USING DIRECT FED MICROBIALS AND EXOGENOUS FIBROLYTIC ENZYMES TO ENHANCE FIBER DIGESTIBILITY OF DAIRY COW DIETS

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

DAYANE NOGUEIRA LOBÃO DA SILVA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Dr. Noah B. Litherland and Dr[a]. Márcia Endres

June 2015

Acknowledgements

It has been such a tremendous learning experience to be part of the Graduate Program at the University of Minnesota. It has helped me to develop as a person and as a professional. I feel lucky and honored that I had the opportunity to work with and learn from so many incredible people that helped me during this journey.

Foremost, I want to thank my adviser Dr. Noah Litherland for believing in my work and for giving me the opportunities to work on different research projects. Also, I want to thank you for teaching me to work independently and as a team player.

My sincere thanks also go to my co-adviser Dr[a] Marcia Endres who has taken me under her arm and guide me throughout my Ph.D studies. Marcia, you love what you do that's what makes you such a great adviser. Thank you so much for everything!

I want to thank my co-advisers Drs. Jim Linn and Douglas Mashek for sharing their expertise.

Furthermore, I would like to thank all of my fellow labmates, especially Kelly Froehlich, Alex Gander and Jose Alvarez for helping me with sample collection and laboratory analysis. Also, to thank my friend and labmate Paula Basso for her companionship. I will miss our talks about transition cows and coffee breaks.

I could not forget the help from the University of Minnesota Dairy Research and Teaching Facility staff for helping with sampling collection during research projects. Thanks to all of you!

I also appreciate my sponsors: DuPont and AB Vista.

Last and most important, I want to thank my family for being supportive and for their unconditional love. Especially, I want to thank my beloved husband, Airton for

being such a great life partner and completing my life. Thank you for all your support and love!

Dedication

To my mother and father for making me to believe on my dreams.

To my beloved husband Airton for being always on my side.

To my daughter Livia for motivating me to conclude my studies.

Table of Contents

List of Tables

List of Figures

Introduction

Feed cost represents 40 to 60% of the total cost of production in dairy farms (Bozic et. al., 2012), and because of that nutritionists are constantly in search of alternatives to increase animals' feed utilization which can be accomplished by enhancing dietary digestibility of feedstuffs. Increasing fiber digestibility is a common practice as an attempt to reduce feed costs and ensure greater financial returns. Forages continue to be the most important components of the diets fed to ruminant animals even under intensive concentrate feeding systems (Beauchemin et al., 2013). However, the energy availability from forages is significantly limited by large quantities of fiber materials forming plant cell walls and as a consequence it limits feed intake and animal performance (Jung and Allen, 1995). To increase efficiency and precision of dairy cattle nutrition, novel feeding and feed ingredient technologies need to be continually implemented on farm in order to reduce operation costs and meet nutrient demands for greater milk production. As profit margins for milk production shrink, farmers must become more conscious of the importance of maximizing feeding efficiency.

Ruminant animals and microbes have evolved together in a symbiotic relationship in which microorganisms are able to ferment the polysaccharides of plant cell walls that are resistant to mammalian enzymatic hydrolysis (Knapp et al., 2014).The symbiotic relationship fills a niche, and the conversion of complex plant carbohydrates to energy is beneficial to both the host animal and the microbial symbionts. Even though fiber can be extensively digested by rumen microbes, the plant cell wall components digestibility can be limited by depots of lignin, a highly branched polyphenolic macromolecule resistant to chemical and biological degradation (Jung and Deetz, 1993). How the other materials in

the plant cell wall components are arranged within its structure can also determine digestibility of the plant (Van Soest, 1967).

Dairy producers aim to procure alternatives that provide the lowest cost source of nutrients that most closely matches the nutrient requirements of the dairy cow. Fiber digestion limitations have motivated producers to feed higher starch diets. Dietary starch plays an important role in the diet of high producing dairy cows, providing an energy-dense substrate especially critical during early lactation when glucose requirements are high (Van Vuuren et. al., 2009). However, dietary starch concentration can be challenging and lead to consequences that impair the rumen ecosystem (Enermark, 2008) leaving cows at greater risk of developing sub-acute ruminal acidosis (SARA), a common digestive disorder frequently caused by feeding a diet containing highly fermentable carbohydrates, with inadequate physically effective fiber required for adequate rumen buffering (Plaizier et al., 2008).

Methods that increase fiber digestion are likely to play a role in improving energy availability of ruminant diets and reducing feed costs (Vinici et al., 2003). Not just health concerns, but also increases in feed prices (Gencoglu et al., 2010) have prompted producer's interest to feed direct fed microbials (DFMs) and exogenous fibrolytic enzymes (EFE) which can be an alternative to enhance feed utilization by improving fiber digestibility, and increasing energy utilization per unit of feed, culminating in a reduction of feed costs (Beauchemin et al., 2008). Supplementation of DFMs and EFE is likely to increase fiber digestibility therefore, enabling nutritionists to formulate more flexible diets and increase the inclusion of forages in final rations without jeopardizing energy available for milk production. Increases in plant cell wall digestion due to DFMs

and EFE supplementation can reduce diet cost as the amounts of starch (provided by expensive cereal grains) are likely to be reduced whereas feed efficiency increases with a net effect of increased profit margins. Moreover, the increases in fiber digestibility might promote better energy balance for cows and be advantageous during the transition period (3 weeks before and 3 weeks after calving), decreasing body fat mobilization and reducing the incidence of metabolic diseases during this critical time of a cow's life.

Chapter 1: Review of Literature

a) Plant cell wall and dietary fiber

The metabolism of carbohydrates by ruminal microorganisms results in the production of volatile fatty acids which in turn, supply around 60 – 80% of the host animals' total caloric requirements. The conversion of complex plant polysaccharides, resistant to mammalian enzymatic hydrolysis, to energy is beneficial to both the host animal and the microbial symbionts (Knapp et al., 2014). In this context, forages continue to be the most import component of the diets fed to ruminant animals, even under intensive concentrate feeding systems. Animal performance, however, is greatly correlated to voluntary feed intake which is negatively affected by plant cell wall concentration when animals consume high-forage diets (Jung and Allen, 1995). Cell walls affect intake by contributing to ruminal fill which is critically determined by its concentration and rate of passage parameters (Jung and Allen, 1995).

Plant cell walls are composed of four major polymeric building components: three polysaccharides - cellulose, hemicellulose and pectin, and the polyphenol lignin (Glass et al. 2013). However, plant cell walls structure configuration and composition vary depending on plant tissue, age and cell type, and also within each cell wall layer (Ding et al., 2006). In fact, cellulose is the most abundant biopolymer on earth. Plants produce around 180 billion tons of this biopolymer per year worldwide (Festucci-Buselli et al., 2007). Cellulose is a water-insoluble β-glucan consisting of a liner molecular of up to 15,000 D-anhydroglucopyranose residues linked by a β-(1→4) bond. Anhydrocellobiose is the repeating unit of cellulose which is linked to glucose moieties that rotate 180º with respect to their immediate adjacent molecules. The microfibrils are organized on parallel

way to each other and consist of crystalline regions, and cellulose molecules are tightly together. Microfibrils can also be less organized and form paracrystalline (amorphous) sections (Sticklen, 2008; Paloheimo et al, 2011).

Hemicellulose or xyloglucans are a group of heterogeneous polysaccharides with branched chains consisting of various sugars units, and also closely associated cross-linking with cellulose and lignin. Hemicellulose structure is much more complex than cellulose, and it is named according to the main sugar monomer unit in its backbone structure. For instance, xylans are polymers with D-xylose units in the main chain and those with D-mannose, L-arabinose and D-galactose are referred to as mannans, arabinans and galactans, respectively. Xylan is the major component of hemicellulose, and can account for 20 - 35 % of plant cell wall material of annual plants such as grasses and cereals which makes this biopolymer the second most abundant renewable source on earth after cellulose. The main chain of xylan is composed of 1,4- β linked D-xylopyranose units which are shorten the cellulose chains. Typically, arabinoxylan is the common xylan found on cereal and grasses, but xylan can also contain ferulic acid and p-coumaric acid attached to the arabinofuranose structures (Sjöström, 1993, Sticklen, 2008, Paloheimo et al, 2011).

Pectin forms another group of heteropolysaccharides composed mainly of galacturonic acid residues from four major structural classes: homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II and xylogalacturonan (Carpita et al., 2002). Its major role on plant cell walls structure is to form a jelly-like matrix, which unites the other components of the plant cell walls together (Sticklen, 2008). The pectin and hemicellulose polysaccharides, and the aromatic polymer lignin, interact with the

cellulose fibrils, creating an inflexible structure strengthening the plant cell wall along with covalent cross-links, which are thought to be involved in limiting cell grow and reducing cell wall biodegradability (Paloheimo et al, 2011).

Plant cell wall or fiber digestibility by animals depends on the association of its major components, cellulose and hemicellulose with the primary factor lignin controlling nutrient availability (Van Soest, 1967). Lignin is a highly branched polyphenolic macromolecule strongly resistant to chemical and biological degradation and can account for about 10 - 25% of total plant dry matter. Lignin is linked in a network to cellulose and xylose with ester, phenyl and covalent bonds with an important role in protecting the plants against invasion by pathogens and insects (Mosier et al., 2005; Sticklen, 2008). Lignin is the most important single fiber component limiting nutrient animal accessibility to nutrients; however, digestibility of plant cell wall is more regulated by how the components of the cell wall are arranged than by its proportions (Van Soest, 1967; Jung and Deetz, 1993). Both cellulose and hemicellulose are slowly digested by rumen microbes, but can be completely digested in the absence of lignin (Weimer, 1996).

From an animal feeding standpoint, fiber instead of plant cell wall is a term used to define a nutritional, not a chemical or plant anatomical concept (Mertens, 1993). Mertens (1989) defines fiber as the "indigestible and slowly digesting, or incompletely available, fractions of feeds that occupies space in the gastrointestinal tract" which defines fiber as insoluble components of plants. The definition of fiber in animal nutrition was proposed by Van Soest (1967) who developed the neutral detergent fiber (NDF) method, a significant advancement for the nutritional characterization of feedstuffs; NDF includes cellulose, hemicellulose and lignin as part of its fraction. This also includes the

acid detergent fiber (ADF) that measures only lignin and cellulose. Neutral detergent fiber is the method that best separates structural from nonstructural carbohydrates in plants (NRC, 2001). Basically, this methodology divides plant cell walls into two categories: a readily digestible fraction and an incompletely digestible fraction isolated as fiber components (hemicellulose, cellulose and lignin) (Van Soest 1967). Later on, due to starch and protein contamination concerns, the NDF method was improved by adding heat stable amylase and sodium sulfite steps, respectively, and it was named amylase-treated NDF (aNDF) (Undersander et al., 1993). It is important to notice that the NDF method does not consider pectin as fiber component. Although pectin is present in plant cell walls, this polysaccharide is readily fermented in the rumen and thought to be completely available to the ruminant.

Fiber concentration of feed material is a critical component of animal nutrition. The concentration and digestibility of NDF in feeds is closely related to the variation in dry matter digestibility (DMD) which determines how much nutrient is available to the animal. Neutral detergent fiber has a negative relationship with DMD which is related to the energy in the feed available to the animal and so is linked to animal performance (Mertens et. al., 2002). Moreover, NDF fraction plays an important role in rumen health. It stimulates the appropriate motility of the rumen to promote rumination, secretion of saliva to regulate ruminal pH, and development of the ruminal mat that optimizes the fermentation processes (Tafaj et al., 2004; Zebeli et al., 2012). The effectiveness of fiber in meeting ruminant minimum requirements is measured not only by the aNDF measurement itself but within combination with fiber's physical properties, such as particle size. This effectiveness has been traditionally referred as the ability of fiber to

maintain milk fat production and animal health. Effective fiber is divided into two different concepts: physically effective NDF (peNDF) and effective NDF (eNDF). The peNDF is related to physical characteristics of fiber (primarily particle size) that influence chewing activity and the biphasic nature of ruminal contents (buoyant mat of large particles on a pool of liquid and small particles). Also, peNDF supply additional buffering to the rumen and help to modify rumen pH. The eNDF is related to the sum total ability of a feed to replace forage or roughage in a ration. The peNDF can range from 0 when NDF in a feed stimulates no chewing to 1 when NDF promotes maximum chewing activity and can be measured by both animal physiological responses, pef = [min. of chewing per kg of NDF in the test feed] / [min of chewing per kg of NDF in long grass hay]) (Mertens, 1997), and laboratory procedures which consider the peNDF as product of physical effectiveness factor (pef) measured by the proportion of feed retained on a 1.18 mm (Mertens, 1997; Kononoff et al., 2003) or) or 8 mm (Lammers et al., 1996) sieve and multiplying it by the NDF content of a feed (i.e., peNDF = pef × NDF; Armentano and Pereira, 1997). According to Mertens (1997) calculations using physiological responses of dairy cows, the requirement for peNDF equals 22% of ration DM to maintain an average ruminal pH of 6.0 and 20% of ration DM to maintain the milk fat percentage of early to midlactation Holstein cows at 3.4%.

The goal of analyzing the particle size of ingredients and diets is an attempt to measure the particle size distribution of the feed cows are actually consuming. Although various methods are available to measure particle size of diets, the newest Penn State Particle Separator (PSPS) which has three sieves (19-mm, 8-mm and 4-mm) and a bottom pan has been designed to mimic the complex laboratory method, and it is the most current

on-farm tool used to estimate the peNDF of feed (Heinrichs, 2013). Briefly, a representative 3 pints sample of feedstuff is place on the upper sieve of the PSPS and then stacked on the top of the two other sieves and bottom pan. The PSPS is placed on a flat surface and shaken in one direction 5 times then rotated one-quarter turn. The process is repeated 7 times making a total of 8 sets and 40 shakes. The amount remaining on each sieve is then weighted and the percentage of each sieve is calculated. The peNDF is calculated by adding the percentage of feed on the top three sieves (all > 4-mm) and multiplying the value by the NDF content of the feedstuff (Heinrichs, 2013).

