed. T. B. Mepham.) pp. 399-436. (Elsevier Science Publishers: Amesterdam).

Arambel, M. J., and B. A. Kent. 1990. Effect of yeast culture on nutrient digestibility and milk yield response in early and midlactation dairy cows. J. Dairy Sci. 73:1560-1563.

Arambel, M. J., R. D. Wiedmeier, and J. L. Walters. 1987. Influence of donor animal adaptation to added yeast culture and/or *Aspergillus oryzae* fermentation extract on in vitro rumen fermentation. Nutr. Rep. Int. 35:433.

Association of American Feed control Officials. 1999. Inc. C. P. Frank, Georgia, Department of Agriculture, Plant Food, Feed and Grain Division, Capital Square, Atlanta, GA.

Babuchowski, A., L. Laniewska-Moroz, and I. Warmanska-Radyko. 1999. Propionibacteria in fermented vegetables. Le Lait. 79:113-124.

Bassett, J. M. 1978. Endocrine factors in the control of nutrient utilization: ruminants. Proceed. Nutr. Soc. 37:273-280.

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

Baumgard, L. H., B. A. Corl, D. A. Kwyer, A. Saebo, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. 278:R179-R184.

Bayourthe, C., R. Moncoulon, and M. Vernay. 1993. Effect of protected fat on ruminal and total nutrient digestibility of sheep diets. J. Anim. Sci. 71:1026-1031.

Beam, S. W., and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56:133-142

Beam, S. W., and W. R. Butler. 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81:121-131.

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

Bergen, W. G., and D. B. Bates. 1984. Ionophores: their effect on production efficiency and mode of action. J. Anim. Sci. 58:1465-1483.

Bergman, E. N. 1973. Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Veterinarian 63:341-382.

Bergman, E. N., R. P. Brockman, and C. F. Kaufman. 1974. Glucose metabolism in ruminants: comparison of whole body turnover with production by gut, liver and kidneys. Federation Proceed. 33:1849-1854.

Bergman, E. N., and J. E.Wolff. 1971. Metabolism of volatile fatty acids by liver and portal-drained viscera in sheep. Amer. J. Physiol. 221:586-592.

Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on live triglyceride concentration and early lactation. J. Dairy Sci. 75:1914.

Bines, J. A., and J. C. Hart. 1984. The response of plasma insulin and other hormones to intraruminal infusion of VFA mixture in cattle. Can. J. Anim. Sci. 64 (Suppl.):304.

Block, E., J. E. Nocek, W. P. Kautz, and J. A. Z. Leedle. 2000. Direct fed microbial and anionic salt supplementation to dairy cows fed 21 days pre- to 70 days postpartum. J. Anim. Sci. 78(Suppl. 1):304.

Block, S. S. W. R. Butler, R. A. Ehrhardt, A. W. Bell, M. E. Amburgh, Y. R. Boisclair. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. J. Endocrinol. 171:339-348.

Bloom, S. R., and A.V. Edwards. 1985b. Pancreatic neuroendocrine responses to butyrate in conscious sheep. J. Physiol. (Lond.) 364:281

Borcherding, J. R., and K. Wanner. 1987. Product report: Megalac. Successful Farming 85:54.

Borrebaek, B., K. Halse, B. Tveit, H. L. Dahle, and L. Ceh. 1990. Plasma glucose, ketone bodies, insulin, glucagons, and enteroglucagon in cows: Diurnal variations related to ketones levels before feeding and to the ketogenic effects of feeds. Acta Vet. Scand. 31:5-15.

Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964-2971.

Burroughs, W., W. Woods, S. A. Ewing, T. Greig, and B. Theurer. 1960. Enzyme addition to fattening rations. J. Anim. Sci. 19:458.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function dairy cattle. J. Dairy Sci. 72:767.

Callaway, E. S., and S. A. Martin. 1997. Effects of *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Cassie, E. A., H. Rulquin, G. B. Huntington. 1994. Effect of mesenteric vein infusion in primiparous Holstein cows. J. Dairy Sci. 77:3296-3303.

Castaneda-Gutlerrez, E., Overton, T. R., Butler, W. R., and D. E. Bauman. 2005. Dietary supplements of tow doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J. Dairy Sci. 88:1078-1089.

Chen, G. C., and J. B. Russell. 1991. Effect of monensin and a protonophore on protein degradation, peptide accumulation and deamination by mixed ruminal microorganisms in vitro. J. Anim. Sci. 69:2169-2203.

Chew, P. P., R. E. Erb, J. F. Fessler, C. J. Callahan, and P. V. Malven. 1979. Effect of ovariectomy during pregnancy and of prematurely induced parturition on progesterone, estrogens and calving traits. J. Dairy Sci. 62: 557-566.

Christensen, J. O., R. R. Grummer, F. E. Rasmussen, and S. J. Bertics. 1997. Effects of method of delivery of propylene glycol on plasma metabolites of feed restricted cattle. J. Dairy Sci. 80:563-568.

Church, D. C. 1993. The ruminant animal digestive physiology and nutrition. Waveland Press Inc., Illinois, USA.

Colenbrander, V. F., R. J. Grant, and G. Schaaf. 1988. Milk production and feed intake of dairy cows fed Lactobacillus inoculated alfalfa silage. Appl. Agric. Res. 3:55-59.

Cotta, M. A., and J. B. Russell. 1997. Digestion of nitrogen in the rumen. A model for metabolism of nitrogen compounds in gastrointestinal environments. Page 380-423 in Gastrointestinal Ecosystem and Fermentations, Vol. 1. R. I. Mackie and B. White, ed. Chapman and Hall, New York, NY.

Counotte, G. H. M., R. A. Prins, R. H. A. M. Janssen, and M. J. A. DeBie. 1981. Role of Megasphaera elsdenii in the fermentation of DL-[2^{13}] lactate in the rumen of dairy cattle. Appl. Environ. Microbiol. 42:649-655.

Cummins, C. S., and J. L. Johnson. 1986. Genus1. Propionibacterium. In P.H.A. Sneath, N.S. Mair, and J.G. Holt (Ed.), Bergey's manual of systemic bacteriology. Vol. 2. Williams and Wilkins, Baltimore, MD.

Dann, H. M., J. K. Drackley, G. C. McCoy, M. F. Hutjens, and J. E. Garrett. 2000. Effects of yeast culture (*Saccharomyces cerevisiae*) on prepartum intake and postpartum intake and milk production and milk production of Jersey cows. J. Dairy Sci. 83: 123-127.

Danfaer, A. 1994. Nutrient metabolism and utilization in the liver. Livest. Prod. Sci. 39:115-127

Danfaer, A., V. Tetens, and N. Agregaard. 1995. Review and an experimental study on the physiological and quantitative aspects of gluconeogenesis in lactating ruminants. Comp. Biochem. Physiol. 111B:201-210.

Davidson, C. A. 1998. The isolation, characterization, and utilization of Propionibacterium as a direct-fed for beef cattle. M. S. Thesis. Oklahoma State Univ., Stillwater.

Davis, A. J., I. R. Fleet, J. A. Goode, M. H. Hamon, F. M. Maule Walker, and M. Peaker. 1979. Changes in mammary function at the onset of lactation in the goat: correlation with hormonal changes. J. Physiol. 288.33-44.

Davis, S. R., and Collier, R. S. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. J. of Dairy Sci., 68:1041-1058.

Dawson, K.A. 1993. Current and future role of yeast culture in animal production; a review of research over the last seven years. Page 269 in Biotechnology in the Feed Industry. T.P. Lyons ed. Alltech Tech. Publ., Nicholasville, KY.

Dawson, K.A., K. E. Newman, and J. A. Boling. 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. J. Anim. Sci. 68:3392.

Dawson, K. A., and D. M. Hopkins. 1991. Differential effects of live yeast on the cellulolytic activities of anaerobic ruminal bacteria. J. Anim. Sci. 69 (Suppl. 1): 531 (Abstr.).

De Jong, A. 1982. Patterns of plasma concentrations of insulin and glucagon after intravascular and intraruminal administration of volatile fatty acids in the goat. J. Endocrinol. 92:357 (Abstr.).

Demigne, C., C. Yacoub, C. Morand, C. Remesy. 1991, Interactions between propionate and amino acid metabolism in isolated sheep hepatocytes. British Journal of Nutrition. 65:301-307.

Dennis, S. M., T. G. Nagaraja, and E. E. Bartley. 1981. Effects of lasalocid or monensin on lactate-producing or using rumen bacteria. J. Anim. Sci. 52:418-426.

De Vries, M. J., and R. F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83:62-69.

Dirksen, G. 1969. Acidosis. In: A. T. Phillipson (Ed.) Physiology of Digestion and Metabolism in the Ruminant. P. 612-625. Oriel Press Limited, New Castle upon Tyne, England.

Dijkstra, J. 1994. Production and absorption of volatile fatty acids in the rumen. Livest. Prod. Sci. 39:61-69.

Doreau, M., A. Ferlay, and Y. Elmeddah. 1993. Organic matter and nitrogen digestion by dairy cows fed calcium salts of rapeseed oil fatty acids or rapeseed oil. J. Anim. Sci. 71:499-504.

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

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long chain fatty acid metabolism in liver of dairy cow during the periparturient period. J. Dairy Sci. 84(E. suppl.):E100-E112.

Duffield, T. 1997. Effects of a monensin controlled released capsule on energy metabolism, health and production in lactating dairy cattle. Thesis dissertation, University of Guelph, Guelph, ON. Canada.

Duffield, T. F., D. Sandals, K. E. Lissemore, B. W. McBride, J. J. Lumsden, P. Dick, and R. Bagg. 1998b. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. J. Dairy Sci. 81:2866-2873.

Duffield, T. F., Sandals, K. E. Leslie, K. Lissemore, S. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1999b. Effect of a monensin-controlled release capsule on cow health and reproductive performance. J. Dairy Sci. 82:2377-2384.

Edgerton, L. A., H. D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid, and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451-458.

Edwards, I. E. 1991. Practical uses of yeast culture in beef production; insight into its mode of action. Page 51 in Biotechnology in the Feed Industry. T.P. Lyons ed. Alltech Tech. Publ., Nicholasville, KY.

Edwards, I., T. Mutsavanga, J. Topps, and G. Paterson. 1991. Effect of supplemental yeast culture on patterns ofruminal fermentation and performance of intensively fed bulls. Page 329 in Biotechnology in the Feed Industry. T.P. Lyons ed. Alltech Tech. Publ., Nicholasville, KY.

Eicher, R. A. Liesegang, E. Bouchard, A. Tremblay. 1999. Effect of cow-specific factors and feeding frequency of concentrate on diurnal variations of blood metabolites in dairy cows. Amer. J. Vet Res. 60:1493 -1499.

Elam, N. A., J. F. Gleghorn, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears, and S. M. Younts-Dahl. 2003. Effects of live culture of Lactobacillus acidophilus (strains NP45 and NP51) and Propionibacterium freudenreichii on performance, carcass and intestinal characteristics, and Escherichia coli strain O157 shedding of finishing beef steers. J. Anim. Sci. 81:2686-2698.

Emery, R. S., J. S. Liesman, and T. H. Herdt. 1992. Metabolism of long chain fatty acids by ruminant liver. J. Nutr. 122:832-837.

Erasmus, L. J., P. M. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056-3065.

Erasmus, L. J., I. Smith, A. Muller, and D. O'Hagan. 1999. Effects of lasalocid on performance of lactating dairy cows. J. Dairy Sci. 82:1817-1823.

Erdman, R. A., and B. K. Sharma. 1989. Effect of yeast culture on milk yield and composition in dairy cows. J. Dairy Sci. 72:1929.

Fahey, Jr., G. C., and L. L. Berger. 1988. Carbohydrate Nutrition of Ruminants. In: D. C. Church (Ed.) The Ruminant animal: Digestive Physiology and Nutrition. Pp 269-295. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

FDA, 2003. Office of Regulatory Affairs: compliance Policy Guides. Sec. 689.100 Direct-Fed Microbial Products (CPG 7126:41) Available: http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg689-100.html.

FDA, 2004. FDA approves rumensin for milk production efficiency in dairy cattle (M-I-04-7). Available: http://www.cfsan.fda.gov/~ear/mi-04-7.html.

Ferguson. J. D., L. A. Lee and D. T. Galligan. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on lactation performance of dairy cattle. J. Dairy Sci. 70 (Suppl. 1). 207 (Abstr.).

Firkins, J. L., A. N. Hristov, M. B. Hall, G. A. Vargas, and N. R. St-Pierre. 2006. Integration of ruminal metabolism in dairy cattle. J. Dairy Sci. 89(E. Suppl.):E31-E51.

Fisher, L. J., J. D. Erfle, G. A. Lodge, and F. D. Sauer. 1973. Effects of propylene glycol or glycerol supplementation of the diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. Can. J. Anim. Sci. 53:289-296.

Flores-Galaza, R. A., B. A. Glatz, C. J. Bern, and L. D. Van Fossen. 1985. Preservation of high moisture corn by microbial fermentation. J. Food Prot. 48:48:407-411.

Forbes, J. M., and J. P. Barrio. 1992. Abdominal chemo- and mechanosensitivity in ruminants and its role in the control of food intake. Exp. Physiol. 77:27-50.

Formigoni, A., M. C. Cornil, A. Prandi, A. Mordenti, A. Rossi, D. Portetelle, and R. Renaville. 1996. Effect of propylene glycol supplementation around parturition on milk yield, reproduction performance and some hormonal and metabolic characteristics in dairy cows. J. Dairy Res. 63:11-24.

Forsberg, N. E., R. L. Baldwin, and N. E. Smith. 1985a. Roles of glucose and its interactions with acetate in maintenance and biosynthesis in bovine mammary tissue. Journal of Dairy Sci. 68:2544-2549.

Francisco, C. C., C. S. Chamberlain, D. N. Waldner, R. P. Wettemann, and L. J. Spicer. 2002. Propionibacteria Fed to Dairy Cows: Effects on Energy Balance, Plasma Metabolites and Hormones, and Reproduction. J. Dairy Sci. 85:1738-1751.

Freetly, H. C., and C. L. Ferrel. 1998. Net flux of glucose, lactate, volatile fatty acids and nitrogen metabolites across the portal drained viscera and liver of pregnant ewes. J. Anim. Sci. 76:3133-3145.

Gabai, G., G. Gozzi, F. Rosi, I. Andrighetto, and G. Bono. 2002. Glucose or essential amino acid infusions in late pregnant and early lactating Simmental cows failed to induce a leptin response. J. Vet. Med. A 49:73-80.

Ghorbani, G. R., D. P. Morgavi, K.A. Beauchemin, and J .A. Z. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. J. Anim. Sci. 80:1977-1986.

Glass, T. L., M. P. Bryant, and M. J. Wolin. 1977. Partial purification of ferredoxin Ruminococcus albus and its role in pyruvate metabolism and reduction of nicotinamide adenine dinucleotide by H_2. J. Bacteriol. 131:463-472.

Goad, D.W., C. L. Goad, and T. G. Nagaraja. 1998. Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. Southwest Kansas Branch: Agric. Exp. Sta. Rep. Prog. 555. Cattle Feeder's day.

Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260 -1268.

Gomez-Basauri, J., M. B. deOndarza, and J. Sialiano-Jones. 2001. Intake and milk production of dairy cows fed lactic acid bacteria and mannanoligosaccharide. J. Dairy Sci. 84 (Suppl. 1):283.

Gottschalk, G. 1986. Propionate and Succinate Fermentation. In: Gottschalk, G. (Ed.) Bacterial Metabolism. Pg 242-244. Springer-Verlag, New York.

Grappin, R., Beuvier, Y. Bouton, and S. Pochet. 1999. Advances in biochemistry and microbiology of Swiss-type cheeses. Le Lait. 79:3-22.

Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K.V. Nurmela. 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251-1261.

Grohn, Y. T., S. W. Eicker, V. Ducrocq, and J. A. Hertl. 1998. Effect of diseases on culling of Holstein dairy cows in Newyork State. J. Dairy Sci. 81:966-978.

Grovum, W.L. 1995. Mechanisms explaining the effects of short chain fatty acids on feed intake in ruminants.-osmotic pressure, insulin, and glucagons. In: ruminant

physiology: Digestion, Metabolism, Growth and Reproduction (Englehardt, W. V. Leonhard-Marek, S., Breves, G. and Geisecke, D., eds), pp. 173-197. Ferdinand Enke Verlag, Stuttgart, Germany.

Grum, D. E., J. K., Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuezen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.

Grummer, R. R. 1993. Etiology of related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Grummer, R. R., J. C. Winkler, S. J. Bertics, and V. A. Studer. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. J. Dairy sci. 77:3618-3623.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition cow. J. Anim. Sci. 73:2820-2833.

Grummer, R. R., 1987. Influence of prilled fat and calcium salts of palm oil fatty acids on ruminal fermentation and nutrient digestibility. J. Dairy Sci. 71:117.

Hanson, T. L., and T. J. Klopfestein, 1979. Monensin protein source and protein levels in growing steers. J. Anim. Sci. 48:474-479.

Harfoot, C. G., and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. Pages 285 – 322 In the Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier Appl. Sci., London, England.

Harmon. D. L. 1992. Impact of nutrition on pancreatic exocrine and endocrine secretion in ruminants. A review. J. Anim. Sci. 70:1290-1301.

Harris, B.Jr., D. E. Dorminey, W. A. Smith, H. H. Van Horn and C. J. Wilcox. 1992. Effects of feather meal at two protein concentrations and yeast culture on production parameters in lactating dairy cows. J. Dairy Sci. 75:3524-3530.

Harris, B., and D. W. Webb. 1990. The effect of feeding a concentrate yeast culture to lactating dairy cows. J. Dairy Sci. 73(Suppl. 1): 266 (Abstr.).

Harrison, G. A., R. W. Hemken, K. A. Dawson, R. J. Harmon, and K. B. Barker. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci. 71:2967-2975.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump, D.K. Beede, M. J. VandeHaar, L. H. Kilmer, J. K. Drackley, D. J. Carrol, G. A. Varga, and S. S. Donkin. 1999. Prediction equations for dry matter intake of transition cows fed diets that vary in nutrient composition. J. Dairy Sci. (Supp. 1):1.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci. 85:3430-3443.

Herbein, J. H., R. W. van Maanen, A.D. McGilliard, and J. W. Young. 1978. Rumen propionate kinetics in growing cattle fed isoenergetic diets. J. Nutr. 108:994-1001.

Herdt, T. N. 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet. Clin. North Am. Anim. Pract. 16:215-230.

Herdt, T. H. 2002. Gastrointestinal physiology and metabolism. Postabsorptive nutrient utilization. Pages 303-303 in Textbook of Veterinary Physiology. Third Edition. J. Cunningham. W. B. Saunders Company.

Hettinga, D. H., and G. W. Reinbold. 1972. The Propionic acid bacteria – A review. II Metabolism. J. Milk Food technol. 35:358-372.

Higginbotham, G. E., D. L. Bath, and L. J. Butler. 1993. Effect of feeding an *Aspergillus oryzae* extract on milk production related responses in a commercial dairy herd. J. Dairy Sci. 76: 1484-1489.

Higginbotham, O. E., C. A. Collar, M.S. Asektube, and D. L. Bath. 1994. Effect of Yeast culture and *Aspergillus oryzae* extract on milk yield in a commercial dairy herd. J. Dairy Sci. 77:343-348.

Higginbotham, G., J. Merriam, and E. DePeters. 2001. Effect of live yeast versus yeast culture and related parameters in early lactation cows. J. Dairy Sci. (Suppl. 1): 281 (Abstr.).

Hoover, H., and S. R. Stokes. 1991. Balancing carbohydrates on product for optimum rumen microbial yield. J. Dairy Sci. 74:3630-3644.

Huhtanen, P. J., R. Blauwicked, I. Saastamoineu. 1998. Effect of intraruminal infusions of propionate and butyrate two different protein supplements on milk production and blood metabolites in dairy cows receiving grass silage-based diet. J. Sci. Food Agric 77:213-222.

Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York.

Hungate, R. E. 1988. Introduction: The ruminant and the rumen. P. 1-20. In. P. N. Hobson (Ed.). The rumen microbial ecosystem. Elsevier Applied Science, London, United Kingdom.

Huntington, G. B. 2000. High-starch rations for ruminant production discussed. Feedstuffs. 12 – 13:23

Hurtaud, C., H. Rulquin, and R. Verite. 1998a. Effects of graded duodenal infusions of glucose on yield and composition of milk from dairy cows.1. Diet based on corn silage. J. Dairy Sci. 81:3239-3247.

Hurtaud, C., S. Lemosquet, H. Rulquin. 2000. Effect of graded duodenal infusions of glucose on yield and composition of milk from dairy cows. 2. Diets based on grass silage. J. Dairy Sci. 83:2952-2962

Hurtaud, C., H. Rulquin, and R. Verite. 1998b. Effects of level and type of energy sources (Volatile fatty acids or glucose) on milk yield, composition and coagulating properties in dairy cows. Reprod. Nutr. Dev. 38:315-330.

Ingvartsen, K. L, and J. B. Anderson. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. J. Dairy Sci. 83:1573-1597.

Isichei, C. O., and W. G. Bergen. 1980. The effect of monensin on the composition of abomasal N flow in steers fed grain and silage rations. J. Anim. Sci. 51(Suppl. 1): 371 (Abstr.).

Istasse, L., N. A. MacLeod, E. D. Goodall, E. R. Orskov. 1987. Effects on plasma insulin of intermittent infusions of propionic acid, glucose or casein into the alimentary tract of non lactating cows maintained on a liquid diet. Br. J. Nutr. 58:139 – 148.

Janes, A. N., T. E. C. Weekes, and D. G. Armstrong. 1985. Insulin action and glucose metabolism in sheep fed on dried-grass or ground, maize-based diets. Br. J. Nutr. 54:459-471.

Jaquette, R. D., A. H. Rakes, and W. J. Croom. 1988. Effects of body condition and protein on milk fat depression in early lactation dairy cows. J. Dairy Sci. 71:2123-2134.

Jones, R. G., and D. H. Williamson. 1984. Alterations in mammary gland blood flow and glucose metabolism in the lactating rat induced by short term starvation and refeeding. Bio. Sci. Repr. 4:421-426.

Johnson, J. C., P. R. Utley, B. G. Mullinix, and A. Merrill. 1988. Effects of adding fat and lasalocid to diets of dairy cows. J. Dairy Sci. 71:2151-2165.

Kanegasaki, S, and H. Takaliashi. 1967. Function of growth factor for rumen microorganisms. 1. Nutritional characteristics of *Selenomonas ruminantium*. J. Bacteriol. 93:456-463.

Karr, K. J., K. R. McLeod, K. A. Dawson, R. E. Tucker and G. E. Mitchell, Jr. 1991. Influence of yeast culture and/or monensin on nitrogen flow and rumen fermentation in sheep. Page 337 in Biotechnology in the Feed Industry. T. P. Lyons ed. Alltech Tech. Publ., Nicholasville, KY.

Karsli, M. A., and Russell, J. R. 2001. Effects of some dietary factors on ruminal microbial protein synthesis. Turk J. Vet. Anim. Sci. 25:681-686.

Kellems, R. O., A. Lagerstedt, and M. V. Wallentine. 1990. Effect of feeding *Aspergillus oryzae* fermentation or *Aspergillus oryzae* plus yeast culture plus mineral and vitamin supplement on performance of Holstein cows during complete lactation. J. Dairy Sci. 73:2922-2928.

Kim, S. W., D. Standorf, H. RomanRosario, M. T. Yokoyama, and S. R. Rust. 2001a. Potential use of Propionibacterium acidipropionici, strain DH42 as a direct-fed microbial strain for cattle., Michigan State Univ. Beef cattle, sheep and forage systems research and demonstration report, Michigan State Univ., East Lansing.

Kim, S. W., S. R. Rust, H. Roman-Rosario, and M. T. Yokoyama. 2001b. In vitro effects of *Propionibacterium acidipropionici*, strain DH42 on fermentation characteristics of rumen microorganisms, Michigan State Univ. Beef cattle, sheep and forage systems research and demonstration report, Michigan State Univ., East Lasing.

Kmet, V., H. J. Flint, and R. J. Wallace. 1993. Probiotics and manipulation of rumen development and function. Arch. Anim. Nutr. 44:1-10.

Knapp, J. R., H. C. Freetly, B. L. Reis, C. C. Calvert, and R. L. Balwin. 1992. Effects of somatotropin and substrates on patterns of liver metabolism in lactating dairy cattle. J. Dairy Sci. 75:1025 -1035.

Koch, J. E., H. I. Hong, D. M. Osbakken, and M. I. Friedman. 1998. Temporal relationships between eating behavior and liver adenine nucleotides in rats treated with 2,5-Anhydro-D-Mannitol. Amer. J. Physiol. 274:R610-R617.

Knowlton, K. F., T. E. Dawson, B. P. Glenn, G. B. Huntington, R. A. Erdman. 1998. Glucose metabolism and milk yield of cows infused abomasally or ruminally with starch. J. Dairy Sci. 81:3248 – 3258.

Krehbiel, C. R., S. R. Rust, G. Zhang, and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81(E. Suppl. 2): E120-E132.

Kung, L., E. M. Kreck, R.S. Tung, A. O. Hession, A. C. Shepherd, M. A. Cohen, H. E. Swain, and J .A. Z. Leedle. 1997. Effects of a live yeast culture and enzyme on in vitro ruminal fermentation and milk production. J. Dairy Sci. 80:2045-2051.

Kung, L. 2001. Direct-fed microbials for dairy cows, Page 22-28 in Proc. 12th Annual Florida Rumin. Nutr. Symp., Univ. of Florida, Gainesville.

Kujawa, M. 2003. Profitability of yeast culture in today's environment. Pag 5-9 in Proc. 2003. Penn State Dairy Cattle Nutrition Workshop, Grantville, PA.

Kunz, P. L., J. W. Blum, C. Hart, H. Bickel, and J. Landis. 1985. Effects of different energy intakes before and after calving on food intake, performance and blood metabolites in dairy cows. Anim. Prod. 40:219-231.

Lean, I. J., M. Curtis, R. Dyson, and B. Lowe. 1994. Effects of sodium monensin on reproductive performance of dairy cattle. I. Effect on conception rates, calving to conception interval, calving to heat and milk production in dairy cows. Aust. Vet. J. 71:273-277.

Leavens, H., H. Deluyker, Y. H. Schukken, L. De Meuleteester, R. Vondermeersch, E. De Mueleneere, and A. De Kruif. 1997. Influence of parity and stage of lactation on somatic cell count in bacteriologically negative dairy cows. J. Dairy Sci. 80:3219-3228.

Leedle, J. A. Z. 1991. Microbiology and Physiology of the Rumen and Its Responses to Different Feeding Strategies: The Bovine Proceedings. No. 23. p 3-7.

Lemosquet, S., N. Rideau, H. Rulquin, P. Faverdin, J. Simon, and R. Verite. 1997. Effect of a duodenal glucose infusion on the relationship between plasma concentrations of glucose and insulin in dairy cows. J. Dairy Sci. 80:2854-2865.

Lemosquet, S., S. Rigout, A. Bach, H. Rulquin, and J. W. Blum. 2004. Glucose metabolism in lactating cows in response to isoenergetic infusions of propionic acid or duodenal glucose. J. Dairy Sci. 87:1767-1777.

Lindsay, D. B. 1978. Gluconeogenesis in ruminants. Biochemical Society Transactions. 6:1152-1156.

Leonard, M.,and E. Block. 1997. Effects on nutrient and hormonal profile of long term infusions of glucose or insulin plus glucose in cows treated with recombinant bovine somatotropin before peak milk yield. J. Dairy Sci. 80:127-143.

Liefers, S. C., R. F. Veerkamp, M. F. te Pas, C. Delavaud, Y. Chilliard, and T. van der Lende. 2003. Leptin concentrations in relation to energy balance, milk yield, intake, liveweight, and estrus in dairy cows. J. Dairy Sci. 86:799-807.

Linehan, B., C. C. Scheifinger, and M. J. Wolin. 1978. Nutritional requirements of *Selenomonas ruminantium* for growth on lactate, glycerol or glucose. Appl. Environ. Microbiol. 35:317 (Abstr.).

Lomax, M. A. G. D. Baird, G. B. Mallinson, and H. W. Symonds. 1979. Differences between lactating dairy cows in concentration and secretion rate of insulin. J. Biochem. 180:281-289.

Lomax, M. A., and G. D. Baird. 1983. Blood flow and nutrient exchange across the liver and gut of the dairy cows: effects of lactation and fasting. Brit. J. of Nutr. 49:481-496.

Lowe, L. B., G. J. Ball, B. R. Carruthrers, R. C. Bobos, G. A. Lynch, P. J. Moate, P. R. Poole, and S. C. Valentine. 1991. Monensin controlled release intra-ruminal capsule for the control of bloat in pastured dairy cattle. Aust. Vet. J. 68:17-20.

Lynch, H. A., and S. A. Martin. 2002. Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on in vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 85:2603-2608.

Maciel, S. M., Chamberlain, C. S., Wettemann, R. P., and Spicer, L. J. 2002. Dexamethasone influences endocrine and ovarian functions in dairy cattle. J. Dairy Sci. 84:1998-2009.

Mackie, R. I., and F. M. C. Gilchrist. 1978. Microbiological and chemical changes in rumen during the stepwise adaptation of sheep to high concentrate diets. J. Agric. Sci. Cam. 90:241-254.

Mackie, R. I., and F. M. C. Gilchrist. 1979. Changes in lactate-producing and lactate utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high-concentrate diet. Appl. Environ. Microbiol. 39:422-430.

Mackie, R. I., F. M. C. Gilchrist, and S. Heath. 1984. In vivo study of ruminal microorganisms influencing lactate turnover and its contribution to volatile fatty acid production. J. Agric. Sci. 103:37.

Machmuller, A., C. R. Soliva, and M. Kreuzer. 2003. Effect of coconut oil and defaunation treatment on methanogenesis in sheep. Reprod. Nutr. Dev. 43:41-55.

Majdoub, L., M. Beylot, M. Vermorel, and I. Ortigues-Marty. 2003. Propionate supplementation did not increase whole body glucose turnover in growing lambs fed rye grass. Reprod. Nutr. Dev. 43:357-370.

Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. Lacey Vankapen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune response during the peripartum period and its ramification on dairy cow and calf health. J. Dairy Sci. 81:585-595.

Martin, S. A., and D. J. Nisbet. 1990. Effects of *Aspergillus oryzae* fermentation extract on fermentation of amino acids, bermudagrass and starch by mixed ruminal microorganisms in vitro. J. Anim. Sci. 68:2142.

Martin S. A. and D. J. Nisbet. 1992. Symposium: Effect of direct-fed microbial on rumen microbial fermentation. J. Dairy Sci. 75:1736-1744.

Mashek, D. G., and R. R. Grummer, 2003. Feeding pre-fresh transition cows; Should we maximize feed intake or minimize feed intake depression? J. Dairy Sci. 86 (Suppl. 1):11 (Abstr.)

Mcallister, T. A., and K. J. Cheng. 1996. Microbial strategies in the ruminal digestion of cereal grain. Anim. Feed Sci. Technol. 62:29-36.

McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan, 2002. Animal Nutrition. 6th ed. Pearson Education Limited, Edinburgh Gate, Harlow, Essex, England.

McDougall, S., L. Young, and F. M. Anniss. 2004. Production and health of pasture-fed dairy cattle following oral treatment with the ionophore lasalocid. J. Dairy Sci. 87:-2967-2976.

McGilliard, M. L., and C. C. Stallings. 1998. Increase in milk yield of commercial dairy herds fed a microbial an enzyme supplement. J. Dairy Sci. 81: 1353-1357.

Mead, L. J., and G. A. Jones. 1981. Isolation and presumptive identification of adherent epithelial bacteria ("epimural" bacteria) from ovine rumen wall. Appl. Environ. Microbiol. 41:1020-1028.

Melendez, P., A. Donovan, C. A. Risco, M. B. Hall, R. Littell, and J. Goff. 2002. Metabolic responses of transition Holstein cows fed anionic salts and supplemented at calving with calcium and energy. J. Dairy Sci. 85:1085-1092.

Merry, R. J., and D.R. Davies. 1999. Propionibacteria and their role in biological control of aerobical spoilage in silage. Le Lait 79:149-164.

Miller-Webster, T., W. H. Hoover, and Holt. 2002. Influence of yeast culture on ruminal microbial metabolism in continuous culture. J. Dairy Sci. 85:2009-2014.

Miyoshi, S., J. L. Pate, and D. L. Palmquist. 1995. Productive, reproductive, and metabolic responses to daily administration of propylene glycol in early lactation. J. Dairy Sci. 78 (Suppl. 1):306 (Abstr.).

Mutsvangwa, T., J. P. Walton, J. C. Plaizer, T. F. Duffield, R. Bagg, P. Dick, G. Vessie, and B. W. McBride. 2002. Effects of a monensin controlled-release capsule or

premix on attenuation of subacute ruminal acidosis in dairy cows. J. Dairy cows. J. Dairy Sci. 85:3454-3461.

Nagaraja, T. G., T. B. Avery, E. E. Bartley, S. J. Galitzer, and A. D. Dayton. 1981. Prevention of lactic acidosis in cattle by lasalocid or monensin. J. Anim. Sci. 53:206-216.

Nagaraja, T. G., S. M. Dennis, S. J. Galitzer, and D. L. Harmon. 1986. Effect of lasalocid, monensin and thipeptin on lactate production from in vitro rumen fermentation of starch. Can. J. Anim. Sci. 60:120-139.

Nagaraja, T. G., C. J. Newbold, C. J. Van Nevel, and D. I. Demeyer, 1997. Manipulation of ruminal fermentation. Pages 523-632 in The rumen microbial ecosystem. Second edition. P. N. Hobson and C. S. Steward. Blackie Academic and Professional.

Nelson, D. L., and M. M. Cox. 2000. Oxidation of fatty acids. Pages 598-622. In Lehninger Principles of biochemistry. Third edition. Worth Publishers, New York, NY 10010.

Newbold, C. J., P. E. V. Williams, N. McKaijn. A. Walker, and R .J. Wallace. 1990. The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. Proc. Nutr. Soc. 49(1): 47A.

Newbold, C. J., R. Block, and R. J. Wallace. 1991. Influence of autoclaved or irradiated *Aspergillus oryzae* fermentation extract on fermentation in the rumen simulation technique (Rusitec). J. Agric Sci. (Camb.) 116:159-162.

Newbold, C. J., R. J. Wallace, and F. M. McIntosh. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. Br. J. Nutr. 76:249-261.

Nielsen, N. I., K. L. Ingvartsen, and T. Larsen. 2003. Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR- fed dairy cows. J. Vet. Med. A Physiol. Pathol. Clin. Med. 50:88-97.