The NRC (2001) recommends a minimum of 25% to 28% dietary NDF, 75% of which should be supplied by forages. Essentially fiber is added to ruminant's diets to promote adequate rate of passage and digestion which results in rumen stability, and a harmonic symbiosis between host and microorganisms with greater feed efficiency. Dietary fiber contributes to formation of the ruminal mat, and it works as an effective first-state separator between the gas layer and liquid layer (Zebeli et al., 2012). Fiber mat increases the time allowed for digestion by filtration and trapping potentially small particles that could easily escape the fermentation process (Weidner and Grant, 1994). However, a very high level of NDF in the ration can reduce intake and animal performance. For instance, Allen and Bradford (2009) reported that several studies in the literature showed a decrease in dry matter intake (DMI) of up to 4 kg/d when diet NDF content was increased from 25 to 35% by substituting concentrates for forages. It is important to notice that NDF produces some filling effect in a diet which can also be determined primarily by the initial bulk density of feed and their filling effects over time in the rumen (Allen and Bradford, 2009). However, according to Allen (2000) increasing

diet NDF content by increasing non-forage fiber source (NFFS) such as soy hulls, beet pulp, cottonseeds, corn gluten feed and distiller's grains has shown little effect on DMI. As feed intake increases in early lactation, control of feed intake is dominated by ruminal distention and the extent to which ruminal distention limits feed intake is linearly related to milk yield (Voelker Linton and Allen, 2007). Therefore, techniques to increase fiber digestibility and thus animal performance are desirable and should be closely investigated to increase feed utilization.

Rumen microbial population's ability to degrade fiber can be affected by type of forage, harvest, and fermentation or processing methods (Galloway et al., 1991). Factors such as hybrid selection, maturity at harvest, starch content and length of fermentation prior to feeding can impact corn silage digestibility and subsequent milk yield. For instance, Johnson et al. (1999) reported greater DMI and increased milk production ranging from 0.2 to 2.0 kg/d when cows were fed mechanically processed corn silage vs. non processed corn silage. Moreover, Oba and Allen (1999) reported significant increases in milk production (41.7 vs. 38.9 kg/d) of dairy cows when diets containing either brown midrib corn silage (49% NDFD) or normal corn silage (39.4% NDFD) were fed.

Dairy nutritionists and dairy producers are constantly seeking strategies to both increase the digestibility of forage and also reduce its variability in digestibility as these factors are likely to play a role on animal performance. Oba and Allen (1999) suggested that each unit-increase in neutral detergent fiber digestibility (NDFD) was associated with 0.17 kg increase in DMI and a 0.25 kg increase in 4% fat-corrected milk. Furthermore, these authors also suggested that digestibility of NDF should be measured more routinely to assess forage quality effects on animal performance. Moreover, DMD

and NDFD of forages are not constant and feed additives that increase DMD, NDFD, and therefore DMI are needed to increase cow's performance and increase feed efficiency.

b) Non-fiber carbohydrates and dietary starch

Carbohydrates are broadly classified as either nonstructural or structural. Structural carbohydrates comprise those found in plant cell walls and nonstructural carbohydrates (NSC) are found inside the cells of plants. Nonstructural carbohydrates are the major source of energy for high producing dairy cows and include sugar, starches, organic acids and fructans (NRC, 2001). However, from a ruminant feeding perspective, non-fibrous carbohydrates (NFC) is mostly considered by nutritionists when formulating rations for dairy cows. Non-fibrous carbohydrates include pectin as one of its components as this structural polymer is rapidly and completely fermented by microorganisms in the rumen. Plant NFC can be calculated as: NFC = 100 - (%NDF + %CP + %Fat + %Ash) (Grant, 2005; NRC, 2001). Due to rumen health concerns such as acidosis, the maximum concentration of NFC should be limited to 32-42 percent of the total ration dry matter (DM) (Nocek, 1997). According to NRC (2001) the optimal concentrations of NFC in diets of high producing dairy cows are associated with: 1) starch ruminal digestion rates and its impacts on fiber digestibility; 2) the impact on volatile fatty acids (VFA), rumination and salivation due to replacement of NDF by NFC; 3) starch digestion location; 4) animal physiological state and differences in DMI; 5) conservation as well as processing methods used to increase NFC digestion.

Starch and sugar are the major components of NFC plant fraction. Sugars, the second major component of NFC after starch, are highly water soluble carbohydrates supplying a rapid source of energy to rumen microbes which may alter the rumen microbial ecology to increase fiber digestion (Chamberlain et al., 1993). However, dietary situations influence the optimum feeding rate of between 2.5 and 5% of supplemental sugar (Broderick and Radloff, 2004; Firkins et al., 2008). Compared to starch and structural carbohydrates, microbes spend less energy to reduce sugars to smaller units (Golder et al., 2012) which means that rumen microbes can utilize sugars at faster rates, and use this energy to grow more rapidly and increase their capability to degrade more fiber. Rate of carbohydrate fermentation can be beneficial in same extent as it might result in more effective capture of rumen degradable protein (RDP) and increase the supply of metabolizable protein (MP) (Broderick and Radloff, 2004). In addition, sugars can also be converted to VFA that can be absorbed through the rumen in a short period of time and then be used as energy source by the host animal (Nafikov and Beitz, 2007). For instance, sucrose (a disaccharide composed of the monosaccharides glucose and fructose that makes up 70% of the cane molasses supplementations for cattle) is largely fermented in the rumen and has been associated with greater favorable effects on the ruminal environment, especially for fiber digestion compared with starch. Also, molasses sugar has been related with increased molar yields of acetate and butyrate (Broderick and Radloff, 2004; Hall and Weimer, 2007; Oelker et al., 2009).

Starch is the major component of the NFC fraction in dairy cattle diets, and its optimal concentration in diets is a function of several factors, including the intrinsic degradability of the starch source; processing method such as fine grinding, steam flaking

or ensiling; amount of soluble protein; NDF content; and feeding method. Commonly, dietary starch recommendations range between 23 to 30% of ration dry matter (DM) depending on forage content of the diet (Grant, 2005) with its total-tract digestibility in dairy cows ranging from 70 to 100% (Firkins et al., 2001; Ferraretto et al., 2013). Dietary starch of high yielding dairy cattle can frequently bypass ruminal fermentation and 1 to 5kg/d of starch can became available for degradation and absorption in the lower gastrointestinal tract (McCarthy et al., 1989; Aldrich et al. 1993). However, starch might also totally bypass gastrointestinal digestion and end up in the fecal material. In this scenario, a portion of the fecal material can be sieved and the amount of grain caught by the screen in addition with measurements of the dietary starch concentration and in vitro starch digestibility of feedstuffs can determine how rations should be adjusted to avoid incomplete digestion and excessive grain in feces (Hall, 2002).

Feeding diets differing in starch and NFC concentrations as a prepartum feeding strategy for optimal postpartum intakes and better health performance has been more controversial than clearly defined. The transition period defined 3 weeks before calving and 3 weeks after calving is the most stressful time of a cows' life. Although energy demand increases in late gestation and early lactation, feed intake typically decreases (Grummer, 1995). Rapid growth of the fetus can account for the decrease in prepartum intakes due to extra abdominal compression and reduction in the rumen capacity, and after calving feed intake continually increases and peak around 9 – 13 weeks of lactation (Kertz et al., 1991). The period from parturition until peak milk production is the most critical phase for a dairy cow (Schingoethe et al, 1993). In fact, maintenance and pregnancy energetic requirements of dairy cattle increase 23% during the last month

prepartum (Moe et al., 1972), whereas feed intake typically decreases 30% (Grummer, 1995), leading to considerable adipose tissue mobilization and increases in non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) circulation (Overton and Waldron, 2004). Blood concentration of NEFA can be used as a suggestive of energy deficit at prepartum and as an index of lipid mobilization during postpartum (Duffield, 2000). However, primiparous and multiparous cows present a different pattern in blood metabolites (Meikle et al., 2004; Santos et al., 2001).

Adequate nutritional management during the transition period is critical and should minimize health related problems and maximize future lactation performance (Douglas et al., 2006). Overfeeding energy during prepartum and high NFC diets have been associated with greater decreases in DMI, energy intake and gluconeogenesis resulting in increased rates of postpartum lipolysis and more risk of developing diseases such as fatty liver (Minor et al., 1996; Douglas et al., 2006; Janovick et al., 2011). However, Rabelo et al. (2003) supported the opposite and pinpointed that feeding high starch diets prepartum might be an advantageous management practice because it adapts a cow's rumen papillae to high concentration diets that will be fed in early lactation. Moreover, Van Vuuren et al. (2010) reported greater DMI for dairy cows fed high-starch diets compared with cows fed low-starch, and attributed those results to the greater palatability of high-starch diets.

During early lactation dietary starch plays an important role in the diet of high producing dairy cows, providing an energy-dense substrate especially critical during periods when glucose requirements are high (Van Vuuren et. al., 2009) but cows struggle to meet these demands due to low DMI and high milk yields, often leading to postpartum

energy balance deficit (Spurlock et. al., 2012). However, glucose availability for direct absorption is low in ruminants due to high carbohydrate fermentation rates in the rumen (Baird et al., 1980). In fact, starch is efficiently fermented by microorganisms in the rumen to short-chain fatty acids which from these propionate, valerate, and isobutyrate can be utilized as glucogenic precursor for net synthesis of glucose to fulfill high priority of lactose in the mammary gland. Nevertheless, propionate is known to be the most abundant of the three glucogenic acids (15 - 40%) and by far the predominant substrate for gluconeogenesis in ruminants for lactose demand. High producing dairy cows produce over 2 kg of lactose daily and have specific requirement for glucose especially during early lactation. Failure in supplying glucose may not only lead to decreases in milk yields but also can cause disorders in fat metabolism occasioning direct and indirect health problems (Knegsel et al., 2005). The mammary gland utilizes 60 to 85% of the total glucose used in lactating ruminants, lactose synthesis by itself accounts for 50 to 85% of mammary glucose utilization (Annison, 1974). Glucose requirements and status are critically dependent on state of lactation and level of milk production in dairy cattle, which is closely related to endogenous glucose production supported by endocrine changes during peak lactation (Hammon et al., 2010; Reynolds 1995). Decreases in tissue responsiveness to insulin and reduction in glucose uptake into insulin-sensitive organs (muscle and adipose tissue) is part of homeorhetic changes that take place after parturition and thus favors glucose uptake into the mammary gland (Komatsu et al., 2005). Insulin resistance ensures that body reserves can occur to support mammary gland requirements (Bauman, 2000).

Nevertheless, in spite of being a key nutrient of dairy cow diets during early lactation by providing an energy-dense substrate, dietary starch concentration can be challenging and lead to consequences that might impair the rumen ecosystem (Enermark, 2008). High-producing dairy cows fed high-starch diets are commonly prone to development of SARA, frequently caused by feeding a diet containing highly fermentable carbohydrates, with inadequate physically effective fiber required for adequate rumen buffering, resulting in daily episodes of low ruminal pH (Plaizier et al., 2008; Zebeli et al. 2008). There is a discrepancy in the literature regarding exact threshold of pH to correspond to the presence of SARA (Zebeli and Zebeli et al., 2012). However, the duration which pH remains below 5.6 which is detrimental to ruminal epithelium and VFA absorption (Russel and Wilson, 1996; Plaizier et al., 2008) or 5.8 which is harmful to ruminal cellulolytic bacteria (Gabel et al., 2002; Zebeli et al., 2008) for longer than 3 or 4 h/d, respectively, are safer indicators of SARA. Drops in ruminal pH can negatively impact rumen NDF digestibility since fibrolytic bacteria have low tolerance to low pH and their growth ceases at pH below 5.8 (Kaufman et.al 1980; Meissner et al., 1996). It is estimated that the prevalence of this digestive disorder range from 19 to 29% in lactating dairy cows (Garrett et al., 1997; Krause and Oetzel, 2006). Low forage diets promote less rumination rates, resulting in lower secretion of saliva, and this may decrease the buffering capacity in the rumen leading to low ruminal pH (Maekawa et al., 2002; Beauchemin et al., 2008). Also, rapidly fermentable carbohydrate diets result in greater VFA accumulation which leads to low ruminal pH , caused also by the instability in the rumen microbial populations that results when the balance between lactate-producing bacteria and lactate-utilizing bacteria is disrupted contributing to the risk of SARA

(Nagaraja and Titgemeyer, 2007). Rumen digestive disorders can also disrupt the host's inner homeostasis triggering the activation of acute phase response (APR) (Zebeli and Metzler-Zebeli, 2010). Moreover, it has been demonstrated that high culling rates, fluctuating DMI, and laminitis are clinical manifestations of SARA which may be exacerbated in primiparous cows because they have not previously had long-term exposure to a highly fermentable lactation diet (Mirzaei Alamouti et.al., 2009). Results from Beauchemin et al. (1997) suggested that the effects of low fiber diets on intake differ for younger and older cows, as well as effects of diets on digestibility. Parity is an important concern when feeding cows as feed intake and meal patterns differ between primiparous and multiparous animals and they are usually grouped and managed differently (Grant et al., 1995).

Animal welfare and production performance concerns in addition with recent increases in feed costs, especially grains, and consequent reductions in income over feed cost have encouraged dairy producers to use moderate starch diets (Gencoglu et al., 2010 and Ferraretto et al., 2011). Replacing a portion of the diet's concentrate for ingredients with greater fiber content can lower the price of final diets and also decrease disturbances in rumen metabolism (Staple, 2007; Zebeli and Metzler-Zebeli, 2012). Special attention should be given to early lactating cows who already undergo tremendous metabolic challenges during the transition from late gestation to early lactation (Bell, 1995), and introduction of high-starch diets during this period might present an extra challenge to periparturient cows that are already struggling to adjust to several metabolic changes (Drackley, 1999). Feeding a moderate starch diet with elevated fiber may promote increased fiber digestibility while maintaining the energy density necessary to maintain

milk production and meet the higher energetic requirements of high-producing cows. However, offering diets in excess of fiber and low rapidly fermentable carbohydrates may decrease feed intake and lower the efficiency of feed use (Yang and Beauchemin, 2006). Therefore, if moderate-starch diets are fed, then it seems reasonable that the fiber digestibility should be boosted thus right amounts of energy can still be provided to cows to sustain high milk production, and meet their energetic requirement. Increases on dietary fiber concentration can be compensated by boosting fiber digestibility to a maximum. Direct fed microbials have generally been supplemented to animals during periods of stress or low DMI with the assumption that establishment of beneficial microorganism populations in the digestive tract will decrease or prevent pathogenic organism establishment (NRC, 2001). Moreover, supplementation of enzyme feed additives with fibrolytic properties can also offer a choice for increasing fiber digestibility and improving ruminal energy utilization (Chung et al., 2012).

c) Direct fed microbials

Feeding antibiotics to animals was prohibited in the European Union (EU) in 2006 due to concerns over increasing bacterial antibiotic resistance in humans, as sub-therapeutic doses of antibiotics had been used in feed to promote growth and maintain health in farm animals (Prieto et al., 2014). The growing concern regarding the use of antibiotics in animal production has increased the interest of exploring alternatives to antimicrobial feed additives (Martin et al., 1999). Direct fed microbials, traditionally referred to as “probiotic” are live or viable naturally occurring organisms commonly used as supplements in animal production with the goal to confer a health benefit to the host

by including improved establishment of beneficial gut microflora (Fuller, 1999; NRC, 2001). Different probiotic strains have been shown to successfully improve growth performance and to reduce enteric disease in pigs, poultry, ruminants and cultured fish (Balcázar et al., 2006; Gaggìa et al., 2010).

Probiotics have been used in animal and human nutrition to promote health for over a century (Metchnikoff and Chalmers, 1910; Edens, 2003). Once they populate the digestive tract, these microorganisms play an important role in the health, growth, and development of the host (O'Hara and Shanahan, 2006). In fact, the inclusion of supplements such as DFMs have been a common practice in dairy cows diets, especially during periods of stress or low DMI (Nocek and Kautz, 2006), with the theory that those beneficial microorganism populations can establish themselves in the animal digestive tract and decrease or prevent pathogenic organism establishment and return gut function more quickly (NRC, 2001).

Direct fed microbials have three primary ways to influence ruminants: 1) as an additive for silage or haylage or as a preservative for hay; 2) to replace or decrease the use of antibiotics in stressed cattle; 3) to enhance feed efficiency and increase milk production in dairy cows and body weight gain in beef cattle (Yoon and Stern, 1995).The term DFMs has included specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Beharka and Nagaraja, 1993; Sullivan and Martin, 1999; Knowlton et al., 2002). The most common DFMs interventions of ruminal fermentation to promote desirable intestinal micro flora, improve nutrient utilization and stabilize pH to promote rumen health, include the supplementation of fungal cultures (*Aspergillus oryzae*, *Saccharomyces cerevisiae*) and lactate producing (*Enterococcus*) and lactate-utilizing

(*Proprionibacterium*) bacterial species as well as *Bifidobacterium spp*., and *Bacillus spp* (NRC 2001; Beauchemin 2003 and FAO, 2013).

A diversity of mechanisms and theories have been suggested to clarify the rumination fermentations and improvements in performance when ruminants are fed fungal-based DFMs. However, production responses attributed to yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and increased flow of microbial protein from the rumen (Martin and Nisbet, 1992; Newbold et al., 1996). For instance, *Aspergillus oryzae*, a particular fungal strain, has produced a range of different results when included into lactating cow diets. Most of the results have been positive responses by improving digestibility of various fiber fractions (Martin and Nisbet, 1992). Fungi cultures are thought to produce significant levels of cellulases and xylanases which are known to increase fiber digestion in ruminant (Akin and Borneman, 1990). *Saccharomyces cerevisiae* fungal strain had been successfully used as an alternative to antimicrobial feed additives for over 2 decades (Ramsing et al., 2009). This fungal strain has been known to increase dietary nutrient availability and milk production by promoting cellulolytic, proteolytic, and lactate-utilizing bacteria in the rumen (Harrison et al., 1988; Callaway and Martin, 1997). It has been verified that *Saccharomyces cerevisiae* increases DMI and improves immunologic status, resulting in higher milk production and lower milk somatic cell count (SCC) of dairy cows in early lactation (Zaworski et al., 2014). Furthermore, supplementation of *Saccharomyces cerevisiae* has been shown to improve immune function in dairy calves, broilers, and pigs (Magalhães et al., 2008; Gao et al., 2009; Shen et al., 2009) by activating the innate and adaptive immune response (Jensen et al., 2008).

Fungal species are also commonly fed to animals as a blend with bacterial species such as *Enterococcus* strains which have been selected for raising nadir pH in the rumen, and increasing daily mean rumen pH (Nocek et al., 2002; Oetzel et. al., 2007). *Enterococcus* strains in a combination with yeast product have enhanced lactation cow performance by increasing milk protein percentage and improving health status by decreasing the number of antibiotic treatment which might be a promising alternative to enhance performance of transition dairy cows (Oetzel et al., 2007). The combination of bacterial and fungal strains has also been supplemented to feedlot cattle fed a high-grain diet as an attempt to decrease the risk of acidosis and improve feed digestion. Beauchemin et al. (2003) showed that although *Enterococcus faecium* caused only small shifts in microbial ecosystem when supplemented to feedlot cattle fed high grain diets, it appeared to be metabolically active.

Proprionibacterium as a DFM form is also commonly fed to animals, and is also known to improve ruminal function. Ghorbani et al. (2002) reported that steers receiving both lactate-utilizing *Propionibacterium* and lactate-producing *Enterococcus* had higher ruminal concentrations of acetate, and some of the blood variables measured indicated a reduced risk of metabolic acidosis. Although the mode of action of DFMs in the rumen is not completely understood, the presence of lactate-producing bacteria is thought to help the ruminal microflora adapt to the presence of lactic acid (Ghorbani et al., 2002), whereas the presence of lactate-utilizing bacteria is thought to prevent accumulation of lactate (Martin and Nisbet, 1992).

The use of *Bacillus* strains, frequently fed to animals as DFMs, has also been drawing attention over the years due to its benefits on animal health and fiber

digestibility. While once considered soil microbes, *Bacillus* species are now commonly used as probiotics in animal and human diets as their commensal nature has gradually been realized (Cutting, 2011). For instance, *Bacillus pumilus* (BP) a spore-forming gram-positive bacterial species which has also been found in bovine ruminal fluid (Degrassi et al., 1995), has successively proved to effectively produce xylanase with the genetic machinery necessary for the metabolism of xylan (Kanehisa et al., 2014; Kanehisa et al., 2000). Xylanase can break down xylan and the bonds that link lignin to cellulose (Poorna and Prema, 2007).

Xylan is the second most abundant biopolymer renewable source on earth after cellulose (Wong et al., 1988; Benini et al., 2001). Microbial xylanases (especially endo-β -1, 4-xylanase, EC 3.2.1.8) are critical enzymes for xylan hydrolysis, and display promising potential for application in the food, animal feed, paper, and pulp industries (Poorna and Prema, 2006; Badhan et al., 2007; Battan et al., 2007). *Bacillus pumilus'* capacity to degrade lignocellulosic material (Degrassi et al., 1995), has led to a discussion of the benefits that *Bacillus pumilus* might have in enhancing feed utilization and increasing animal feed efficiency as well as animal performance. *Bacillus* spores display a potential promise to enhancing animal performance due to their ability to degrade fiber materials.

For instance, *Bacillus subtilis* has been successfully used as probiotics. It has shown improvement in growth performance in chickens (Samanya and Yamauchi, 2002; Chen et al., 2009) and white shrimp (Liu et al., 2009). Kritas et al. (2006) found that *B. subtilis* combined with *Bacillus licheniformis* increased the milk yield in ewes, and *B. subtilis natto* altered rumen fermentation pattern in calves (Sun et al., 2013).

Furthermore, Peng et al. (2011) reported that *B. subtilis natto* fermentation products effectively increased lactation performance of early lactation dairy cows possibly by altering the rumen fermentation pattern without any negative effects on blood metabolites. Along with potential promise to enhance fiber digestibility, *Bacillus* spores also are well-known for their antimicrobial activity against a range of gram-positive and some gram-negative bacteria due, at least in part, to the production of bacteriocins (Cutting, 2011; Prieto et al., 2012; Prieto et al., 2014). In fact, *Bacillus* antimicrobial activity has been shown against *Salmonella and E. coli*, common microorganisms capable of causing clinical disease mainly in pigs and humans (EFSA, 2008). It is believed that in animals the oligosaccharides derived from cell wall digestion resist the attack of digestive enzymes, and are able to reach the colon, where they work as 'prebiotics' supporting proliferation of beneficial microflora such as *Bifidobacterium* and *Lactobacillus* spp., and at the same time suppressing the growth of pathogenic bacteria such as *Salmonella*, *Clostridium*, *Campylobacter* and *Escherichia coli* (Thammarutwasik *et al.,* 2009).

It is relevant to consider that dietary changes influence populations of ruminal microbes, which can be highly specific to the ruminant host, and if properly manipulated can improve the nutritional management of ruminants (Mullins et al., 2013). Evidence support that rumen microbial populations adapt to dietary changes (Ramirez et al., 2012). Modern animal production should effectively take advantage of feed additives as rumen manipulators to increase animal productivity. Despite the range of promising benefits of *Bacillus pumilus* including enhanced fiber digestibility and increased feed efficiency, there is still a lack of information on the effects of its supplementation to ruminant

animals. Research should consider investigating the benefits of relevant DFMs such as *Bacillus pumilus* that can improve animal performance and economic returns.

d) Exogenous fibrolytic enzymes

Increase in feed prices, especially grains, and declines in enzyme costs have prompted interest in using enzyme additives in dairy cattle diets to increase nutrient utilization, and assure profitability and sustainability of animal production activity (Beauchemin et al., 2008). Methods that increase fiber digestion are likely to play a role in improving energy availability of ruminant diets and reducing feed costs (Vinici et al., 2003), as forage digestibility continues to limit the intake of available energy by ruminants, and correspondingly, contributes to excessive nutrient excretion by livestock (Beauchemin et al., 2013). Forages contain about 30 to 70 percent NDF, and NDF digestibility in the ruminant digestive tract is typically less than 65 percent for North American diets (with about 50 percent NDF degradability in the rumen), but can be considerably higher for some grasses and grass silage-based diets (Tas et al., 2005; Huhtanen et al., 2009)

Ruminal energy utilization can be increased by enzyme feed additives with fibrolytic properties (Chung et al., 2012), as these products increase the quantity of enzymes that are available to digest fiber materials in the rumen to enhance utilization of fibrous feedstuff (Vinici et al., 2003). In general, enzyme supplementation provides more flexibility when formulating a diet since the ingredient quality or animal digestibility capacity can be manipulated (Paloheimo et. al, 2011). Enzymes can enhance feed

utilization use by increasing the rate and extent of pre-ingestive, ruminal, and postruminal fiber hydrolysis, digestion, and degradation, by increasing the ruminal passage rate, by increasing ruminal microbial numbers and/or attachment, by stimulating ruminal microbes, and by decreasing digestive fluid viscosity (Adesogan et al., 2014; Morgavi et al., 2000)

Plant cell wall digestion is complex as its three major polysaccharides building components cellulose, hemicellulose and pectin are cross-linked with lignin, a polyphenolic macromolecule strongly resistant to chemical and biological degradation (Glass et al. 2013). Plant cell walls are also linked with enzymes, structural proteins, and proteoglycans, forming an intricately linked network that provides strength and durability to its structure (Popper et al., 2011). Therefore, numerous enzymes are required in the process of plant cell wall digestion (Stichlen, 2008).

The non-starch polysaccharide (NSP) enzymes in feed have traditionally been classified according to the IUB Enzyme Nomenclature (Bairoch, 2000) and belong to the glycosyl hydrolases (EC 3.2.1.x). This classification is based on both the reaction type and substrate specificity, e.g. β-glucanases hydrolyzing β-glucan, and xylanases acting on xylan. Most of the glycosyl hydrolases are endo-acting enzymes, cutting in the middle of the polymer chain and rapidly reducing viscosity (Paloheimo et. al, 2011). Four classes of enzymes are involved in the biodegradation of cellulose, with the EC number based on the type of reaction catalyzed by the enzyme. Endoglucanases (EC 3.2.1.4) hydrolyze cellulose to gluco-oligosaccharides. Cellobiohydrolases (EC 3.2.1.91) release cellobiose from crystalline cellulose. Glucosidases (EC 3.2.1.21) degrade the oligosaccharides to glucose. Exoglucanases release glucose from cellulose and gluco-oligosaccharides

(Sticklen, 2008). The biodegradation of the xylan backbone, the major component of hemicellulose, depends on two classes of enzymes. Endoxylanases (EC 3.2.1.8) are able to cleave the xylan backbone into smaller oligosaccharides, which can then be degraded further to xylose by xylosidases (EC 3.2.1.37). Both classes of enzymes, as well as their encoding genes, have been characterized from many organisms.

Celluloses and xylanases are produced by free-living and gut microorganisms and have also been found from algae, protozoa, snails, crustaceans and seeds of terrestrial plants (Woodward, 1984; Sunna and Antranikian, 1997; Dornez et al., 2009). The β – glucanases (cellulases) and xylanases have been used as feed additives for over 20 years and their ability to improve the feed conversion ratio and weight gain of monogastric animals (poultry and pigs) has been demonstrated in numerous publications (Paloheimo et. al, 2011).