Nielsen, N. I., and K. L. Ingvarsten. 2004. Propylene glycol for dairy cows: A review of the metabolism of propylene glycol and its effect on physiological parameters, feed intake, milk production, and risk of ketosis. Anim. Feed Sci. Technol. 115: 191-213.

Nocek, J. E., and W. P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health and performance of pre- and postpartum dairy cows. J. Dairy Sci. 89:260-266.

Oba M. and M. S. Allen. 2003. Dose-response of intraruminal infusion of propionate on feeding behavior of lactating cows in early or midlactation. J. Dairy Sci. 86:2922-2931.

Oddy, V.H., J. M. Gooden, G. M. Hough, E. Teleni, E., and E. F. Annison. 1985. Partitioning of nutrients in merino ewes. II. Glucose utilization by skeletal muscle, the pregnant uterus and the lactating mammary gland in relation to whole body glucose utilization. Austral. J. Biol. Sci. 38:95-108.

Ogimoto, K., and S. Imai. 1981. Atlas of rumen microbiology. Japan Scientific Societies Press, Tokyo.

Oscar, T. P., and J. W. Spears. 1990. Incorporation of nickel into ruminal factor F_{430} as affected by monensin and formate. J. Anim. Sci. 68:1400-1404.

Oshio, S.,I. Tahata, and H. Minato. 1987. Effect of diets differing in ratios of roughage to concentrate on microflora in the rumen heifers. J. Gen. App. Microbiol. 33:99-111.

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

Palmquist, D. L., and H. R. Conrad. 1978. High fat rations for dairy cows. Effect on feed intake, milk and fat production and plasma metabolites. J. Dairy Sci. 61:890-901.

Parrot, T. D. 1997. Selection of Propionibacterium strains capable of utilizing lactic acid from in vitro models. M.S. Thesis, Oklahoma State Univ., Stillwater.

Patton, R. S., C. E. Sorenson, and A. R. Hippen. 2004. Effects of dietary glucogenic precursors and fat on feed intake and carbohydrate status of transition dairy cows. J. Dairy Sci. 87:2122-2129.

Paynter, M. J. B., and S. R. Elsden. 1970. Mechanism of propionate formation by *Selenomonas ruminantium*, a rumen micro-organism. J. Gen. Microbiol. 61:1-7.

Perlman, D. 1978. Vitamins. P. 303-326. In. A. H. Rose (Ed.), Economic microbiology, Vol. 2. Primary products of metabolism. Academic press, Inc., London.

Phipps, R. H., J. I. D. Wilkinson, L. J. Jonker, M. Tarrant, A. K. Jones, and A. Hodge. 2000. Effect of monensin on milk production of Holstein-Friesian dairy cows. J. Dairy Sci. 83:2789-2794.

Piva, G., S. Belladonna, G. Fusion, and F. Scald. 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76:2717-2722.

Pickett, M. M., M. S. Piepenbrink, and T. R. Overton. 2003. Effects of propylene glycol or fat drench on plasma metabolites. Liver composition, and production of dairy cows during the periparturient period. J. Dairy Sci. 86:2113-2121

Plata, F. P. S. S. Gonzalez, G. Mendoza, and R. Barcena. 1993. Effect of a yeast culture (*Saccharomyces cerevisiae*) on nutritive value of oat straw based diets fed to Holstein steers. J. Anim. Sci. 71 (Suppl. 1); 288. (Abstr.).

Prenge, R. W., C. L. Davis, and J. H. Clarke. 1978. Propionate production in the rumen of Holstein steers fed either a control or monensin supplemented diet. J. Anim. Sci. 46:1120-1124.

Pusztai, A., G. Grant, T. P. King, and E. M. W. Clarke. 1990. Chemical Probiosis. In: Haresing, W. and D. J. A. Cole (Ed.). Recent Advances in Animal Nutrition. P 47. Butterwoth-Heinemann Ltd. Ltd. Linacre House, Jordan Hill, Oxford OX2 8DP, England.

Putman, D. E., C. G. Schwab, M. T. Socha, N. L. Whitehouse, N. A. Kierstead, and B.D. Garthwaite. 1997. Effect of yeast culture in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. J. Dairy Sci 80:374-384.

Quigley, J. D., and R. N. Heitmann. 1991. Effects of propionate infusion and dietary energy on dry matter intake in sheep. J. Anim. Sci. 69:1178-1187.

Rajala, P. J., and Y. T. Grohn. 1998. Effect of dystocia, retain placenta, and metritis on milk yield in dairy cows. J. Dairy Sci. 81:3172 -3181.

Reinbold, R. S., C. A. Ernstron, and C. L. Hansen. 1985. Temperature, pH, and moisture profiles during cooling of 29.0 kg stirred-curd cheddar cheese blocks. J. Dairy Sci. 75: 2071.

Reynolds, C. K., G. B. Huntington, H. F. Tyrell, and P. J. Reynolds. 1988. Net portal-drained visceral and hepatic metabolism of glucose, L-lactate, and nitrogenous compounds in lactating Holstein cos. J. Dairy Sci. 71:1803-1812.

Reynolds, C. K., P. C. Aikman, D. J. Humphries, and D. E. Beever. 2000. Splanchnic metabolism in transition dairy cows. J. Dairy Sci. 83(Suppl. 1):257. (Abstr.)

Richardson, L. F., A. P. Raun, E. L. Potter, C. O. Cooley, and R. P. Rathmacher. 1976. Effect of monensin on rumen fermentation in vitro and in vivo. J. Anim. Sci. 43:657-664.

Rigout, S., S. Lemosquet, J. E. van Eys, J. W. Blum and H. Rulquin. 2002. Duodenal glucose fluxes and lactose synthesis in grass silage-fed dairy cows. J. Dairy Sci. 85:595-606.

Rigout, S., C. Hurtaud, S. Lemosquet, A. Bach, and H. Rulquin. 2003. Lactational effect of propionic acid and duodenal glucose in cows. J. Dairy Sci. 86:243-253.

Rode, L. M., W. Z. Yang, and K. A. Beauchemin. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. 82:2121-2126.

Rook, J. A. F. 1979. The role of carbohydrates metabolism in the regulation of milk production. Proceed. of the Nutri. Soc. 38:309-314.

Russell, J. B. 1987. A proposed model of monensin action in inhibiting rumen bacterial growth; effects on ion flux and proton motive force. J. Anim. Sci. 64:1519-1525.

Russell, J. B., and R. J. Wallace. 1988. Energy Yielding and Consuming Reactions. In: P. N. Hobson (Ed.) The Rumen Microbial Ecosystem. Pp 185-215. Elsevier Applied Science, London and New York.

Russell, J. B., and S. A. Martin. 1984. Effect of various methane inhibitors on the fermentation of amino acids by mixed rumen microorganisms in vitro. J. Anim. Sci. 59:1329-1338.

Russell, J. B., and H. J. Strobel. 1989. Effect of ionophore on ruminal fermentation. Appl. Environ. Microbiol. 55:1-6

Ryan, J. P., B. F. Leek, and K. Jacques. 1993. The complementary effects of *Saccharomyces cerevisiae* yeast culture (Yea Sacc^{1026}) and Yucca schidigera extract (Solid De-Oderase) on ruminal metabolism in sheep. J. Anim. Sci. 71 (Suppl. 1); 287. (Abstr.).

Sano, H., Y. Terashima, and T. Seushu. 1989. Insulin secretory response to feeding in sheep fed a diet supplemented with calcium, potassium and sodium propionate. Jpn. J. Zootech. Sci. 60:70 (Abstr.).

Sano, H. S. Motsunobu, M. Nakagawa, and Y. Tereshina. 1990. Insulin responsiveness to glucose and tissue responsiveness to insulin over the feeding cycle in sheep. J. Anim, Sci. 68:3736-3741.

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

Sano, H., S. Hayakawa, A. Takahashi, and Y. Tereshima. 1995. Plasma insulin and glucagons responses to propionate infusion into femoral and mesenteric veins in sheep. J. Anim. Sci. 73:191-197.

Sartin, T. L., K. A. Cummins, R. J. Kemppainen, R. Cannes, D. G. McClary and J. C. Williams. 1985. Effect of propionate infusion on plasma glucagons, insulin and growth hormone concentrations in lactating dairy cows. J. Dairy Sci. 109:348-354.

Sauer, F. D., J. K. G. Kramer, and W. J. Cantwell. 1989. Antiketogenic effects of monensin in early lactation. J. Dairy Sci. 72:436-442.

Schelling, G. T. 1984. Monensin mode of action in the rumen. J. Anim. Sci. 58:1518-27.

Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. J. Anim. Sci. 70:1271-1282.

Schneider, P. L., J. Downer, and W. Chalupa. 1987. Early lactation response to ruminally inert dietary fat. J. Dairy Sci. 70 (Suppl. 1):220 (Abstr.).

Schultz, L. H. 1971. Management and nutritional aspects of ketosis. J. Dairy Sci. 54:962-973.

Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. J. Dairy Sci. 59:1254.

Schwab, C. G., M. T. Socha, and N. L. Whitehouse. 1993. Opportunities for rumen protected lysine and methionine in lactating dairy cow nutrition. Page 3 in Proc. Rhone-Poulenc Anim. Nutr. Symp. In conjunction with 29th Can. Feed Inc. Assoc./Univ. Guelph Nutr. Nutr. N.A., Mississauga, ON, Canada.

Seal, C. J., and C. K., Reynolds. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutr. Res. Rev. 6:185-208.

Seal, C. J. and D.S. Parker. 1994. Effect of intraruminal propionic acid and infusion on metabolism of mesenteric and portal drained viscera in growing steers fed a forage diet. I. Volatile fatty acids, glucose and lactate. J. Anim. Sci. 72:1325-1334.

Seal, C. J., and D. S. Parker. 1995. Effect of intraruminal propionic acid infusion on metabolism of mesenteric- and portal viscera in growing steers fed a forage diet. II. Ammonia, urea, amino acids and peptides. J. Anim. Sci. 74:245-256.

Sharma, H. R., J. R. Ingalls, and J. A. McKirdy. 1978. Replacing barley with protected tallow rations of lactating cows. J. Dairy Sci. 61:574-583.

Sheldrake, R. F., R. J. T. Hoare, and G. D. McFregor. 1983. Lactation stage, parity, and infection affecting somatic cell, electrical conductivity, and serum albumin in milk. J. Dairy Sci. 66:542-547.

Shepherd, A. C., and D. K. Combs. 1998. Long-term effects of acetate and propionate on voluntary intake by midlactation cows. J. Dairy Sci. 81:2240-2250.

Subiyatno, A., D.N. Mowat, and W.Z. Yang. 1996. Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. J. Dairy Sci. 79:1436-1445.

Slyter, L. L. 1976. Influence of Acidosis on Rumen Function. J. Anim. Sci. 43:910-929.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger, 1985. Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci. 68:1531-1553.

Spicer,, L. J., R. K. Vernom, W. B. Tucker, R. P. Wettemann, J. F. Hogue, and G. D. Adams. 1993. Effects of inert fat on energy balance, plasma concentration of hormones, and reproduction in dairy cows. J. Dairy Sci. 76:2664-2673
.

Spicer, L. J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-1 in dairy cows: relationships among energy balance, body condition, ovarian activity, and estrus cycle behavior. J. Dairy Sci. 73:929

Starnes, S. R. J. W. Spears, M. A. Froetschel, and W. J. Croom. 1984. Influence of monensin and lasalocid on mineral metabolism and ruminal urease activity in steers. J. Nutr. 114:518.

Stein, D. R., D. T. Allen, E. B. Perry, J. C. Bruner, K. W. Gates, T. G. Rehberger, K. J. Mertz, D. A. Jones, and L. J. Spicer. 2006. Effects of feeding Propionibacteria to dairy cows on milk yield, milk components and reproduction. J. Dairy Sci. 89:111-125.

Steinhour, W. D., and D. E. Bauman. 1988. Propionate metabolism. A new interpretation. Page 238 in Aspect of digestive physiology in ruminants A. Dobson and M.J. Dobson, eds. Cornell Univ. Press, Ithaca, NY

Stock, R. A., S. B. Laudert, W. W. Stroup, E. M. Larson, J. C. Parrott, and R. A. Britton. 1995. Effect of monensin and tylosin combination on feed intake variation of feedlot steers. J. Anim. Sci. 73:39-44.

Storm, E., and E. R. Orskov. 1983. The nutritive value of rumen microorganism in ruminant. 1. Large scale isolation and chemical composition of rumen microorganisms. Br. J. Nutr. 50:463-470.

Studer, V. A., R. R. Grummer, S. J. Bertics, and C. K. Reynolds. 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cow. J. Dairy Sci. 76:2931-2939.

Sutton, J. D., I. C. Hart, S. V. Morant, E. Schuller, and A. D. Simmonds. 1988. Feeding frequency for lactating cows: Diurnal patterns of hormones and metabolites in peripheral blood in relation to milk-fat concentration. Br. J. Nutr. 60:265-275.

Swartzlander, J. H. 1994. Selection, establishment and in vivo denitrification of Propionibacterium strain in beef. M.S. Thesis, Oklahoma State Univ., Stillwater.

Swatz, D. L., L. D. Muller, G. W. Rogers, and G. A. Varga. 1994. Effect of yeast culture on performance of lactating dairy cows: A field study. J. Dairy Sci. 77: 3073-3080.

Swinney-Floyd, D. L. 1997. The impact of inoculation with Propionibacterium on ruminal acidosis. Ph.D. Thesis, Oklahoma State Univ., Stillwater.

Symonds, M. E., Bird, J. A., Clarke, L., Gate, J., Lomax, M. A. 1995. Nutrition, temperature, and homeostasis during perinatal development. Exper. Physiol. 80: 907-940.

Thomas, P. C., and P. A. Martin. 1988. The influence of nutrient balance on milk yield and composition. Page 97-118 in Nutrition and lactation in the dairy cow. P. C. Garnsworthy, ed. Butterworths, London, UK.

Thatcher, W. W., de la Sota, E. J-P. Schmitt, T. C. Diaz, L. Badinga, F. A. Simmen, C. R. Staples, and M. Drost. 1996. Control and management of ovarian follicles in cattle to optimize fertility. Reprod. Fertil. Dev. 8:203-217

Trenkle, A. 1978. Relation of hormone variations to nutritional studies and metabolism of ruminant. J. Dairy Sci. 61:281-293.

Van Maanen, R.W., J. H. Herbein, A.D. McGilliard, and J. W. Young. 1978. Effects of monensin on in vivo rumen propionate production and blood glucose kinetics in cattle. J. Nutr. 108:1002-1007.

Van Soest, P. J. V. 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press, Ithaca, New York.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, L. T. Chapin, and R. L. Fogwell. 1988. Association between energy balance and luteal function in lactating cows. J. Dairy Sci. 71:1063-1072.

Wedegaertner, T. C., and D. E. Johnson. 1983. Monensin effects on digestibility, methanogenesis and heat increment of a cracked corn-silage diet fed to steers. J. Anim. Sci. 57:168-185.

Weekes, T. E. C. 1991. Hormonal control of glucose metabolism. In 'Physiological Aspects of Digestion and Metabolism in Ruminants'. Eds. T. Tsuda, Y. Sasaki and R. Kawashima) pp. 183-200. (Academic Press Inc.: San Diego, New York, Boston, London, Sydney, Tokyo and Toronto).

Weidmeier, R. D., M. J. Arambel, and J. L. Walters. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and digestibility. J. Dairy Sci. 70:2063-2068.

Weimer, P. J. 1998. Manipulating ruminal fermentation. A microbial ecological perspective. J. Anim. Sci. 76:3114-3122.

Weiss, W. P., and B. A. Amiet. 1990. Effects of lasalocid on performance of lactating dairy cows. J. Dairy Sci. 73:153-162.

Williams, P. E. C. A. G. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.

Williams, P. E., A. Walker, and J. C. MacRae 1990. Rumen probiosis: the effect of addition of yeast culture (viable yeast (*Saccharomyces cerevisiae*) plus growth medium) on duodenal protein flow in wether sheep. Proc. Nutr. Coc. 49:128 (Abstr.).

Wohlt, J. E. A. D. Finkelstein, and C.H. Chung. 1991. Yeast culture to improve intake, nutrient digestibility, and performance by dairy cattle during early lactation. J. Dairy Sci. 74:1395-1400.

Wolin M. J., and T. L. Miller. 1988. Microbe-Microbe Interactions. In: P. N. Hobson (Ed.). The Rumen Microbial Ecosystem. Pp 343-359. Elsevier Applied Science, London and New York.

Wood, H. G. 1981. Metabolic Cycles in the fermentation by propionic acid bacteria. In: Current topics in cellular regulation. 18:255-289.

Wood, H. G., and F. W. Leaver. 1953. Carbon dioxide turnover in the fermentation of 3, 4, and 6 carbon compounds by the propionic acid bacteria. Biophys. Acta. 12:207-222.

Woolford, M. K. 1975. The significance of Propionibacterium species and micrococcus lactilyticus to the ensiling process. J. Appl. Bacteriol. 39:301-306.

Yang, C. M. J., J. B. Russell. 1993. The effect of monensin supplementation on ruminal ammonia accumulation in vivo and the numbers of amino acids-fermenting bacteria. J. Anim. Sci. 71:3470-3476.

Yang, W. Z., K. A. Beauchemin, D. D. Vedres, G. R. Ghorbani, D. D. Colombatto, and D. P. Morgavi. 2004. Effects of direct-fed microbial supplementation on ruminal acidosis, digestibility, and bacterial protein synthesis in continuous culture. Anim Feed Sci. Technol. 114:179-193.

Zhou, B. 2002. Effects of diamond V- XP yeast culture on milk production and milk components in early lactation. DA016-s, Diamond V Biological and Fermentation Engineering Technologies Co. LTD, Xiangai, China.

CHAPTER III

EFFECT OF FEEDING YEAST AND PROPIONIBACTERIA ON MILK YIELD AND COMPONENTS AND REPRODUCTION IN HOLSTEIN COWS

Abstract

To determine the effect of supplemental feeding of Diamond V-XP Yeast Culture (XPY) alone or in combination with Propionibacteria strain P169 on milk production and milk components, body weight (BW), body condition score (BCS), days to first and second ovulations, milk glucose and plasma glucose and insulin, 31 primiparous (PP) and multiparous (MP) Holstein cows were fed one of three dietary treatments between 2 wk prepartum to 30 wk postpartum: 1) Control (n=10), fed a corn silage-based total mixed ration (TMR); 2) XPY (n=11), fed Control TMR plus XPY (at 56 g/head/d); and 3) P169+XPY (n=10), received Control TMR plus XPY plus P169 (at 6 x 10^{11} cfu/ head/d). After parturition, daily milk weights were recorded, and milk samples were collected twice weekly for milk component analyses. Daily uncorrected milk, solids-corrected milk (SCM) and 4% fat-corrected milk (4%FCM) production tended to be affected by dietary treatment such that milk production for cows (averaged across PP and MP cows) fed P169+XPY was 8-12% and 3-5% greater than Control and XPY cows, respectively. The percentage of milk fat was greater (P=0.01) in Control than XPY and P169+XPY groups. Milk lactose percentage was affected by diet x parity (P=0.0001) with P169+XPY fed MP cows having greater lactose levels than Control and XPY MP

cows; milk lactose in PP cows did not differ (P=0.20) among diet groups. Diet x parity tended (P=0.06) to affect milk protein percentage such that milk protein did not differ in MP cows but decreased in PP cows fed XPY compared to the other groups. Percentage of SNF tended to be greater in P169+XPY fed MP cows than Control and XPY MP cows (diet x parity; P=0.10). Body condition score did not differ among groups and averaged 2.61, 2.51 and 2.73±0.16 for Controls, XPY, and P169+XPY. Change in body weight postpartum was influenced by treatment x parity x week (P<0.0061) such that XPY exhibited a greater recovery of wk 1 body weight than Control and P169+XPY MP cows. In contrast to MP cows, Control PP cows experienced greater recovery of wk 1 BW than their counterparts. Interval to first and second postpartum ovulation did not differ among the groups. Milk samples during sequential p.m. and a.m. milkings were collected during a 2-wk period (wk 23 and 24 of lactation) from PP and MP cows, and blood samples were collected hourly during a 15-h post-feeding interval during wk 27 of lactation from only MP cows. Milk glucose was affected (P=0.01) by dietary treatment such that both PP and MP cows fed P169+XPY had 28% greater milk glucose levels (25±1 mg/dL) than Control cows and 32% greater milk glucose levels than XPY-fed cows. Diurnal plasma glucose concentration (59±1 mg/dL) was not affected by diet in MP cows. Plasma insulin levels were affected (P=0.01) by dietary treatment and time such that plasma insulin levels in MP cows fed P169+XPY (0.86±0.05 ng/mL) were 34% and 30% greater than in MP cows fed Control and XPY diets, respectively, at wk 27 of lactation. Milk glucose and plasma insulin responses to P169+XPY feeding suggest that P169+XPY supplementation might have enhanced gluconeogenesis and increased glucose uptake by

the mammary gland in Holstein cows, and thus may hold potential as a natural direct-fed microbial to enhance lactational performance.

Introduction

The transition from late gestation to early lactation (also called the periparturient period) is regarded as one of the most challenging periods of a cow's production cycle, and imposes profound physiologic and metabolic changes in preparation for parturition and lactation. These changes often disrupt the homeostatic mechanisms of the cow because they occur in a matter of days, and sometimes hours, as the liver adapts from a minimal systemic glucose demand to an overwhelming demand for glucose (DeFrain et al., 2005). During early lactation, many high producing dairy cows exhibit a state of negative energy balance (Spicer et al., 1990), because of decreased dry matter intake (Bertics et al., 1992; Grummer, 1993) and high glucose requirements of the mammary gland (Bell, 1995). When the cow is in negative energy balance, energy output in the form of milk exceeds energy input in the form of feed (DeFrain et al., 2004). The energy requirements for these biological events are eventually met through an increase in combination of feed intake and mobilization of body energy reserves (i.e., lipolysis) to offset energy requirements by various physiological activities (Vallimont et. al., 2001; Banos, et al., 2005). An animal in prolonged negative energy balance exhibits cummulative body energy loss and becomes prone to health and reproductive problems, and considerable financial loss (De Vries and Veerkamp, 2000; Veerkamp et al., 2000). Increasing the proportion of concentrates above 55–60% of total ration dry matter (DM) to provide higher energy density may result in problems such as rumen acidosis, milk fat depression and possibly higher incidences of displaced abomasum, milk fever, ketosis,

and retained placenta (Clark and Davis, 1980). Any factor that would improve prepartum DMI or increase the rate of increase of DMI postpartum, should be beneficial for increased milk production and/or reduced metabolic problems. Increases in glucose supply to transition cows can reduce blood non-esterified fatty acids (NEFA) and β-hydroxy butyric acid (BHBA) concentrations, and prevent associated metabolic disorders like ketosis and fatty liver (Burhans and Bell, 1998).

The availability of glucose to the mammary gland has an important impact on milk yield because lactose is the major osmoregulator in mammary gland uptake of water (Lemosquet et al., 2004). The mammary gland utilizes 60 to 85% of the total glucose available in lactating ruminants, and lactose synthesis accounts for 50 to 85% of mammary glucose utilization (Knowlton et al., 1998). In ruminants, often less than 10% of the glucose utilized is absorbed from the lower gastrointestinal tract. Therefore, gluconeogenesis must supply more than 90% of glucose (Amaral et al., 1990). Based on the growing concern regarding the use of antibiotics in animal production, there is much interest in exploring alternatives to antimicrobial feed additives (Martin et al., 1999). One recent example of this is feeding supplemental P169 to lactating dairy cows which caused an increase in ruminal propionate, milk lactose and milk production (Stein et al., 2006). Ruminal propionate produced by Propionibacteria is the single most important substrate for gluconeogenesis (Drackley et al., 2001). Propionate also spares glucogenic amino acids in gluconeogenesis and consequently decreases overall animal energy supply (Van Soest, 1994). Propionate inhibits hepatic lipid oxidation and it is antiketogenic (Drackley, 1999). Unlike other glucogenic precursors, propionate is not down-regulated by insulin and liver uptake of propionate is preferential and highly efficient (Bergman, 1990). In

addition, propionate has a positive effect on ruminal mucosal development, leading to enhancement of glucose precursor flow into the circulation (Arieli et al., 2001).

Estimates by Seal and Reynolds (1993) indicate that propionate supplies 32% to 73% of glucose demands. The efficiency of utilization for maintenance of propionic acid is 0.86 vs. 0.59 for acetate and 0.76 for butyrate (McDonald et al., 2002). The theoretical efficiency of propionate as a source of energy for ATP/100 g nutrient is 108% compared to glucose (McDonald et al., 2002). Both drenching (Grummer et al., 1994) and feeding propylene glycol (Christensen et al., 1997) have decreased NEFA and increased plasma insulin and increased ruminal propionate concentrations, all of which are beneficial to combating the extent and duration of negative energy balance, fatty liver and ketosis. Effects of propylene glycol are partially mediated through increases in ruminal propionate (Grummer et al., 1994; Christensen et al., 1997), providing reasons to feed propionate during the transition period. Alternatively, feeding Propionibacteria strain 169 (P169) to Holstein dairy cows to increase rumen propionate (Stein et al., 2006) also increases plasma leptin (Francisco et al., 2002), plasma glucose and insulin (Aleman et al., 2007), milk lactose and milk production (Stein et al., 2006).

Yeast cultures have been fed to dairy cattle for more than 60 yr with varied responses (Schingoethe et al., 2004). In some studies, yeast cultures improve DMI (Williams et al., 1991; Wohlt et al., 1991; Dann et al., 2000) and milk production (Wang et al., 2001), whereas other studies (Arambel and Kent, 1990; Soder and Holden, 1999) found no response to yeast cultures. In vitro experiments have reported that in some cases, *S. cerevisiae* culture favorably alters the mixed ruminal microorganism fermentation and stimulated lactate uptake and cellulose digestion (Nisbet and Martin,

1991; Callaway and Martin, 1997). Miller-Webster et al. (2002) found that yeast culture increased molar percentage of propionic acid, reduced acetic acid and lowered acetate/propionate ratio. Even though the effects of *S. cerevisiae* are not always consistent (Martin and Nisbet, 1992), several modes have been proposed regarding the stimulatory effects of yeast culture on ruminal fermentation (Lyons et al., 1993). The effect of yeast culture and propionibacteria might differ, and/or the combination of these two feed additives might have an advantage over the use of either feed additive alone. However, no studies have evaluated the glucogenic precursor biogenerator, P169, in conjunction with yeast culture. Therefore, the present study was conducted to test the hypothesis that manipulation of microflora by supplemental feeding a yeast culture alone or in combination with Propionibacteria would have metabolic benefits for transition cows and improve milk yield and components, as well as body weight and body condition scores via alterations in plasma metabolites and hormones of dairy cows.

Materials and Methods

Experimental Design and Sample Collection

Approximately two weeks before expected parturition to 30 wk postpartum, 31 multi- and primiparous Holstein cows were randomly assigned to one of three dietary groups and stratified based on estimated calving date and previous year's lactation averages of the multiparous cows and current predicted transmitting ability (PTA's) of the primiparous cows. One group received the TMR ration (control diet) throughout the course of the study (Control, n=10); a second group received the control diet plus 56 g/d of Diamond V-XP yeast (XPY, n=11; Diamond V Mills Inc. Cedar Rapids, Iowa); and a third group received the control diet with XPY plus $6x10^{11}$ cfu/head/d dose of

Propionibacteria strain 169 (P169+XPY, n=10). P169 was isolated from rumen fluid collected from ruminally cannulated Holstein dairy cows at the OSU Dairy Cattle Center (Davidson, 1998), and was prepared by Agtech Products Inc. (Waukesha, WI) as a viable freeze dried product using maltodextran as a carrier. The previous 305-d mature equivalent (ME) milk yield for the Control (n=6), XPY (n=5), and P169+XPY (n=5) groups of multiparous cows averaged 11063±753, 12028±753, and 11593±753 kg, respectively and did not differ (P=0.68) among treatment groups. The genetic merit for the primiparous cows of the Control (n=4), XPY (n=6), P169+XPY (n=5) groups averaged 402±92, 359±84 and 348±92 kg PTA, respectively and did not differ (P=0.91) among treatment groups. 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 among the treatment groups. Cows had free access to water and were provided with ad-libitum feed. Cows were fed the XPY and P169+XPY via mixing the XPY and/or P169 with a small amount of TMR (4.5 kg/hd) once per day (a.m.). To assure a rapid and complete consumption of the "treatment" TMR, all cows in each group were brought to the feed alley at the same time and allowed to consume the small amount of "treatment" TMR prior to the main a.m. TMR feeding. The TMR was composed of corn silage, alfalfa hay, whole cottonseed, corn gluten feed, Megalac-R and concentrate (Table 1). Energy concentration of the diet was formulated to support daily milk production of 50 kg (NRC, 2001). Cows were fed twice daily at 0900 and 1600. On average, cows in the Control, XPY, and P169+XPY groups had treatment initiated 13±3, 15±3, and 18±3 d prior to calving, respectively. One Control and one XPY multiparous cows were taken out of the study because of health reasons. The calving window extended over a 5-month

period (November 26, 2003 to April 18, 2004). The Julian calendar day that cows in each group calved averaged 40±15, 43±15, and 34±15 for the Control, XPY, and P169+XPY groups, respectively, and did not differ (P=0.99) among treatment groups.

Cows were milked twice daily at 0430 and 1630 h and a.m. and p.m. milk yields were recorded. Daily (sum of a.m. and p.m.) milk production was used to calculate average weekly milk yield per cow. Milk production was expressed three ways: 1) uncorrected milk 2) fat-corrected milk fat (4%FCM), and 3) solids-corrected milk (SCM). Fat corrected milk was derived using the equation 0.4 x milk, kg/d + 15 x fat, kg/d (NRC, 2001). Solids corrected milk was calculated using formula (Tyrell and Reid, 1965), SCM = 12.3 (total fat, kg) + 6.56 (kg, SNF) - 0.0752 (total kg, uncorrected milk). Milk samples were collected twice weekly (sequential a.m. and p.m. milkings) for analyses and quantification of protein, fat, lactose, somatic cell count (SCC), milk urea nitrogen (MUN) and solids-not-fat (SNF) levels (Heart of America DHIA, Manhattan, KS). Using the following equation, urinary excretion (g/d) = 0.0259 (BW)(MUN) was calculated as previously described (Davidson et al., 2003) to assess the impact of dietary treatments on nitrogen excretion.

Blood was collected once weekly (between 0630 and 0830 h) via coccygeal venipuncture from parturition to 25 wk postpartum. After blood collection in vacutainer tubes containing EDTA, blood was transported on ice to the lab and centrifuged at 1200 x g for 15 min at 4 °C. Plasma was harvested and stored at -20°C until subsequent analysis of concentrations of plasma progesterone (P_4). Weekly body weights were recorded from wk 1 to 25 at the time of blood collection. Body condition scores (BCS; 1=emaciated, 5=obese; Sniffen and Ferguson, 1995) were measured at four stages postpartum: 0–30 d

(Stage 1), 30–90 d (Stage 2), 90–150 d (Stage 3), and greater than 150 d postpartum (Stage 4).

Additional Sample Collection

Milk samples during sequential p.m. and a.m. milking were collected during a two week period (wk 23 and 24 of lactation) for evaluation of treatment effects on milk glucose concentrations. In addition, hourly blood samples were collected during a 15-h post-feeding interval during wk 27 of lactation to evaluate the effect of treatment on diurnal plasma glucose and insulin concentration in multiparous cows only. Multiparous cows were cannulated via the jugular vein one day prior to blood sample collection. Cows were sampled starting at 1 h prior to the a.m. feeding and continued through 15 h post-feeding. Plasma was harvested for determination of glucose and insulin concentrations.

Laboratory Analyses

Feed samples were collected weekly for monthly composite analyses (Forage One, DHI Forage Testing Laboratory, Ithaca, NY). Plasma P_4 concentrations were determined using a solid-phase ^{125}I radioimmunoassay (RIA) Kit (Coat-A-Count, Diagnostic Products Corp., Los Angels, CA) as previously described (Francisco et al., 2002). Intraassay and interassay coefficients of variation averaged 6.4% and 12.4%, respectively.

Plasma and milk concentrations of glucose were determined as previously described (Aleman et al., 2007) using colorimetric glucose kits based on the hexokinase coupled with glucose-6-phosphate dehydrogenase enzymatic reaction (Thermo Electron Corporation, Louisville, CO). The intraassay and interassay coefficients of variation for

plasma glucose were 2.9 and 5.8%, and were 5.3 and 10.2% for milk glucose, respectively.

Plasma concentrations of insulin were determined by using solid-phase insulin RIA Kit (Micromedia Insulin Kit, ICN Biomedicals Inc., Costa Mesa, CA) as previously described (Francisco et al., 2002). The intraassay and interassay coefficients of variation were 9.5 and 14.1%, respectively.

Statistical Analyses

Milk production and components were analyzed as a completely randomized block design for repeated measures, utilizing the MIXED model of SAS with the main effect of dietary treatment (Control, XPY and P169+XPY), week postpartum, parity and their interactions. The model of the covariate structure for repeated measurements was an autoregressive with lag equal to one. If any interaction was significant, simple effects were analyzed using slice option for the LSMEANS statement. Conversely, main effects were analyzed using LSMEANS with the DIFF option if the interaction was not significant.

Plasma glucose and plasma insulin were analyzed as a completely randomized design for repeated measures, utilizing the MIXED model of SAS with main effect of dietary treatment (Control, XPY and P169+XPY), time (hour) of collection and their interaction. Milk glucose concentrations were analyzed as a completely randomized design for repeated measures, utilizing the MIXED model of SAS with main effect of dietary treatment (Control, XPY and P169+XPY), parity, time (day and week) of collection and their interaction.

Results

Milk Production

Uncorrected Milk Production

Daily milk production was influenced by week (P=0.0001) and parity (P=0.0001), and tended (P=0.08) to be affected by dietary treatment. No interactions were significant (P=0.33). Daily milk production averaged across primiparous and multiparous cows, was 33.9±2.5, 36.6±2.0, and 38.0±2.1 kg/d for Control, XPY, and P169+XPY groups, respectively, over the 30 wk of lactation. Cows fed P169+XPY exhibited an 11.9% (P=0.03) and 3.7% (P=0.43) increase in milk production above Control and XPY groups, respectively, over the 30 wk of lactation. Milk production in XPY cows was 7.9% (P=0.13) above control cows during the same interval. Milk production by multiparous and primiparous cows fed P169+XPY was consistently greater than control groups from wk 3 throughout 30 wk of lactation (Figure 1). As expected, daily milk production was 33.6% greater (P=0.0001) in multiparous than in primiparous cows and averaged 41.1 and 31±2.0 kg/d, respectively, over the 30 wk of lactation.