Filamentous fungi are among the most efficient degraders of plant biomass and used as the main source to produce commercial enzymes used to degrade plant cell walls (Kubicek et al., 2009). The most commonly used organism for commercial production of cellulases is *Trichoderma reesei* (Hypocrea jecorina); however, other filamentous ascomycete fungi in particular *Aspergillus niger* and *N. crasa* have been addressed as fiber digester microorganisms (Glass et al. 2011). Regarding the bacterial commercial preparation, fibrolytic enzymes are derived basically from *Bacillus* species (Sunna and Antranikian 1997). Fungi and bacteria microorganisms carrier specific genes that encode a variety protein that act as fibrolytic enzymes under cellulose, xylanase – inducing condition and synergically contribute to plant cell wall destruction (Sunna and Antranikian 1997; Glass et al, 2011). The wide range of enzymes produced by

Aspergillus, for the degradation of plant cell wall polysaccharides, is crucial to the food and feed industry. Recently, several *Aspergillus spp*. have received increased interest as hosts for heterologous protein production. The black aspergilli have a number of characteristics which make them ideal organisms for industrial applications, such as good fermentation capabilities and high levels of protein secretion (Davies, 1994). Large amounts of cellulolytic enzymes (cellulases and hemicellulases) are also secreted by *Trichoderma reesei* which increase the capacity to utilize this microorganism in numerous industrial applications for degradation of plant cell wall polysaccharides (Kumar et al., 2008)

However, efficacy of fibrolytic enzymes in improving animal's performance while increasing feed conversion in ruminants has been variable (Beauchemin et al., 2008). The variability of responses to exogenous fibrolytic enzymes can be attributed to the durations of feeding period, stage of lactation that cows are fed, and inappropriate choice of enzymes with lack of sufficient potency and specificity for improving digestibility under ruminal conditions (Adesogan et al. 2014). It is recommended that feeding trial should be followed only after previous in vitro model evaluation of EFE on ruminal temperature and pH conditions. In order to optimize enzyme activity it needs to be placed in an ideal media with proper temperature, pH and have comparable subtract. According to a study mentioned on a literature review by Adenogan et al. (2014) an evaluation of 18 commercial EFE showed that 78 and 83% of them exhibited optimal endoglucanase and xylanase activities, respectively, at 50°C, and 77 and 61% had optimal activities at pH 4 to 5, respectively, indicating that most would likely act suboptimally in the rumen.

Ariola et al. (2011) reported that multiparous or primiparous cows fed low-concentrate diets treated with fibrolytic enzymes had a better performance than cows fed untreated high-concentrations diets, which leads to the conclusion that greater forage proportions can be fed to dairy cows without jeopardizing milk production, lowering the cost of diets and reducing risk of rumen acidosis. Enzyme additives increase the rate of fiber digestion, which can provide more digestible energy to the animal for growth or milk production (Beauchemin et al., 2008). Dairy animals usually calve for the first time at about 24 months of age as this maximizes economic benefit (Hoffman and Funk, 1992). However, animals are not yet physically mature at this stage; they require nutrients for their own continued growth in addition to that of their developing calf (Coffey, 2006). Supplementing primiparous cows with fibrolytic enzymes before calving can boost energy supply for growth during prepartum resulting in greater periparturient energy balance in addition to increasing their adaptation to enzyme additives before lactating starts and assuring greater enzyme responses during their first lactation.

Enzyme additives provided by the manufacturers are usually powdered products that need to be diluted into water daily and then applied to ingredients to be fed to cows (Yang et al., 2000). It might be an inconvenience due to the additional on-farm labor and uncertainty if the right dose of EFE is being delivery to the diets. This is an important concern that should be raised since factors as level of enzyme provided and method of application can account for inconsistent responses of enzyme supplementation (Zinn and Salinas, 1999). Technologies that increase the efficacy of fibrolytic enzyme ensuring the delivery of an accurate dose of this product play a role in improving cows' performance responses. Fibrolytic enzyme preparation ready to be used, and delivered through a feed

ingredient such as molasses-based liquid feed (MLF), for instance, might reduce on-farm labor and increase the acceptance of this new technology by producers while decreasing the errors of enzyme preparation. Moreover, MLF is designed to provide sugar into diets and also improve particle adhesion. This ingredient feature can help to enhance binding of the enzyme with the feed substrate, which may increase the resistance of enzyme to proteolysis in the rumen. According to Morgavi et al. (2000) applying a solution of enzymes to the feed allows the enzyme to bind to substrate, which increases the resistance of the enzymes within the rumen.

The use of EFE to improve forage quality can also replace other expensive strategies that increase fiber digestibility such as treatments with physical agents such as heat, steam, and pressure, or with chemicals such as acids, alkalis, NH3, and ozone. Those alternatives are likely to require a bigger investment of capital and have essentially a high energy intensive utilization for physical methods such as steam or pressure explosion, the potential of pelleting, chopping, or grinding which might result in limiting salivary buffering of ruminal acids. Moreover, the corrosive and/or hazardous nature of chemicals such as NH3 and NaOH might add potential for excessive DM losses following hydrolysis (Adenogan et al., 2014; Lynch et al., 2013).

Should you have a brief conclusion? Maybe not needed.

Chapter 2: Determination of the effect of *Bacillus pumilus* on rumen fermentation patterns, diet digestibility, and lactation performance in multiparous dairy cows fed low or high starch concentration both pre- and postpartum

D. N Lobão da Silva*, Z. Sawall*, E. Galbraith†, T. Parrott‡, M. I. Endres*, N. B. Litherland*

*Department of Animal Science, University of Minnesota, St. Paul 55108.

† DuPont Nutrition and Health, Waukesha, WI.

‡ DuPont Industrial Biosciences, Waukesha, WI.

Overview

The objective of this study was to investigate the effects of *Bacillus pumilus* 8G-134 (**BP**) supplementation on total tract nutrient digestibility and milk yield of postpartum dairy cows fed low (20%, **LS**) or high (27%, **HS**) starch diets. We hypothesized that BP, a spore forming Gram-positive bacteria, would increase production of ruminal xylanase and increase fiber digestion, dry matter intake (**DMI**), and performance of cows fed LS and HS diets postpartum. Forty-eight (n=12/treatment) multiparous cows were randomly assigned to a 2 × 2 factorial design (pre- and postpartum starch [low vs. high] with supplementation of either BP carrier or BP postpartum). Factors combined resulted in 4 treatments: 1) LS pre- and postpartum + BP carrier postpartum (**LSCO**); 2) LS pre- and postpartum + BP postpartum (**LSBP**); 3) HS pre- and postpartum + BP carrier postpartum (**HSCO**); 4) HS pre- and postpartum + BP postpartum (**HSBP**). Low starch and HS diets were designed to vary in starch

concentration which was adjusted by replacing corn silage with ground corn. *Bacillus pumilus* carrier or BP were top dressed on the total mixed ration (**TMR**) once daily to provide approximately $5x10^9$ CFU/cow/d from calving until 112 day in milk (**DIM)**. Data were analyzed using the MIXED procedure of SAS. Prepartum, cows maintained DMI at 1.7 and 1.8 % of body weight for LS and HS treatments, and had similar serum non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHBA**), glucose, and haptoglobin concentrations. There was no change in apparent total-tract neutral detergent fiber digestibility (**NDFd**) but dry matter digestibility (**DMd**) tended to be 4.4 % greater for LS treatment. Ruminal acetate concentration tended to be 2.6 % greater and butyrate concentration was 1.5 % lower for cows fed LS diet. Postpartum DMI was unaffected by starch or BP; however, NEFA concentration was lower, and BHBA concentration tended to be lower for cows fed BP compared to cows that did not receive supplementation. Milk yield, 3.5% FCM, and milk components were similar among treatments, except milk protein which was greater for HS diets. Results indicated that BP supplementation during early lactation decreased body lipid mobilization; therefore further research is needed to investigate the use of BP as a feeding strategy during the early postpartum period.

Key words: *Bacillus pumilus*, starch, transition cow.

Introduction

Dietary starch plays an important role in the diet of high producing dairy cows, providing an energy-dense substrate especially critical during early lactation when glucose requirements are high (Van Vuuren et al., 2009) but cows struggle to meet these demands due to low DMI and high milk yields, often leading to postpartum energy balance deficit (Spurlock et. al., 2012). However, high-producing dairy cows fed high-starch diets are commonly prone to development of SARA, a common digestive disorder frequently caused by feeding a diet containing highly fermentable carbohydrates, with inadequate physically effective fiber (Plaizier et al., 2008). One strategy that could be used to reduce the severity of SARA is to reduce starch and increase fiber content of the diet.

In addition to health concerns, recent increases in feed cost, especially grains, and consequent reductions in income over feed cost have encouraged dairy producers to use moderate starch diets (Gencoglu et al., 2010 and Ferrarretto et al., 2011). Replacing a portion of the diet's concentrate for ingredients with greater fiber content can lower cost of diets and also decrease disturbances in rumen metabolism (Staple, 2007; Zebeli and Metzler-Zebeli, 2012). Special attention should be given to early lactating cows who already undergo considerable metabolic challenges during the transition from late gestation to early lactation (Bell, 1995), and introduction of high-starch diets during this period presents an extra challenge to periparturient cows that are already adjusting to metabolic changes (Drackley, 1999).

Feeding a moderate starch diet with elevated fiber may promote increased fiber digestibility while maintaining the energy density necessary to maintain milk production

and meet the higher energetic requirements of high-producing cows. Supplements such as DFMs have generally been supplemented to animals during periods of stress or low DMI with the assumption that establishment of beneficial microorganism populations in the digestive tract will decrease or prevent pathogenic organism establishment and according to Nocek and Kautz (2006), the inclusion of DFMs in dairy cow diets has become a common practice.

Bacillus pumilus (**BP**) is a spore-forming Gram-positive bacterial species that has been found in bovine ruminal fluid (Degrassi et al., 1995). While once considered soil microbes, *Bacillus* species are now commonly used as probiotics in animal and human feed and their commensal nature has gradually been realized (Cutting, 2011). Strains of this species have demonstrated high resistance to environmental stresses and production of xylanases which break down xylan and the bonds that link lignin to cellulose (Degrassi et al., 1998; Poorna and Prema 2006). Xylan is the second most abundant biopolymer renewable source on earth after cellulose (Wong et al., 1988; Benini et al., 2001). Microbial xylanases (especially endo-β -1, 4-xylanase, EC 3.2.1.8) are critical enzymes for xylan hydrolysis, and display promising potential for application in the food, animal feed, paper and pulp industries (Poorna and Prema, 2006; Badhan et al., 2007; Battan et al., 2007). In this study, *Bacillus pumilus* 8G-134, a strain originating from the rumen was chosen, as draft genome sequencing analysis indicated that this strain possesses the necessary genetic machinery for the metabolism of xylose (Figure 1), and it has also been demonstrated that the strain has the capacity to utilize xylose *in vitro* (API 50 CHB media and test kit, BioMerieux, Marcy l'Etoile, France; data not shown). Furthermore, *Bacillus* species might offer practical advantages as animal probiotics including the ability to form

spores which can tolerate the harsh environment of the GIT and are capable of germinating and proliferating within the intestine (Casula et al., 2002; Tam et al., 2006; Cutting, 2011). In addition, *Bacillus* spores are well-known for their antimicrobial activity (Cutting, 2011).

Despite the range of possible benefits that BP might confer as a DFM, such as enhanced fiber digestibility and increased feed efficiency, there is a lack of information on the effects of supplementing this novel DFM species during early and mid-lactation of dairy cows fed similar diets or diets varying in nutrients. Therefore, the objective of this study was to investigate the effects of *Bacillus pumilus* strain 8G-134 supplementation on the total tract nutrient digestibility and milk yield of postpartum dairy cows (early lactation to 112 DIM) fed low (20%, **LS**) or high (27%, **HS**) starch diets. We hypothesized that BP supplementation would increase NDF and DM digestibility and consequently increase feed conversion leading to better energy balance and increased milk yield for both LS and HS fed cows compared to cows not supplemented with BP.

Materials and Methods

Cows and Treatments

Forty-eight (n=12/treatment) multiparous Holstein and Holstein cross (Holstein × Montbéliarde × Swedish Red) dairy cows were blocked by lactation, body condition score (BCS), and body weight, and assigned to a 2 × 2 factorial design (pre- and postpartum starch [low vs. high] and supplementation of either an inert limestone carrier or BP + carrier postpartum). Factors combined resulted in 4 treatments: 1) LS pre and postpartum + inert limestone carrier postpartum (**LSCO**); 2) LS pre and postpartum + BP postpartum (**LSBP**); 3) HS pre and postpartum + inert limestone carrier postpartum

(**HSCO**); 4) HS pre and postpartum +BP postpartum (**HSBP**). *Bacillus pumilus* 8G-134 was added by top dressing 28 g/d of powder product on the TMR once daily to provide $5x10^9$ colony-forming units (CFU)/head per day of live BP strain (DuPont Nutrition and Health, Waukesha, WI) from calving to 112 DIM. Low starch and HS diets (Table 1) were designed to vary in starch concentration which was adjusted by replacing corn silage with ground corn resulting in 2 pre- and postpartum diets; LS (12.1%) pre- and (18.7%) postpartum or HS (20.1%) pre- and (27.1%) postpartum. From d 42 to d 22 prepartum cows received a common far-off diet (Table 1). All diets were formulated using CNCPS dairy software (Version 6.1; Cornell University, Ithaca, NY) to supply adequate NE_L and metabolizable protein for 650-kg dry cows 280 d in gestation prepartum and a 650-kg cow producing 40 kg of milk with fat concentration of 3.5% postpartum. Diets were fed at *ad libitum* rate (to ensure 10% feed refusals). Starch concentrations for HS diets were lower than anticipated due to changes in corn silage during the study; however, a difference of 6 to 7 percentage units of starch was maintained between LS and HS pre- and postpartum diets.

Animal Housing and Management

This experiment was carried out from September 2011 to May 2012 at the University of Minnesota Dairy Teaching and Research Unit (St. Paul). All experimental procedures were conducted under an approved protocol by the Institutional Animal Care and Use Committee of the University of Minnesota. Throughout the experiment, cows were housed in individual tie-stalls with rubber-filled mattresses and bedded with sawdust in a mechanically ventilated barn. Water was available *ad libitum* in each stall.

Cows were fed once daily during the dry period (1200 h) and twice daily after calving (0300 and 1100 h). After calving cows were milked twice daily (0200 and 1400 h).

Sample Collection and Preparation

Feed Collection and Analysis. Individual ingredients used on dry and lactation diets (Table 1) were sampled weekly, frozen at −20°C and composited monthly on a wet weight basis, dried in a 60°C forced air oven for 48 h (or until static weight was achieved) and then ground through a 1-mm screen in a Wiley mill (Thomas-Wiley, Philadelphia, PA). Dried samples were analyzed at Dairyland Laboratories (St. Cloud, MN) using wet chemistry methods. Monthly averages of the nutrient composition of individual ingredients were used in the CNPS dairy model to calculate the nutrient composition of the diets. Organic matter concentration of feed and feces was calculated as the difference between DM content and ash content. Ash content was determined using method 942.05 (AOAC International, 2000). Crude protein was determined using method 990.03 (AOAC International, 2000). Heat-stable, α-amylase treated, sodium sulfite NDF for feed ingredients and fecal samples was determined using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY) based on procedures described by Van Soest et al. (1991). Acid detergent fiber was determined using method 973.18 (AOAC International, 2000). Lignin was determined using method 973.18, and ADF-insoluble CP was determined by method 973.18 (AOAC International, 2000) and ether extract was determined by method 920.39 (AOAC International, 2000).

DMI and Nutrient Intake. Daily individual cow DMI was measured from 42 d prior to calving to 112 DIM. Feed offered and refused were measured daily and recorded

electronically. Dry matter intake was calculated by correcting as-fed offered minus refusals and multiplied by weekly diet DM. Energy balance both pre- and postpartum was calculated for each cow using equations described by NRC (2001). Net energy intake was determined by multiplying DMI by the calculated mean NE_L density of the diet. The NE_L 3 × maintenance was predicted from total digestible nutrients according to the NRC (2001). The NE_L value for forages was adjusted with the Van Soest variable discount method (Dairy One, 1999). Weekly means for individual cows were used as model inputs for prepartum DMI, and BW was used for prepartum EB calculations. Postpartum EB was calculated using inputs of weekly averages of DMI, BW, milk yield and components.

BW and BCS. Individual cows were weighed weekly at 1600 h from wk – 6 through 16 relative to calving. The weekly BCS was assigned on 0.25-unit increments (Ferguson et al., 1994) and averages of 3 trained investigators from wk – 6 through 16 relative to calving were reported.

Milk Yield and Composition. Cows were milked twice daily (0200 and 1400 h), individual milk yields were recorded at each AM and PM milking from d 1 through 112 DIM. Individual cow milk samples were taken weekly, preserved (800 Broad Spectrum Microtabs II; D and F Control Systems Inc., San Ramon, CA) until next day analysis using mid-infrared procedures (method 972.16; AOAC International, 2000) for fat, protein, lactose, SCC, and MUN.

Apparent Total-Tract NDF Digestibility. To determine apparent total-tract NDF and DM digestibility at d – 14, 7, 14 and 84 relative to calving, TMR samples were collected once daily on the same day as 2 fecal grab samples per cow (at 0800 h and 1600 h) were collected. The TMR were composited by treatment and fecal samples were

composited by cow on a wet weight basis, dried at 60^0C for 48 hr, and ground through a 1-mm screen in a Wiley mill. Samples of TMR and feces were used to determine DM and NDF digestibility using acid-insoluble ash as an internal marker as described by Block et al. (1981).

Blood Collection and Analyses. Blood samples were collected weekly on d - 28, -21, -14, -7, 7, 14, 21, and 28 relative to calving at 0800 h. Approximately 10 mL of blood was collected from the coccygeal vein into an evacuated serum tube (serum separator, Becton Dickinson Vacutainer systems, Franklin Lakes, NJ), centrifuged at 2,000 x g for 20 min immediately after sample collection, and frozen at - 20°C until analysis. Serum NEFA concentrations were determined using a NEFA C kit (Wako Diagnostic, Richmond, VA). Serum glucose concentrations were quantified by enzymatic reaction (Stanbio Laboratory, Boerne, TX). Serum calcium concentrations on d -7 and d 1 relative to calving were measured using a calcium (Arsenazo) reagent set (Point Scientific, Inc., Canton, MI). Serum haptoglobin concentrations were determined by a colorimetric procedure as described by Hulbert et al. (2011). Absorbance for NEFA, glucose, calcium, and haptoglobin assays was quantified using a microplate spectrophotometer (Eon TM, BioTek Instruments Inc., Winooski, VT). Serum BHBA concentrations were quantified using the Precision Xtra® ketone monitoring system direct electrochemical test (Abbot Laboratories Inc., Abbott Park, IL). Briefly, a droplet of serum was placed on a ketone test strip containing the enzyme β-hydroxybutyrate dehydrogenase, which oxidizes BHBA to acetoacetate. The enzyme β-hydroxybutyrate dehydrogenase reduces nicotinamide adenine dinucleotide (NAD+) to NADH. The NADH is then reoxidized to NAD+ by an electron transfer mediator molecule. The electrical current generated by this

conversion is measured by the meter and is directly proportional to the BHBA concentration (Oetzel and McGuirk, 2007).

Rumen Fluid. Rumen fluid samples were collected on d -14, 7, 14, and 84 relative to calving at 0900 h via oral-esophageal tube technique (OST) at 200 cm insertion depth (Shen et. al., 2012) described with details by Duffield et al. (2004). Immediately after sampling, pH was measured using a pH meter 345 (pH meter 345^{TM}, Corning Inc., New York City, NY); samples were then placed in liquid N and transferred to - 20^{0}C freezer on the same day until further analysis. Rumen fluid samples were analyzed for VFA and ammonia N by Dairyland Laboratories Inc. (St. Cloud, MN). Volatile fatty acids were quantified using high pressure liquid chromatography technique, and ammonia N determined by distillation.

Statistical Analysis

Data were analyzed using SAS (version 9.2 SAS Institute, Cary, NC). Prepartum and postpartum data were analyzed separately. Prepartum data were analyzed as a completely randomized design using MIXED models procedures of SAS. Model included effects of breed, starch concentration, time and interaction of starch concentration by time.

Postpartum data were analyzed as a randomized complete block design with a 2 X 2 factorial arrangement of treatments using MIXED models procedure of SAS. Model included effects of breed, starch concentration, BP, time, and interactions between starch, BP, and time. On pre- and postpartum periods repeated measures over time were modeled with autoregressive [AR (1)], and denominator degrees of freedom were estimated using

Kenwards-Rogers method. Single measurements were modeled with autoregressive [AR (1)], and denominator degrees of freedom were estimated using Satterthwaite methods. Least squares means for starch, BP, time and all interactions were separated by use of PDIFF statement when the overall F-test was $P \leq 0.05$. Trends were indicated when $P \leq 0.10$.

Results and Discussion

Results during the prepartum period are reported as the main effects of dietary starch concentration, whereas results during postpartum phase are reported as the main effects of dietary starch concentration, BP supplementation, and interactions between dietary starch concentration and BP.

Prepartum DMI, Nutrient Intake, BW, BCS and Blood Metabolites

We did not observe any effect of starch on prepartum DMI which averaged 1.71 and 1.81 (as % of BW) for LS and HS, respectively (Tables 2 and 3). Prepartum EB was unaffected by dietary starch concentration ($P = 0.11$). This could be due to the combination of numerically greater DMI and NE_L for HS versus LS prepartum diets. Prepartum EB expressed as a percentage of requirements was also similar ($P = 0.12$) between treatments. There were no effects of starch diet concentration on BW and BCS.

Neither prepartum concentration of NEFA nor BHBA were affected by dietary starch. However, both NEFA ($P < .001$) and BHBA ($P < .001$) increased as parturition approached, and reached their peak on d -7 (Figure 2). Prepartum concentration of glucose was similar ($P = 0.34$) between LS and HS treatments, and decreased weekly ($P < .001$) as calving approached (Figure 2). Prepartum concentrations of haptoglobin were

similar ($P = 0.97$) for LS and HS; however, HS had greater ($P < 0.05$) concentration on wk -2 (Figure 2). Concentration of calcium on wk -1 relative to calving was not affected by dietary starch and averaged 10.6 and 11.3 mg/dL for LS and HS, respectively.

Maintenance and pregnancy energetic requirements of dairy cattle increase 23% during the last month prepartum (Moe et al., 1972), whereas feed intake typically decreases 30% (Grummer, 1995), leading to considerable adipose tissue mobilization and increases in NEFA and BHBA circulation (Overton and Waldron, 2004). Results for DM and energy intakes when prepartum HS diets are fed have been contradictory. Minor et al. (2001) reported that feeding diets with greater NFC during prepartum was associated with greater decreases in DM and energy intakes; however, Rabelo et al. (2003) supported the opposite and pinpointed that feeding HS diets prepartum might be an advantageous management practice because it adapts a cow's rumen papillae to high concentration diets that will be fed in early lactation. In terms of blood metabolites, results generated in this study concur with Smith et al. (2008), where no effects on NEFA and BHBA blood concentrations for cows fed low or high starch diets with differences of 11% of starch between diets during 21 d prepartum were observed. Regarding glucose concentration, results from our study agree with Kunz et al. (1985) who indicated that glucose concentrations remain stable or increase slightly during the pre-fresh transition period regardless of energy intake. However, Rabelo et al. (2005) reported that concentration of glucose was greater for dairy cows fed higher versus lower concentration of starch prepartum.

Prepartum Rumen Fluid and Apparent Total-Tract Digestibility

Rumen fluid sampling revealed no differences in pH or ammonia concentrations for LS and HS diets (Table 4). Ruminal acetate concentration tended to be greater (P = 0.06), and butyrate 1.45% lower (P = 0.02) for LS treatment compared to HS. Prepartum DMI on wk -2, measured at same wk that rumen fluid and fecal grab samples were collected, did not differ (P = 0.28) between LS and HS treatments; however it was 1.25 kg/d numerically greater for HS treatment which probably explains the tendency (P = 0.07) of 4.35% lower DMd for HS compared to LS due to greater rate of passage. Prepartum NDFd was not affected (P = 0.77) by dietary starch concentration. Prepartum NDF intake averaged 4.4 and 4.9 kg/d for LS and HS, respectively, but was unaffected (P = 0.43) by starch concentration.

Ruminal pH averages were greater than what we expected for both treatments, which could potentially be attributed to sampling method. However, rumen fluid samples were collected using OST technique at 200 cm insertion depth, and according to Shen et al. (2012) this is the optimal depth to reach the central rumen and obtain representative rumen fluid with minimal saliva contamination. Moreover, OST is simpler, quicker and less invasive (Duffield et al., 2004) compared to rumenocentesis (rumen puncture) and rumen cannulation. In our study rumen fluid samples were collected only once a day 20 to 21 h (prepartum cows) after feeding. This sampling intensity may not have been sufficient to exhibit more subtle changes in rumen fermentation pattern due to differences in dietary starch concentrations. Differences in acetate concentration between treatments might be attributed to the 5.4% greater NDF and lower starch content of LS compared to HS diets during the prepartum period. It was expected that HS would decrease fiber

digestibility, since high starch diet concentration results in decreases in rumen pH and fibrolytic bacterial growth is negatively affected (Kaufman et.al 1980), thus less acetate would be available in the rumen to be absorbed. Our results for changes in butyrate agree with Rabelo et al. (2001) who fed greater NFC to prepartum cows but disagree with the changes in propionate reported in the same study.

Colostrum Yield and Calf Birth

There was no effect of prepartum dietary starch concentration in colostrum yield ($P = 0.44$) or calf birth weight ($P = 0.19$). Colostrum yield was 5.69 and 6.63 ± 0.87 kg and calf birth weight was 43.62 and 41.38 ± 1.22 kg for LS and HS treatments, respectively.

Postpartum Rumen Fluid and Apparent Total-Tract Digestibility

Early postpartum rumen fluid samples, wk 1 and 2, revealed no effects of starch, BP or interactions of starch x BP on pH or ammonia concentration (Table 5). Early postpartum VFA and acetate concentrations were greater ($P = 0.04$) for cows fed LS compare to HS; however, no changes or interactions were observed for BP and starch x BP. Early postpartum propionate and butyrate were unaffected by starch or BP, and no interaction between starch x BP were observed among treatments. Regarding A:P ratio, LS tended ($P = 0.08$) to be greater due to greater acetate for LS treatments. Lechartier et al. (2011) also reported lower A:P ratio value for LS diets compared with HS; however, no effect on VFA range. Rumen fluid results on wk 12 revealed no effects of starch, BP

or starch x BP neither in ammonia nor VFA (acetate, propionate and butyrate). Ratio of A:P were identical among treatments.

Early postpartum DMI, wk 1 and 2, were similar among treatments; however, small differences along with differences in NDF content of LS and HS diets resulted in a tendency ($P = 0.09$) of greater NDF intake (kg/d) for LS treatments compared to HS treatments. Digestibility of NDF was unaffected by starch, BP or starch x BP. Moreover, LSCO and LSBP diets had 9% greater NDFd compared with HSCO and HSBP. Furthermore, changes in NDFd (%) were not enough to cause changes in NDFd (kg/d), and it was unaffected by starch, BP and starch x BP. Dry matter digestibility (%) was similar among treatments, as well DMd (Kg/d). Mid-lactation DMI, on wk 12, tended to be greater ($P = 0.06$) for LSCO and HSCO compared with LSBP and HSBP, respectively; whereas DMd (%) was only affected by starch, and LS tended ($P = 0.07$) to have greater digestibility compared with HS, whereas DMd (kg/d) was greater for LSCO and HSCO compared to LSBP and HSBP. We expected supplemented BP cows to have greater ruminal fluid ammonia, pH and changes in rumen fermentation patterns representative of greater fiber digestion.

Theoretically, some bacterial DFMs may prevent a decline in rumen pH by decreasing lactic acid production and increasing the utilization of lactic acid by some microbes (Nocek et al., 2002; Chaucheyras et al. 1996). However, Spriet et al. (1987) found no changes in pH, the concentration of ammonia and VFA or the apparent digestibility of protein or organic matter when *Bacillus* spp. was fed to cannulated pigs, whereas Peng et al. (2011) found no changes in ruminal pH, ammonia N, but ruminal acetate was greater for dairy cows fed other type of *Bacillus*, such as *Bacillus subtilis*

natto compared with control treatment. For our study, it needs to be considered that once daily feeding combined with moderate and high starch diets from ground shelled corn might have influenced the responses that we found. Nocek et al. (2002) suggested that alterations in diurnal ruminal pH profile when feeding high forage diets which support greater pH are generally more conductive to enhancing ruminal DM digestion when feeding a DFM (combination of *Enterococcus faecium, Lactobacillus plantarum,* and *Saccharomyces cerevisiae*). In addition, ruminal pH profile differences do not necessarily indicate similar ruminal bacterial compositions, and milk fat depression can occur at intermediate ruminal pH (Palmonari et al., 2010).