Solids-Corrected Milk (SCM)

Solids-corrected milk production was influenced by week (P=0.01) and parity (P=0.001), and tended (P=0.09) to be affected by dietary treatment and parity x week (P=0.09) with no other main effect or interaction being significant (P=0.15). Solids-corrected milk production, averaged across primiparous and multiparous cows, was greater in the P169+XPY (38.0±2 kg/d) treatment cows than XPY (36.2±2 kg/d) and Control cows (35.3±2 kg/d) during the 30-wk study. P169+XPY cows exhibited 8%

(P=0.05) and 5% (P=0.15) greater SCM than Control and XPY cows, respectively. There was no significant difference (P=0.50) in SCM between XPY and Control cows (Figure 2). Daily SCM production in primiparous cows significantly increased from wk 1 through wk 5 and then constant throughout the remainder of the 30 wk lactation period (Figure 3). Daily SCM production in multiparous cows significantly increased from wk 1 through wk 6, after which a significant decrease in milk production was seen during wk 7 and wk 18 of lactation (Figure 3)

4% Fat Corrected Milk (%4 FCM)

Daily 4% fat-corrected milk (FCM) was altered by parity (P=0.0001), week (P=0.03) and tended (P=0.06) to be affected by dietary treatment. No interactions were significant (P≥0.26). Daily 4%FCM production averaged across primiparous and multiparous cows, was greater (P=0.02) in P169+XPY (36.2±2.05 kg/d) vs. Control (33.5± 2.0 kg/d) cows during the 30 wk study. There was no significant difference in 4%FCM between the P169+XPY and XPY (35.1±2.02 kg/d) cows or between XPY and Control cows (Figure 4). During the 30 wk study, 4%FCM in P169+XPY cows was 8.1% greater than in Control cows and the XPY treatment cows had 4.8% greater 4%FCM than Control cows. Daily 4%FCM production averaged across parity significantly increased from wk 1 to wk 5 of lactation after which there was no significant decrease or increase throughout the remainder of the 30 wk of lactation (Figure 5).

Milk Components

Milk Fat Percent

The percentage milk fat was altered by treatment (P=0.01), week (P=0.02), and parity (P=0.0001) but not their interactions. Averaged over 30 wk of lactation, percentage

milk fat averaged 4.55, 4.14 and 4.21±0.10 % for Control, XPY and P169+XPY groups, respectively. Cows fed Control diet exhibited a 9% (P=0.005) and 7.5% (P=0.02) increase in milk fat % above XPY and P169+XPY treatment cows, respectively, over the 30 wk of lactation. There was no significant difference (P=0.62) between XPY and P169+XPY treatment cows (Figure 6). The percent milk fat averaged 4.52 and 4.07±0.08 % for primiparous and multiparous cows, respectively. Milk fat percentage decreased significantly from wk 1 to 4 with no further significant change during the subsequent weeks in all groups of cows (Figure 7).

Milk Lactose Percent

The percentage of lactose in milk was influenced by treatment (P=0.03), week (P=0.0001), parity (P=0.0001), and parity x treatment interaction (P=0.0001) such that milk lactose levels in P169+XPY multiparous cows (4.79±.03%) were increased (P=0.0001) above their respective Control (4.63±0.03%) and XPY (4.57±0.03%) groups (Figure 8). Percentage milk lactose averaged 4.92, 4.99, and 4.89 ± 0.03% for Control, XPY and P169+XPY primiparous, respectively, and did not differ among groups. Weekly milk lactose percentage increased from wk 1 to 6 and remained unchanged in the subsequent weeks.

Milk Protein Percent

Percentage milk protein was influenced by week (P=0.0001) and parity (P=0.0001) with a tendency to be influenced by treatment x parity (P=0.06). Averaged across all treatment groups, weekly milk protein percentage significantly decreased from wk 1 to 4, remained constant and then increased gradually from wk 18 through wk 30 (Figure 9). Milk protein percentage was 3.3% greater (P=0.04) in both Control

(3.1±0.03%) and P169+XPY (3.1±0.03%) primiparous cows vs. XPY (3.0±0.03%) primiparous cows, but these differences were not evident among Control (2.9± 0.03%), P169+XPY (2.9±0.03%) and XPY (2.9±0.03%) multiparous cows (Figure 10).

Milk Solids-Not-Fat Percent

The percentage SNF in milk was influenced (P=0.001) by both week of lactation and parity with a tendency to be influenced by treatment (P=0.10) but not other main effects (P=0.50) or interactions (P=0.20). Percentage of SNF tended (P=0.10) to be greater in P169+XPY (8.69 ± 0.03%) cows (averaged across PP and MP cows) than XPY (8.57±0.03%) cows but did not differ from control cows (8.58 ± 0.03%) (Figure 11). Percentage SNF averaged 8.86 and 8.37±0.03% for primiparous and multiparous cows, respectively.

Milk Urea Nitrogen (MUN)

Levels of MUN were affected by week (P=0.0001) and parity (P=0.0001), but not treatment (P=0.93), treatment x week (P=0.76) or parity x week (P=0.83). However, there was a trend (P=0.09) for treatment x parity and treatment x parity x week interaction. Averaged over 30 wk of lactation, MUN levels were 13.7, 13.8 and 13.6±0.3 mg/dL for Controls, XPY and P169+XPY groups, respectively. Multiparous cows had greater (P=0.0001) MUN levels (14.7±0.2 mg/dL) than primiparous cows (12.7±0.2 mg/dL). Levels of MUN in primiparous cows significantly increased from wk 1 through wk 8 and remained constant throughout the remainder of the 30 wk lactation period (Figure 12). Levels of MUN in multiparous cows significantly increase from wk 1 through wk 12 and significant decreased between wk 21 and 27 of lactation (Figure 12). The MUN was lower (P=0.07) in Control (12.2±0.4 mg/dL) primiparous cows vs. XPY (13.2±0.4

mg/dL) but not (P=0.30) P169+XPY (12.6±0.4 mg/dL) primiparous cows. There was no difference in MUN levels among treatment groups in multiparous cows (Figure 13).

Urinary Nitrogen Excretion

Urinary nitrogen excretion was affected by parity (P=0.0001) and week (P=0.0001) but not by treatment (P=0.59) or their interactions (P=0.38). Averaged over 30 wk of lactation, urinary nitrogen excretion averaged 220.6, 230.1 and 227.2±6.7 g/d for Control, XPY and P169+XPY groups, respectively, and did not differ (P=0.59). Multiparous cows had greater (P=0.0001) urinary nitrogen excretion (264.9±5.6 g/d) than primiparous cows (186±5.1 g/d). The general trend was for a significant increase in urinary nitrogen excretion levels between wk 1 to wk 8 of lactation in both primiparous and multiparous cows (Figure 14). However, after wk 8, there was no further increase in urinary nitrogen excretion in primiparous cows, but in multiparous cows specific effects (i.e., increases and decreases) varied depending on the week of lactation (Figure 14).

Somatic Cell Counts

Somatic cell counts (SCC) were altered by treatment (P=0.01), week (P=0.009) and parity (P=0.02) but not (P=0.42) their interactions. Specifically, SCC in P169+XPY group was increased above that of Control and XPY groups between wk 4 and 8 and wk 14 and 30 (Figure 15). Averaged over 30 wk of lactation, SCC averaged 134±27.5 x 10^3, 190±25.6 x 10^3, and 254±27.2 x 10^3 per mL for Control, XPY and P169+XPY groups, respectively. Multiparous cows (229.71±22.7 x 10^3) exhibited a greater SCC than primiparous cows (156±21.2 x 10^3/mL).

Body weight changes (%)

Analysis of weekly BW changes expressed as % of wk 1 revealed that BW changes were influenced by week (P=0.0001), treatment x parity (P=0.0008), treatment x week (P=0.01), and treatment x parity x week interaction (P=0.006). There was also a trend for BW change to be influenced by treatment (P=0.08). The XPY multiparous cows exhibited a greater (P=0.05) recovery of wk 1 BW vs. Control and P169+XPY multiparous cows during the 25 wk of lactation, recovering, 105, 98, and 102±1.2 %, respectively. Nadir BW occurred between wk 2 and 4 for the multiparous cows; percentage BW loss from wk 1 averaged 0.8, 4.8 and 7.1±1.2% for XPY, Control and P169+XPY multiparous cows, respectively. Control primiparous cows exhibited a greater (P=0.007) recovery of wk 1 BW than P169+XPY and XPY primiparous recovering 105, 99 and 102±1.2%, respectively. Nadir BW occurred at wk 3 for the primiparous cows; percentage BW loss from wk 1 averaged 3.7, 5.5 and 5.6±1.2%, for the Control, P169+XPY and XPY primiparous cows, respectively (Figure 16).

Body Condition Score (BCS)

BCS was altered by parity (P=0.0001), not by treatment (P=0.63), stage (P=013), or treatment x stage of lactation (P=0.49) or treatment by parity (P=0.67). BCS was measured at four stages postpartum: 0–30 d, 30–90 d, 90– 50 d, and greater than 150 d postpartum. BCS ranged from 1.5 to 4 and averaged 2.61, 2.51 and 2.73±0.16 for Controls, XPY, and P169+XPY groups, respectively. BCS averaged 2.79, 2.50, 2.50 and 4.69±0.13 in stage 1 through 4, respectively. BCS was 29% greater (P=0.0001) in primiparous (3.1±0.13) than multiparous (2.2±0.13) cows.

Postpartum Interval to Ovulation

Average days to first and second ovulations (i.e., first and second rise in plasma $P_4 \geq 0.5$ ng/mL) were not significantly influenced by treatment, parity, or treatment x parity (P=0.19). Averaged across parity groups, days to first and second ovulations were 35 and 69; 45 and 64; and 34 and 65±6 in Control, XPY and P169+XPY.

Milk Glucose

Milk glucose concentration was affected (P=0.01) by dietary treatment but not parity (P=0.80), wk, or their interactions (P=0.70). Both primiparous and multiparous cows receiving P169+XPY had 28% greater (P=0.05) milk glucose levels than the control cows and 32% greater (P=0.05) milk glucose levels than the XPY-fed cows (Figure 17). Milk glucose concentrations did not differ between wk 23 (20.9 ± 0.8 mg/dL) wk 24 (21.0±0.8 mg/dL) or between multiparous (20.5±0.9 mg/dL) and primiparous (21.4±0.8 mg/dL) cows.

Plasma Glucose

Plasma glucose concentration at wk 27 was not affected (P=0.15) by dietary treatment, time or treatment x time interaction (Figure 18). Plasma glucose concentrations in multiparous cows fed Control, XPY and P169+XPY averaged 59, 58 and 61±1 mg/dL, respectively.

Plasma Insulin

Plasma insulin concentration was affected (P=0.01) by dietary treatment and time, but not their interaction (P=0.75). Plasma insulin concentration in cows fed P169+XPY had 34% and 30% greater (P=0.01) plasma insulin concentrations than cows fed Control and XPY TMR, respectively, and averaged 0.64, 0.66 and 0.86±0.05 ng/mL for Control,

XPY and P169+XPY cows, respectively. Plasma insulin concentration increased with time after feeding more rapidly in cows fed P169+XPY. The first significant increase in plasma insulin occurred at 6 h post-feeding vs. 12 and 10 h in Control and XPY- fed cows, respectively (Figure 19).

Discussion

The present experiment was designed to determine if feeding of Diamond V-XP Yeast alone or in combination with Propionibacteria strain P169 to lactating cows could alter milk production, milk components, BW, BCS and(or) metabolic and reproductive functions. Feeding P169+XPY in this study increased actual milk, SCM and 4%FCM production by 8-12% above Control cows, but only 3-5% above XPY-fed cows. This response was consistent with that demonstrated in a previous study (Nocek and Kautz, 2006) that involved direct-fed microbial supplementation of yeast combined with two strains of *Enterococcus faecium* bacteria. Stein et al. (2006) reported that feeding P169 along with XPY to MP and PP cows fed a sorghum-silage based TMR (vs. a corn silage TMR as in the present study) increased 4%FCM production by 8.5% compared to XPY-fed Controls. Francisco et al. (2002) also feeding MP cows a sorghum-silage TMR but without supplemental yeast reported that 4%FCM production (a 1.9% numeric increase) was not significantly affected by P169 supplementation during the first 12 wk of lactation. Therefore, feeding Propionibacteria in conjunction with yeast culture may enhance the ability of P169 to increase 4%FCM production. Stimulatory factors for rumen bacteria, such as B vitamins or branched-chain VFA (Higginbotham et al., 1994) and growth factors, such as malate (Nisbet and Martin, 1991) are present in yeast culture, and their absence may have contributed to the lack of milk production response in

Francisco et al. (2002). Whether the difference in silage type (i.e., corn vs. sorghum) used in the different studies affect the lactational response to XPY and (or) P169 is unclear. Feeding XPY alone in the present study did not significantly alter 4%FCM production (a 4.8% numeric increase). However, the effect of *S. cerevisiae* on milk production is not always consistent (Martin and Nisbet, 1992) increasing milk production in some studies (William et al., 1990; Wohlt et al., 1991; Piva et al., 1993; Putman et al., 1997; Kujawa, 2003), but not in other studies (Erdman and Sharma, 1989; Arambel and Kent, 1990; Higginbotham et al., 1994; Swartz et al., 1994; Kung et al., 1997; Dann et al., 2000). Numbers of animals per group was small (i.e., 4-6) and thus additional studies with a greater number of animals will be needed to confirm results of the present study. Regardless, the numeric increase in 4%FCM production induced by XPY in the present study (i.e. 4.8%) was within the response range of previous reports (Schingoethe et al., 2004). Beneficial effects of yeast culture vary with factors such as age of cow, differences in experimental conditions including stage of lactation, type of diet, amount and duration of yeast fed, environmental conditions (i.e., heat stress), and animal factors, and thus, further studies will be required to define the exact conditions that provide the optimal response to yeast culture and (or) P169 supplementation.

Propionibacteria likely increases milk production because of an increased supply of glucogenic precursors caused by changes in rumen fermentation (Kim et al., 2001; Stein et al., 2006). Previously, Aleman et al., (2007) reported that glucose concentrations in plasma samples collected weekly for 25 wk was 9% greater in PP cows fed low-dose (6 x 10^{10} cfu) P169 versus Control PP cows, but glucose concentrations in MP cows did not differ among treatments. Plasma glucose measured during 15-h post-feeding at 27 wk of

lactation did not significantly differ among Control, XPY and P169+XPY MP cows in the present study. Similarly, Francisco et al. (2002) found no effect of feeding P169 (low dose) on plasma glucose concentrations measured weekly during wk 1 to 12 of lactation in MP dairy cows. The lack of a detectable change in plasma glucose observed in the present study may be due in part to the fact that plasma insulin concentrations increased faster in response to feeding in P169+XPY-fed than Control and XPY-fed MP cows. Alternatively, it is possible that the metabolic status especially for the high producing P169+XPY MP cows impacted whether glucose concentrations are altered by increased ruminal propionate. Infact, Stephenson et al. (1997) reported that monensin treated cows had greater glucose concentrations before but not after calving. Therefore, changes in glucose might have gone undetected because of the greater demand for glucose by high producing P169+XPY fed MP cows. Previously, insulin concentrations were greater in PP cows fed high-dose P169 vs. Controls and in MP cows fed high-dose P169 vs. low-dose P169 during wk 1 to 25 (Aleman et al., 2007), but insulin did not differ between P169 (low dose) fed and Control MP cows during early lactation (Francisco et al., 2002). Feeding or infusion of propionate transiently increases both glucose and insulin concentrations in cattle (Subiyatno et al., 1996; Oba and Allen, 2003) and sheep (Sano et al., 1995), and these increases are short-lived (i.e. < 120 min). As observed in the present study, Sutton et al. (1988) and Borrebaek et al. (1990) observed diurnal variation in concentrations of glucose in peripheral blood of cows that were fed twice daily. Nielson et al. (2003) on the other hand did not observe variation in plasma glucose concentration within 24 h, but in this particular study, cows were fed TMR four times daily. Therefore, these studies indicate that frequent blood sample collections are necessary to detect

transient increases in plasma glucose concentrations in response to propionate infusions or feeding. However, the response may also depend on the basal diet fed, dose of P169 given, and physiological condition of animals.

Cows fed P169+XPY had significantly greater milk glucose concentrations than Control and XPY-fed cows of the present study. Previously, glucose and propionate treatments increased glucose-6-P levels in milk (Rigout et al., 2003), and glucose levels in milk tended to increase during C3 treatment (Lemosquet et al., 2004). Rigout et al. (2002) reported that increased glucose appearance rates favor increased glucose uptake by the mammary gland and, therefore, increased lactose production. Consistent with this notion, the P169+XPY fed MP cows had significantly greater milk lactose % than Control and XPY-fed MP cows. Stein et al. (2006) observed that milk lactose percentage was significantly greater in P169-fed MP cows than Control cows during a 25 wk study. Higher milk lactose content of cows supplemented with P169+XPY than Control or XPY-fed cows in the present study were likely due to increased delivery of glucose to the mammary gland.

Effects of yeast culture on milk components have been inconsistent, and few attempts have been made to determine or review the mechanisms responsible for the beneficial effects (Krehbiel et al., 2003). Various researchers reported no effects of yeast culture (Arambel and Kent, 1990; Wohlt et al., 1991) or P169 (Francisco et al., 2002) on milk fat percentage. Similarly, Dann et al. (2000) reported concentrations of fat, protein, lactose, total solids, and MUN, as well as SCC, were not significantly affected by yeast culture. In a study conducted by Harris and Webb (1990), milk fat and milk protein percentages were increased in cows supplemented with yeast culture. In the present study, milk fat

percentage was lower in both P169+XPY and XPY vs. Control cows. These data parallel a previous study by Stein et al. (2006), where high dose P169-fed cows had lower milk fat percentage than Control MP cows. Other studies that involved supplementation of cows with a combination of DFM demonstrated a lower milk fat percentage or milk fat depression (McGilliard and Stallings, 1998; Nocek and Kautz, 2006), while other others (Higginbothan et al., 1994; Wohlt et al., 1998; Francisco et al., 2002) showed no response. Increased plasma insulin in the P169+XPY vs. XPY and Control groups of the present study indicates a possibility that the milk fat depression observed in P169+XPY group could be from insulin-induced shortages of lipid precursors due to increased adipose tissue lipogenesis. It is also possible that the milk fat depression observed in both P169+XPY and XPY groups are due to dilution caused by increased milk yields vs. Control cows. It is important to note that a decrease in milk fat concentration without a decline in milk yield is not necessarily a negative consequence of using a feed additive, because there is an increasing interest in producing milk with lower fat content (Rode et al., 1999). However, further study will be required to understand causes of the variable response of milk components to P169 and yeast.

In the present study, P169+XPY treatment did not alter milk protein percentage during the first 30 wk of lactation, whereas XPY alone decreased (by 3.2%) milk protein percentage in PP not MP cows. Interestingly, XPY alone increased MUN in PP but not MP cows. Previously, both High-dose and Low-dose P169-fed MP cows tended to increase milk protein percentage above Control MP cows during a 25 wk study (Stein et al., 2006). Francisco et al. (2002) reported an increase in percentage of milk protein but only during wk 1 of lactation after P169 supplementation vs. Control. Ruminal

propionate infusion led to an increase milk protein percentage or protein yield (Huhtanen et al., 1998; Rigout et al., 2003). Propionate spares glucogenic amino acids in gluconeogenesis (van Soest, 1994), however, urinary nitrogen excretion was not altered by feeding P169+XPY in the present study. Whether the addition of P169 present in P169+XPY treatment resulted in improved flow of microbial protein to the lower intestinal tract to prevent decreased milk protein levels seen in XPY-fed cows or improved glucose concentration or both will require further elucidation. The effect of feeding yeast culture on milk protein has varied, with most studies reporting no changes in milk protein levels (Arambel and Kent, 1990; Dann et al., 2000) whereas, others report increases (Putman et al., 1997) in milk protein levels. Different environmental conditions among studies may impact microbial protein flow (Bach et al., 2005).

In the present study, a tendency for increased percentage of SNF in the P169+XPY vs. XPY and Control cows was observed. Stein et al. (2006) reported that the percentage of SNF was greater in cows fed both High-dose and Low-dose P169 vs. Control TMR. Similarly, Francisco et al. (2002) reported increased percentage of SNF in cows fed P169 vs. Control during the first week of lactation. Increases in milk SNF in these studies is likely due to increases in milk components such as milk lactose (this study; Stein et al., 2006) or protein (Francisco et al., 2002; Stein et al., 2006) percentage. Higginbotham et al. (1994) reported lower percentages of lactose and SNF for cows fed yeast culture plus *A. oryza*e vs. Control cows. Therefore, differences in feed components, dairy management, or other environmental factors may contribute to differences in which milk components change in response to a given dietary supplement.

MUN is thought to reflect the concentration of BUN (Broderick and Clayton, 1997), and therefore may serve as an index of inefficient N utilization in lactating dairy cows (Baker et al., 1995). MUN levels in the present study were not affected by feeding P169+XPY but increase MUN levels were seen in PP not MP fed XPY. This supports the findings of Francisco et al. (2002) who reported no effect of P169 on MUN levels during wk 1 to 12 of lactation. However, the greater MUN levels in MP vs. PP cows parallels Stein et al. (2006) findings, and this likely reflects differences in body growth, milk production, and DMI (Godden et al., 2001). The present study further extends these observations to urinary nitrogen excretion: MP cows had greater urinary nitrogen excretion than PP cows, and the various TMR treatments had no effect on urinary nitrogen excretion.

The measurement of SCC is used worldwide as an indicator of subclinical mastitis (Leavens et al., 1997). Because no international standard exists for the definition of intramammary infections (IMI), methods used to define udder health status vary, and the guidelines for diagnosis on an IMI have changed over time. For example, in 1967, International Dairy Federation bulletin considered an udder normal when the SCC was less than 500 x 10^3 cells/mL (Leavens et al., 1997). Factors such as parity, stage of lactation, and milk production have been associated with variation in SCC (Sheldrake et al., 1983). In the present study, feeding February-calving cows P169+XPY increased SCC above those in Control and XPY-fed cows during wk 4-7 and wk 14-30. In contrast, Stein et al. (2006) using fall (September) calving cows reported lower SCC in both High-dose P169 and Low-dose P169 cows than Control cows. Therefore, season of calving

may influence P169 effects on SCC but further studies will be required to verify this suggestion.

Lactating dairy cows first partition metabolizable energy toward milk production, then body condition gain and finally to reproductive functions. In the present study, the XPY MP cows exhibited a greater recovery of wk 1 BW vs. Control and P169+XPY cows. It is possible that the MP cows fed XPY alone partitioned more of their energy for body growth than the other groups, and therefore less energy was available for lactation. Unlike the MP cows, the Control PP cows exhibited a greater recovery of wk 1 BW than P169+XPY and XPY PP cows. Similarly, Stein et al. (2006), observed that Control PP cows exhibited a greater recovery of wk 1 BW than High-dose PP cows. Again it is possible that the PP cows fed P169+XPY partitioned more of their energy for more milk production than the other groups, therefore, less energy was available for recovery of their wk 1 BW. Jersey cows supplemented with yeast culture (*S. cerevisiae*) lost BW less rapidly postpartum than non-supplemented cows (Dann et al., 2000). In fact, during the postpartum period, Jersey cows that consumed the Control diet lost 23.4 kg more BW during the first 42 days in milk than did cows that consumed yeast culture. In addition, Francisco et al. (2002) and Stein et al. (2006) showed no significant difference in average days to first ovulation in P169 treated vs. Control groups, and the present study agrees with these results. Thus, increased milk production from P169+XPY supplementation had no adverse effect on reproductive performance as measured by days to first and second ovulation.

Conclusions

Supplementation of P169 in combination with XPY during early and mid-lactation increased actual milk yield, SCM and 4%FCM production by 8-12% (above Controls) in both PP and MP cows. Unlike milk fat percentage that was decreased in both XPY and P169+XPY cows, protein percentage was not affected by the P169+XPY supplementation. Milk lactose and milk glucose were both greater in P169+XPY MP cows than Control and XPY fed MP cows. Although 15-h post-feeding fluctuations in plasma glucose did not significantly differ among Control, XPY and P169+XPY groups, the lack of an effect on glucose may be due, in part to the fact that insulin concentrations increased faster in P169+XPY fed cows of the present study. These results might be attributed to greater glucose demand and greater gluconeogenesis in high producing P169+XPY cows vs. cows in the other treatment groups. Despite the increase in milk production observed from P169+XPY vs. Control, no significant changes in reproductive performance or BCS were observed suggesting that the increase in milk production was compensated for by improved metabolism. Because animal numbers were small in the present study, more research is needed on larger commercial dairy farms before recommendations to dairy producers are made.

Literature Cited

Aleman, M, D. R. Stein, D. T. Allen, E. Perry, K. V. Lehloenya, T. G. Rehberger, K. J. Mertz, D. A. Jones, and L. J. Spicer. 2007. Effects of feeding two levels of Propionibacteria to dairy cows on plasma hormones and metabolites. J. Dairy Res. 74:146-153.

Amaral, D. M., J. J. Veenhuizen, J. K. Drackley, M. H. Cooley, A. D. McGilliard, and J. W. Young. 1990. Metabolism of propionate, glucose, and carbon dioxide as affected by exogenous glucose in dairy cows at energy equilibrium. J. Dairy Sci. 73:1244-1254.

Arambel, M. J., and B.A. Kent. 1990. Effect of yeast culture on nutrient digestibility and milk yield response in early to midlactation dairy cows. J. Dairy Sci. 73:1560-1563.

Arieli, J. E., J. E. Vallimont, A. Aharoni, and G. A. Varga. 2001. Monensin and growth hormones effects on glucose metabolism in prepartum cow. J. Dairy Sci. 84:2770-2776.

Bach, A, S. Calsamiglia, and M.D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88 (Suppl.1): E9-E21.

Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. J. Dairy Sci. 78:2424-2434.

Banos, G., M. P. Coffey, and S. Brotherstone. 2005. Modeling daily energy balance of dairy cows in the first three lactations. J. Dairy Sci. 88:2226-2237.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567-590.

Bertics, S. J., R.R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglycerides concentration in early lactation. J. Dairy Sci. 75:1914-1922.

Borrebaek, B., K. Halse, B. Tveit, H. L. Dahle, and L. Ceh. 1990. Plasma glucose, ketone bodies, insulin, glucagon and enteroglucagon in cows: Diurnal variations related to ketones levels before feeding and to the ketogenic effects of feeds. Acta Vet. Scand. 31:5-15.

Broderick, G. A and M. K. Clayton. 1997. A statistical evaluation of nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964-2971.

Burhans, W. S., and A. W. Bell. 1998. Feeding the transition cow. Pages 247-258 in Cornell Nutr. Conf., Ithaca, NY.

Callaway, E.S., and S.A. Martin. 1997. Effect of *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Christensen, J. O., R. R. Grummer, F. E. Rasmussen, and S. J. Bertics. 1997. Effects of method of delivery of propylene glycol on plasma metabolites of feed restricted cattle. J. Dairy Sci. 80:563-568.

Clark, J. H., and C. L. Davis. 1980. Some aspects of feeding high producing cows. J. Dairy Sci. 63:873-882.

Dann, H. M., J. K. Drackley, G.M. McCoy, M.F. Hutjens, and J.E. Garrett. 2000. Effects of yeast culture (*Saccharomyces cerevisiae*) on prepartum and postpartum intake and milk production of Jersey cows. J. Dairy Sci. 83:123-127.

Davidson, S., B. A. Hopkins, D. E. Diaz, S. M. Bolt, C. Brownie, V. Fellner, and L. W. Whitlow. 2003. Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early lactation Holstein cows. J. Dairy Sci. 86:1681-1689.

DeFrain, J. M., A. R. Hippen, K. F. Kalscheus, and P. W. Jardon. 2004. Feeding glycerol to transition dairy cow: Effect on blood metabolites and lactation performance. J. Dairy Sci. 87:4195-4206.

DeFrain, J. M., A. R. Hippen, K.F. Kalscheur, and R.S. Patton. 2005. Effects of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. J. Dairy Sci. 88:983-993.

De Vries, M. J., and R. F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variable and fertility. J. Dairy Sci. 83:62-69.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84:100-112.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier. J. Dairy Sci. 82:2259-2273.

Erdman, R. A., and B. K. Sharma. 1989. Effect of yeast culture and sodium bicarbonate on milk yield and composition in dairy cows. J. Dairy Sci. 72: 1929-1932.

Francisco, C. C., C. S. Chamberlain, D. N. Waldner, R. P. Wettemann, and L. J. Spicer. 2002. Propionibacteria Fed to Dairy Cows: Effects on Energy Balance, Plasma Metabolites and Hormones, and Reproduction. J. Dairy Sci. 85:1738-1751.

Godden, S. M., K. D. Lissemore, D. F. Kelton, K. E. Leslie, J. S. Walton, and J. H. Lumsden. 2001. Relationship between milk urea concentrations and nutritional management, production, and economic variables in Ontario dairy herds. J. Dairy Sci. 84:1128-1139.

Grummer, R. R. 1993. Etiology of related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Grummer, R. R., J. C. Winkler, S. J. Bertics, and V. A. Studer. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. J. Dairy Sci. 77:3618-3623.

Harris, B., and D. W. Webb. 1990. The effect of feeding a concentrate yeast culture to lactating dairy cows. J. Dairy Sci. 73(Suppl. 1): 266 (Abstr.).

Higginbotham, G.E., C.A. Collar, M.S. Aseltine, and D.L. Bath. 1994. Effect of yeast culture and *Aspergillus oryzae* extract on milk yield in a commercial dairy herd. J. Dairy Sci. 77:343-348.

Huhtanen, P. J., R. Blauwicked, I. Saastamoineu. 1998. Effect of intraruminal infusions of propionate and butyrate two different protein supplements on milk production and blood metabolites in dairy cows receiving grass silage-based diet. J. Sci. Food Agric 77:213-222.

Kim, S. W., H. Romano-Rosario, M. T. Yokoyama, and S. R. Rust. 2001. In vitro effects of Propionibacterium acidipropionici, strain DH42 on fermentation characteristics of rumen microorganism. Michigan State Univ. Beef cattle, sheep and forage system research and demonstration report, Michigan State Univ. East Lansing.

Knowlton, K. F., T. E. Dawson, B. P. Glenn, G. B. Huntington, and R. A. Erdman. 1998. Glucose metabolism and milk yield of cows infused abomasally or ruminally with starch. J. Dairy Sci. 81:3248-3258.

Kujawa, M. 2003. Profitability of yeast culture in today's environment. Page 5-9 in Proc 2003. Penn State Dairy Cattle Nutrition Workshop, Grantville. PA.

Krehbiel, C. R., S. R. Rust, G. Zhang, and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminants diets, performance response and mode of action. J. Anim. Sci. 81:E120-E32.

Kung, L., E. M. Kreck, R.S. Tung, A. O. Hession, A. C. Shepherd, M. A. Cohen, H. E. Swain, and J .A. Z. Leedle. 1997. Effects of a live yeast culture and enzyme on in vitro ruminal fermentation and milk production. J. Dairy Sci. 80:2045-2051.

Leavens, H., H. Deluyker, Y. H. Schukken, L. De Meuleteester, R. Vondermeersch, E. De Mueleneere, and A. De Kruif. 1997. Influence of parity and stage of lactation on somatic cell count in bacteriologically negative dairy cows. J. Dairy Sci. 80:3219-3228.

Lemosquet, S., S. Rigout, A. Bach, H. Rulquin, and J. W. Blum. 2004. Glucose metabolism in lactating cows in response to isoenergetic infusions of propionic acid or duodenal glucose. J. Dairy Sci. 87:1767-1777.

Lyons, T. P., K. A. Jacques, and K. A. Dawson. 1993. Miscellaneous products from yeast, pages 293-324 in *The Yeasts* (eds. A.H. Rose and J.S. Harrison). Vol. 5. Academy Press, New York.

Martin, S. A., S. F. Al-Khaldi, J. D. Evans, and H. M. Sullivan. 1999. Potential for manipulating gastrointestinal microflora: Alternatives to antibiotics and ionophores. Recent Res. Dev. Microbiol. 3:441-454.

Martin, S. A., and D. J. Nisbet. 1992. Effect of direct fed microbials on rumen microbial fermentation. J. Dairy Sci. 75:1736-1744.

McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan, 2002. Animal Nutrition. 6th ed. Pearson Education Limited, Edinburgh Gate, Harlow, Essex, England.

McGilliard, M. L., and C. C. Stallings. 1998. Increase in milk yield of commercial dairy herds fed a microbial an enzyme supplement. J. Dairy Sci. 81: 1353-1357.

Miller-Webster, T., W. H. Hoover, and M. Holt. 2002. Influence of yeast culture on ruminal microbial metabolism in continuous culture. J. Dairy Sci. 85:2009-2014.

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

Nielsen, N. I.; K. L. Ingvartsen, and T. Larsen. 2003. Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR- fed dairy cows. J. Vet. Med. A Physiol. Pathol. Clin. Med. 50:88-97.

Nisbet, D.J., and S.A. Martin. 1991. Effect of *Saccharomyces cerevisiae* on lactate utilization by the ruminal bacterium *Selenomonas ruminantium*. J. Anim. Sci. 69:4629-4633.

Nocek, J. E., and W.P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health and performance of pre- and postpartum dairy cattle. J. Dairy Sci. 89:260-266.

Oba, M., and M. Allen. 2003. Extent of hippomania caused by propionate infusion is related to plasma glucose concentration in lactating dairy cows. J. Nutr. 133:1105-1112.

Piva, G., S. Belladonna, G. Fusconi, and F. Sicbaldi. 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76:2717-2722.

Putman, D. E., Schwab, M. T. Socha, N. L. Whitehouse, N. A. Kierstead, and B. D. Garthwaite. 1997. Effect of yeast in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. J. Dairy Sci. 80: 374-384.

Rigout, S., C. Hurtaud, S. Lemosquet, A. Bach, and H. Rulquin. 2003. Lactational effect of propionic acid and duodenal glucose in cows. J. Dairy Sci. 86:243-53.

Rigout, S., S. Lemosquet, J.E. van Eys, J.W. Blum, and H. Rulquin. 2002. Duodenal glucose fluxes and lactose synthesis in grass silage-fed dairy cows. J. Dairy Sci. 85:595-606

Rode, L. M., W. Z. Yang, and K. A. Beauchemin. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci 82:2121-2126.