According to Russell and Wilson (1996), increases in ruminal pH boost fiber digestion, and even though Padaria et al. (2014) characterized *Bacillus pumilus* as being an efficient fiber-degrading spore due to its ability for the production of extracellular cellulase, perhaps, due to no changes in ruminal pH in our study, differences in fiber digestibility were not seen among treatments. Moreover, we expected that cows supplemented with BP would have enough microbial xylanase production to increase considerably the breakdown of xylan, and the bonds that link lignin to cellulose, increasing the fiber surface area and boosting fiber digestibility of the diets.

Changes in fiber digestibility when *Bacillus* strains are fed have produced a range of results in different species. Saylor et al. (1993) reported a reduction in pasted vents in broilers fed a probiotic containing *Bacillus pumilus* (1×10^9 CFU/kg of diet) compared with broilers fed the control diet which suggested improved digestibility. However, Kornegay and Risley (1996) found no effects of a mixture of *Bacillus sp.* including *B. pumilus* on digestibility coefficients of DM, NDF, and ADF when fed to growing pigs.

We found intriguing that NDFd (%) was greater for HS on wk 1 and 2, but lower on wk 12 compared with LS treatments (Table 5). We attributed these results to the increases of almost 10 kg/d in DMI and increases in starch intake and also rate of passage from early to mid-lactation period. Fiber digestibility in the rumen is the result of the competitive processes of digestion and rate of passage; increases in DMI can decrease retention time of feed particles in the rumen and decrease digestibility (Huhtanen et al., 2006). Digestibility of NDF is a key predictor of dairy cow intake and performance (Jung and Allen, 1995; Oba and Allen, 2000), and digestion of NDF in the rumen can be negatively affected by levels of starch due to reduced rumen pH (Meissner et al.,1996). Van Vuuren et al. (2010) found that high-starch diets significantly decreased NDF and OM digestibilities. Perhaps, during early lactation, starch intake by HS fed cows was not enough to diminish digestibilities; however, the increase of 10 kg/d of DMI and certainly increases in starch intake from early to mid-lactation caused the reduction in %NDFd.

Postpartum Blood Metabolites

Results for this section are presented in Table 6. Postpartum NEFA concentration was unaffected by starch ($P = 0.29$), but lower ($P = 0.05$) for BP supplemented treatments. Cows assigned to LSBP had 52.2 µEq/L lower NEFA concentration compared with LSCO and HSBP had 161 µEq/L lower NEFA concentration compared with HSBP. Moreover, NEFA concentration was consistently greater for LSCO and HSCO compared with LSBP and HSBP, respectively, throughout the 28 d after calving (Figure 3). Similarly, lower NEFA concentrations for cows supplemented with BP, reflected in a tendency ($P = 0.07$) of lower BHBA concentrations for the same treatments. Cows assigned to LSBP tended to have 2.5 mg/dl lower BHBA concentration than

LSCO, and HSBP had 6.5 mg/dl lower BHBA concentration than HSCO. Furthermore, BHBA concentration was consistently greater for LSCO and HSCO compared with LSBP and HSBP, respectively, throughout the 28 d after calving (Figure 3). Postpartum glucose concentration was similar across treatments, unaffected by starch ($P = 0.56$), BP ($P = 0.49$), or interaction of starch x BP ($P = 0.66$), and constant during early postpartum (Figure 3). No changes on postpartum haptoglobin were observed among treatments; concentration was not affected by starch ($P = 0.22$), BP ($P = 0.66$), or interaction of start x BP ($P = 0.11$); however, LSCO, LSBP and HSCO reached a peak on wk 2 when HSBP had its lowest concentration (Figure 3). Concentration of calcium was similar among treatments, and was not affected by starch ($P = 0.31$), BP ($P = 0.47$), or interaction of starch x BP ($P = 0.57$).

The increased energetic demands of lactation result in mobilization of fat reserves into the circulation in the form of NEFA, and hepatic ketogenesis via incomplete oxidation of NEFA indeed explain increases in BHBA concentration (Bell, 1995). Lower NEFA concentration for cows supplemented with BP suggested that this DFM can be used as a feeding strategy to improve immune function and decrease incidence of common metabolic disorders such as fatty-liver that affects almost half of all dairy cows immediately after parturition. Fatty liver is characterized by accumulation of triacylglycerols (TAG) in the hepatocytes arising from a negative energy balance after parturition (Bauchart et al. 1998). Serum NEFA concentrations have been found to have a strong relationship with mediators of immune responses such as TNF- alfa and fatty liver at 12 d postpartum (Ametaj et al., 2005). We hypothesized that BP supplementation would increase diet energy availability and improve feed conversions as a result of

increase in diet fiber digestibility leading to lower negative energy balance consequently lower lipid mobilizations for both LS and HS. Mechanistically, increases in % NDFd observed in both LS and HS diets supplemented with BP might have contributed to differences in postpartum energy status resulting in lower NEFA and BHBA concentrations. These results disagree with Peng et al. (2012), who found no differences in blood metabolites when a *Bacillus subtilis* based-DFM product was fed to lactating cows; however, supplemented cows had linearly decreased NEFA concentration, and the same cows also had greater milk yield and feed efficiency compared with control cows. It is possible that the lower NEFA and BHBA concentrations observed here may be attributed to differences between *Bacillus subtilis natto* and *Bacillus pumilus* DFM strains.

Early Lactation and Mid-Lactation DMI, EB, BW, BCS, Milk Yield and Components and Feed Efficiency

Results for this section are all presented in Tables 7 and 8 and Figures 4 and 5. No effects of starch ($P = 0.16$), BP ($P = 0.25$) or starch x BP interaction ($P = 0.98$) in DMI during the 28 d postpartum were observed among the treatments. Dry matter intake as percentage of body weight was also similar among treatments. Energy balance was not affected by dietary starch levels ($P = 0.25$), BP supplementation ($P = 0.51$) or starch x BP interaction ($P = 0.71$); however, cows from all treatments remained in a negative energy balance through 28 DIM. Body weight, BCS and BCS changes were similar among treatments. However, BCS change tended ($P = 0.09$) to be lower for LS compared to HS, and there was an interaction ($P = 0.02$) between starch x BP; whereas BCS change was

greater negatively for LSBP compared to LSCO, change was greater for HSCO compared with HSBP. As expected MUN was lower (P = 0.01) for HSCO and HSBP compared to LSCO and LSBP, but no other effects of starch, BP or starch x BP interaction during the 28 d were observed. Yields of 3.5% FCM were similar among treatments and were unaffected by starch (P = 0.35), BP (P = 0.67) or starch x BP interaction (P = 0.62); Although 3.5% FCM yield was 2.7 kg/d numerically greater for HSBP cows compared with HSCO, the study design did not have enough statistical power to report a significant effect. During postpartum early lactation, 28 DIM, no effects of starch (P = 0.16), BP (P = 0.25) or starch x BP interaction (P = 0.98) were observed on DMI. As percentage of BW, DMI was not affected by postpartum dietary starch concentration (P = 0.45), BP (P = 0.29) or starch x BP interaction (P = 0.33) and averaged 2.91, 2.86, 3.24, 3.47 for LSCO, LSBP, HSCO, and HSBP, respectively.

Dry matter intake throughout 112 DIM was unaffected by starch amount (P = 0.86) or BP (P = 0.57) but there was a tendency (P = 0.08) for cows in treatments LSBP and HSCO compared to treatments LSCO and HSBP to have lower DMI, which resulted in a greater but not significant increase in feed efficiency due to similar 3.5% FCM. During 112 DIM, EB, and EB as percentage of BW were affected by starch concentration (P = 0.04 and P = 0.03, respectively), but unaffected by BP or starch x BP interaction. Energy balance and EB as percentage of BW was lower (P = 0.04 and P = 0.03, respectively), for LS compared with HS diets. Body weight and BCS changes during the 112 DIM tended to be greater (P = 0.06 and P = 0.06, respectively), for LSBP and HSCO compared to LSCO and HSBP. No changes in milk yield, 3.5% FCM and milk components were observed due to dietary starch levels, BP or starch x BP interaction,

except for milk protein (kg/d) which was greater ($P = 0.05$) for HS diets, and an interaction was found for starch x BP. Cows that did not receive BP supplementation had greater milk protein yield (kg/d) compared to LSBP. Milk urea nitrogen was lower ($P = 0.05$) for HS diets but unaffected by BP supplementation; in addition, no interactions were observed for starch x BP.

Our hypothesis was that BP supplementation would increase NDF and DM digestibilities and result in greater milk yield for LS and HS fed cows compared with no supplemented cows during early lactation. No adjustments on the dose of BP were made as the DMI increased from calving to 112 DIM, which may explain why we did not see any effects of BP in DMI, milk components and yield, or feed efficiency as we expected; however a reduction in NEFA and tendency toward a reduction in BHBA was notable. Sun et al (2012) fed a *Bacillus* – based DFM during a 70-d period and noticed that supplementation linearly increased milk production, 4% FCM and ECM, as well as milk fat, protein and lactose yield, and results were attributed to capability of the *Bacillus* used on their study to promote growth of total ruminal bacteria. Regarding the effects of dietary starch concentration, Van Vuuren et al. (2010) reported greater DMI for dairy cows fed high-starch diets compared with cows fed low-starch, and attributed those results to the greater palatability of high-starch diets. Gencoglu et al. (2010) reported the opposite with increases in DMI for cows fed reduced starch diets compared to normal starch diets with greatest differences during wk 3 to 6 of the study. Feeding diets varying in dietary starch concentration did not affect milk yield in studies by Dann et al. (2008) and Gencoglu et al. (2010) which drives the conclusion that LS diets might be preferable over HS diets, as rumen pH tends to be greater with reduced likelihood of developing

SARA compared to HS diets. Our results for greater milk protein yield coupled with lower MUN results for HS were similar to results found by Ferrarretto et al. (2010). These results can be related to greater starch intakes with greater rumen energy availability which is likely to increase ruminal microbial protein synthesis (Huntington, 1997; NRC, 2001). We have no explanation for the observed greater milk protein yields for the non-supplemented LS and HS treatments (Table 8); we expected instead to have an increase in ruminal fermentation efficiency with greater yield of microbial protein for BP supplemented cows.

Disease Incidence

Health events are presented in Table 9. Five cows were removed from the study due to health complications (reproduction problem, DA surgery, leg injury), resulting in 43 cows completing the study.

Conclusions

Prepartum diets differing in 6% starch concentration had no effects on blood metabolites during the prepartum period; however, LS tended to increase rumen fluid acetate concentration.

Bacillus pumilus 8G-134 supplementation could be advantageous when fed to early lactation cows due to a reduction in NEFA and tendency to a reduction in BHBA, which demonstrates a decrease in body lipid mobilization. Increases in DMI as DIM increased and consequently a reduction of rumen feed retention time might have diminished the benefits of BP on fiber digestion and no changes in % NDFd were seen at

mid-lactation. It should be emphasized that no adjustments on the dose of BP were made as the DMI increased from calving to 112 DIM. Further research is needed to evaluate the use of BP as a feeding strategy during peripartum period and also the potential benefits of adjusting BP dose supplementation according to increases in DMI as cows progress through lactation.

Chapter 3: Effect of *Bacillus pumillus* 8G-134 supplementation on rumen patterns, diet digestibility and lactation performance of primiparous cows fed low or high starch diets

D. N. Lobão da Silva*, M. I. Endres*, E. Galbraith†, T. Parrott†, N. B. Litherland‡

*Department of Animal Science, University of Minnesota, St. Paul 55108.

† DuPont Industrial and Biological Sciences, Waukesha, WI.

Overview

The objective of this study was to investigate the effects of *Bacillus pumilus* (**BP**) supplementation on the total tract nutrient digestibility, rumen fermentation parameters and milk yield of primiparous postpartum dairy cows fed low (20%, **LS**) or high (27%, **HS**) starch diets. We hypothesized that BP, a spore forming gram-positive bacteria, would increase production of xylanase and increase fiber degradation, dry matter intake (**DMI**), and milk yield of primiparous cows fed LS and HS diets. Forty-eight (n=12/treatment) nulliparous cows were randomly assigned to a 2 × 2 factorial design (pre- and postpartum starch [low vs. high] and combination of low or high starch with BP carrier or BP postpartum). Factors combined resulted in 4 treatments: 1) LS pre- and postpartum + BP carrier postpartum (**LSCO**); 2) LS pre- and postpartum + BP postpartum (**LSBP**); 3) HS pre- and postpartum + BP carrier postpartum (**HSCO**); 4) HS pre- and postpartum + BP postpartum (**HSBP**). Data were analyzed using the MIXED procedure of SAS. During prepartum, heifers had similar DMI at 1.8 % of body weight for LS and HS treatments, and similar serum non-esterified fatty acids (**NEFA**), β-

hydroxybutyrate (**BHBA**), glucose and haptoglobin concentrations. There was no change in apparent total-tract NDFd or DMd, and all rumen fluid parameters were similar, except for butyrate which was 1.2 % greater for HS diets. During the first 28 DIM all cows experienced negative energy balance (NEB) and NEFA and haptoglobin concentrations were similar among treatments; however, BHBA concentration tended to be lower for controls compared to BP treatments. Early postpartum DMI was similar among treatments, but apparent total-tract NDFd was 4.4 and 8.5% greater for LS and HS diets supplemented with BP, respectively, which tended to increase milk yield, 3.5% FCM and 3.5% ECM by 13.9%, 14.9% and 15.2% for HS diets, respectively, whereas increases in LS supplemented diets were less apparent. During the 112 d of BP supplementation, DMI and milk fat yield increased, and milk protein yield tended to increase for both LS and HS diets. Moreover, BP tended to improve 3.5% FCM yield by 4.3% and 8.9% for LS and HS, respectively. Results indicated that BP supplementation to primiparous cows can increase fiber digestibility and milk yield resulting in potential increases in profitability.
Key word: *Bacillus pumilus*, dietary starch, primiparous cow.

Introduction

Bacillus species, well-known for their antimicrobial activity, offer advantages as an animal probiotic as their spores can tolerate the harsh environment of the GIT and are capable of germinating and proliferating within the intestine (Tam et al., 2006; Cutting, 2011). *Bacillus pumilus* (**BP**), which has already been isolated from bovine ruminal fluid, produces xylanase which breaks down xylan and the bonds that link lignin to cellulose (Degrassi et al., 1995; Poorna and Prema, 2006). Moreover, this particular spore forming gram-positive bacteria has shown potential for application in the food, animal feed, paper, and pulp industries (Poorna and Prema, 2006; Badhan et al., 2007; Battan et al., 2007).