Sano, H., Narahara, A. Takahashi, and Y. Terashima. 1995. Plasma insulin and glucagons responses to propionate infusion into femoral and mesenteric veins in sheep. J. Anim. Sci. 73: 191-197.

Schingoethe, D. J., K. N. Linke, K.F. Kalscheur, A. R. Hippen, D.R. Rennich, and I. Yoon. 2004. Feeding efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87:4178-4181.

Seal, C.J., and C.K. Reynolds. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutr. Res. Rev. 6:185-208.

Sheldrake, R. F., R.J.T. Hoare, and G. D. McFregor. 1983. Lactation stage, parity and infection affecting somatic cell, electrical conductivity, and serum albumin in milk. J. Dairy Sci. 66:542-547.

Soder, K.J., and L.A. Holden. 1999. Dry matter intake and milk yield and composition of cows fed yeast prepartum and postpartum. J. Dairy Sci. 82:605-610.

Sniffen, D., and J. Ferguson. 1995. Body condition scoring guide. Church and Dwight Co., Inc., Princeton, NJ. Pages 1-12.

Spicer, L.J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-I in dairy cows: Relationship among energy balance, body condition score, ovarian activity and estrous behavior. J. Dairy Sci. 73:929-937.

Stein, D.R., D.T. Allen, E. B. Perry, J. C. Bruner. K.W. Gates, T.G. Rehberger, K.J. Mertz, D.A. Jones, and L.J. Spicer. 2006. Effect of feeding Propionibacteria to dairy cows on milk yield, milk components and reproduction. J Dairy Sci. 89:111-125.

Stephenson, K. A., I. J. Lean, M. L. Hyde, M. A. Curtis, J. K. Garvin, and L. B. Lowe. 1997. Effects of monensin on the metabolism of periparturient dairy cows. J. Dairy Sci. 80:830-837.

Subiyatno, A., D.N. Mowat, and W.Z. Yang. 1996. Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. J. Dairy Sci. 79:1436-1445.

Sutton, J. D., I. C. Hart, S. V. Morant, E. Schuller, and A. D. Simmonds. 1988. Feeding frequency for lactating cows: Diurnal patterns of hormones and metabolites in peripheral blood in relation to milk-fat concentration. Br. J. Nutr. 60:265-275.

Swatz, D. L., L. D. Muller, G. W. Rogers, and G. A. Varga. 1994. Effect of yeast culture on performance of lactating dairy cows: A field study. J. Dairy Sci. 77: 3073-3080.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48: 1215-1223.

Vallimont, J. E., G. A. Varga, A. Arieli, T. W. Cassidy, and K. A. Cummins. 2001. Effects of prepartum somatotropin and monensin on metabolism and production of periparturient Holstein dairy cows. J. Dairy Sci. 84:2621-2621.

Van Soest, P. J. V. 1994. Nutritional ecology of the ruminant. 2nd ed. Comstock, Ithaca, NY.

Veerkamp, R. F., J. K. Oldenbroek, H. J. Van der Gaast, and J. H. J. Van der Werf. 2000. Genetic correlation between days until start of luteal activity and milk yield, energy balance and live weight. J. Dairy Sci. 83:577-583.

Wallace, R. J. 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: Progress and problems. J. Anim. Sci. 72:2992-3003.

Wang, Z., M. L. Eastridge, and X. Quip. 2001. Effects of forage neutral detergent fiber and yeast culture on performance of cows during early lactation. J. Dairy Sci. 84:204-212.

Williams, P. E. V., C. A. G. Taut, G. M. Innes, and C. J. Newbold. 1990. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in rumen of steers. J. Anim. Sci. 69:3016-3026.

Williams, P. E. C. A. G. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016.

Wohlt, J. E., A. D. Finkelstein, and C. H. Chung. 1991. Yeast culture to improve intake, nutrient digestibility and performance by dairy cattle during early lactation. J. Dairy Sci. 74:1395-1400.

Table 1. Ingredient and Nutrient Composition of the Control Lactation Diet (DM basis)

Ingredient	%	Nutrient	
Corn Silage	25.23	DM, % (as fed)	53.39
Alfalfa (RFV 180)	20.75	CP, %	16.5
Grain mix	38.23	ADF, %	26.90
Whole Cottonseed	6.53	NDF, %	39.60
Corn Gluten Feed	7.98	NE_l, Mcal/kg	1.65
MEGALAC®-R*	1.28		
		Ca, %	0.91
Lactation Cow Grain Mix		P, %	0.37
Ground Corn	60.7	Mg, %	0.35
Wheat Midds	15.8		
Soybean Meal	14.3	K, %	1.62
Extruded/Expeller SB Meal	5.6		
Calcium Carbonate	1.0	Na, %	0.33
Sodium Bicarbonate	1.0		
		S, %	0.21
Magnesium Oxide	0.5		
Salt – White	0.5		
Zinpro 4-plex **	0.1	Zn, ppm	50.38
Lactating premix	0.5		
		Fe, ppm	271.63
		Cu, ppm	14.88
		Mn, ppm	51.63
		Mo, ppm	1.15

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

** Zinpro Corp.,(Eden Prairie, MN); Zinpro 4 - plex contains:
Zinc – 2.58%, Mn – 1.43, Cu – 0.90%, Co – 0.18%, Methionine – 8.21%, Lysine – 3.8%

Figure 1. Effect of feeding XPY and P169+XPY on uncorrected milk production through 30 wk of lactation in Holstein cows. Within treatment groups, data were averaged across parity groups (Controls, n=10; XPY, n=11; P169+XPY, n=10). Means (±SEM) differed (P=0.08) among treatment groups.

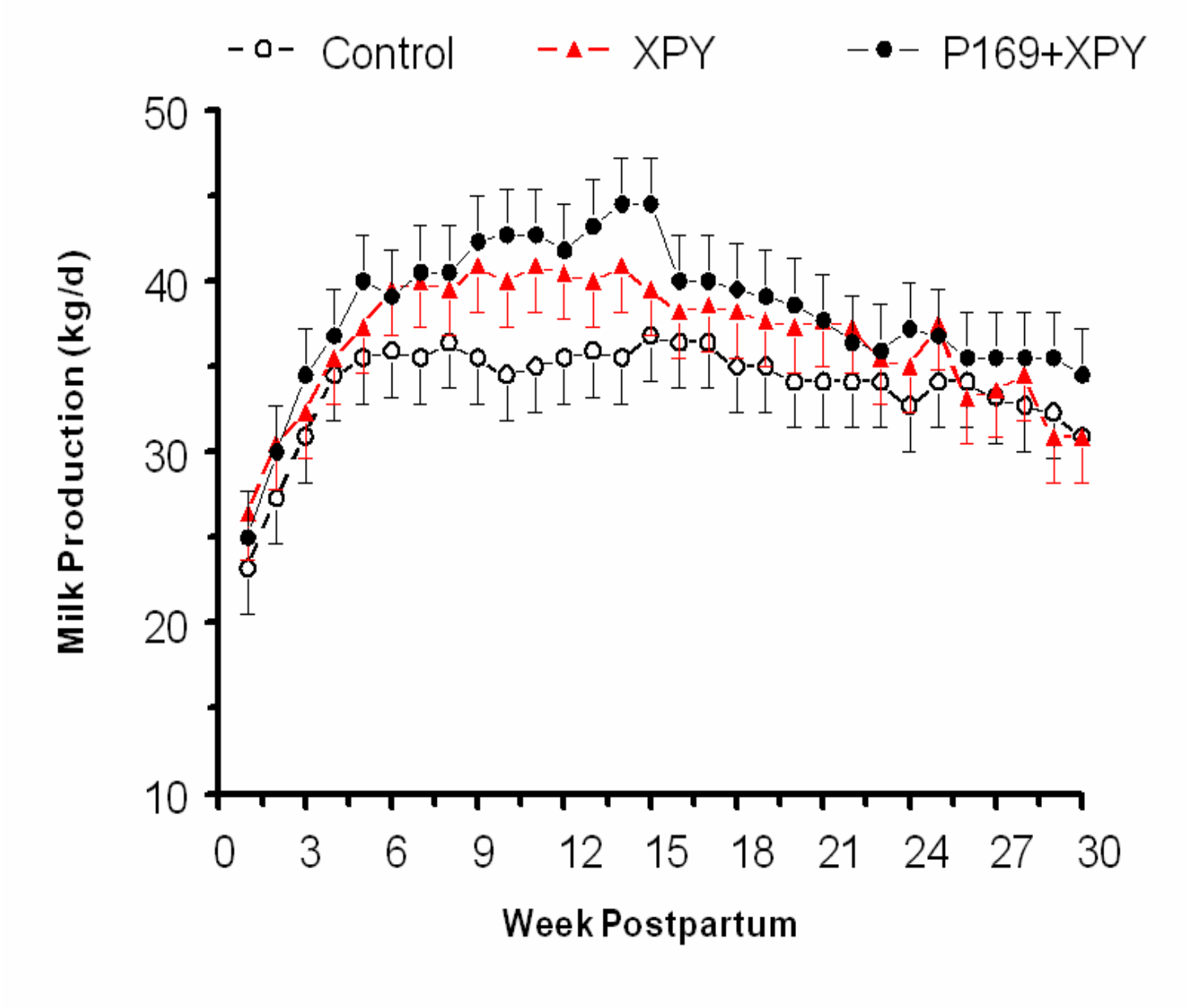

Control
XPY
P169+XPY
Milk Production (kg/d)
50
40
30
20
10
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 2. Effect of feeding XPY and P169+XPY on solids corrected milk (SCM) production through 30 wk of lactation in Holstein cows. Within treatment groups, data were averaged across parity groups (Controls, n=10; XPY, n=11; P169+XPY, n=10). Means (±SEM) differed (P=0.09) among treatment groups.

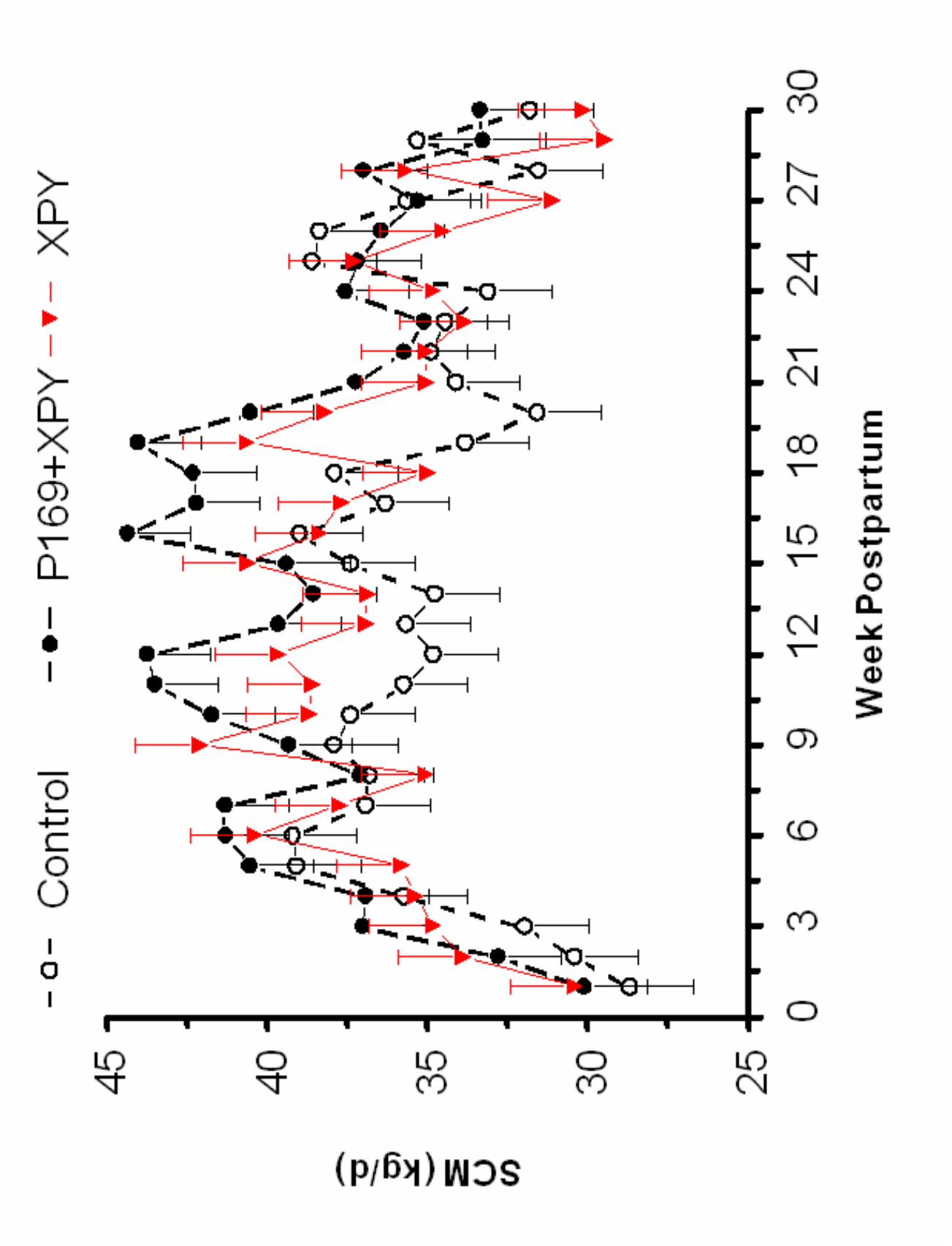

Control
P169+XPY
XPY
SCM (kg/d)
Week Postpartum
45
40
35
30
25
0
3
6
9
12
15
18
21
24
27
30

Figure 3. Effect of parity x week interaction on solids-corrected milk in primiparous (n = 15) and multiparous (n = 16) Holstein cows. Within parity, data were averaged across treatments. Means (±SEM) were affected by parity x week interaction (P<0.09).

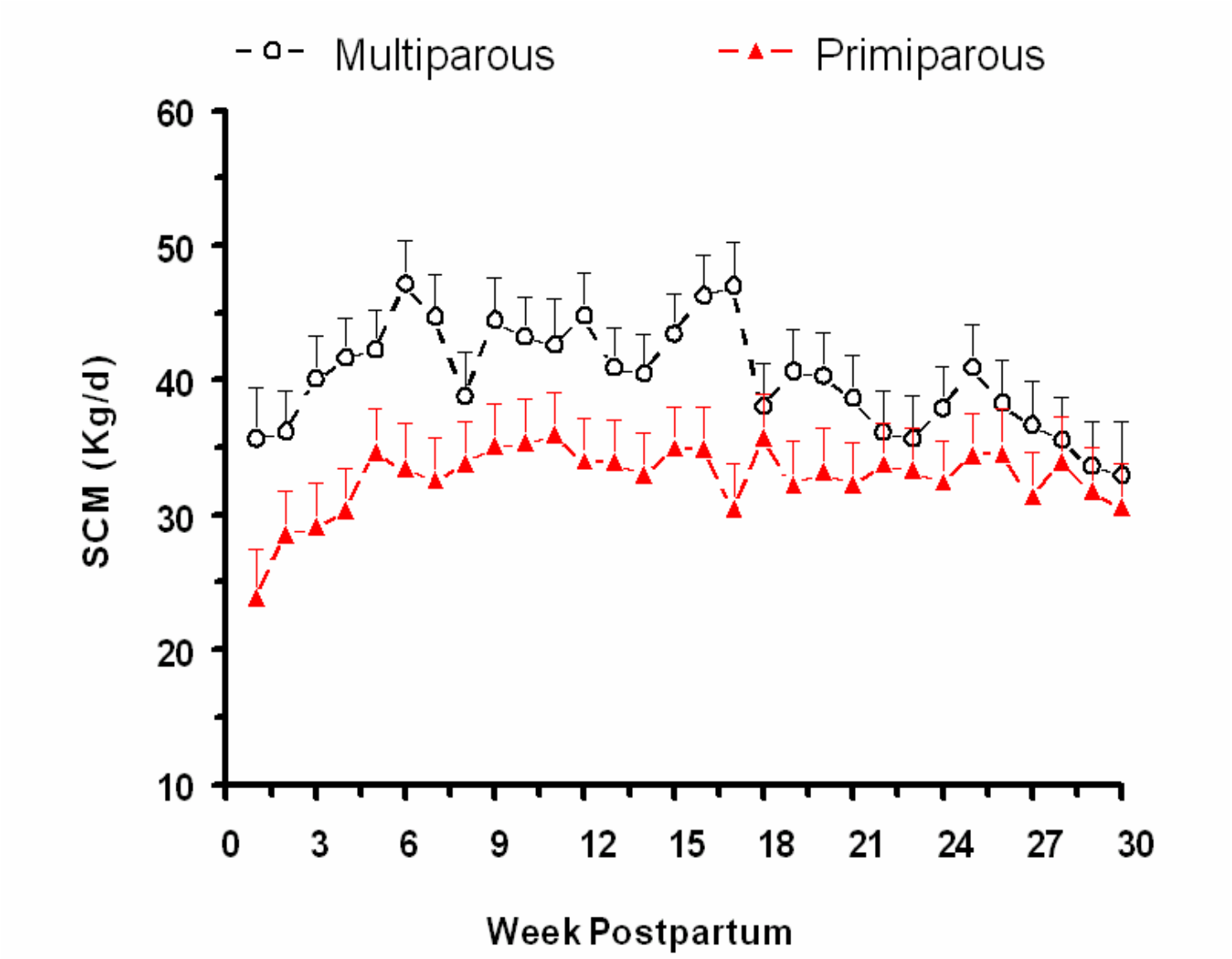

Multiparous
Primiparous
SCM (Kg/d)
60
50
40
30
20
10
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 4. Effect of feeding XPY and P169+XPY on 4%FCM through 30 wk of lactation in Holstein cows. Within treatment groups, data were averaged across parity groups (Controls, n=10; XPY, n=11; P169+XPY, n=10). Means (±SEM) differed (P=0.06) among treatment groups.

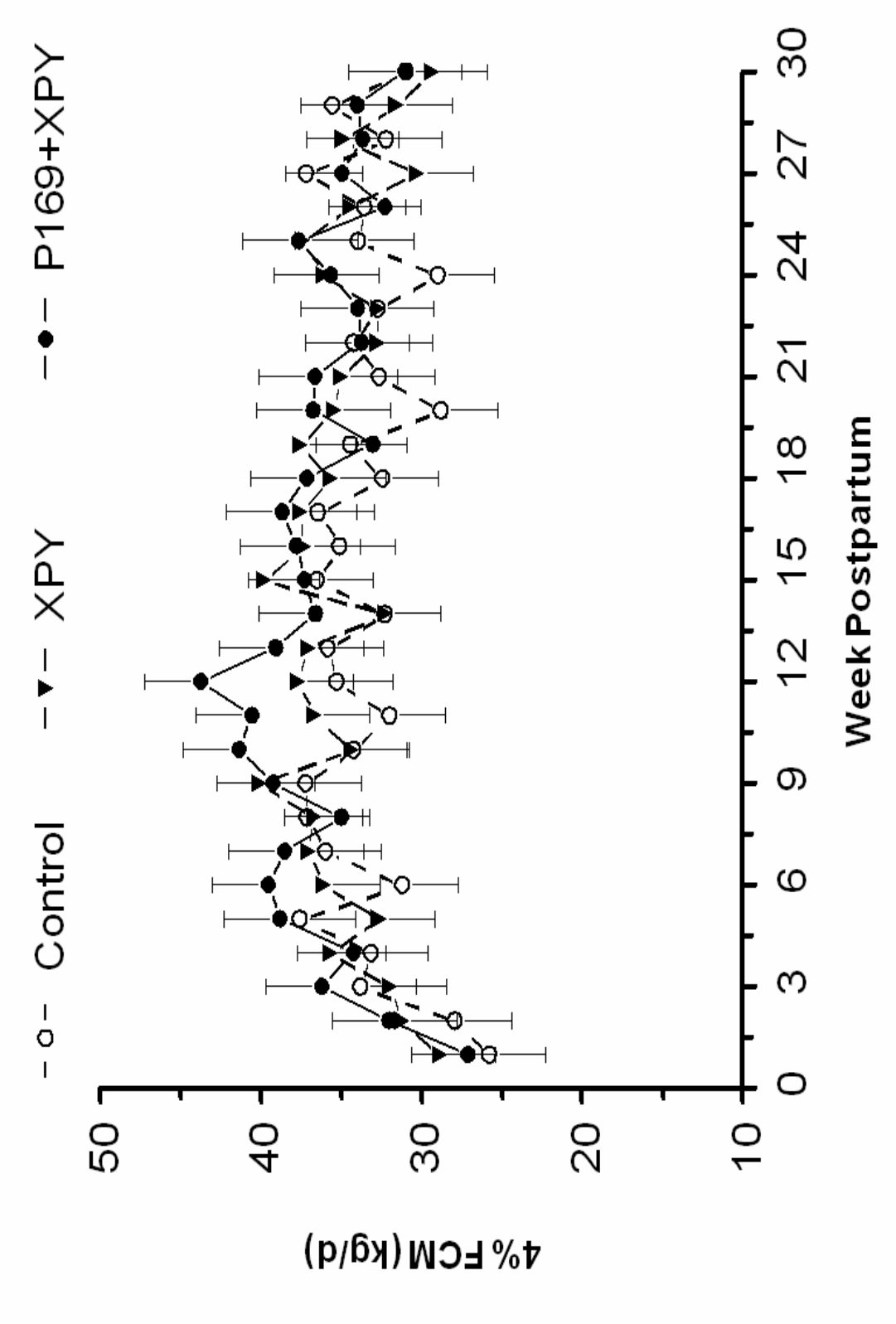

Control
XPY
P169+XPY
4% FCM (kg/d)
50
40
30
20
10
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 5. 4%FCM production during wk 1 to 30 of lactation in multiparous and primiparous cows fed Control (n=10) and XPY (n=11) and P169+XPY (n=10) rations. Data were pooled across treatment and parity. Means (±SEM) were affected by week of lactation (P=0.03).

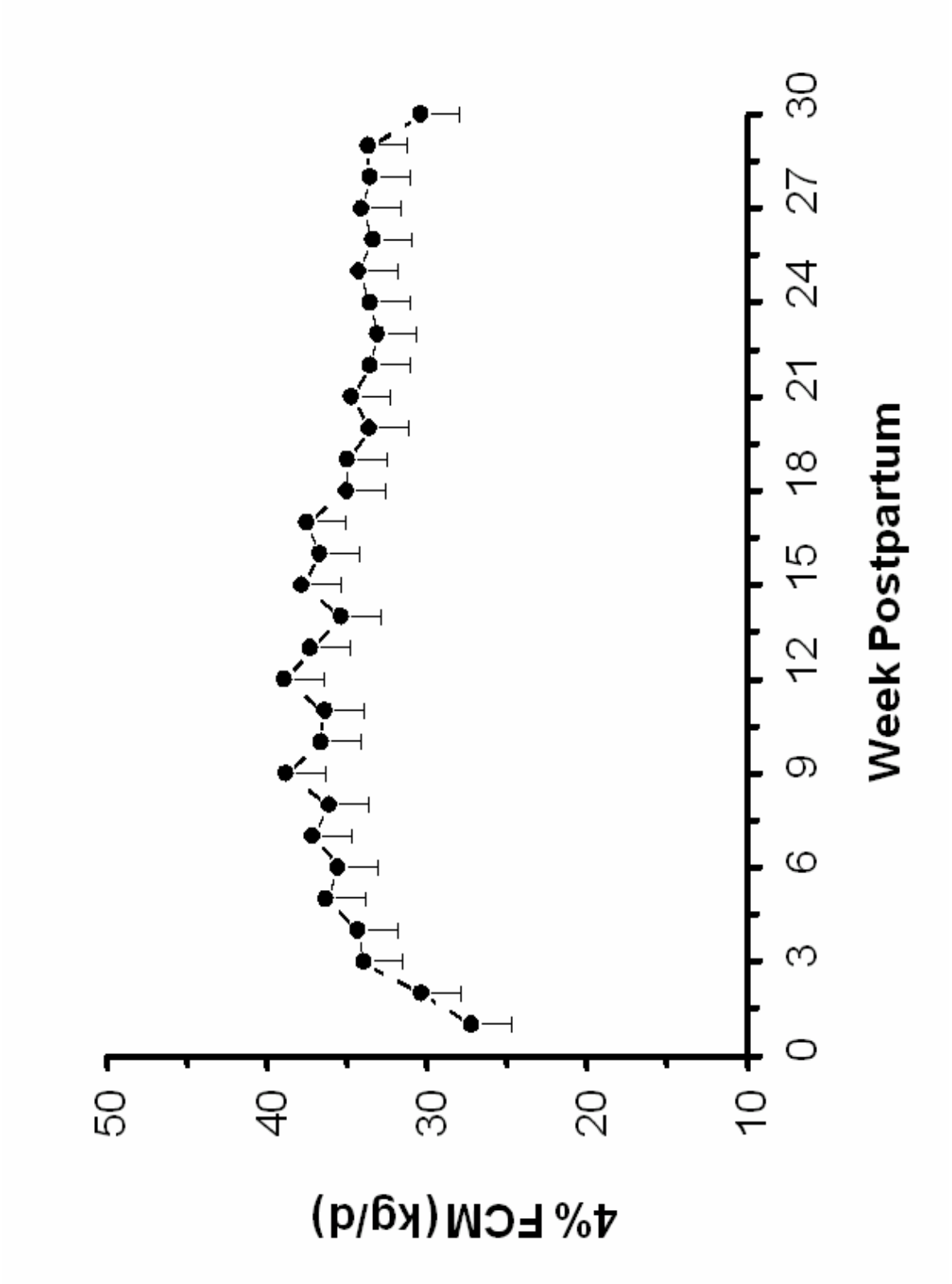

4% FCM (kg/d)
50
40
30
20
10
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 6. Milk fat percent in multiparous and primiparous cows fed Control (n=10) and XPY (n=11) and P169+XPY (n=10) rations during wk 1 to 30 of lactation. Data from cows were pooled across parity and wk. Asterisk (*) indicates mean differs from XPY and P169+XPY means (P=0.01)

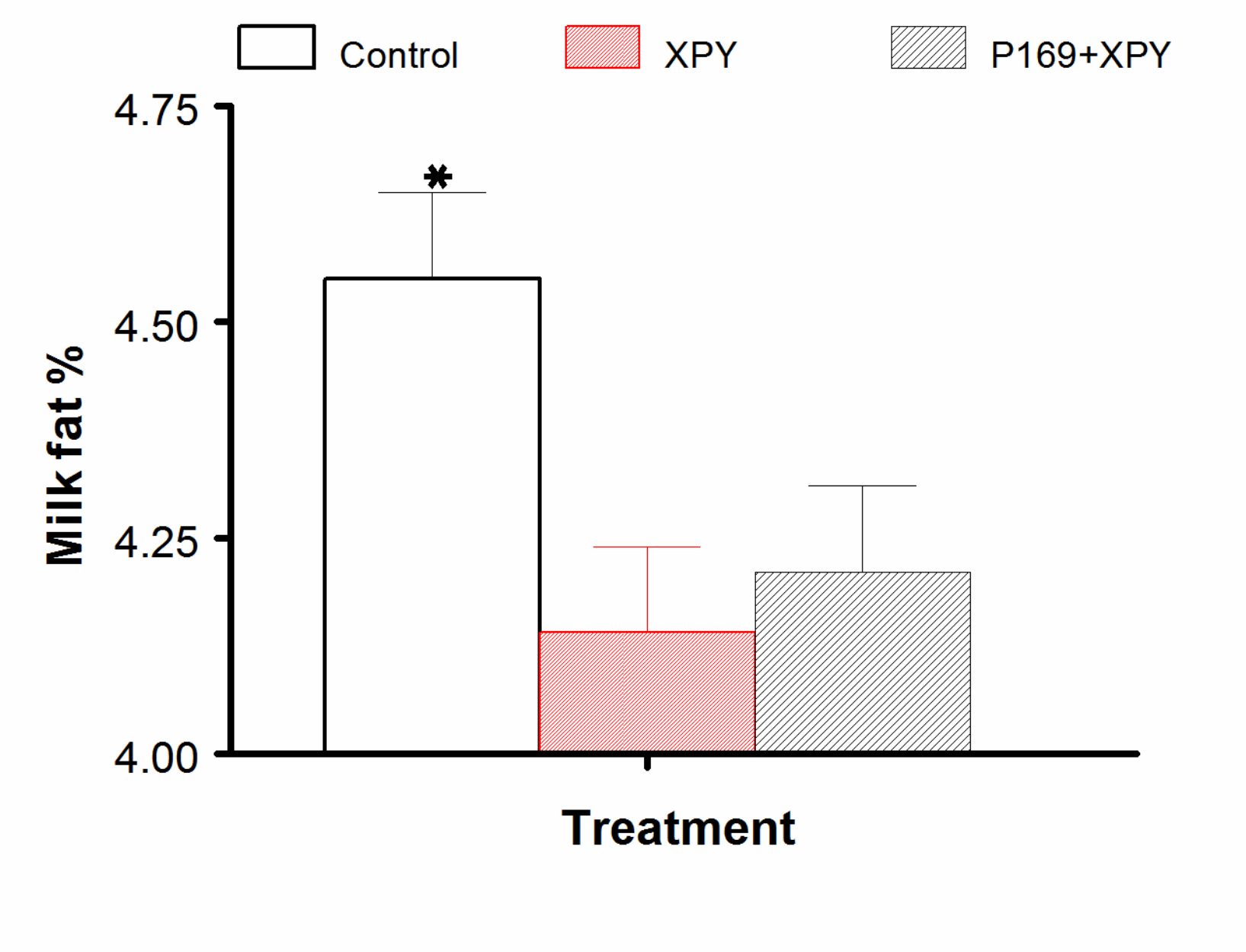

Control
XPY
P169+XPY
4.75
4.50
4.25
4.00
Milk fat %
*
Treatment

Figure 7. Milk fat percentage during wk 1 to 30 of lactation in multiparous (n=16) and primiparous (n=15) cows fed Control (n=10), XPY (n=11) and P169+XPY (n=10) rations. Data from cows were pooled across treatment and parity. Means (±SEM) were affected by week of lactation (P=0.02).

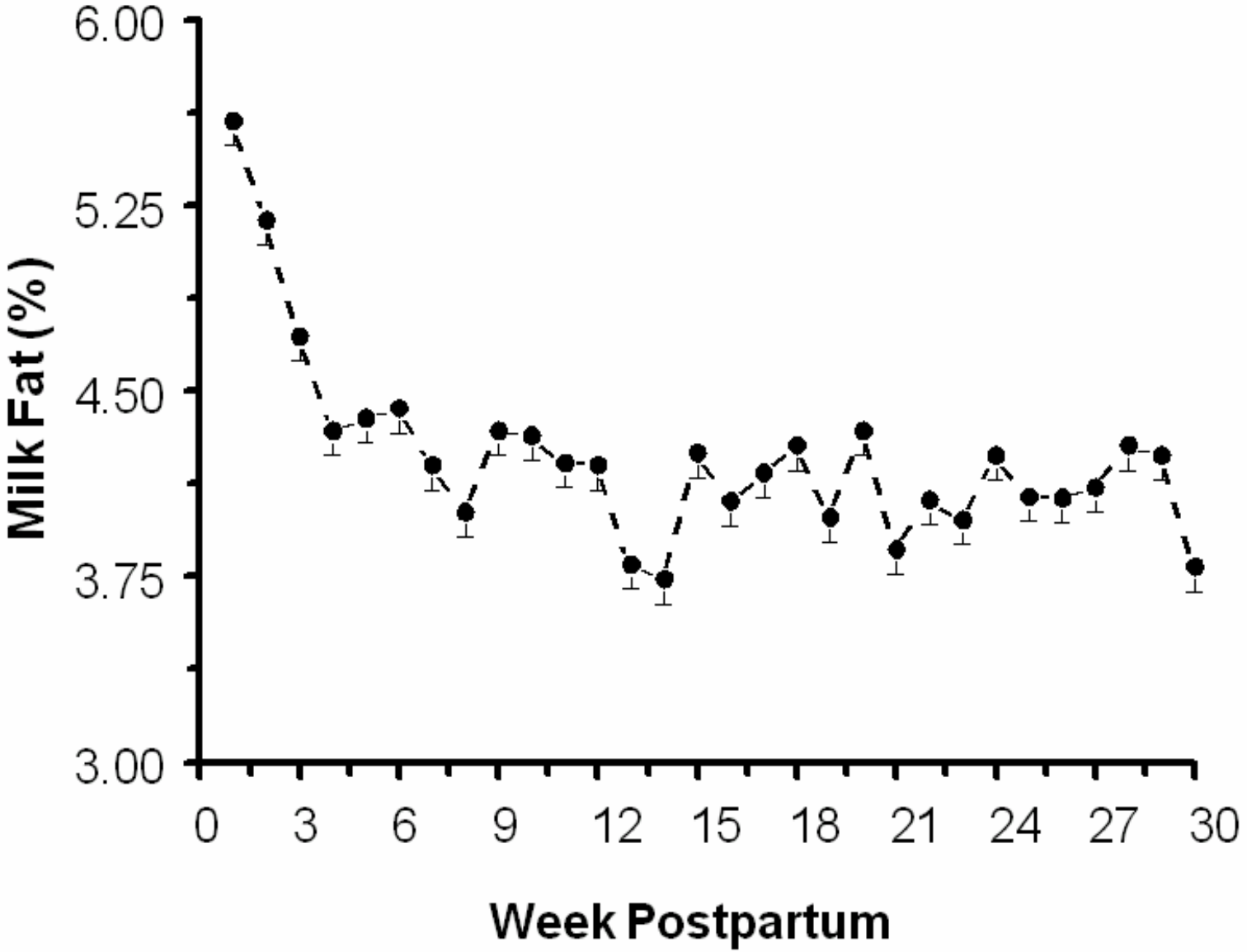

6.00
5.25
4.50
3.75
3.00
Milk Fat (%)
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 8. Effect of feeding XPY and P169+XPY on milk lactose percentage during 30 wk of lactation. (Treatment x Parity interaction). [a,b,c] Means without a common letter differ (P<0.0001).

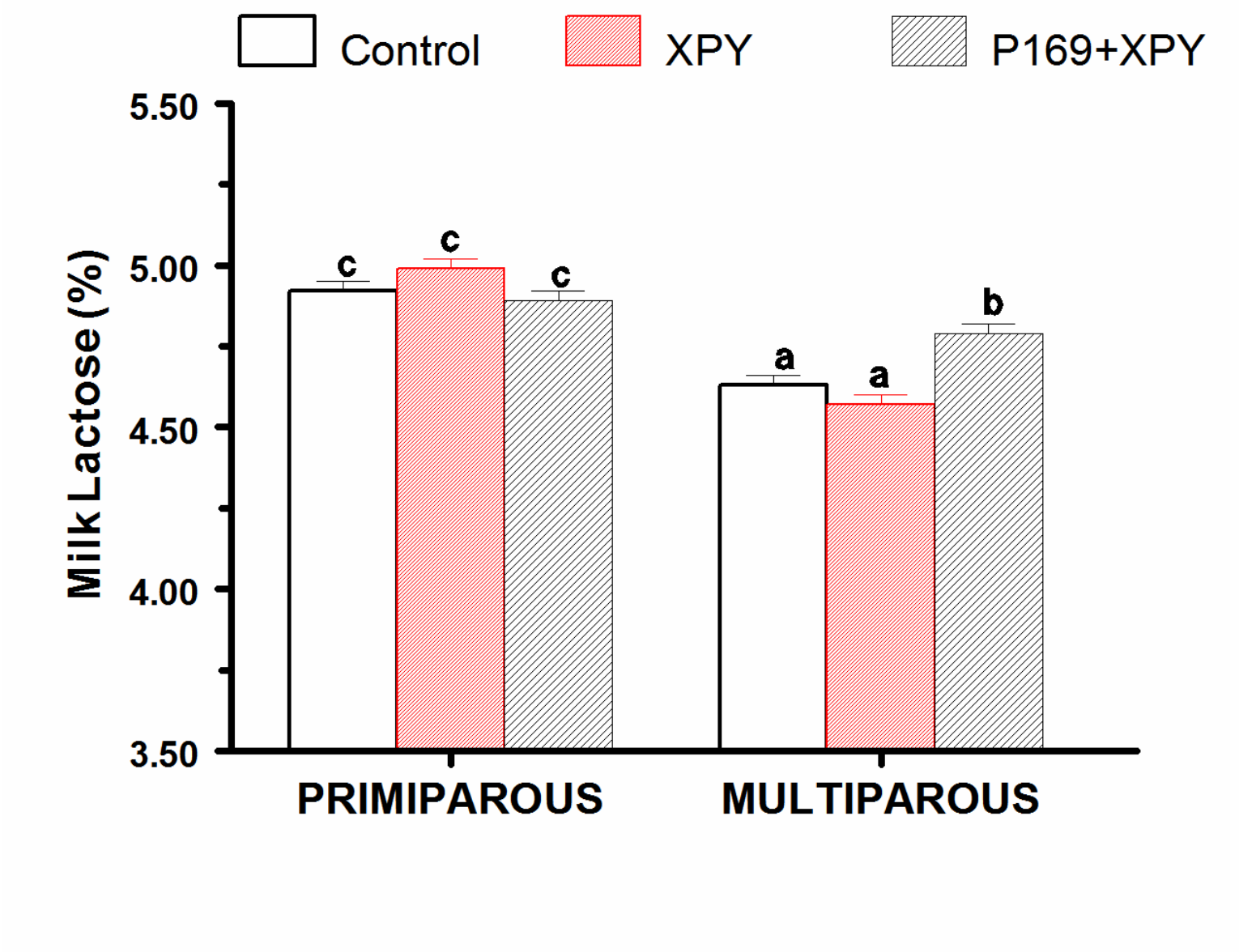

Control
XPY
P169+XPY
Milk Lactose (%)
5.50
5.00
4.50
4.00
3.50
c
c
c
a
a
b
PRIMIPAROUS
MULTIPAROUS

Figure 9. Effect of feeding XPY and P169+XPY on milk protein percentage during wk 1 to 30 of lactation in multiparous (n=16) and primiparous (n=15) cows fed Control (n=10), XPY (n=11) and P169+XPY (n=10) rations. Data from cows were pooled across treatment and parity. Means (±SEM) were affected by week of lactation (P=0.0001).

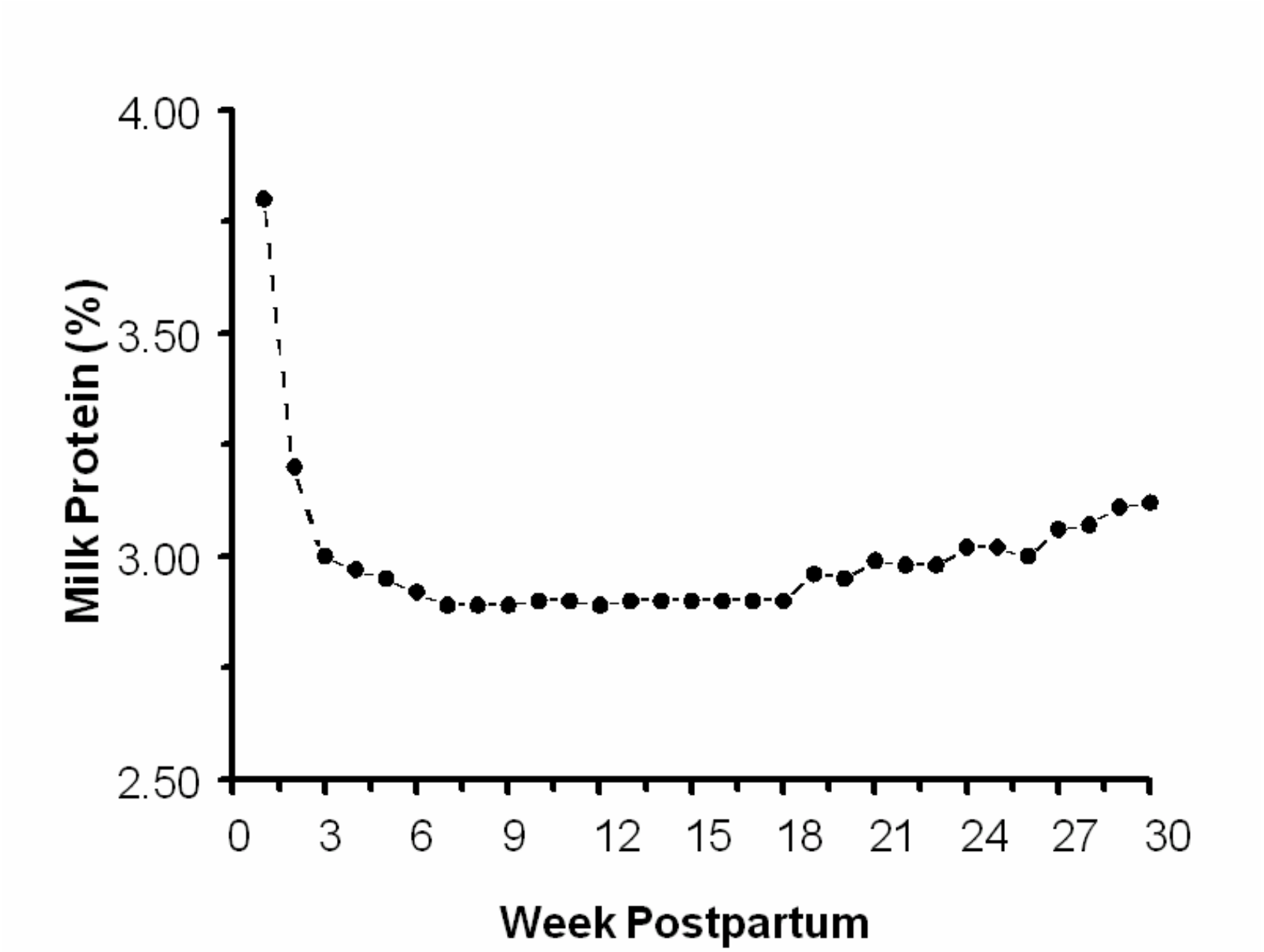

4.00
3.50
3.00
2.50
Milk Protein (%)
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 10. Effect of feeding XPY and P169+XPY on milk protein percentage (Treatment x Parity interaction). a,b,c Means without a common letter differ (P=0.05).

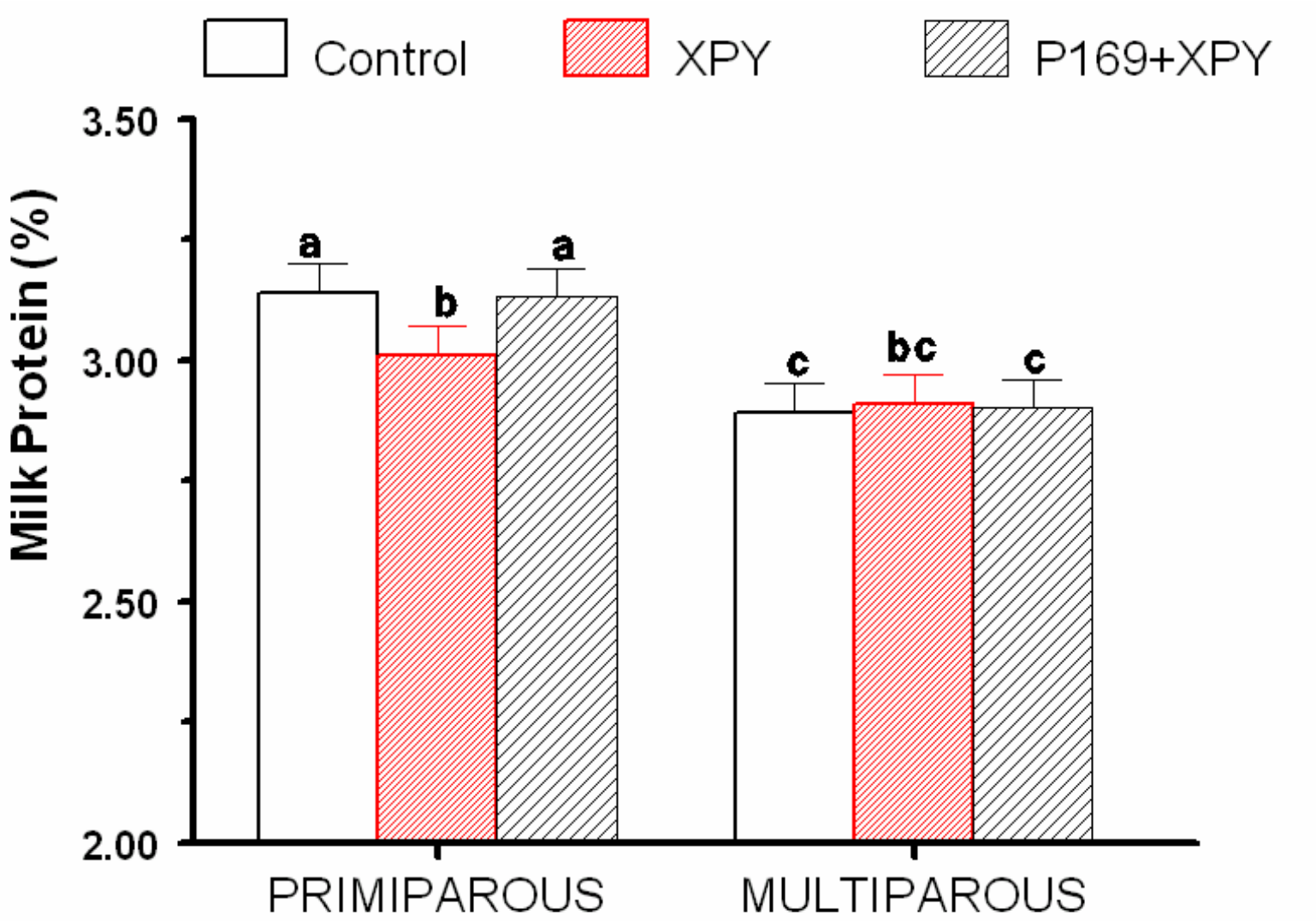

Control
XPY
P169+XPY
Milk Protein (%)
3.50
3.00
2.50
2.00
a
b
a
c
bc
c
PRIMIPAROUS
MULTIPAROUS

Figure 11. Effect of feeding XPY and P169+XPY on milk solids-not fat percentage during wk 1 to 30 of lactation averaged across multiparous and primiparous cows fed Control (n=10), XPY (n=11) and P169+XPY (n=10) rations. Means (±SEM) tended (P=0.10) to differ among treatment groups.

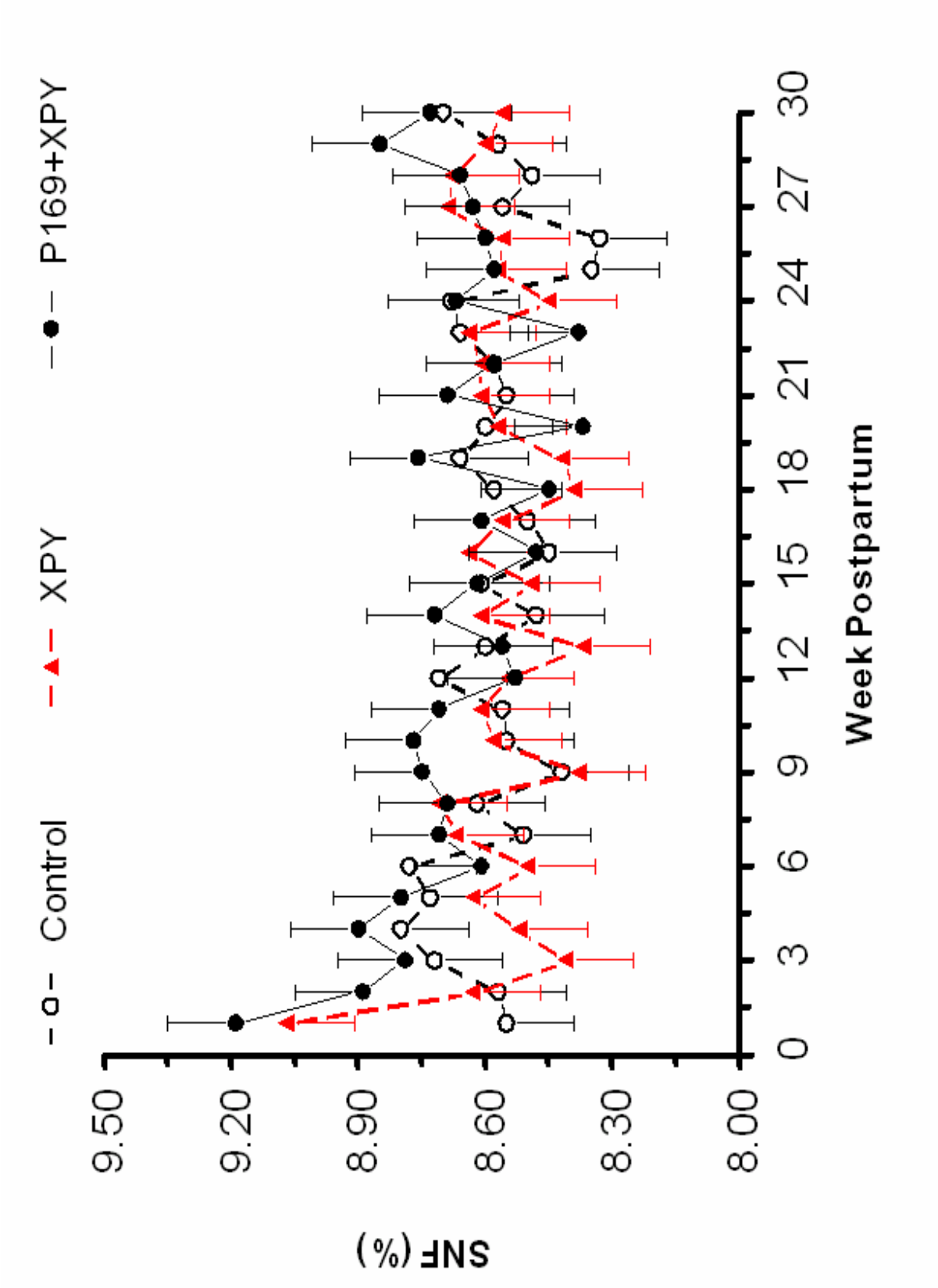

Control
XPY
P169+XPY
SNF (%)
9.50
9.20
8.90
8.60
8.30
8.00
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 12. Effect of feeding XPY and P169+XPY on milk urea nitrogen during 30 wk of lactation. Top Panel: milk urea nitrogen in multiparous cows (n =16) fed Control (n = 6) and XPY (n = 5) and P169+XPY (n = 5) rations from wk 1 to 30. Bottom Panel: milk urea nitrogen for primiparous cows fed Control (n = 4) and XPY (n = 6) and P169+XPY (n = 5) rations from wk 1 to 30. Means (±SEM) tended (P=0.09) to be affected by treatment x parity x week interaction.

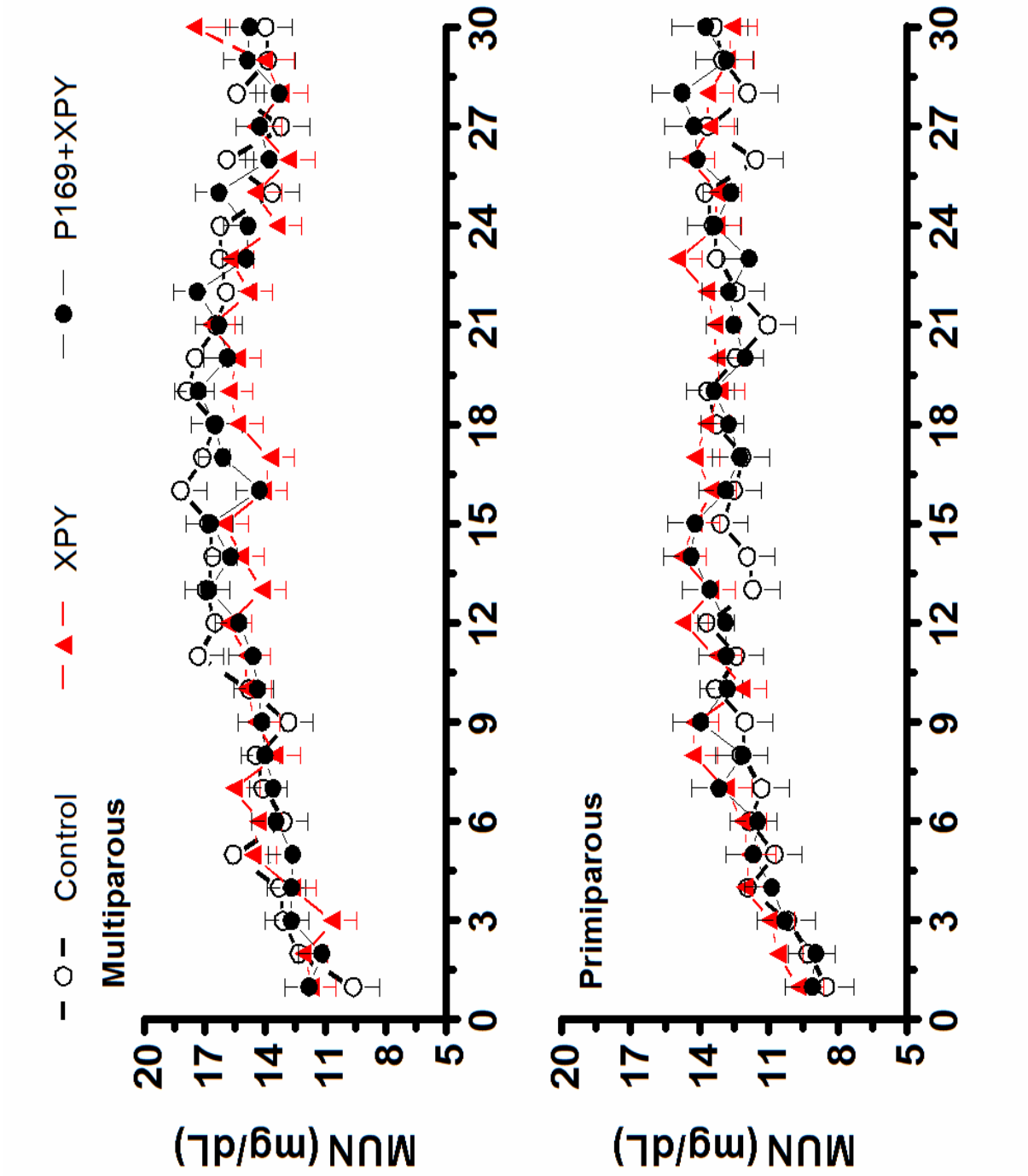

Control
XPY
P169+XPY
Multiparous
Primiparous
MUN (mg/dL)
MUN (mg/dL)
20
17
14
11
8
5
0
3
6
9
12
15
18
21
24
27
30

Figure 13. Effect of feeding XPY and P169+XPY on milk urea nitrogen (Treatment x Parity interaction). a,b,c Means without a common letter differ (P=0.05).

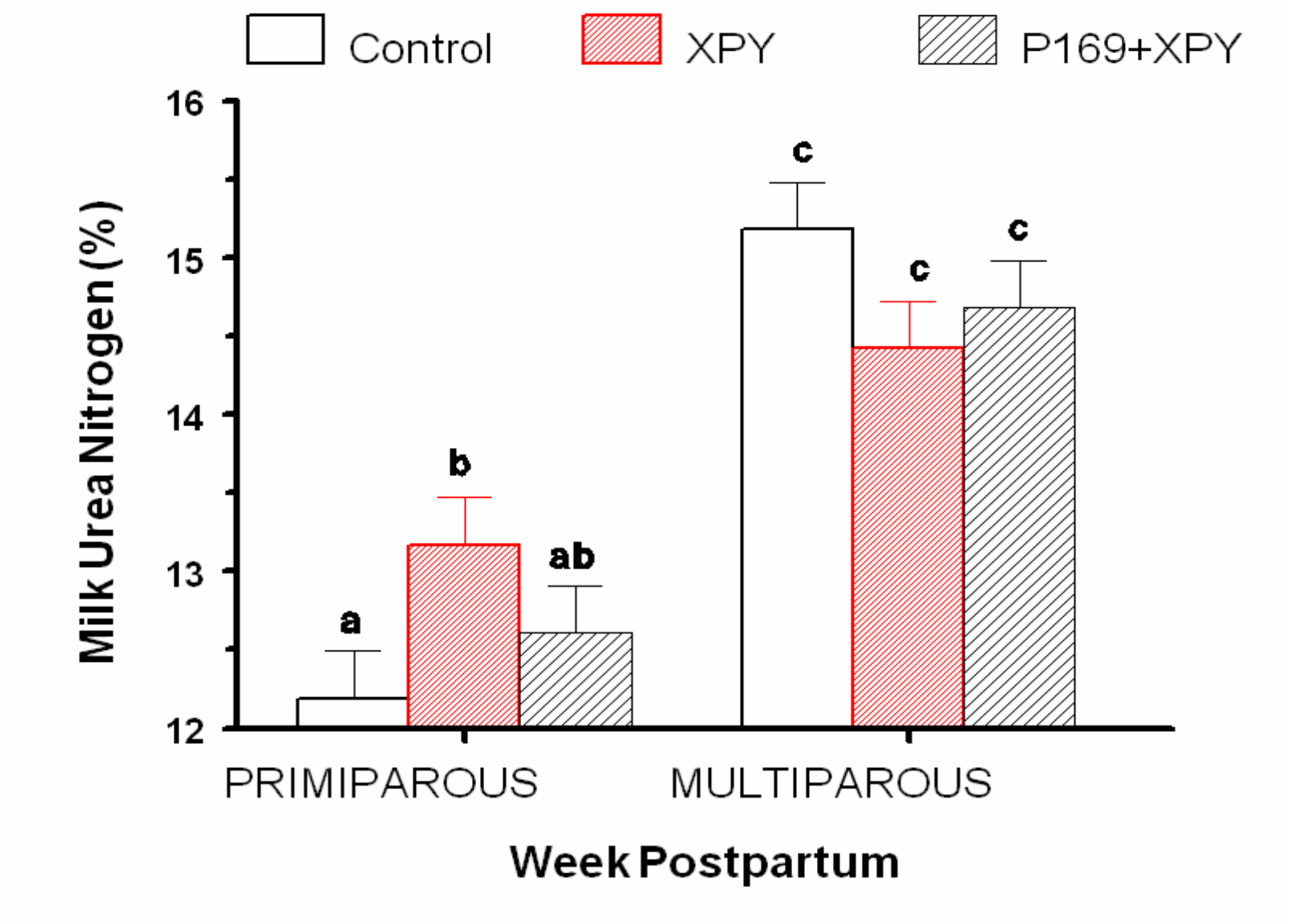

Control
XPY
P169+XPY
Milk Urea Nitrogen (%)
16
15
14
13
12
a
b
ab
c
c
c
PRIMIPAROUS
MULTIPAROUS
Week Postpartum

Figure 14. Changes in urinary nitrogen excretion during wk 1 to 30 of lactating multiparous (n =16) and primiparous (n=15) cows fed a Control (n=10), XPY (n=11) and P169+XPY (n=10) rations. Data were pooled across treatments within multiparous and primiparous groups. Means (±SEM) were affected by parity (P=0.0001)

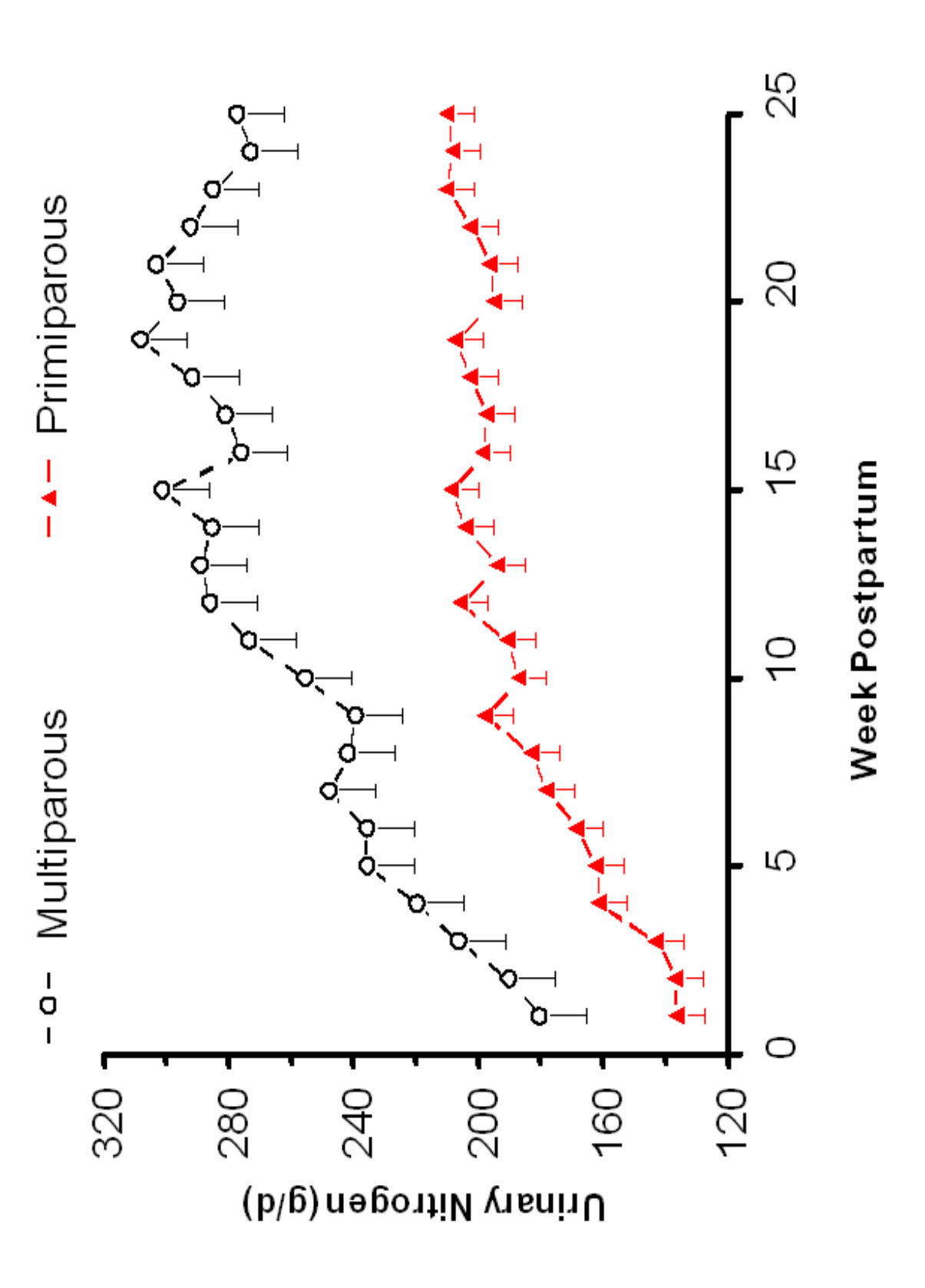

Multiparous
Primiparous
Urinary Nitrogen (g/d)
320
280
240
200
160
120
0
5
10
15
20
25
Week Postpartum

Figure 15. Effect of feeding XPY and P169+XPY on somatic cell counts (SCC x 10^3/ml) during wk 1 to 30 of lactating cows fed a Control (n=10), XPY (n=11) and P169+XPY (n=10) rations. Data were pooled across parity within treatments. Means (±SEM) were different (P=0.01) among treatment groups.

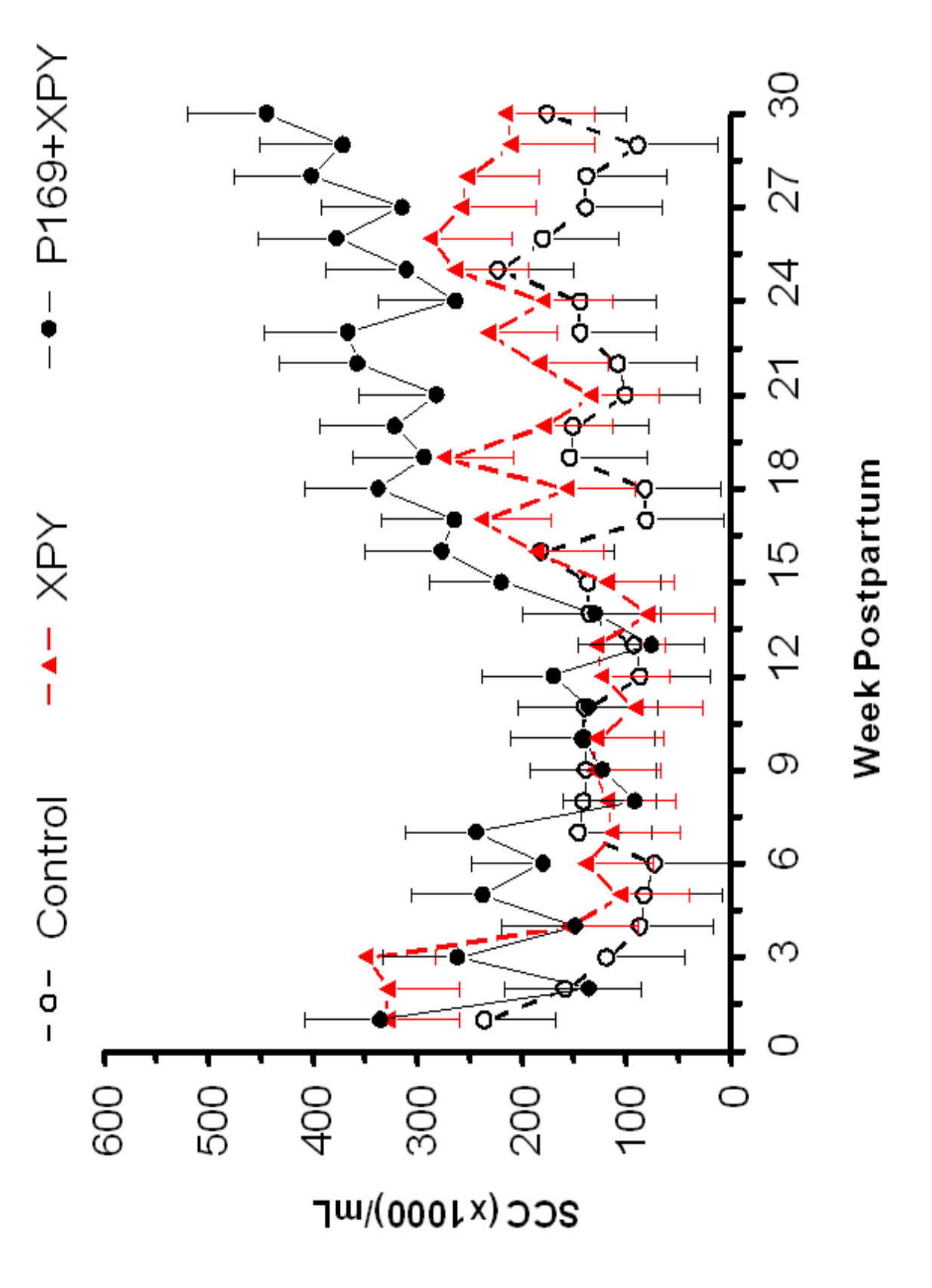

Control
XPY
P169+XPY
SCC (x1000)/mL
0
100
200
300
400
500
600
0
3
6
9
12
15
18
21
24
27
30
Week Postpartum

Figure 16. Effect of feeding XPY and P169+XPY on weekly body weight changes during 25 wk of lactation. Top Panel: Weekly body weight in multiparous cows (n=16) fed Control (n=6) and XPY (n=5) and P169+XPY (n=5) rations from wk 1 to 25. Bottom Panel: Weekly body weight for primiparous cows fed Control (n = 4) and XPY (n=6) and P169+XPY (n=5) rations from wk 1 to 25. Means (±SEM) were affected (P=0.006) by treatment x parity x week interaction.