Dietary starch recommendations can be as high as 30 % of total DMI (Grant 2005). However, health concerns due to inadequate physically effective fiber that can lead to sub-acute ruminal acidosis (SARA) (Plaizier et al., 2008) and recent increases in feed cost, especially grains, have encouraged dairy producers to opt for moderate starch diets (Gencoglu et al., 2010). Ruminal disturbance such as SARA may be exacerbated in primiparous cows because they have not previously had long-term exposure to a highly fermentable lactation diet (Mirzaei Alamouti et al., 2009). Dairy animals usually calve for the first time at about 24 months of age as this maximizes economic benefit (Hoffman and Funk, 1992). Animals are not yet physically mature at this stage, therefore they are in a differing metabolic state to that experienced by multiparous cows as they require nutrients for their own continued growth in addition to that of their developing calf (Coffey, 2006). Results from Beauchemin et al. (1997) suggested that the effects of low

fiber diets on intake differ for younger and older cows, as well as effects of diets on digestibility.

Research evaluating the effects of decreased dietary starch concentration in order to decrease ruminal disturbance in primiparous cows is scanty and deserves further investigation. Nevertheless, if lower starch diets are fed to primiparous cows as an attempt to reduce health disturbances then it seems reasonable that fiber digestibility should be boosted thus right amounts of energy can still be provided to the animals to sustain growth, milk production, and therefore meet their energetic requirements. A recent study conducted by our group (unpublished data) has shown that BP successfully decreased body lipid mobilization in multiparous cows during early lactation due to its potential benefits, such as enhancement of fiber digestibility; however, the benefits of this direct fed microbial (**DFMs**) was diminished as lactation advanced due to high DMI of multiparous cows and no adjustments of the DFMs dose. Since parity is an important concern when feeding cows as feed intake and meal patterns differs between primiparous and multiparous animals (Grant et al., 1995) and they are usually grouped and managed differently, we recognized that it was also valid to investigate the effects of BP when fed to animals in their first lactation, and whether the potential benefits could be extended beyond the early lactation phase for primiparous cows. There are no studies showing the benefits that this novel DFM might have in primiparous cows' performance from early lactation to approximately 100 DIM. Therefore, the objective of this study was to investigate the effects of BP supplementation on the total tract nutrient digestibility and milk yield of postpartum primiparous dairy cows (early lactation to 112 DIM) fed low (20%, **LS**) or high (27%, **HS**) starch diets. Forty-eight (n=12/treatment) nulliparous cows

were randomly assigned to a 2 × 2 factorial design (pre- and postpartum starch [low vs. high] and supplementation of either BP carrier or BP postpartum). Factors combined resulted in 4 treatments: 1) LS pre- and postpartum + BP carrier postpartum (**LSCO**); 2) LS pre- and postpartum + BP postpartum (**LSBP**); 3) HS pre- and postpartum + BP carrier postpartum (**HSCO**); 4) HS pre- and postpartum + BP postpartum (**HSBP**). We hypothesized that BP supplementation would increase NDF and DM digestibility and consequently improve feed conversion leading to better energy balance and increased milk yield for both LS and HS fed cows compared to cows not supplemented with BP.

Materials and Methods

Cows and Treatments

Forty-eight (n=12/treatment) nulliparous Holstein and Holstein cross (Holstein × Montbéliarde × Swedish Red) cows were blocked by future mature 305 ME, BCS, body weight, and randomly assigned to a 2 × 2 factorial design (pre- and postpartum starch [low vs. high] and combination of low and high starch with *Bacillus pumilus* [**BP**] carrier or BP postpartum). Factors combined resulted in 4 treatments: 1) LS pre and postpartum + BP carrier postpartum (**LSCO**); 2) LS pre and postpartum + BP postpartum (**LSBP**); 3) HS pre and postpartum + BP carrier postpartum (**HSCO**); 4) HS pre and postpartum +BP postpartum (**HSBP**). *Bacillus pumilus* was added by topdressing 28 g/d of powder product on TMR once daily to provide $5x10^9$ colony-forming units (CFU)/head per day of live BP strain (DuPont Industrial and Biological Sciences, Waukesha, WI) from calving to 112 DIM. Low starch and HS diets (Table 10) were designed to vary in starch concentration which was adjusted by replacing corn silage with ground corn resulting in 2 pre- and postpartum diets; LS (12.1%) pre- and (18.7%) postpartum or HS (20.0%) pre-

and (27.1%) postpartum. From d 42 to d 22 prepartum heifers received a common far-off diet (Table 10). All diets were formulated using CNCPS dairy software (Version 6.1; Cornell University, Ithaca, NY) to supply adequate NE_L and metabolizable protein. Diets were fed at *ad libitum* rate (to ensure 10% feed refusals). Starch concentrations for HS diets were lower than anticipated due to changes in corn silage along the trial; however, a difference of 6 to 7 percentage units of starch concentration were maintained between LS and HS pre and postpartum diets. Crude protein concentration of dry cows diets were higher than anticipated, but similar among treatments.

Animal Housing and Management

The study was conducted at the University of Minnesota Dairy Teaching and Research Center. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Throughout the experiment, animals were housed in individual tie-stalls with rubber-filled mattresses and bedded with sawdust in a mechanically ventilated barn. Water was available *ad libitum* in each stall. Animals were fed once daily prepartum (1200 h) and twice daily postpartum (0300 and 1100 h). After calving cows were milked twice daily (0200 and 1400 h).

Sample Collection and Preparation

Feed Collection and Analysis. Individual ingredients used on dry and lactation diets (Table 10) were sampled weekly, frozen at −20°C and composited monthly on a wet weight basis, dried in a 60°C forced air oven for 48 h (or until static weight was achieved) and then ground through a 1-mm screen in a Wiley mill (Thomas-Wiley,

Philadelphia, PA). Dried samples were analyzed at Dairyland Laboratories (St. Cloud, MN) using wet chemistry methods. Monthly averages of the nutrient composition of individual ingredients were used in the CNCPS dairy model to calculate the nutrient composition of the diets. Organic matter concentration of feed and feces was calculated as the difference between DM content and ash content. Ash content was determined using method 942.05 (AOAC International, 2000). Crude protein was determined using method 990.03 (AOAC International, 2000). Heat-stable, α-amylase treated, sodium sulfite NDF for feed ingredients and fecal samples was determined using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY) based on procedures described by VanSoest et al. (1991). Acid detergent fiber was determined using method 973.18 (AOAC International, 2000). Lignin was determined using method 973.18. ADF-insoluble CP was determined by method 973.18 (AOAC International, 2000) and ether extract was determined by method 920.39 (AOAC International, 2000).

DMI and Nutrient Intake. Daily individual cow DMI was measured from 42 d prior to expected calving to 112 DIM. Feed offered and refused were measured daily and recorded electronically. Dry matter intake was calculated by correcting as-fed offered minus refusals and multiplied by weekly diet DM. Energy balance both pre- and postpartum was calculated for each animal using equations for primiparous cows described by NRC (2001). Net energy intake was determined by multiplying DMI by the calculated mean NE_L density of the diet. The NE_L 3 × maintenance was predicted from total digestible nutrients according to the NRC (2001). Maintenance NEL (Mcal) was calculated as $BW^{0.75} \times 0.080$. Pregnancy requirements for NEL (Mcal) were calculated as [(0.00318 × day of gestation – 0.0352) × (calf birth weight/45)]/0.218. Requirements of

NEL for milk production were calculated as (0.0929 × fat %) + (0.0547 × protein %) + (0.0395 × lactose %) (NRC, 2001). Since animals were primiparous, retained energy (RE) required during pregnancy was calculated using equation 11–2 in NRC (2001). The equation for calculation of RE was: RE = 0.0635 × {0.891 × [(0.96 × current BW). The NE_L value for forages was adjusted with the Van Soest variable discount method (Dairy One, 1999). Weekly means for individual animal were used as model inputs for prepartum DMI, and BW were used for prepartum EB calculations. Postpartum EB was calculated using inputs of weekly averages of DMI, BW, milk yield and components.

BW and BCS. Individual animals were weighed weekly at 1600 h from wk – 6 through 16 relative to calving. The BCS was weekly assigned on 0.25-unit increments (Ferguson et al., 1994) and averages of 3 trained investigators from wk – 6 through 16 relative to calving were reported.

Milk Yield and Composition. Cows were milked twice daily (0200 and 1400 h), individual milk yields were recorded at each AM and PM milking from d 1 through 112 DIM. Milk samples from individual cows were taken weekly, preserved (800 Broad Spectrum Microtabs II; D and F Control Systems Inc., San Ramon, CA) until next day analysis using midinfrared procedures (AOAC International, 2000) for fat, protein, lactose, SCC, and MUN.

Apparent Total-Tract NDF Digestibility. TMR samples were collected once daily on the same day as 2 fecal grab samples per cow were collected at 0800 h and 1600 h on d – 14, 7, 14 and 84 relative to calving to determine apparent total-tract NDF and DM digestibility. Fecal samples were composited by cow on a wet weight basis, dried at 60^0C for 48 hr, and ground through a 1-mm screen in a Wiley mill. Analysis of TMR, and fecal

samples included DM and NDF digestibility using acid-insoluble ash as an internal marker as described by Block et al. (1981).

Blood Collection and Analyses. Blood samples were collected weekly on d - 28, -21, -14, -7, 1, 7, 14, 21, 28 relative to calving at 0800 h. Approximately 10 mL of blood was collected from the coccygeal vein into an evacuated serum tube (serum separator, Becton Dickinson Vacutainer systems, Franklin Lakes, NJ), centrifuged at 2,000 x g for 20 min immediately after sample collection, and frozen at - 20^0C until analysis. Serum NEFA concentrations were determined using a NEFA C kit (Wako Diagnostic, Richmond, VA). Serum glucose concentrations were quantified by enzymatic reaction (Stanbio Laboratory, Boerne, TX). Serum calcium concentrations on d -7 and d 1 relative to calving were measured using a calcium (Arsenazo) reagent set (Point Scientific, Inc., Canton, MI). Serum haptoglobin concentrations were determined by a colorimetric procedure as described by Hulbert et al. (2011). Absorbance for NEFA, glucose, calcium, and haptoglobin assays were quantified using a microplate spectrophotometer (Eon ™, BioTek Instruments Inc., Winooski, VT). Serum BHBA concentrations were quantified using the Precision Xtra® ketone monitoring system direct electrochemical test (Abbot Laboratories Inc., Abbott Park, IL).

Rumen Fluid. Rumen fluid samples were collected on d -14, 7, 14, and 84 relative to calving at 0800 h via oral-esophageal tube technique (OST) at 200 cm insertion depth (Shen et. al., 2012) described with details by Duffield et al. (2004). Immediately after sampling, pH was measured using a pH meter 345 (pH meter 345 ™, Corning Inc., New York City, NY), samples were then placed in liquid N and transferred to - 20^0C freezer on the same day until further analysis. Rumen fluid samples were

analyzed for VFA and ammonia N by Dairyland Laboratories Inc., (St. Cloud, MN). Volatile fatty acids were quantified using high liquid chromatography (HPLC) technique, and ammonia N determined by distillation.

Statistical Analysis

Data were analyzed using SAS (version 9.2 SAS Institute, Cary, NC). Prepartum and postpartum data were analyzed separately. Prepartum data were analyzed as a completely randomized design using MIXED models procedures of SAS. Model included effects of breed, starch concentration, time and interaction of starch concentration by time.

Postpartum data were analyzed as a randomized complete block design with a 2 x 2 factorial arrangement of treatments using MIXED models procedure of SAS. Model included effects of breed, starch concentration, BP, time, and interactions between starch, BP, and time. On pre-and postpartum periods repeated measures over time were modeled with autoregressive [AR (1)], and denominator degrees of freedom were estimated using Kenwards-Rogers method. Single measurements were modeled with autoregressive [AR (1)], and denominator degrees of freedom were estimated using Satterthwaite methods. Least squares means for starch, BP, time and all interactions were separated by use of PDIFF statement when the overall F-test was $P \leq 0.05$. Trends were indicated when $P \leq 0.10$.

Results and Discussion

Results during prepartum period are reported as the main effects of dietary starch concentration, whereas results during postpartum phase are reported as the main effects of dietary starch concentration, BP supplementation, and interactions between dietary starch concentration and BP.

Prepartum DMI, Nutrient Intake, BW, BCS and Blood Metabolites

Results for this section are presented in Tables 11 and 12. We did not observe any effects of starch on prepartum DMI. Dry matter intake (% of BW) also was not affected by prepartum dietary starch concentration, and averaged 1.81 and 1.83 for LS and HS, respectively. Prepartum EB was unaffected by dietary starch concentration ($P = 0.26$). Prepartum EB expressed as a percentage of requirements was also similar ($P = 0.25$) between treatments. There were no effects of starch diet concentration on BW and BCS ($P = 0.29$ and $P = 0.73$, respectively).

Neither prepartum concentration of NEFA ($P = 0.79$) nor BHBA ($P = 0.78$) were influenced by starch dietary levels. Whereas concentrations of NEFA increased ($P < 0.001$) as parturition approached, and peaked on wk -1, BHBA concentrations were greater ($P = 0.05$) on wk -3 (Figure 6). Prepartum concentrations of glucose were similar ($P = 0.16$) between LS and HS treatments, and weekly decreased ($P < 0.001$) as calving approached (Figure 6). Prepartum concentrations of haptoglobin were similar ($P = 0.73$) for LS and HS, and similar ($P < 0.22$) during the 3 wk of prepartum (Figure 6).

Concentration of calcium on wk -1 relative to calving was not affected by dietary starch and averaged 10.6 mg/dl and 11.3 mg/dl for LS and HS, respectively.