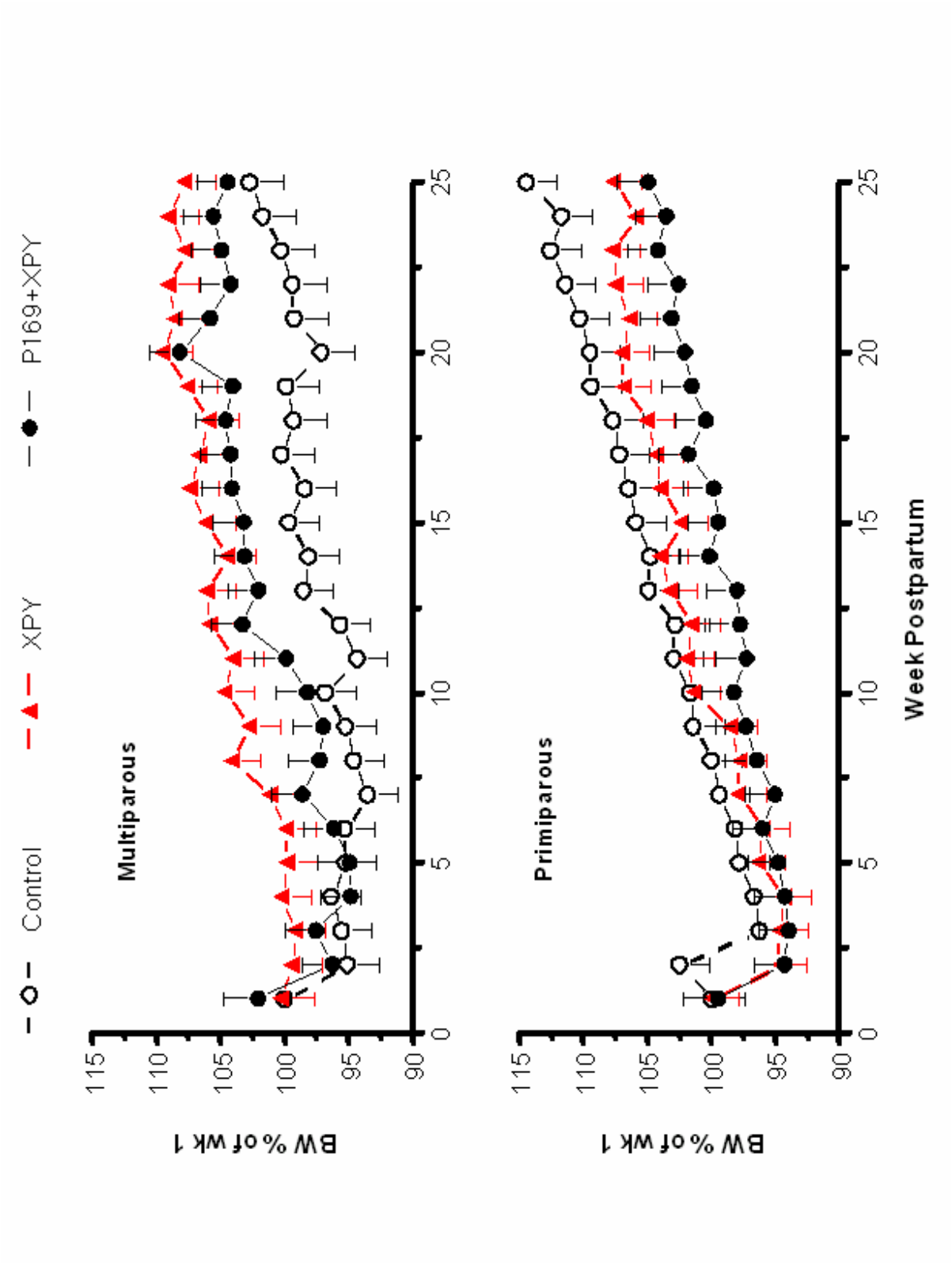

Control
XPY
P169+XPY
Multiparous
Primiparous
BW % of wk 1
Week Postpartum
115
110
105
100
95
90
0
5
10
15
20
25

Figure 17. Milk glucose concentrations between wk 23 and 24 after feeding Control, XPY or P169+XPY rations. Milk samples were collected at sequential a.m. and p.m. milkings on one day during two sequential weeks. Asterisk (*) mean differs from Control and XPY means (P=0.01).

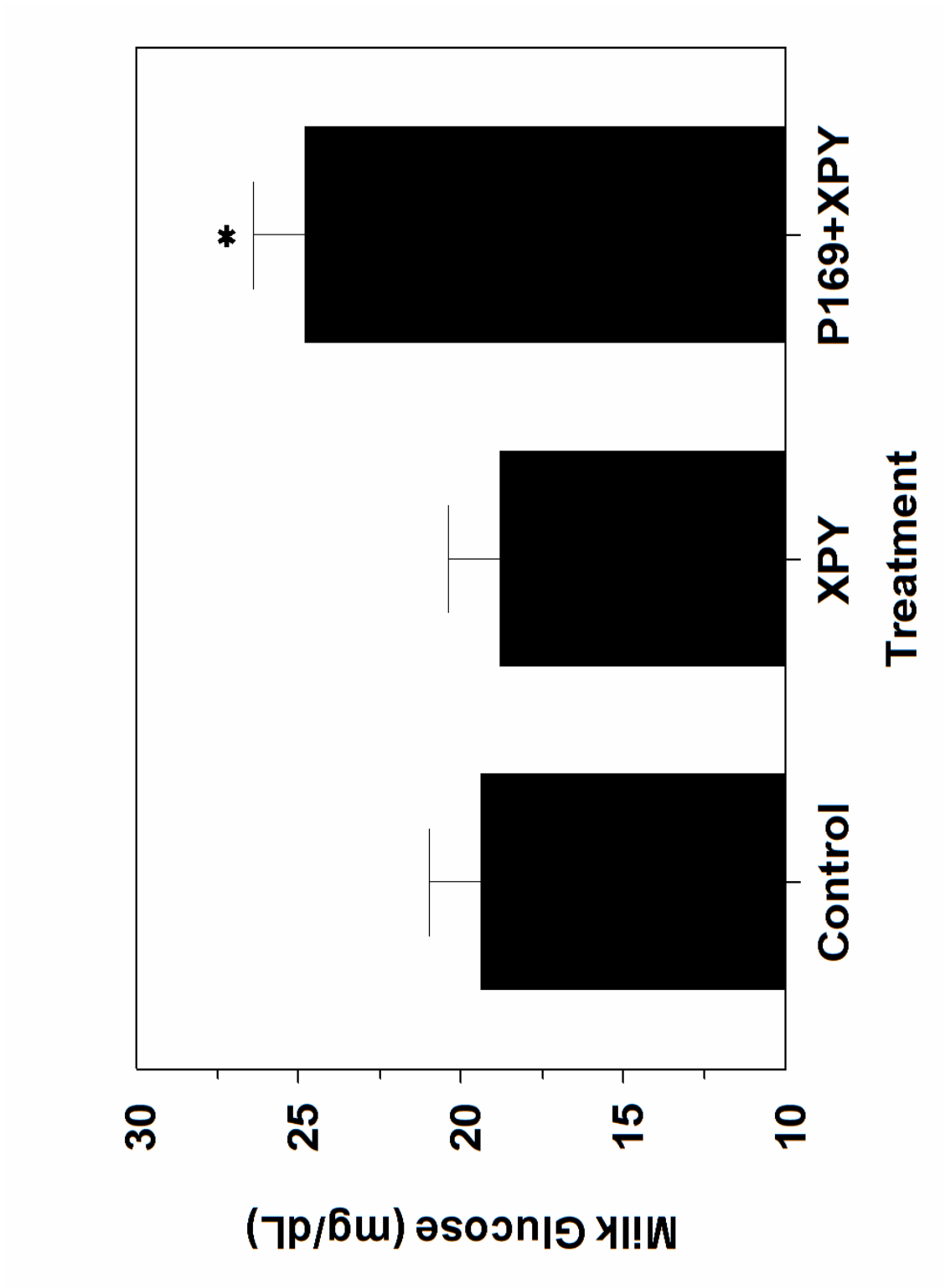

Milk Glucose (mg/dL)
30
25
20
15
10
*
Control
XPY
P169+XPY
Treatment

Figure 18. Diurnal changes in plasma glucose concentrations after feeding Control, XPY or P169+XPY rations on wk 27 of lactation. No significant effect of treatment was observed (P=0.15).

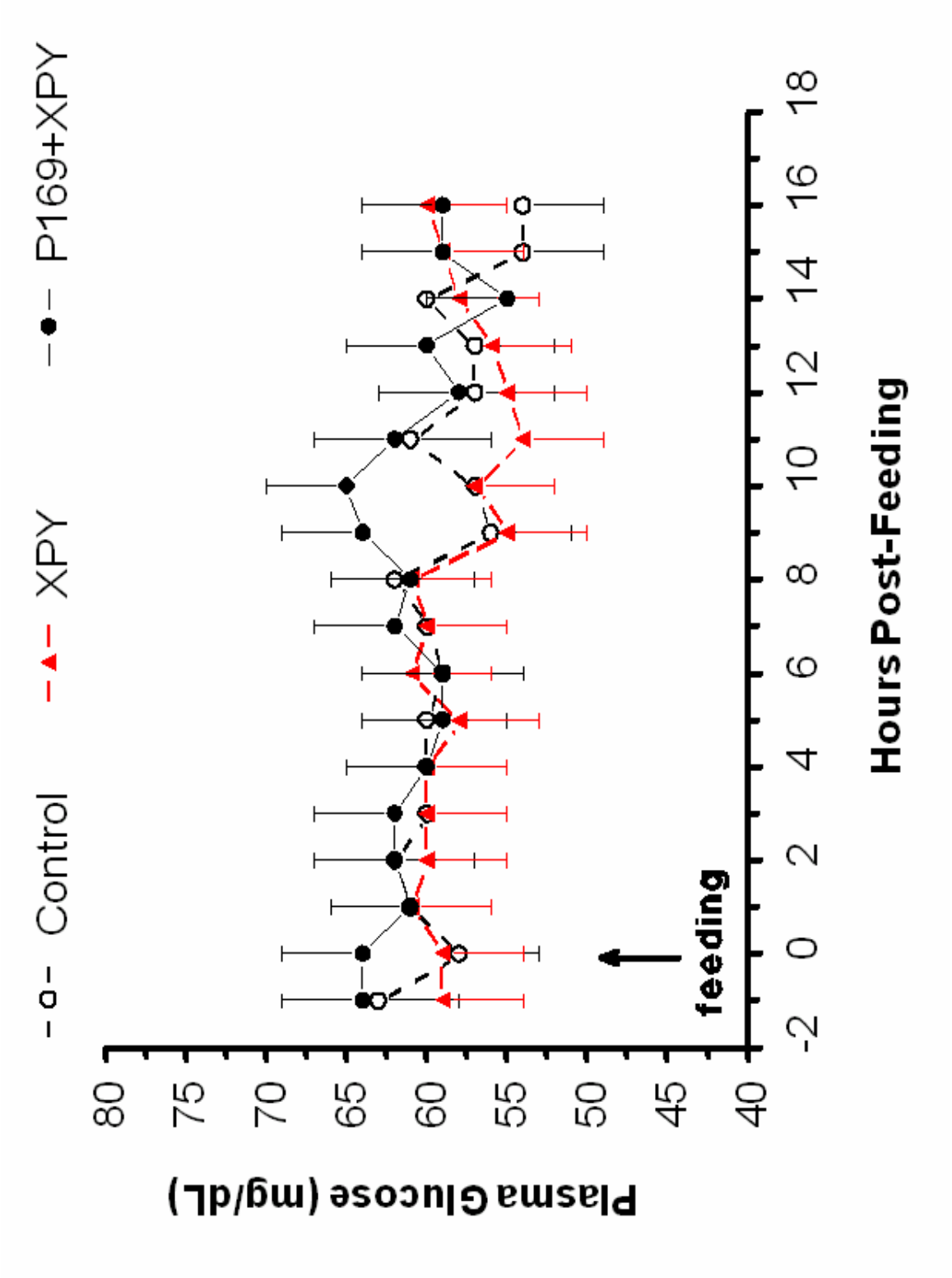
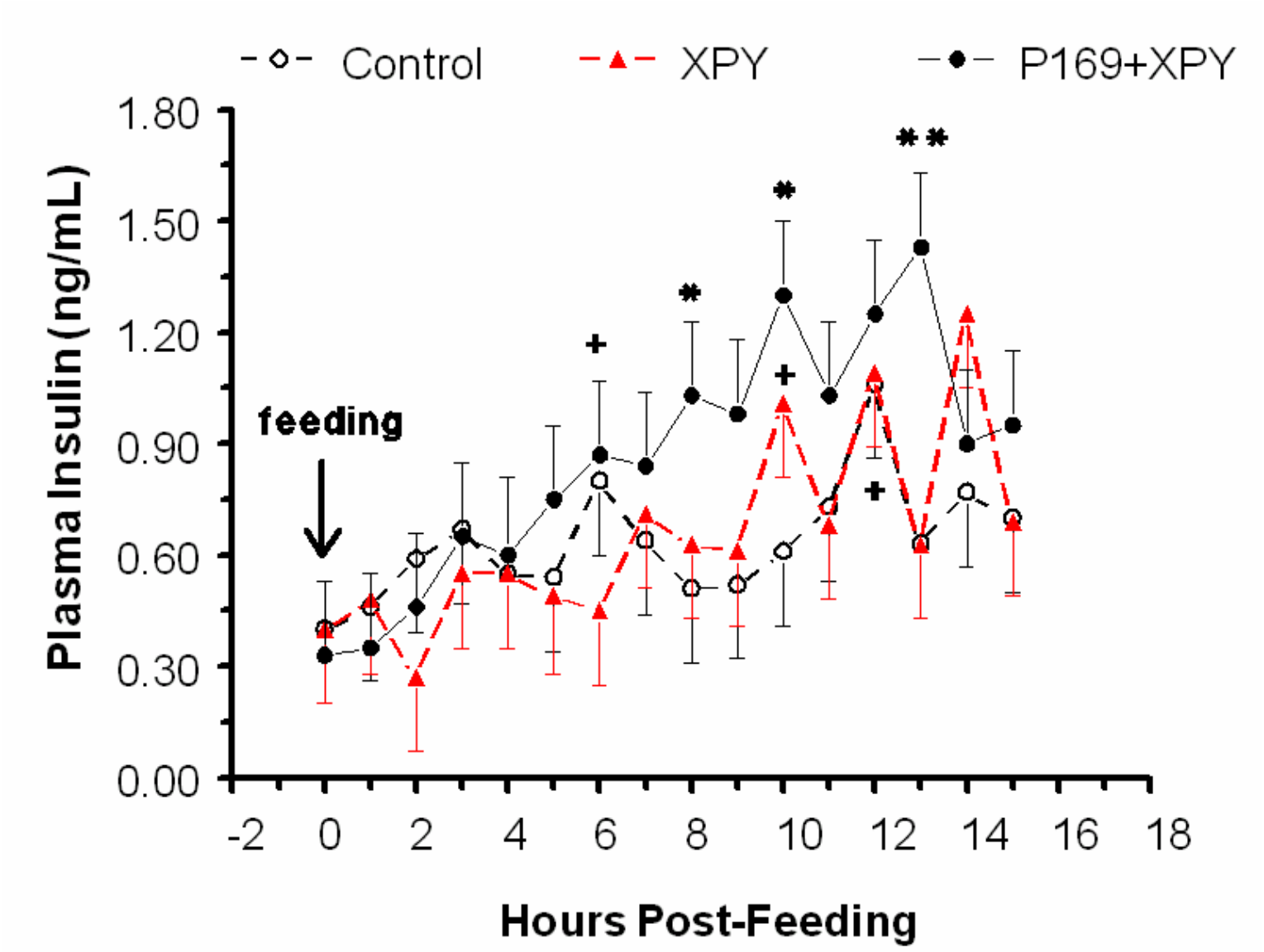

Control
XPY
P169+XPY
Plasma Glucose (mg/dL)
80
75
70
65
60
55
50
45
40
feeding
-2
0
2
4
6
8
10
12
14
16
18
Hours Post-Feeding

Figure 19. Diurnal changes in plasma insulin after feeding Control, XPY or P169+XPY rations on wk 27 of lactation. Asterisk (*) indicates mean differs (P=0.01) from its respective Control value. Plus (+) indicates first mean to differ (P=0.05) from 0 h (treatment x time; P=0.05).


Control
XPY
P169+XPY
Plasma Insulin (ng/mL)
1.80
1.50
1.20
0.90
0.60
0.30
0.00
feeding
-2
0
2
4
6
8
10
12
14
16
18
Hours Post-Feeding

CHAPTER IV

EFFECTS OF FEEDING YEAST CULTURE AND PROPIONIBACTERIA TO BEEF STEERS ON DIGESTION

Abstract

An experiment was designed to evaluate the effects of feeding yeast culture (XPY), Propionibacteria (P169) or their combination on feed intake, ruminal and postruminal digestion, fermentation profiles, duodenal nutrient flow and microbial nitrogen flow of steers fed sorghum based silage total mixed ration (TMR). Twelve ruminally and duodenally cannulated Angus x Hereford steers (initial BW=537.8±31.9 kg) were randomly assigned (based on their BW) to one of the four treatments as follows: 1) Control (n=3), fed a sorghum silage-based TMR; 2) XPY (n=3), fed Control TMR plus XPY (at 56 g/head/d; n=3); 3) P169 (n=3), Control TMR plus P169 (at 6 x 10^{11} cfu/head/d); and 4) P169+XPY (n=3), Control TMR plus P169+XPY (at 6 x 10^{11}/head/d and 56 g/head/d, respectively). Each of the two periods lasted for 21 d: d 1 to 15 for adaptation and d 16 to 21 for fecal, duodenal, ruminal and blood sample collection. Steers were individually fed each daily treatment via mixing it with a small amount of TMR (4.5 kg/head) once per day (a.m.), allowed about 2 h to consume this small amount and then fed additional TMR ad-libitum. XPY tended to decrease ($P \leq 0.07$) total organic matter, ADF and N intake and decreased (P=0.05) NDF intake. However, XPY tended to increase ($P \leq 0.10$) total tract digestibilities of OM, N, NDF, ADF in Controls but not

P169-fed steers. Ruminal digestibility, duodenal flow, microbial N synthesis, fluid dilution rate and particulate passage rates for OM, N, NDF, ADF were not affected ($P \geq 0.11$) by dietary treatments. Feeding P169 ($P=0.07$) increased propionate by 14% above Control and XPY steers and decreased ($P=0.05$) acetate: propionate concentrations by 15% compared to Control fed steers. Plasma glucose and insulin concentrations were not affected ($P \geq 0.35$) by dietary supplementation. High plasma glucose concentrations indicate that these steers were well fed. These results imply that feeding P169 might alter ruminal metabolism toward increased propionate without affecting feed intake, nutrient digestibilities, duodenal flow, rumen kinetics or microbial nitrogen synthesis by beef steers. Although feeding XPY improved total tract digestibilities of OM, N, ADF, and NDF, XPY decreased nutrient intake of these well fed steers. Additional studies with larger numbers of experimental units and a longer adaptation period are warranted.

Introduction

Insufficient DMI during early lactation of many high producing dairy cows predisposes the animals to a state of negative energy balance (Spicer et al., 1990). Ruminal propionate is the single most important substrate for gluconeogenesis (Drackley et al., 2001; Kung, 2001). Estimates by Seal and Reynolds (1993) indicate that propionate supplies 32 to 73% of glucose demands. Both drenching (Grummer et al., 1994) and feeding propylene glycol (Christensen et al., 1997) have decreased NEFA and increased plasma insulin and ruminal propionate concentrations, all of which are beneficial to combating the extent and duration of negative energy balance, fatty liver and ketosis. Effects of propylene glycol are partially mediated through the observed increases in

ruminal propionate (Grummer et al., 1994; Christensen et al., 1997), providing a reason to feed propionate during the transition period. Also, feeding Propionibacteria strain 169 (P169) in Holstein dairy cows has been shown to increase plasma leptin (Francisco et al., 2002) and increased ruminal propionate levels (Stein et al., 2006).

Little evidence exists in the literature regarding the effects of feeding propionate on DMI, digestibility and duodenal bacterial nitrogen flow as well as microbial protein synthesis. However, previous studies have demonstrated that ruminal propionate infusion decreased DMI during early and mid lactation (Shepherd and Combs, 1998; Oba and Allen, 2003) and decreased in-vitro fiber digestibility (Akay and Dado, 2001). Francisco et al. (2002) reported that cows fed supplemental P169 had improved energy balance at wk 1 of lactation and had lower DMI per kg BW than Control cows between wk 3 and 12 of lactation. Raeth-Knight et al. (2007) reported no effect of supplementing mid-lactation cows with DFM products containing *Lactobacillus acidophilus* and *Propionibacteria freudenreichii* on cow performance, diet digestibility, or rumen fermentation.

Yeast cultures have been fed to dairy cattle for more than 60 yr with varied responses (Schingoethe et al., 2004). In some studies, yeast cultures improve DMI (Williams et al., 1991; Wohlt et al., 1991; Dann et al., 2000) and milk production (Wang et al., 2001), whereas other studies (Arambel and Kent, 1990; Soder and Holden, 1999) showed no response to yeast cultures. In vitro experiments have reported that in some cases, *S. cerevisiae* culture favorably altered the mixed ruminal microorganism fermentation and stimulated lactate uptake and cellulose digestion by pure cultures of predominant bacteria (Nisbet and Martin, 1991; Callaway and Martin, 1997). Even though the effects of *S. cerevisiae* are not always consistent (Martin and Nisbet, 1992),

several modes have been proposed regarding the stimulatory effects of yeast culture on ruminal fermentation (Lyons et al., 1993; Wallace, 1994).

Recently, Stein et al. (2006) feeding P169 in conjunction with yeast culture, reported 8% increase in milk production, increased ruminal propionate and increased milk protein percentage by Holstein dairy cows compared to the Controls. Therefore, whether P169+XPY (Diamond V-XP) improves the flow of microbial cell protein to the duodenum, spares glucogenic amino acids or both to increase milk protein, will require further studies.

The objective of our proposed experiment was to determine whether yeast culture, P169 or their combination would improve DMI, ruminal fermentation and (or) site of digestion, microbial protein synthesis as well as duodenal bacterial nitrogen flow in beef cattle.

Materials and Methods

Animals and Treatments

This experiment was conducted at the Nutrition Physiology Research Center, Stillwater, OK, in accordance with an approved Oklahoma State University Animal Care and Use Committee protocol. Twelve ruminally and duodenally cannulated Angus x Hereford crossbred steers (initial BW=537.8±31.9 kg) were stratified by weight and randomly allotted to 1 of 4 treatments in a Completely Randomized Design experiment consisting of two periods. During the experiment, steers were housed in individual indoor pens (3 m x 4 m) with ad-libitum access to fresh water. Treatments included: 1) Control, fed a sorghum silage-based total mixed ration (TMR); 2) XPY, fed Control TMR plus XPY (at 56 g/head/d); 3) P169, Control TMR plus P169 (at 6 x 10^{11} cfu/head/d); and 4)

P169+XPY, Control TMR plus P169 and XPY (at 6 x 10^{11}/head/d and 56 g/head/d, respectively). Steers were fed each treatment daily via mixing it with a small amount of TMR (4.5 kg/head) once per day (a.m.), allowed about 2 h to consume this small amount and then fed additional TMR ad-libitum. Individual feed bunks were used to monitor feed intake.

Experimental period was 21 d in length. Day 1 through 15 was the diet adaptation period and d 16 through 21 was the sampling period. On d 1 of each period, animals were weighed and were placed in 3 m x 4 m pens. Ten days prior to duodenal digesta sampling, gelatin capsules containing 7.5 g of chromium oxide (indigestible marker) were placed directly in the rumen at 0800 and 1700 (15 g/steer/d) to enable prediction of fecal output (Merchen, 1988). The steers were moved into individual metabolism stalls two days prior to sampling time of each period.

Sample Collection and Preparation

Diets were weighed out daily and fed to steers on an individual basis following the treatment feeding each morning to allow ad-libitum intake. Samples of the diet were collected at feeding and frozen (-20°C) until dried. Orts were weighed daily, recorded, sampled daily from each steer and sub-sampled at the end of each period and stored frozen (-20°C) until samples were dried in a forced-air oven (60°C) for 72 h and ground in a Wiley mill in order to pass through a 1-mm screen. Diet and orts samples were composited by steer within period.

Approximately 250 mL of duodenal digesta was collected every 6 h on d 16 (0200, 0800, 1400 and 2000 h), on d 17 (0400, 1000, 1600 and 2400 h) and on d 18 (0600, 1200, 1800 and 2400 h) to represent every 2 h over a 24 h time period. Samples

were frozen immediately, freeze dried (lyophilized), ground, and composited by steer within period until further lab analysis.

Ruminal kinetics were evaluated on d 19 and 20. At 0800 h on d 19 of each period, a 200 mL solution containing Co-EDTA was immediately pulse-dosed before the feeding via the ruminal cannula as a fluid dilution marker for determination of fluid passage rate (K_f). About 50 mL of ruminal fluid samples were collected from the ventral rumen of each steer using a suction strainer before dosing (0 h) and at 3, 6, 9, 12, 18, and 24 h after dosing to determine VFA, ammonium N and cobalt concentrations for determining liquid dilution rate. Immediately after straining of the ruminal fluid, the pH of ruminal fluid was determined using a portable pH meter. Eight milliliters of strained ruminal fluid was then acidified with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen (-20 °C) until analysis for VFA content. Ten milliliters of strained ruminal fluid was acidified with 0.5 mL of 6 N HCl and frozen (-20 °C) until NH_3 N analysis. An extra 10 mL of strained ruminal fluid was also frozen immediately after collection for Co concentration determination.

On d 16 through d 20 fecal samples were collected twice daily, frozen (-20 °C), oven dried (60 °C, 72 h), ground (1-mm screen) and stored at room temperature for subsequent analyses. Total ruminal contents were evacuated on d 21. Rumen contents were weighed, mixed thoroughly and sub-sampled in duplicate (approximately 1 kg, as is basis per sample). The control steers' ruminal contents were subdivided and placed into the rumen of the steers in order to minimize carryover effects from period to period (Beauchemin et al., 2003). About 1 kg of ruminal contents was mixed with 1 L of a formaldehyde solution (100 mL of 37% formaldehyde and 9 g NaCl in 900 mL dH_2O)

and stored frozen (-20 °C) for subsequent bacterial isolation to determine purine:N ratio in the ruminal bacteria. Concurrently, subsamples of ruminal contents were weighed, dried (60 °C, 72 h), ground (1-mm screen), and stored at room temperature for further analyses. The 1-kg sample mixture that was mixed with formaldehyde was later refrigerated at 4 °C for at least 24-h, mixed and homogenized in a blender (Waring Products, New Hartford, CT) at high speed for 2 min, and strained through 2 layers of cheese-cloth to remove large particles. The liquid fraction was centrifuged (1500 x g; 10 min, 4 °C) in 250-mL bottles to separate protozoa and feed particles from bacteria. Supernatant was decanted into additional 250-mL bottles and bacteria was pelleted by centrifuging (20,000 x g; 20 min, 4 °C). The supernatant was decanted and discarded, leaving only the bacteria pellet. The bacteria pellet was re-suspended with about 100 mL of 0.9% (wt/vol) NaCl and centrifuged (20,000 x g; 20 min, 4 °C). Through this process, bottles of bacteria from each steer were combined into a single sample. This resulted in each initial bacteria sample receiving 3 rinses. Bacteria were then frozen (-20 °C), lyophilized, and ground with mortar and pestle before analysis.

Blood samples were colleted via coccygeal venipuncture at a time of ruminal sample collection (0 h and 3 h). After blood collection in vacutainer tubes containing EDTA, blood was stored on ice, transported to the lab and centrifuged at 1200 x g for 15 min at 4°C. Plasma was harvested and stored at -20°C until subsequent analysis of concentrations of plasma glucose and insulin.

Laboratory methods

All composited samples (feeds, orts, rumen contents, duodenal digesta, and fecal samples) were analyzed for DM, ash, NDF, ADF, N and acid detergent insoluble ash

(ADIA). Dry matter for all samples was determined by oven drying at 105 °C for 24 h. Ash content was determined by ashing samples at 550 °C for 8 h in a muffle furnace (AOAC, 1990). Nitrogen was determined using a Leco NS-2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Neutral detergent fiber and ADF were determined using the method of Goering and Van Soest (1970). Acid detergent insoluble ash was determined as the residue following complete combustion of the ADF residue (Van Soest et al., 1991).

Duodenal and fecal samples were also analyzed for Cr concentrations to determine digesta flow throughout the gastrointestinal tract. The samples were prepared using the procedure of Williams et al. (1962) and Cr levels determined by an Inductively Coupled Plasma Analyzer (ICP Spectro Analytical Instruments, Fitchburg, MA). Duodenal and bacterial isolates were analyzed for purine levels to determine microbial protein flow using a modified Zinn and Owens (1986) procedure that used a diluted $HClO_4$ to hydrolyze the material containing purines. The perchloric acid (70%) was diluted with water to prepare a solution of 2 M $HClO_4$.

For determination of VFA concentrations, acidified ruminal fluid samples were thawed at room temperature and centrifuged to pellet solids (10 min, at 3,000 x g). Samples of the supernatant (2 ml) were filtered through 0.2 µm filter directly into a 2 ml HPLC auto-sampler vials and capped. Samples were analyzed by a HPLC using a Waters 2690 with a 2410 refractive index detector (Waters Corporation, Milford, MA). Samples were injected into 5 mM H_2SO_4 mobile phase heated to 60°C and separated using a Bio-Rad HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, California). Peak areas were used to determine compound concentration by comparing with external

standards. The external standard solution was prepared volumetrically with glucose, lactic acid, succinic acid, butyric acid, propionic acid and glacial acetic acid. Ruminal NH_3 concentration was determined colorimetrically on a Beckman DU 530 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA; Broderick and Kang, 1980). Non-acidified ruminal fluid samples were thawed and centrifuged at 30,000 x g for 20 min, and the supernatant fluid was analyzed for Co concentration (atomic absorption spectroscopy; Model 4000, Perkin Elmer, Norwalk, CT; with an air plus acetylene flame).

Plasma concentrations of glucose were determined by using colorimetric glucose kits (Thermo Electron Corporation, Louisville, CO) as previously described (Aleman et al., 2007).

Plasma concentrations of insulin were determined by using solid-phase insulin RIA Kit (Micromedia Insulin Kit, ICN Biomedicals Inc., Costa Mesa, CA) as previously described (Francisco et al., 2002).

Calculations and Statistical Analyses

Apparent ruminal digestibility of nutrients was calculated by subtracting nutrient flow at the duodenum from nutrients consumed. Particulate passage was determined by dividing daily ADIA intake by ADIA ruminal fill. Liquid dilution rates were determined using the REG procedure of SAS to regress the natural logarithms of Co concentration against time (Grovum and Williams, 1973). The resulting slopes represented liquid dilution rates. Ruminal liquid turnover time was calculated as the inverse of the dilution rate. Flow (g/d) of bacterial N at the duodenum was estimated by dividing the average

bacterial N:purine ratio of harvested bacteria by the N:purine ratio of the duodenal digesta and multiplying the quotient by the daily N flow at the duodenum (Erasmus et al., 1992). The measurements described the effect of XPY, P169 or their combination on nutrient digestibility and microbial synthesis in the rumen and the resulting nutrient supply to the small intestine.

The feed intake, digestibility, fill, particulate flow rate, ruminal liquid dilution, as well as ruminal liquid volume data were analyzed as a 2 x 2 factorial arrangement of treatments utilizing the MIXED procedure of SAS containing the effects steer, period, XPY, P169 and XPY x P169 interaction. Ruminal pH, VFA, NH_3, plasma glucose and insulin were analyzed as 2 x 2 factorial arrangements of treatments with repeated measures over time. The covariance structure was autoregressive lag=1. The level of significance was set at $P \leq 0.05$ and $P \leq 0.10$ for a trend. If any interaction was significant, simple effects were analyzed using slice option for the LSMEANS statement. Conversely, main effects were analyzed using LSMEANS with the DIFF option if the interaction was not significant.

Results

Intake, fecal output and total tract digestibility

XPY tended to decrease (P≤0.07) OM intake, ADF intake, and N intake and decreased (P=0.05) NDF intake (Table 2). Data showing the effects of diet on fecal output and in-vivo apparent OM, NDF, ADF and N digestibilities are summarized in Table 2. XPY tended to decrease (P≤0.09) fecal OM, NDF and ADF output in Controls but not P169-fed steers. XPY tended to increase (P≤0.10) total tract digestibilities of OM, N, NDF and ADF in Controls but not P169-fed steers (Table 2).

Ruminal digestibility, duodenal nutrient flow, Microbial N synthesis and passage rate

There were no treatment (P≥0.14) effects on ruminal digestibilities and duodenal nutrient flow of OM, N, NDF and ADF (Table 2). However, steers fed XPY alone had numerically greater ruminal digestibilities of OM, N, ADF and NDF vs. other treatment groups. Nitrogen from dietary origin, microbial N synthesis, microbial efficiency (MOEFF) and microbial N as a percentage of intake were not different (P ≥0.11) for all treatments (Table 3).

Ruminal kinetics and ruminal fill

Particulate passage rate of ruminal contents as measured using ADIA as an indigestible marker did not differ (P≥0.27) among treatment groups. Fluid dilution rate and turnover time did not differ (P≥0.35) among treatment groups (Table 4). However, rumen fluid volume was decreased (P=0.05) in steers fed XPY vs. P169 and Control groups. Ruminal contents tended (P≤0.07) to decrease in XPY vs. P169 fed steers (Table 4).

Ruminal pH

Ruminal pH was influenced by time ($P \leq 0.001$) and time x P169 x XPY treatment interaction ($P \leq 0.01$) but not ($P \geq 0.47$) treatment. In general, the pH decreased ($P=0.0001$) with time after feeding, and the lowest (6.08 ± 0.08; $P \leq 0.05$) pH was observed at 12 h in all steers (Figure 1). This difference was most evident in steers receiving P169 with XPY than those fed P169 without XPY (Figure 1). The dietary treatment means across sampling times are presented in Table 5.

Ruminal NH_3N

Ruminal NH_3N was not influenced ($P \geq 0.13$) by feeding P169 or XPY or their interaction ($P \geq 0.99$) Table 5. However, ruminal NH_3N was influenced ($P = 0.0001$) by time of feeding. Ruminal NH_3N concentrations gradually decreased between time 0 and 9 h post-feeding. Thereafter, a significant increase ($P \leq 0.05$) in ruminal NH_3N was seen between 12 and 24 h post-feeding (Figure 2).

Ruminal fermentation

The total and individual VFA concentrations in the rumen are summarized in Table 5. There was no effect of P169 or XPY treatment ($P \geq 0.66$) or their interaction ($P \geq 0.96$), or time x treatment interaction ($P=0.19$) on total concentration of VFA. However, as expected, time after feeding affected ($P=0.0002$) concentrations of total VFA (Figure 3). In general, increases were observed between 0 h and 9 h post-feeding, but no significant changes occurred between 12 and 24 h post-feeding.

Feeding P169 tended ($P=0.07$) to increase propionate concentrations by 14% above Control and XPY-fed steers (Table 5). The ratio of acetate: propionate was lower

(P=0.05; 15%) for P169+XPY than Control and XPY-fed steers (Table 5). In general, acetate: propionate ratio decreased (P=0.05) between 0 and 12 h after feeding and increased thereafter (Figure 4).

Ruminal concentrations of lactate were extremely low and most samples were below the level of detection (0.01 mol/100 ml). Dietary treatments used in this study did not affect the concentrations of acetate, butyrate, valerate, isobutyrate, and isovalerate (Table 5). However, acetate concentration responded with a time x P169 x XPY interaction (P=0.032; Figure 5).

Plasma Glucose

Plasma glucose concentration was not affected (P≥0.32) by P169 or XPY dietary treatments, time (P=0.35) or their interaction (P=0.73). Plasma glucose concentrations in steers fed Control, XPY, P169 and P169+XPY averaged 86.7, 81.2, 82.2 and 79.9±3.8 mg/dL, respectively (Figure 6).

Plasma Insulin

Plasma insulin concentration was not affected by feeding P169 (P=0.39) or XPY (P=0.49), or time (P=0.14), or time x P169 x XPY interaction (P=0.73). Plasma insulin concentration in steers fed Control, XPY, P169 and P169+XPY averaged 1.36, 0.92, 1.35 and 1.41±0.27 ng/ml for Control, XPY, P169 and P169+XPY cows, respectively (Figure 7).

Discussion

In general, the mode of action for supplementation of direct-fed microbials varies based on type of direct-fed microbial and substrate used (Lynch and Martin, 2002), physiological condition of an animal (e.g., stage of lactation), feeding strategy and forage

to concentrate ratio (Kellems et al., 1990). In the present study, feeding yeast culture and Propionibacteria in conjunction with yeast culture decreased OM, N, NDF and ADF intake above Control and P169-fed steers. Feeding Propionibacteria alone did not affect feed intake. Raeth-Knight et al. (2007) feeding direct-fed microbial containing *Lactobacillus acidophilus* and *Propionibacteria freudenreinchii* to Holstein dairy cows in midlactation reported similar results. In contrast, Francisco et al. (2002) reported that feeding Propionibacteria (similar strain, P169) to early lactation Holstein cows decreased DMI per kg of BW. In addition, intraruminal studies have demonstrated that propionate infusion decreased DMI during early to mid lactation (Shepherd and Combs, 1998; Oba and Allen, 2003). The specific effects of propionate on regulating DMI are not clear (Allen, 2000). However, Baile (1971) proposed that propionate receptors in the ruminal region might function to control feed intake. Also propionate is a major stimulant of insulin secretion in cattle (Bines and Hart, 1984). Increased insulin concentrations activates neurons that stimulate anorexic neuropeptides such as proopiomelanocortin that lead to decreased neuropeptide Y and agouti related peptides and subsequently, a decrease in feed intake (Niswender and Schwartz, 2003). Therefore, lack of insulin response to Propionibacteria supplementation might have contributed to lack of response in nutrients intake. Decreased OM, N, NDF and ADF intake by feeding yeast culture is not clear. However, similar studies (Erdman et al., 1989; Kung et al., 1997; Swartz et al., 1994) found no effect of feeding *S. cerevisiae* on DMI while Harris et al. (1992) reported lower DMI in early to midlactation when cows were fed yeast culture. In contrast, Williams et al. (1991) and Wohlt et al. (1991) found that cows fed *S. cerevisiae* produced more milk due to higher feed intake during early lactation. The effects of yeast culture are

not always consistent (Martin and Nisbet, 1992). Some studies reported increased DMI (Huber, 1998) and feed efficiency (Gomez-Alarcon et al., 1990) when yeast was fed during heat stress suggesting the role of yeast in improving appetite during time of stress. This led Arambel and Kent, (1990) to report that yeast culture is best utilized by animals under stress because the nutrient demands are high at that time. Finally, it is also possible that the amount and timing of supplementation of the ration may have not been optimal or long enough to elicit an effect or remove carry-over effects. Erasmus et al. (1992) reported that supplemental yeast increased DMI using 75 d period to eliminate any carry-over effect and allow steers to adapt completely to the yeast culture as opposed to 21 d used in this study. Alternatively, it could be that the Control diet, which was a typical of diet used for lactating cows, provided adequate nutrients for maximum metabolism such that any extra supplementation of P169 or XPY added to a diet did not elicit additional benefit to steers. Infect, Arambel and Kent (1990) reported that yeast increases nutritional value of poor quality forages and high grain diets.

Feed intake affects microbial efficiency because of its effect on passage rate and microbial turnover. Increased levels of intake generally result in increased ruminal dilution rate and decreased turnover time (Vanzant and Cochran, 1994). This decreased turnover time is expected to decrease cellular maintenance costs since the microbes are spending less time in the rumen (Dewhurst et al., 2000). Feeding Propionibacteria, yeast culture or their combination in the present study did not affect particulate passage rate, fluid passage rate or turnover time. Similar observations were reported with *S. cerevisiae* treatment (Williams and Martin, 1990). In ruminants, fluid dilution rate (percentage of fluid volume leaving the rumen per unit time) has been shown to be related to the

efficiency of microbial growth. To date, there is no information on effect of Propionibacteria on ruminal kinetics.

Many cellulolytic bacteria require and use ammonia as their only source of nitrogen (Russell et al., 1992). The accepted adequate level of ruminal ammonia for maximum fiber digestion by ruminal microbes is approximately 2 to 5 mg/100 ml (Satter and Slyter, 1974). The high levels of ruminal ammonia do not always imply the most efficient growth of bacteria. In-fact, Horn and McCollum (1987), reported that ruminal ammonia concentrations are more indicative of the balance between ruminally available energy and degradable protein. Ruminal concentration of NH_3 was not affected by dietary treatment but was affected by sampling time in the present study. Other studies (Harrison et al., 1988; Erasmus et al., 1992) reported decreased ruminal ammonia after yeast culture supplementation and no effect when a mixture of *Lactobacillus acidophilus* and *Propionibacteria freudenreinchii* was fed (Raeth-Knight et al., 2007).

Neither of the treatments influenced the total VFA, concentrations of acetate, butyrate, isobutyrate, isovalerate and valerate. However, steers receiving P169 increased ruminal propionate by 14% compared with the Control and XPY groups. As a result of a higher propionate concentration, the acetate: propionate ratio was decreased by 15% in P169+XPY supplementation versus Control. Williams and Newbold (1990) concluded that lower acetate: propionate ratio when yeast culture is fed is probably the result of increased production of propionate rather than decreased production of acetate. Increase in propionate in P169+XPY-fed steers seen in this study vs. other treatment groups is consistent with the expectation that supplemental yeast culture potentiates Propionibacteria and other rumen lactate utilizing bacteria (e.g., *selenomonas*

ruminantium) to produce more propionate by providing soluble growth factors that benefit the lactate utilizing bacteria. Francisco et al. (2002) reported a non-significant increase in milk production when P169 was included in the diet of early lactating Holstein cows for a period of 12 wk and suggested that a longer adaptation period or higher dose was needed to elicit any effect. Previously, inclusion of Propionibacteria in lactating cow or steer diets increased propionate (Kim et al., 2000; Stein et al., 2006). Other studies also reported that the total VFA, propionate, acetate, butyrate and valerate were increased above Control at all levels (0, 10^3, 10^6, and 10^9 cfu/ml) of inclusion of Propionibacteria P5 (Akay and Dado, 2001). Cellulolytic microorganisms require branched chain VFA as a substrate for growth (Loest et al., 2001a). Harrison et al. (1988) fed *S. cerevisiae* to lactating cows and reported that ruminal pH, acetate concentration and molar acetate to propionate ratio all were lower but molar proportions of propionate and valerate were increased. Wallace and Newbold (1992) further support the idea that the responses of yeast culture are highly variable and apparently influenced by the composition of the diet. For example, when alfalfa hay served as the substrate, neither the *S. cerevisiae* culture nor the live cells had an effect on propionate, butyrate, or acetate: propionate ratio but both concentrations (0.35 and 0.73 g/L) of *S. cerevisiae* live cells and 0.73 g/L of *S. cerevisiae* culture decreased the acetate: propionate ratio (Lynch and Martin, 2002) when soluble starch was the substrate.

In the present study, the ruminal pH averaged across sampling time did not differ among treatments but decreased with time after feeding with nadir pH observed at 12 h after feeding. In support of this study, Ghobani et al. (2002), reported that ruminal pH was not affected by treatment (Propionibacteria, P15, and P15+EF212 (*Enterococcus*

faecium)) compared with the Controls and the lowest pH values occurred between 11 to 13 h after feeding. Also, Kim et al. (2000) feeding *Propionibacterium acidipropionici* and Lactobacillus plantarum to steers made similar observations. Gall et al. (1949) indicated that there were no differences in ruminal pH between ruminant species fed similar basal diet. Gall et al. (1949) further showed that the ruminal pH that was not contaminated with saliva ranged from about 6.3 to 7.3 in both cattle and sheep. Prigge et al. (1984) reported similar results. Therefore, the ruminal pH observed in the current study fall within the acceptable range.

Increased bacterial numbers that lead to greater lactate uptake and increased fiber digestion in the rumen has been one of the most consistently reported effects in animals fed Diamond V-XP, yeast culture (Wiedmeier et al., 1987; Harrison et al., 1988). However, Weidmeier et al. (1987) found with non-lactating cows that feeding *S. cerevisiae* had increased total tract digestibility for crude protein and hemicellullose but not for dry matter or ADF. When *S. cerevisiae* and *A. oryzae* were fed together, total tract digestibility of dry matter, crude protein and hemicellulose were all increased. In the present study, XPY supplementation significantly increased nutrient total tract digestibility. However, nutrient ruminal digestibility was numerically greater in XPY- fed steers compared to other groups. Previously, feeding Propionibacteria strain P5 decreased NDF digestibility when compared to Controls (Akay and Dado, 2001). The decrease in NDF digestibility was attributed to the carrier which had high starch content that accounted for up to 2.8% of the DM. Conversly, Raeth-Knight et al. (2007) reported no effect of feeding *Propionibacteria freudenreinchii* on digestibility. Adams et al. (1981) fed *S. cerevisiae* to steers and wethers and neither ruminal fermentation nor digestibility

was changed. Similarly, Miller-Webster et al. (2002) reported no effect of either yeast culture (Diamond-V XP and A-Max concentrate) on nutrient digestibility parameters. Furthermore, in the latter study and our present study, pH did not drop below 5.8; pH reductions of rumen fluid below 5.8 can cause moderate depression in fiber digestion (Hoover, 1986). It is necessary to determine the type of diet in which the effect of adding yeast culture and Propionibacteria would be most beneficial. What is known from previous research (Sullivan and Martin, 1999) is that *S. cerevisiae* culture has limited effects on the rate and extent of digestion of both alfalfa hay and Coastal Bermuda grass by mixed ruminal microorganisms.

Previous studies have evaluated the effect of yeast culture on microbial protein flow to the duodenum. In sheep, Karr et al. (1991) reported lower flows of microbial N to the duodenum of sheep fed yeast culture. Contrary to this, Williams et al. (1990) reported increase in the flow and absorption of non ammonia nitrogen (NAN) in sheep fed yeast culture supplement and concluded that increased flow of NAN probably could be attributed to microbial protein. Erasmus et al. (1992), fed 10 g/d of yeast culture to lactating dairy cows consuming 35% forage diet that contained 25% wheat straw on DM basis and reported a trend for increased flow of amino acids to the duodenum and protein synthesis in cows that were supplemented with yeast culture compared to the Controls. Contrary to the findings by Erasmus et al. (1992), yeast culture had no effect on flow of amino acids to the duodenum except glycine (Putnam et al., 1997). In the present study, yeast culture, propionibacteria and their combination did not affect microbial nitrogen flow to the duodenum or microbial efficiency.

An increase in propionate concentrations signifies an increase in energy for growth or production. This is based on the fact that propionate is the major glucogenic precursor. Supplementing the diet with Propionibacteria, yeast culture or both in the present study did not increase glucose or insulin concentrations. Previously, Aleman et al. (2007) reported that feeding P169 increased plasma glucose concentration in primiparous cows during early and midlactation. However, glucose concentrations in multiparous cows did not differ among treatments in both lactation stages (Aleman et al., 2007). Similarly, Francisco et al. (2002) found no effect of feeding P169 on glucose and insulin concentrations in early lactating multiparous dairy cows. In cattle (Subiyatno et al., 1998; Oba and Allen, 2003) and sheep (Sano et al., 1993), feeding sources of propionate or infusion of propionate at levels high enough and duration long enough to increase levels of glucose and insulin showed transient increases in glucose and insulin concentrations. Therefore, it is likely that the duration of feeding (2 wk) or frequency of blood sample collection (only twice throughout collection period) or both were not enough to elicit any change in glucose and/or insulin concentrations. Few studies have reported effects of yeast culture on glucose and insulin concentrations. For example, in support to our findings, Piva et al. (1993) and Putman et al. (1997) reported no effects of yeast culture supplementation on the concentrations of plasma glucose and insulin in early lactation dairy cows. Insulin response to propionate infusion depends on the level of nutrients fed to non-lactating, non-pregnant mature ewes (Quigley and Heitmann, 1991). Thus, lower feed intake expressed by P169+XPY compared to the Control steers might have influenced the glucose and insulin response to feeding Propionibacteria and yeast culture or both in the present study. The high plasma glucose concentrations indicate that the

Control diet was well balanced for maximizing production response and this may have influenced the steers' ability to respond to P169 supplementation.

Conclusions

Total organic matter, NDF, ADF and N intake were decreased by feeding XPY vs. P169 and Control dietary treatments. However, total tract digestibilities were greater in XPY vs. P169+XPY and Control fed steers. Ruminal digestibility, duodenal flow, microbial N synthesis, fluid dilution rate and particulate passage rates for OM, N, NDF, and ADF were not affected by dietary treatments. Feeding P169 increased propionate by 14% above Control and XPY steers and decreased acetate: propionate concentrations by 15% compared to Control fed steers. Plasma glucose and insulin concentrations were not affected by dietary supplementation. These results imply that feeding P169 might alter ruminal metabolism toward increased propionate without affecting feed intake, nutrient digestibilities, duodenal flow, rumen kinetics or microbial nitrogen synthesis by beef steers. Although feeding XPY improved total tract digestibilities of OM, N, ADF, and NDF, XPY decreased nutrient intake of these well fed steers. These results suggest that feeding XPY when animals are receiving an adequate and well balanced diet might provide minimum advantage. However, further investigations will be required to assess the effect of feeding P169, XPY or P169 in conjunction with yeast culture using larger numbers of experimental units and longer adaptation periods to allow a complete adaptation to the treatment diets and to eliminate any possible carry-over effect. Moreover, additional studies are warranted to study the effects of feeding P169, XPY or P169 in conjunction with XPY when beef steers are under a different energy status, such as negative energy balance.

Literature Cited

Adams, D. C., M. L. Galyean, H. E. Kiesling, J. D. Wallace, and M. D. Finkner. 1981. Influence of viable yeast culture, sodium bicarbonate, and monensin on liquid dilution rate, rumen fermentation, and feedlot performance in growing steers and digestibility in lambs. J. Anim. Sci. 53:780 (Abstr.).

Akay, V., and R. G. Dado. 2001. Effects of Propionibacterium strain P5 on in-vitro volatile fatty acids production and digestibility of fiber and starch. Turk. J. Vet. Anim. Sci. 25:635-642.

Aleman, M. M., D. R. Stein, D. T. Allen, E. Perry, K. V. Lehloenya, T. G. Rehberger, K. J. Mertz, D. A. Jones, and L. J. Spicer. 2007. Effect of feeding two levels of Propionibacteria to dairy cows on plasma hormones and metabolites. J. Dairy Res. (In Press).

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

AOAC. 1990. Official method of analysis (15th Ed.) K. Helrich (Ed). Association of Official Analytical Chemists Arlington, VA.

Arambel, M. J,. and B. A. Kent. 1990. Effect of yeast culture on nutrient digestibility and milk yield response in early to midlactation dairy cows. J. Dairy Sci. 73:1560-1563.

Baile, C. 1971. Metabolites as feedbacks for control of feed intake receptor sites in goats and sheep. Physiol. Behav. 7:819-826.

Beauchemin, K. A., W. Z. Yang, D. P. Morgavi, G. R. Ghorbani, W. Kautz, and J. A. Z. Leedle. 2003. Effects of bacterial direct fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. J. Anim. Sci. 81:1628-1640.

Bines, J. A., and J. C. Hart. 1984. The response of plasma insulin and other hormones to intraruminal infusion of VFA mixture in cattle. Can. J. Anim. Sci. 64 (Suppl.):304-305.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 67:820-826.

Butler, W.R., and R.W. Smith. 1989. Interrelationships between energy balance, milk production and postpartum reproductive function in cattle. J. Dairy Sci. 72:767-783.

Callaway, E. S., and S. A. Martin. 1997. Effect of *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Christensen, J. O., R. R. Grummer, F. E. Rasmussen, and S. J. Bertics. 1997. Effect of method of delivery of propylene glycol on plasma metabolites of feed-restricted cattle. J. Dairy Sci. 80:563-568.

Dann, H. M., J. K. Drackley, G. M. McCoy, M.F. Hutjens, and J. E. Garrett. 2000. Effects of yeast culture (*Saccharomyces cerevisiae*) on prepartum and postpartum intake and milk production of Jersey cows. J. Dairy Sci. 83:123-127.

DeFrain, J. M., A. R. Hippen, K. F. Kalscheur, and R. S. Patton. 2005. Effects of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. J. Dairy Sci. 88:983-993.

Dewhurst, R. J., D. R. Davies, and R. J. Merry. 2000. Microbial protein supply from the rumen. Anim. Feed Sci. Tech. 85:1-21.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84:100-112.

Erasmus, L. J., P. M. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056-3065.

Erdman, R. A. and B. K. Sharma. 1989. Effect of yeast culture and sodium bicarbonate on milk yield and composition in dairy cows. J. Dairy Sci. 72:1929-1932.

Francisco, C. C., C. S. Chamberlain, D. N. Waldner, R. P. Wettemann, and L. J. Spicer. 2002. Propionibacteria Fed to Dairy Cows: Effects on Energy Balance, Plasma Metabolites and Hormones, and Reproduction. J. Dairy Sci. 85:1738-1751.

Gall, L. S., Burroughs, P. Gerlaugh, and B. H. Edgington. 1949. Rumen bacteria in cattle and sheep on practical farm ration. J. Anim. Sci. 8:441-449.

Ghorbani, G. R., D. P. Morgavi, K.A. Beauchemin, and J .A. Z. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. J. Anim. Sci. 80:1977-1986.

Goering, H. K., and P. J. Van Soest, 1970. Forage fiber analysis (apparatus, reagents, procedures and applications). Agric. Handbook No. 379, ARS. USDA, Washington, DC.

Gomez-Alarcon, R. A., C. Dudas, and J. T. Huber. 1990. Influence of *Aspergillus oryzae* on rumen and total tract digestion of dietary components. J. Dairy Sci. 73:703-710.

Grovum, W. L., and V. J. Williams. 1973. Rate of passage of digesta in sheep. 1. The effect of level of food intake on marker retention times along the small and large intestines. Br. J. Nutr. 29:13-21.

Grummer, R. R., J. C. Winkler, S. J. Bertics, and V. A. Studer. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. J. Dairy Sci. 77:3618-3623.

Harrison, G. A., R. W. Hemken, K. A. Dawson, R. J. Harmon, and K. B. Barker. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial population. J. Dairy Sci. 71:2967-2975.

Harris, B., Jr., D. E. Dorminey, W. A. Smith, H. H. Van Horn, and C. J. Wilcox. 1992. Effects of feather meal a two protein concentrations and yeast culture on production parameters in lactating dairy cows. J. Dairy Sci. 75:3524-3530.

Hoover, W. H. 1986. Chemical factors involved in rumen fiber digestion. J. Dairy Sci. 69:2755-2766.

Horn, G. W., and McCollum, III. 1987. Energy supplementation of grazing ruminants. Proc. Grazing Livestock Nutrition Conference. July 23-24, Jackson, WY. Univ. of Wyoming. Pp. 125-136.

Karr, K. J., R. McLeod, K. A. Dawson. R. E. Tucker, and G. E. Mitchell, Jr. 1991. Influence of yeast culture and/or fermentation on nitrogen flow and rumen fermentation in sheep. Page 377 in Biotechnology in the Feed Industry. T. P. Lyons, ed. Alltech. Publ., Nicholasville, KY.

Kellems, R. O., A. Lagerstedt, and M. V. Wallentine. 1990. Effect of feeding *Aspergillus oryzae* fermentation extract or Aspergillus plus yeast culture plus mineral and vitamin supplement on performance of Holstein cows, during a complete lactation. J Dairy Sci. 73:2922-2928.

Kim, S. W., D. Standorf, H. RomanRosario, M. T. Yokoyama, and S. R. Rust. 2000. Potential use of Propionibacterium acidipropionici, strain DH42 as a direct-fed microbial strain for cattle., Michigan State Univ. Beef cattle, sheep and forage systems research and demonstration report, Michigan State Univ., East Lansing.

Koster, H. H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, I. Abdelgadir, and G. StJean. 1996. Effect of increasing degradable intake protein on intake and digestion of low quality, tallgrass-prairie forage by beef cows. J. Anim. Sci. 74:2473-2481.

Kung, L., E. M. Kreck, R.S. Tung, A. O. Hession, A. C. Shepherd, M. A. Cohen, H. E. Swain, and J .A. Z. Leedle. 1997. Effects of a live yeast culture and enzyme on in vitro ruminal fermentation and milk production. J. Dairy Sci. 80:2045-2051.

Kung, L. 2001. Direct fed microbials for dairy cows. Proceedings, 12th Annual Florida Nutrition Symposium. pp. 2-28.

Loest, C. A., E. C. Titgemeyer, B. D. Lambert, and A. M. Trater. 2001a. Branched chain amino acids for growing cattle limit-fed soybean hull-based diets. J. Anim. Sci. 79:2747-2753.

Lynch, H. A., and S. A. Martin. 2002. Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on in-vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 85:2603-2608.

Lyons, T. P., K. A. Jacques, and K. A. Dawson. 1993. Miscellaneous products from yeast, pages 293-324 in *The Yeasts* (eds. A.H. Rose and J.S. Harrison). Vol. 5. Academy Press, NewYork.

Martin, S.A., and D. J. Nisbet. 1992. Effect of direct fed microbials on rumen microbial fermentation. J. Dairy Sci. 75:1736-1744.

Merchen, N. R. 1988. Digestion, absorption and excretion in ruminants. In: Church DC, editor. The ruminant animal: digestive physiology and nutrition. Englewood Clifs, NJ: Prentice Hall. P 172-209.

Miller-Webster, T., W. H., Hoover, M. Holt and J. E. Nocek. 2002. Influence of yeast culture on ruminal microbial metabolism in continuous culture. J. Dairy Sci. 85:2008-2014.

Newbold, C. J., R. J. Wallace, and F. M. McIntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. J. Anim. Sci. 73:1811-1818.

Nisbet, D. J., and S. A. Martin. 1991. Effect of *Saccharomyces cerevisiae* on lactate utilization by the ruminal bacterium *Selenomonas ruminantium*. J. Anim. Sci. 69:4629-4633.

Niswender, K. D., and M. W. Schwartz. 2003. Insulin and leptin revisited. Adiposity signals with overlapping physiological and intracellular signaling capabilities: Front Neuroendocrinol. Review. 24:1-10.

Oba, M., and M. S. Allen. 2003. Intraruminal infusion of propionate alters feeding behavior and decreases energy intake of lactating dairy cows. J. Nutr. 133:1094-1099.

Piva, G., S. Belladonna, G. Fusconi, and F. Sicbaldi. 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76:2717-2722.

Prigge, E. C., M. J. Baker, and G. A. Varga. 1994. Comparative digestion, rumen fermentation and kinetics of forage diets by steers and wethers. J. Anim. Sci. 59:237-245.

Putman, D. E., C. G. Schwab, M. T. Socha, N. L. Whitehouse, N. A. Kierstead, and B. D. Garthwaite. 1997. Effect of yeast culture in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. J. Dairy Sci 80:374-384.

Quigley, J. D., and R. N. Heitmann. 1991. Effects of propionate infusion and dietary energy on dry matter intake in sheep. J. Anim. Sci. 69: 1178-1187.

Raeth-Knight, M. L., J. C. Linn, and H. G. Jung. 2007. Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cow. J. Dairy Sci. 90:1802-1809.

Russell, J. B., J. D. O'Conner, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets. I. Ruminal fermentation. J. Anim. Sci. 70:3551-3561.

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

Satter, L. D. and L. L. Slyter, 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199-205.

Schingoethe, D. J., K.N. Linke, K.F. Kalscheur, A. R. Hippen, D.R. Rennich, and I. Yoon. 2004. Feeding efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87:4178-4181.

Seal, C. J., and C. K. Reynolds. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutr. Res. Rev. 6:185-208.

Sheperd, A. C., and D. K. Combs. 1998. Longterm effects of acetate and propionate on voluntary intake by midlactation cows. J. Dairy Sci. 81:2240-50.

Soder, K. J., and L. A. Holden. 1999. Dry matter intake and milk yield and composition of cows fed yeast prepartum and postpartum. J. Dairy Sci. 82:605-610.

Spicer, L. J., W. B. Tucker, and G. D. Adam. 1990. Insulin-like growth factor-I in dairy cows: Relationship among energy balance, body condition score, ovarian activity and estrous behavior. J. Dairy Sci. 73:929-937.

Stein, D. R., D. T. Allen, E. B. Perry, J. C. Bruner. K.W. Gates, T. G. Rehberger, K. J. Mertz, D. A. Jones, and L. J. Spicer. 2006. Effect of feeding Propionibacteria to dairy cows on milk yield, milk components and reproduction. J Dairy Sci. 89:111-125.

Subiyatno, A., D. N. Mowat, and W. Z. Yang. 1996. Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. J. Dairy Sci. 79:1436-1445.

Sullivan, H. M., and S. A. Martin. 1999. Effects of *Saccharomyces cerevisiae* culture on in-vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 82:2011-2016.

Swartz, D. L., L. D. Muller, G. W. Rogers, and G. A. Varga. 1994. Effect of yeast cultures on performance of lactating dairy cows: a field study. J. Dairy Sci. 77:3073-3080.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 44:3583-3597.

Vanzant, E. S., and R. C. Cochran. 1994. Performance and forage utilization by beef cattle receiving increasing amounts of alfalfa hay as a supplement to low-quality, tallgrass-prairie forage. J. Anim. Sci. 72:1059-1067.

Wallace, R. J., and C. J. Newbold. 1992. Probiotics for ruminants. Page 317 in Probiotics: The Scientific Basis. R. Fuller, ed. Chapman and Hall, London, UK.

Wallace, R. J. 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: Progress and problems. J. Anim. Sci. 72:2992-3003.

Wang, Z., M. L. Eastridge, and X. Qiu. 2001. Effects of forage neutral detergent fiber and yeast culture on performance of cows during early lactation. J. Dairy Sci. 84:204-212.

Wiedmeier, R. D., M. J. Arambel, and J. L. Walters. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy Sci. 70:2063-2068.

Williams, C. H., D. J. David, and O. Iismaa. 1962. The determination of chromic oxide in faces samples by atomic absorption spectrophotometry. J. Agric. Sci. (Camb.) 59:381-385.

Williams, P. E. V., C. A. G. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in rumen of steers. J. Anim. Sci. 69:3016-3026.

Williams, P. E. V., A. Walker, and J. C. Macrae. 1990. The effects of addition of yeast culture (viable yeast, *Saccharomyces cerevisiae*, plus growth medium) on duodenal flow in wether sheep. Proc. Nutr. Soc. 49:128 A (Abstr.)

Williams, P. E.V., and C. J. Newbold. 1990. Rumen probiosis: the effects of novel microorganisms on rumen fermentation and ruminant productivity. Page 211 in recent advances in Animal Nutrition. W. Haresign and D.J.A Cole, ed. Butterworths, London, Engl.

Wohlt, J. E., A.D. Finkelstein, and C.H. Chung. 1991. Yeast culture to improve intake, nutrient digestibility and performance by dairy cattle during early lactation. J. Dairy Sci. 74:1395-1400.

Zinn, R. A., and F. N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157-166.

Table 1. Ingredient and Nutrient Composition of the Control diet (TMR) fed to beef steers.

Ingredient	**% Diet DM**
Sorghum silage	19.60
Alfalfa	24.54
Whole cottonseed	7.35
Grain mix	47.89
Megalac-R*	0.65
Chemical composition	
DM	65.25
CP	16.72
NDF	40.57
ADF	25.51
Ash	8.1

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

Table 2. Effect of supplementation on intake, fecal output, total tract digestibility, duodenal flow, and ruminal digestibility of steers fed sorghum silage based total mixed ration.

	P169		No P169			P-value		
Item	XPY	No XPY	XPY	No XPY	SEM	P169	XPY	P169 x XPY
BW, kg	530	527	537	547	37	0.87	0.82	0.76
Intake, kg/d								
OM	13.38 ^{y}	13.83 ^{xy}	13.36 ^{y}	14.25 ^{x}	0.46	0.55	0.06	0.52
N	0.389 ^{y}	0.403 ^{xy}	0.388 ^{y}	0.413 ^{x}	0.02	0.65	0.07	0.58
NDF	5.90 ^{b}	6.10 ^{ab}	5.89 ^{b}	6.29 ^{a}	0.19	0.51	0.05	0.49
ADF	3.71 ^{y}	3.82 ^{xy}	3.71 ^{y}	3.96 ^{x}	0.12	0.46	0.06	0.46
Fecal output, kg/d								
OM	4.35 ^{x}	4.10 ^{xy}	3.19 ^{y}	4.60 ^{x}	0.61	0.63	0.30	0.07
N	0.116	0.109	0.085	0.119	0.02	0.41	0.32	0.12
NDF	2.70 ^{x}	2.51 ^{xy}	1.97 ^{y}	2.86^{x}	0.31	0.48	0.20	0.06
ADF	1.89 ^{xy}	1.70 ^{xy}	1.48 ^{y}	2.02 ^{x}	0.23	0.82	0.38	0.09
Duodenal flow, kg/d								
OM	6.56	6.65	5.54	7.54	0.80	0.94	0.21	0.25
True OM	8.83	8.64	8.80	8.30	0.65	0.76	0.57	0.80
N	0.294	0.308	0.241	0.345	0.04	0.83	0.16	0.27
NDF	2.25	2.22	1.98	2.60	0.29	0.85	0.32	0.28
ADF	1.55	1.56	1.40	1.92	0.21	0.61	0.22	0.23
Total tract digestibility, % intake								
OM	68.34 ^{y}	71.03 ^{xy}	75.24 ^{x}	67.61y	3.75	0.53	0.38	0.08
N	70.87 ^{y}	73.59 ^{xy}	77.47 ^{x}	71.07^{y}	2.83	0.45	0.49	0.10
NDF	55.36^{y}	59.59^{xy}	65.33^{x}	54.45^{y}	4.52	0.53	0.40	0.07
ADF	50.00^{xy}	55.63^{xy}	59.78^{x}	48.49^{y}	6.24	0.76	0.52	0.06
Ruminal digestibility, % intake								
OM	52.47	51.71	58.47	45.77	5.82	0.99	0.26	0.32
N	28.44	22.37	37.13	14.09	10.4	0.98	0.16	0.40
True N	65.82	60.29	66.51	56.11	5.15	0.74	0.14	0.64
NDF	62.88	63.45	66.38	57.51	4.74	0.80	0.39	0.33
ADF	58.97	59.27	62.41	50.03	5.56	0.61	0.29	0.27

Supplements included: no supplement (Control - sorghum based TMR); XPY, TMR plus Diamond V-XP Yeast; P169, TMR plus Propionibacteria strain (P169); P169+XPY, TMR plus P169 plus XPY.
SEM = Standard Error of Mean, (n = 6).
^{ab} Means within a row without common superscript (ab) differ ($P \leq 0.05$).
^{xy} Means within a row without common superscript (xy) differ ($0.05 \leq P \leq 0.10$).

Table 3. Effect of feeding XPY, P169 or P169+XPY on N intake and flow of N to the duodenum of beef steers fed sorghum based total mixed ration.

	P169		No P169			Anova P-value		
Item	XPY	No XPY	XPY	No XPY	SEM	P169	XPY	P169 x XPY
N intake, g/d	389.26^{y}	403.42^{xy}	388.33^{y}	413.4^{x}	22.09	0.65	0.07	0.58
Duodenal N flow, g/d	294.70	308.47	241.23	344.99	39.93	0.83	0.16	0.27
Microbial N, g/d	153.00	151.45	113.00	168.53	21.92	0.60	0.23	0.20
Dietary N, g/d*	138.80	157.58	129.23	177.00	19.97	0.79	0.11	0.46
MOEFF g of N/kg**	18.57	19.12	13.05	22.13	3.86	0.74	0.23	0.28
Microbial N, % intake	37.08	38.03	29.51	42.10	5.30	0.73	0.20	0.27

Supplements included: no supplement (Control - sorghum based TMR); XPY, TMR plus Diamond V-XP Yeast; P169, TMR plus Propionibacteria strain (P169); P169+XPY, TMR plus P169 plus XPY.
SEM = Standard Error of Mean, (n = 6).
^{xy} Means within a row without common superscript (xy) differ ($0.05 \leq P \leq 0.10$).
*Contains endogenous and NH_3 nitrogen.
**MOEFF= g of microbial N at the duodenum per kg of true ruminally degraded OM.

Table 4. Effect of feeding XPY, P169 or P169+XPY on ruminal kinetics and capacity of beef steers fed sorghum based silage total mixed ration.

	P169		No P169			Anova P-value		
Item	XPY	No XPY	XPY	No XPY	SEM	P169	XPY	P169 x XPY
Fluid dilution rate, %/h	8.33	8.54	8.91	7.71	0.74	0.86	0.51	0.35
Particulate passage rate, %/h	6.59	6.24	8.25	6.87	1.42	0.27	0.40	0.61
Ruminal contents (kg)	35.56^{xy}	40.27^{x}	32.98^{y}	38.49^{xy}	2.78	0.41	0.07	0.88
Ruminal fluid (L)	29.69^{b}	33.87^{a}	27.75^{b}	32.67^{a}	2.21	0.47	0.05	0.86
Turnover time (h)	12.53	12.15	11.69	13.43	1.11	0.84	0.55	0.35

Supplements included: no supplement (Control - sorghum based TMR); XPY, TMR plus Diamond V-XP Yeast; P169, TMR plus Propionibacteria strain (P169); P169+XPY, TMR plus P169 plus XPY. SEM = Standard Error of Mean, (n = 6).
^{ab} Means within a row without common superscript differ ($P \leq 0.05$).
^{xy} Means within a row without common superscript (xy) differ ($0.05 \leq P \leq 0.10$).

Table 5. Effect of feeding XPY, P169 or P169+XPY on ruminal pH, NH3-N, and VFA to steers consuming sorghum based silage total mixed ration.

	P169		No P169			Anova P-value		
Item	XPY	No XPY	XPY	No XPY	SEM	P169	XPY	P169 x XPY
pH	6.35	6.25	6.32	6.37	0.20	0.65	0.78	0.47
NH_3-N	8.00	8.28	8.14	8.41	0.40	0.46	0.13	0.99
Total VFA, mM	115.88	113.92	113.91	112.38	3.84	0.66	0.66	0.96
	…….mol/100 ml……							
Acetate	72.78	74.78	73.65	76.44	3.01	0.65	0.39	0.89
Propionate	23.30[x]	21.89[x]	20.72[y]	20.42[y]	1.06	0.07	0.43	0.61
Butyrate	12.72	12.53	12.68	12.15	0.65	0.72	0.55	0.77
Valerate	1.47	1.45	1.59	1.47	0.08	0.36	0.41	0.52
Isobutyrate	1.73	1.77	1.84	1.89	0.11	0.28	0.67	0.92
Isovalerate	1.36	1.34	1.48	1.52	0.18	0.24	0.94	0.84
Lactate	0.06	0.14	0.01*	0.26	0.10	0.79	0.12	0.40
Acetate: Propionate	3.23[b]	3.48[b]	3.65[a]	3.79[a]	0.17	0.05	0.29	0.77

Supplements included: no supplement (Control - sorghum based TMR); XPY, TMR plus Diamond V-XP Yeast; P169, TMR plus Propionibacteria strain (P169); P169+XPY, TMR plus P169 plus XPY.
SEM = Standard Error of Mean, (n =6).
[ab] Means within a row without common superscript differ ($P \leq 0.05$).
[xy] Means within a row without common superscript (xy) differ ($0.05 \leq P \leq 0.10$).
*0.01 mol/100 ml = level of detection.

Figure 1. Effect of feeding Propionibacteria strain (P169), Diamond V-XP Yeast (XPY) or both on rumen pH of steers fed sorghum based total mixed ration. Ruminal pH concentrations for P169 x XPY x time interaction (P=0.01) for steers. Top panel: Rumen pH concentrations of steers fed P169 (n=6) or no P169 (n=6) in the absence of XPY. Bottom panel: Rumen pH concentrations of steers fed P169 or no P169 in the presence of XPY.

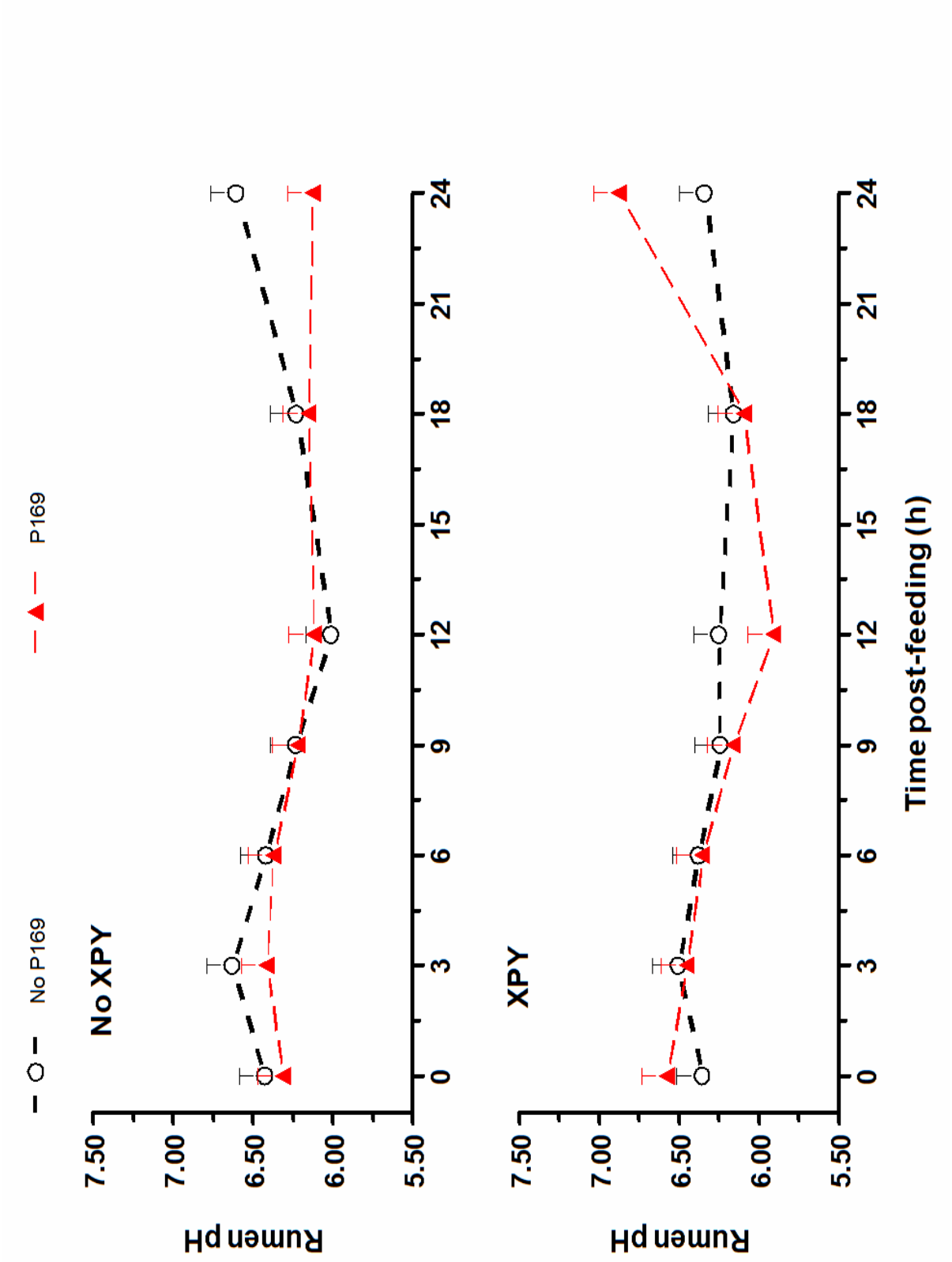

No P169
P169
No XPY
XPY
Rumen pH
Time post-feeding (h)
7.50
7.00
6.50
6.00
5.50
0
3
6
9
12
15
18
21
24

Figure 2. Effect of feeding Propionibacteria strain (P169), Diamond V-XP Yeast (XPY) or both on rumen ammonia nitrogen of steers fed sorghum based total mixed ration. Ruminal ammonia concentrations time effect (P=0.0001) for beef steers.

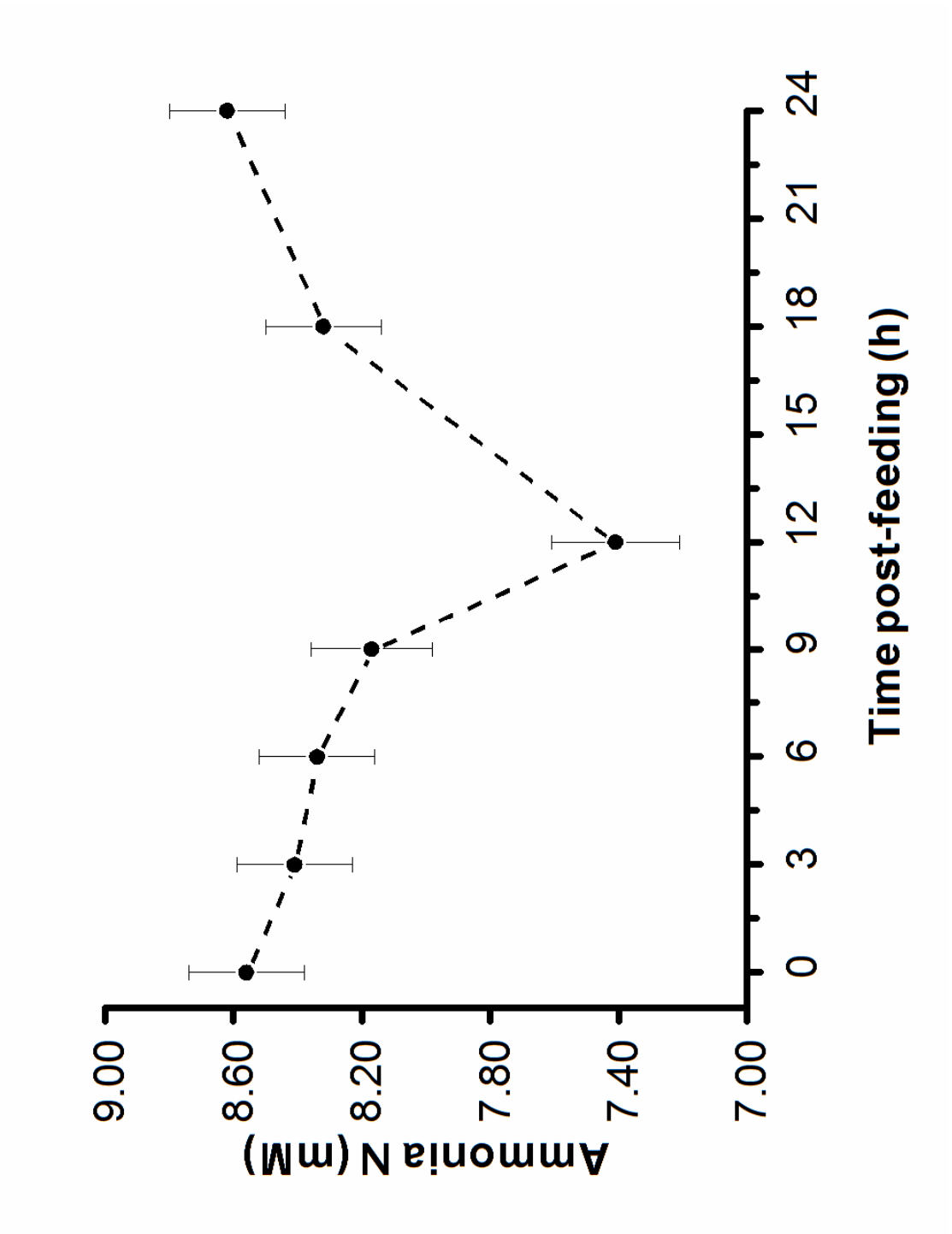

Ammonia N (mM)
9.00
8.60
8.20
7.80
7.40
7.00
0
3
6
9
12
15
18
21
24
Time post-feeding (h)

Figure 3. . Effect of feeding Propionibacteria strain (P169), Diamond V-XP Yeast (XPY) or both on total VFA concentrations of steers fed sorghum based total mixed ration. Total VFA concentrations time effect (P=0.0001) for beef steers.

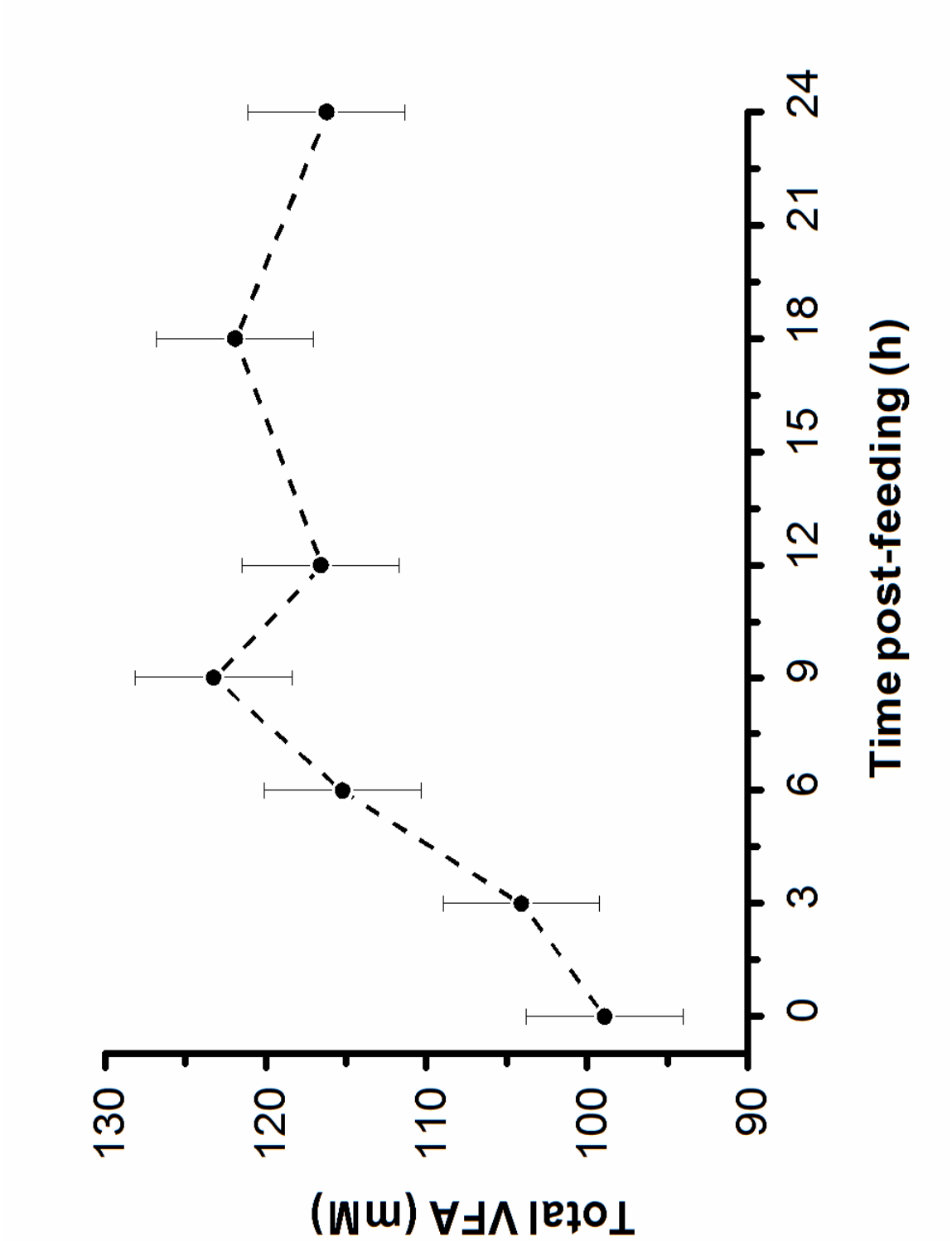

Total VFA (mM)
130
120
110
100
90
0
3
6
9
12
15
18
21
24
Time post-feeding (h)

Figure 4. Effect of feeding Propionibacteria strain (P169), Diamond V-XP Yeast (XPY) or both on acetate to propionate ratio of steers fed sorghum based total mixed ration. Ruminal acetate to propionate ratio time effect (P=0.0001) for beef steers.

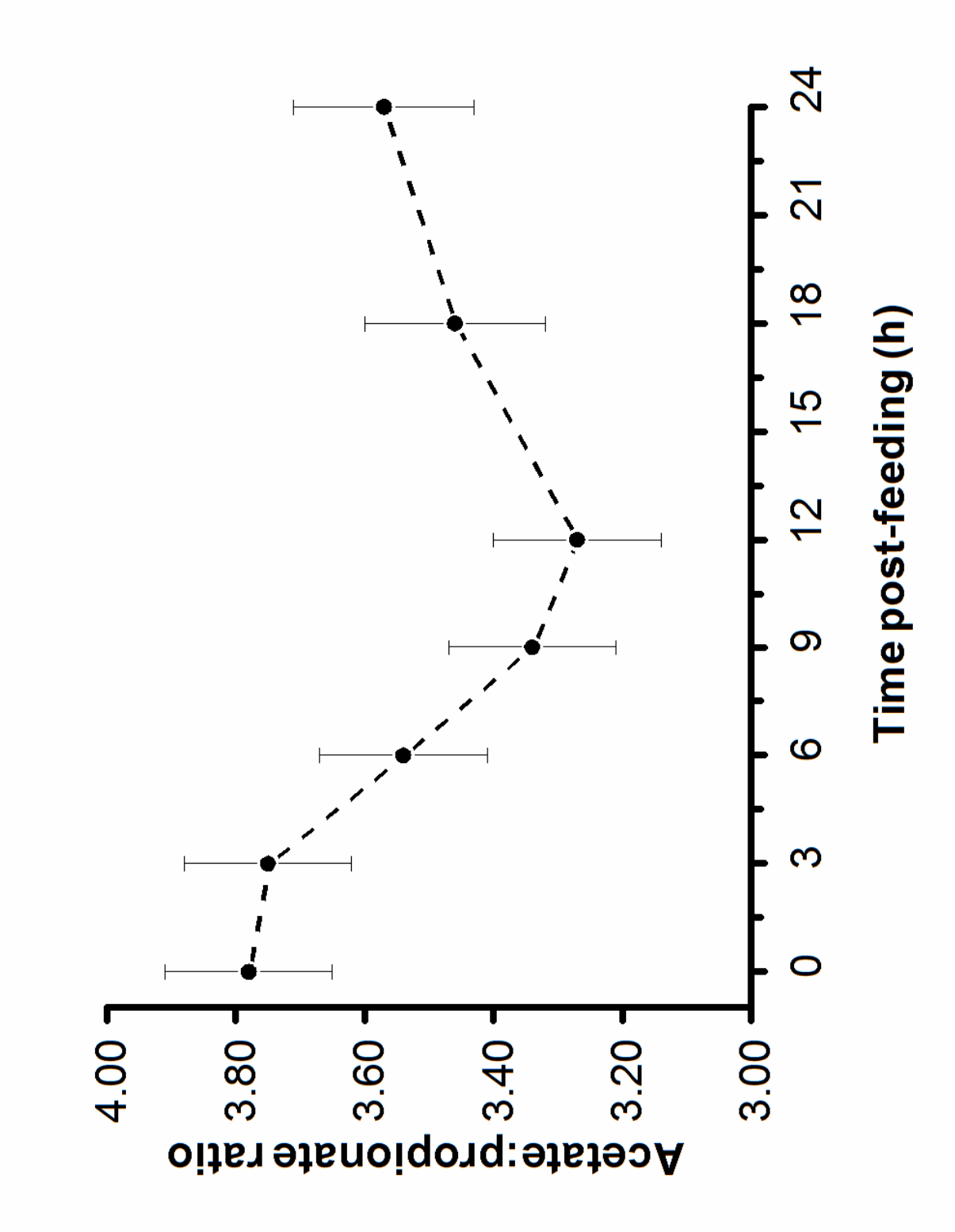

Acetate:propionate ratio
4.00
3.80
3.60
3.40
3.20
3.00
0
3
6
9
12
15
18
21
24
Time post-feeding (h)

Figure 5. Effect of feeding Propionibacteria strain (P169), Diamond V-XP Yeast (XPY) or both on rumen acetate concentration of steers fed sorghum based total mixed ration. Acetate concentrations for P169 x XPY x time interaction (P=0.03) for steers. Top panel: Acetate concentrations of steers fed P169 (n=6) or no P169 (n=6) in the absence of XPY. Bottom panel: Acetate concentrations of steers fed P169 or no P169 in the presence of XPY.

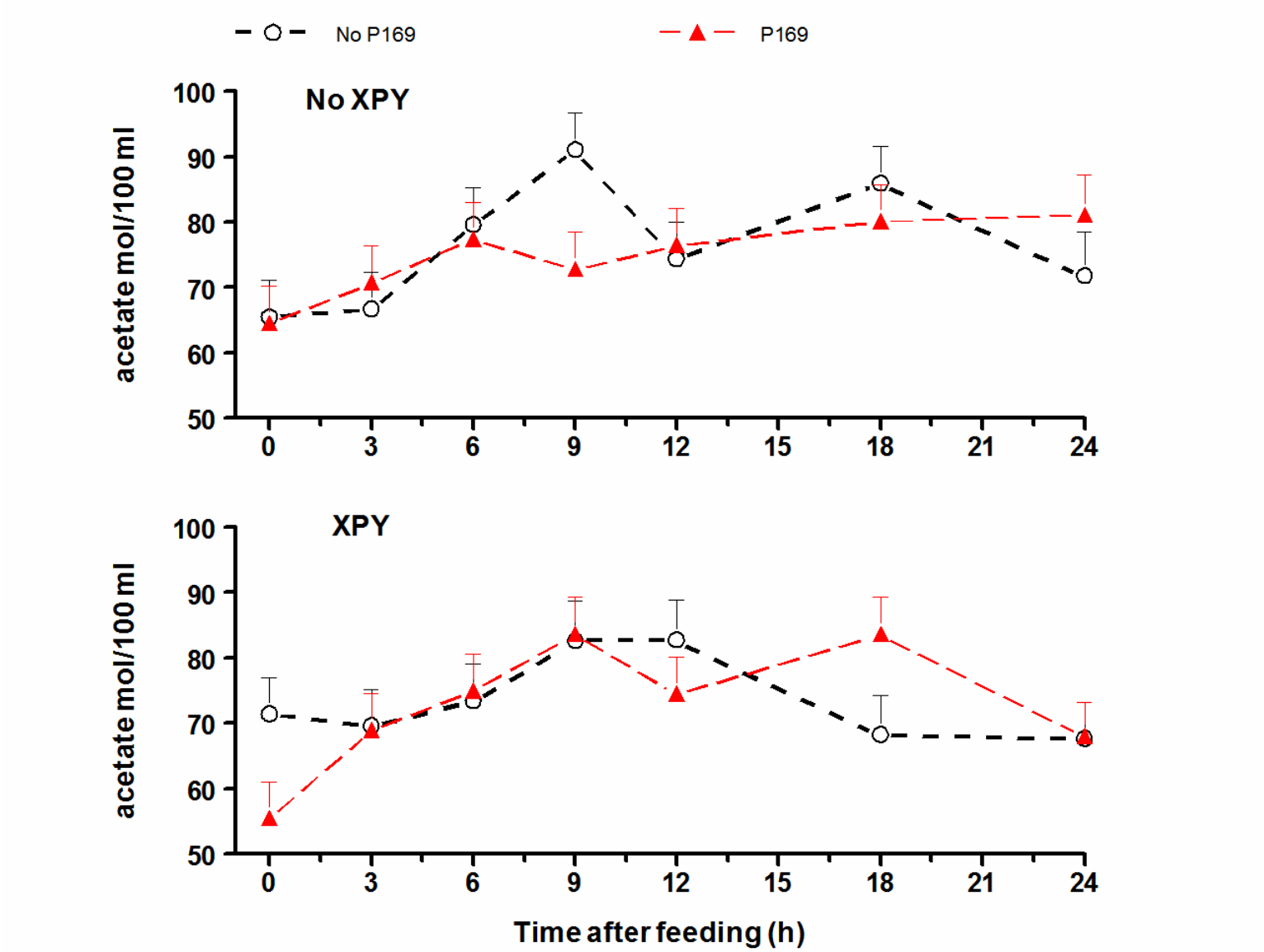

No P169
P169
No XPY
XPY
acetate mol/100 ml
acetate mol/100 ml
100
90
80
70
60
50
0
3
6
9
12
15
18
21
24
Time after feeding (h)

Figure 6. Effect of feeding Pripionibacteria (P169), Diamond V-XP (XPY) or both on plasma glucose concentrations of steers fed sorghum based silage. Within treatment groups, data were averaged across period and time post-feeding. Plasma insulin concentrations did not differ ($P \geq 0.32$) among the treatment groups.

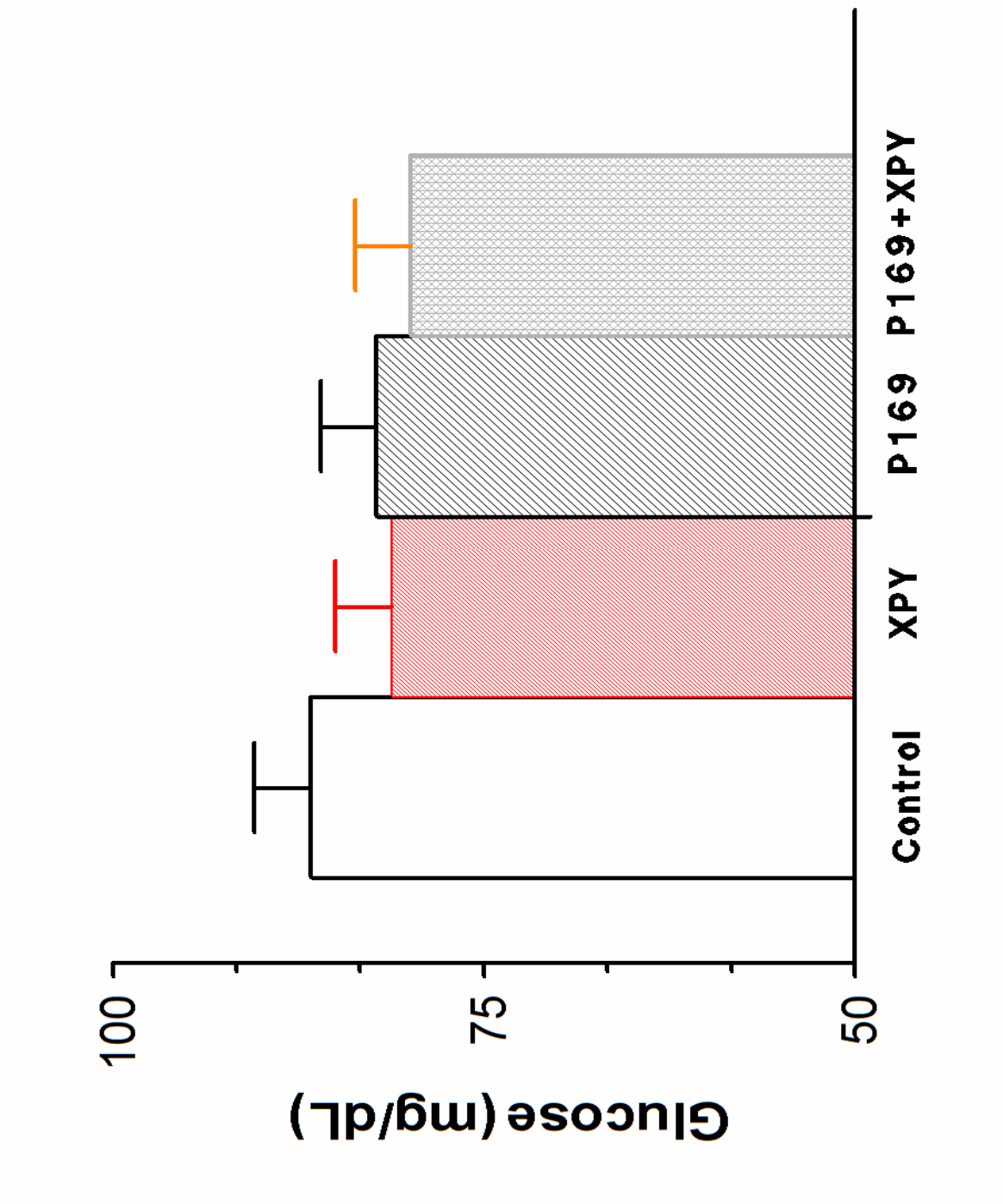
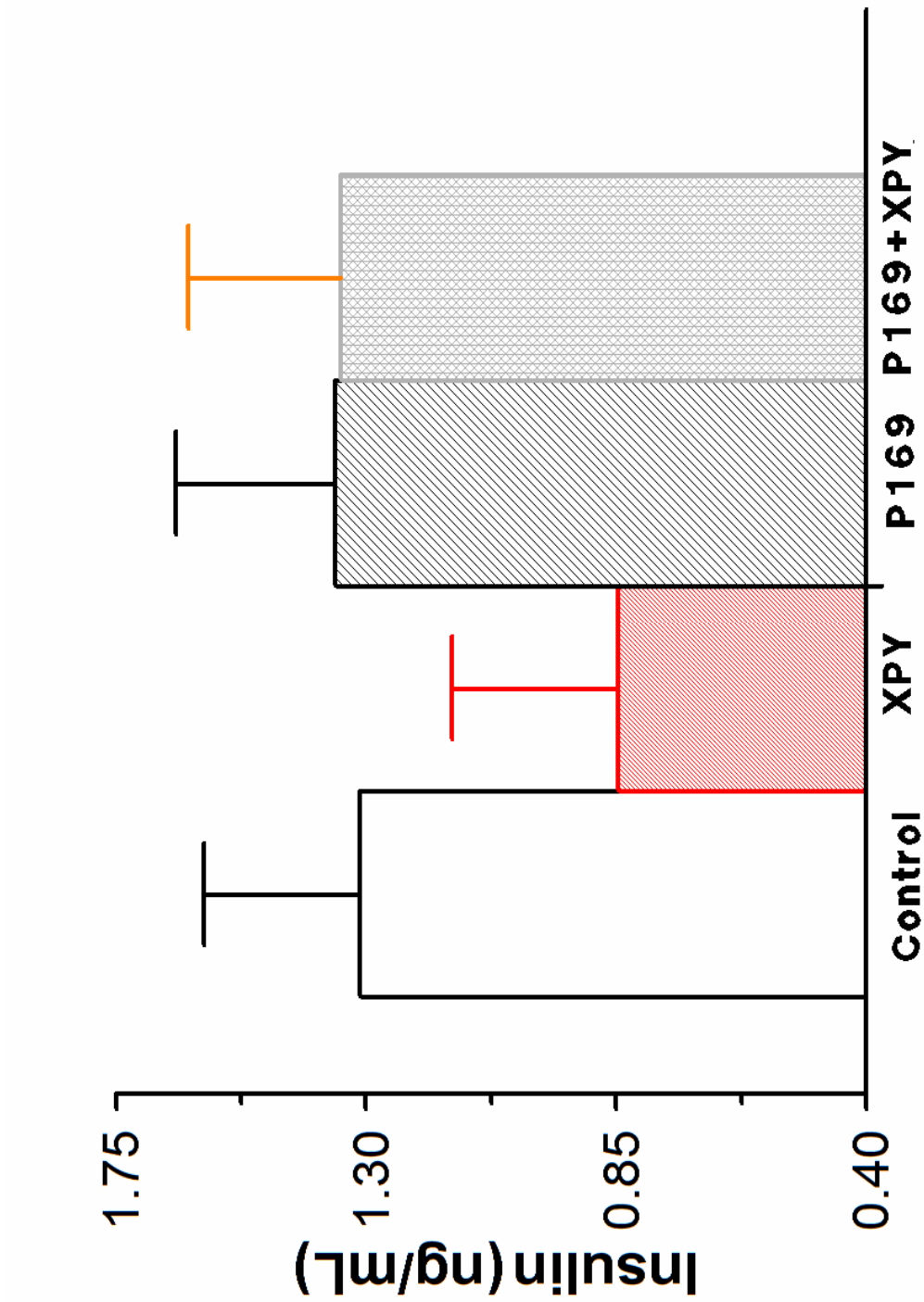

Glucose (mg/dL)
100
75
50
Control
XPY
P169
P169+XPY

Figure 7. Effect of feeding Pripionibacteria (P169), Diamond V-XP (XPY) or both on plasma insulin concentrations of steers fed sorghum based silage. Within treatment groups, data were averaged across period and time post-feeding. Plasma insulin concentrations did not differ ($P \geq 0.39$) among the treatment groups.


Insulin (ng/mL)
1.75
1.30
0.85
0.40
Control
XPY
P169
P169+XPY

VITA

Keneuoe Victoria Lehloenya

Candidate for the Degree of

Doctor of Philosophy

Thesis: A DIRECT-FED MICROBIAL SUPPLEMENTATION TO CATTLE ON MILK YIELD AND COMPONENTS, REPRODUCTION, AND DIGESTION

Major Field: Ruminant Nutrition

Biographical:

Personal Data: Born in Maseru, Lesotho on November 05, 1967.

Education: Graduated from M'abathoana High School, in December, 1984. Earned degree in Bachelor of Science in Agriculture from the National University of Lesotho, Maseru, Lesotho in 1995. Earned Master of Science in Animal Science from Bunda College of Agriculture, Lilongwe, Malawi in 1999. Completed the Requirements for the Doctor of Philosophy in Ruminant Nutrition at Oklahoma State University in July, 2007.

Experience: A Lecturer at the Lesotho Agricultural College and the National University of Lesotho. Faculty representative at the National University of Lesotho. Leardership program- Kellogg foundation fellow.

Professional Memberships: American Society of Animal Science, American Society of Dairy Science Association, OSU Animal Science Graduate Student Organization.

Name: Keneuoe Victoria Lehloenya Date of Degree: July, 2007

Institution: Oklahoma State University Location: OKC or Stillwater, Oklahoma

Title of Study: DIRECT-FED MICROBIAL SUPPLEMENTATION TO CATTLE: EFFECTS ON MILK YIELD AND COMPONENTS, REPRODUCTION AND DIGESTION

Pages in Study: 189 Candidate for the Degree of Doctor of Philosophy

Major Field: Animal Nutrition

Scope and Method of Study: This study was undertaken to determine the effect of manipulating ruminal fermentation via direct feeding of yeast, propionibacteria or their combination to cattle on milk yield and components, reproduction and digestion. Experiment 1: From two wk prior to parturition to 210 d postpartum, 31 primi and multiparous Holstein cows were allocated to three treatments: Control fed corn based silage TMR; XPY, fed control TMR + yeast culture ; P169+XPY, fed control + XPY + P169 . Daily milk yield was recorded and milk samples were analyzed for fat, protein, lactose, solids corrected fat, urea nitrogen, and somatic cell counts. Blood samples were analyzed for progesterone concentrations. Experiment 2: Twelve ruminally and duodenally cannulated steers were assigned to one of the four treatments as described above including, P169 group, fed Control plus P169. Duodenal, ruminal, fecal and blood samples were collected. Intake, ruminal and total tract digestibilities, duodenal flow, fermentation profile, ruminal kinetics and plasma glucose and insulin were measured.

Findings and Conclusions: Feeding P169+XPY increased milk production by 8-12% , but only 3-5% above XPY-fed cows. P169+XPY fed MP cows had significantly greater milk glucose and lactose percentage than Control and XPY-fed MP cows. Plasma insulin concentrations were greater in P169+XPY than XPY and Control-fed MP cows. The XPY fed MP and Control PP cows exhibited a greater recovery of wk 1 BW. There was no significant difference in average days to first ovulation among all groups. We conclude that increased milk production from P169+XPY supplementation via alterations in milk glucose and plasma insulin had no adverse effect on reproductive performance. Experiment 2: Organic matter, NDF, ADF and N intakes were decreased by feeding XPY. However, nutrient flow, ruminal nutrient NDF digestibilities were not affected by dietary treatments. XPY fed steers alone increased total tract nutrient digestibility. Feeding P169 increased propionate by 14% and decreased acetate: propionate concentrations. These results suggest that feeding P169 might alter ruminal metabolism toward increased propionate without affecting feed intake, nutrient digestibilities, duodenal flow, rumen kinetics or microbial nitrogen flow.

ADVISER'S APPROVAL: Dr. Leon J. Spicer