Grummer et al. (1995) found that concentrations of NEFA and BHBA were elevated in primiparous cows that calved at heavier BW and BCS and were likely to affect liver health of animals. We did not observe changes in blood metabolites which might be due to similar BW and BCS between treatments, and moreover, similar DIM during prepartum phase. Available evidence indicates that parity can influence the pattern of change in metabolites, for instance, NEFA were higher in the immediate postpartum period in primiparous cows whereas NEFA tended to be higher in the first month of lactation in multiparous than primiparous cows (Santos et al., 2001; Meikle et al., 2004). Minor et al. (1997) indicated that feeding diets with greater NFC during prepartum was associated with greater decreases in DM and energy intakes; Moreover, it has been proved that over-feeding energy to close-up cows leads to a decrease in gluconeogenesis, declines in DMI and an increase in rate of postpartum lipolysis (Douglas et al., 2007; Janovick et al., 2011) which has been linked to fatty liver (Dann et al., 2005). Fatty liver is a common metabolic dysfunction that affects postpartum dairy cows (Bobe et al., 2004). Janovick et al. (2011) pinpointed that prepartum "overnutrition syndrome" may aggravate insulin resistance that occurs postpartum, contributing to greater mobilization of adipose TAG from body stores and subsequent development of metabolic disorders. Therefore, prepartum energy controlled diets should be recommended during prepartum period. Regarding glucose concentration, results from this study agree with Kunz et al. (1985) who suggested that glucose concentrations remain stable or increase slightly during the pre-fresh transition period.

Prepartum Rumen Fluid and Apparent Total-Tract Digestibility

Results for this section are presented in Table 13. Rumen fluid sampling revealed no differences in pH (P = 0.55) or ammonia concentrations (P = 0.34) for LS and HS diets. Although acetate and propionate were not affected by starch concentration (P = 0.19 and P = 0.72, respectively), butyrate was 1.17 % greater (P = 0.01) for HS treatment compared to LS. Prepartum DMI on wk -2, measured at same wk that rumen fluid and fecal grab samples were collected, did not differ (P = 0.87) between LS and HS treatments. Surprisingly, prepartum NDFD was not affected (P = 0.73) by dietary starch concentration. Prepartum NDF intake was also similar (P = 0.18) between treatments and averaged 4.95 and 4.49 kg/d for LS and HS, respectively.

Rumen fluid pH averages were greater than what we expected for both treatments. However, rumen fluid samples were collected using OST technique at 200 cm insertion depth, and according to Shen et al. (2012) this is the optimal depth to reach the central rumen and obtain representative rumen fluid with minimal saliva contamination. Moreover, OST is simpler, quicker and less invasive (Duffield et al., 2004) compared to rumenocentesis (rumen puncture) and rumen cannulation. However, in our study rumen fluid samples were collected only once a day 20 to 21 h after feeding. This sampling intensity may not have been sufficient to evaluate changes in rumen fermentation pattern due to differences in dietary starch concentrations. It was expected that HS would decrease fiber digestibility, since high starch diet concentration results in decreases in rumen pH and fibrolytic bacteria growth is negatively affected (Kaufman et.al 1980). No changes on the proportions of acetate and propionate agreed with Van Vauuren et al.

(2009) as well the increases in butyrate for heifers fed HS diets. Kreuzer (1986) attributed increases in butyrate when HS was fed to the increases in ruminal protozoa numbers.

Postpartum Rumen Fluid and Apparent Total-Tract Digestibility

Results for this section are presented in Table 14. Early postpartum rumen fluid samples, wk 1 and 2, revealed no effects of starch, BP or interactions of starch x BP in pH. Ammonia concentrations were affected by dietary starch ($P = 0.01$) being greater for LS treatments, but no effects of BP ($P = 0.81$) or interaction of starch x BP ($P = 0.21$) were observed. Early postpartum butyrate concentrations were unaffected by starch or BP, and no interaction between starch x BP were observed among treatments. On the other hand, propionate concentrations were greater ($P = 0.01$) for cows fed HS compared to LS, whereas acetate concentrations were lower ($P = 0.01$). Due to differences in acetate and propionate concentration, A:P ratio tended ($P = 0.08$) to be greater for LS compared to HS treatments; moreover, due to differences in propionate concentration, cows fed BP supplementation had lower ($P = 0.02$) A:P ratio compared with no supplemented cows. Early postpartum DM and NDF intake, wk 1 and 2, although not significant ($P = 0.66$ and $P = 0.60$) were numerically greater for BP supplemented treatments. This might be due to the increase ($P = 0.05$) in NDFd (%) of 4.4% and 8.5% in LS and HS diets, respectively, caused by BP supplementation. Moreover, NDFd (kg/d) tended ($P = 0.09$) to be greater for BP supplemented cows.

Rumen fluid results on wk 12 revealed no effects of starch, BP or interactions of starch x BP neither in pH nor in ammonia concentrations. Whereas acetate concentrations were greater ($P = 0.01$) for LS treatments compared to HS, the opposite was observed for

propionate concentrations (P =0.01). As a result of the changes in acetate and propionate concentrations A:P ratio was also affected by starch (P = 0.01), with greater averages for LS diets. Butyrate concentration was similar across treatments. Dry matter intake on wk 12 revealed no effects of starch; however, it was 15% and 6% greater (P = 0.004) for LS and HS cows fed BP, respectively, compared to control cows. Dry matter digestibility was greater (P = 0.008) for LS compared with HS treatments, but not affected by BP supplementation. Due to changes differences in DMd (%) and DMI, DMd (kg/d) was also greater (P = 0.007) for LS treatments compared to HS.

Total-tract NDFd (%) tended to be greater (P = 0.06) for LS compared to HS treatments, and although BP supplementation improved NDFd (%) by 6% for both LS and HS diets it was so statistically significant (P = 0.56). Moreover, due to greater DMd (%) and DMI for LS diets, DMd (kg) presented similar trends, being greater for LS compared to HS diets.

Direct fed microbials supplemented to ruminants can be categorized into different modes of actions such as: prevention of gastrointestinal tract colonization by pathogens organisms; stimulation of desirable microbial growth in the rumen; stabilization of rumen pH; alteration of ruminal fermentation pattern; enhance nutrient flow postruminally; enhance nutrient digestibility and retention of energy from the diet and improve animal immune response (Yoon and Stern 1995). However, animal responses to DFM have yielded a range of different results in which some strain's confirmed in vitro results but convincing animal data to support this concept are sometime lacking (FAO, 2013). However, there has been some indication that certain bacterial DFM may also have beneficial effects in the rumen (Ghorbani et al., 2002). In especial, bacterial DFM may

help prevent ruminal acidosis, characterized by low ruminal pH and high ruminal concentrations of lactic acid, conditions that can lead to acute metabolic acidosis (Owens et al., 1998).

Despite of minimal or no changes in ruminal parameters in the current study, numerical increases in apparent total-tract digestibilities with BP supplementation were observed. Increases especially in fiber digestibility of diets are always desirable in view of the fact that this increases feed efficiency and decreases cost of production. Numerical increases in NDFd, agreed with our initial hypothesis that BP supplementation would enhance microbial xylanase production sufficiently to increase the breakdown of xylan, and the bonds that link lignin to the cellulose increasing the fiber surface area and boosting fiber digestibility of the diets. Furthermore, increases in NDFd% were not enough to account for changes in DMI during early lactation. It is possible that this was due to the short period (28DIM) that BP was being supplemented; however, it resulted in a longer effect, with increases in DMI at 112 DIM.

Moreover, regarding the lower % NDFd for HS on wk 12, we attribute these results to the increases in DM and starch intakes as lactation progressed. Digestibility of NDF is a key predictor of dairy cow intake and performance (Jung and Allen, 1995; Oba and Allen, 2000), and digestion of NDF in the rumen can be negatively affected by levels of starch due to reduced rumen pH (Meissner et al., 1996). Van Vuuren et al. (2009) found that high-starch diets significantly decreased NDF and OM digestibilities.

Postpartum Blood Metabolites

Postpartum NEFA concentration was unaffected by starch ($P = 0.68$), BP supplementation ($P = 0.55$) and starch x BP interaction ($P = 0.97$). Despite of no changes in NEFA concentrations, cows assigned to BP supplementation tended ($P = 0.06$) to have lower BHBA concentration on the first 28 DIM (Table 15). Postpartum glucose concentrations were similar across treatments, and unaffected by starch ($P = 0.14$), BP ($P = 0.42$), or interaction of starch x BP ($P = 0.37$), and constant during early postpartum (Figure 7). No changes on postpartum haptoglobin were observed across treatments. Concentrations of calcium were also similar across treatments, and not affected by starch ($P = 0.98$), BP ($P = 0.54$), or interaction of starch x BP ($P = 0.40$).

Rapidly increases in energy requirements due to the start of lactation and low intakes lead body fat mobilization to compensate for the energy deficit. The extensive body fat mobilization and the inability to dispose of fatty acids via β -oxidation or the limited capacity to export triacylglycerides (TAG) in the form of very low density lipoproteins (VLDL) from the liver pre-disposes cows to fatty liver and ketosis (Grummer, 1993; Bell, 1995). Circulating NEFA indirectly measures mobilization of TAG from adipose tissue, which is greater in early lactation than in mid lactation (Mashek et al., 2001). Furthermore, postpartum liver total lipid and triacylglycerol concentration were found to be greater in animals overfed energy prepartum indicating that restrict energy diet might be a better strategy during the close-up period (Janotivick et al. 2011). Moreover, on the same study, circulating NEFA for primiparous differed from multiparous cows regarding the concentration and the peaking time; and blood glucose concentration was greater for primiparous compared to multiparous cows when

animals were over and restricted fed. Primiparous cows have relative lower glucose demand when compared to multiparous cows which can be attributed to lower milk yields.

Early Lactation and Mid-lactation DMI, EB, BW, BCS, Milk Yield and Components and Feed Efficiency

Results for this section are all presented in Tables 16 and 17, and Figures 8 and 9. Although DMI was similar among treatments, no effects of starch (P = 0.16), BP (P = 0.25) or interactions of starch x BP (P = 0.98) during the 28 d postpartum were observed, as well as DMI as percentage of body weight. However, cows from all treatments remained in a negative energy balance throughout 28 DIM. Body weight, BCS and BW change were similar among treatments. However, there was an interaction between starch x BP for BCS change, and whereas it tended (P = 0.09) to be lower for LS diets opposite results were seem for HS treatments supplemented with BP. Milk yield (P = 0.10), 3.5% FCM (P = 0.08) and 3.5% ECM (P = 0.08) during the first 28 DIM tended to be greater for cows supplemented with BP, with increases of 13.9 %, 14.9 % and 15.2 %, respectively, for HS diets How about LS diets?. As expected MUN was lower (P = 0.02) for HSCO and HSBP compared to LSCO and LSBP treatments, but no other effects of BP or interactions of starch x BP during the 28 d were observed. Dry matter intake throughout 112 DIM was unaffected by starch levels (P = 0.86); however, BP supplementation increased (P = 0.01) DMI for both LS and HS diets, which also resulted in greater (P = 0.02) DMI as percentage of body weight for the same treatments. Primiparous cows continued in a negative energy balance throughout the 112 days of

trial; however, NEB was affected by dietary starch levels, and values were greater (P = 0.01) for LS treatments compared to HS. Numerical increases in milk yield and milk fat (%) resulted in greater (P = 0.04) milk fat yield (kg/d) for cows supplemented with BP. Moreover, BP supplementation tended (P = 0.06) to increase 3.5 % FCM (kg/d) yields by 4.3% and 8.9% for LS and HS diets, respectively. Milk protein yield (kg/d) also tended (P = 0.06) to be greater for BP treated cows. Milk urea nitrogen tended (P = 0.06) to be lower for HS compared to LS treatments but was unaffected by BP supplementation, and no interactions were observed for starch x BP.

The observed increases in DMI, milk fat and protein and tendency for greater 3.5% FCM for primiparous cows fed BP are exciting. This suggests that BP can be used as an effective probiotic to improve animal performance with enhancement of NDF and DM digestibilities that result in improvements in feed conversions. Sun et al (2012) fed a *Bacillus* – based DFM for a 70-d period and noticed that supplementation linearly increased milk production, 4% FCM and ECM, and well as milk fat, protein and lactose yield, and results were attributed to the capability of the *Bacillus* used on their study to promote growth of total ruminal bacteria.

Regarding the differences in DMI due to dietary starch, Beauchemin et al. (1997) reported that grain source had no effect on DMI of primiparous cows; however, lower concentration of NSC such as starch enhanced feed intake. In their study, starch varied 12.5% units among diets whereas in our study variation of dietary starch was only 6% units. Perhaps, only 6% units difference was not enough to affect intake in primiparous cows, therefore no impact on milk production was seen. Our findings of greater milk protein yield and lower MUN for HS were similar to results found by Ferrarretto et al.

(2010). These results can be related to greater starch intakes with greater rumen energy availability, which are likely to increase ruminal microbial protein synthesis (NRC, 2001; Huntington, 1997). Effects of BP in milk protein yield are likely due to increases in fiber digestibility. According to Clark et al. (1992) increasing diet digestibility has the potential to increase microbial growth, which is often limited by energy availability.

Colostrum Yield and Calf Birth

Colostrum yield and calf birth were similar among treatments (Table 18). There was no effect of prepartum starch dietary concentration in colostrum yield or calf birth.

Disease Incidence

Health events are presented in Table 19. Six cows were removed from the study due to health complications (milk fever, reproduction problem, DA surgery, leg injury), resulting in 42 cows completing the study.

Conclusions

Supplementation of low and high starch primiparous cow diets with *Bacillus pumilus* in early lactation resulted in increases of NFDd of 4.4% and 8.5% units for LS and HS diets, respectively. Moreover, supplementation of BP throughout 112 DIM improved DMI and increased yields of 3.5% FCM, milk fat and protein. These results show that this novel DFM can be used to enhance performance of first lactation cows.

Chapter 4: Effect of Econase on rumen fermentation patterns, diet digestibility and early lactation performance of primiparous dairy cows

D. N Lobão da Silva*, M. I. Endres*, N. B. Litherland*

*Department of Animal Science, University of Minnesota, St. Paul 55108.

Overview

The objective of this study was to examine the effect of adding a fibrolytic enzyme preparation (Econase) through a molasses-based liquid feed (MLF) to diets on digestibility, ruminal patterns and production performance of primiparous cows. Thirty-six animals were blocked by expected calving date, BW, BCS, and randomly assigned to two treatments. Dietary treatments included: 1) control diet pre- and postpartum + 0.5 ml of untreated MLF to each kg of DM (CON); 2) control diet pre- and postpartum + 0.5 ml of enzyme-treated MLF to each kg of DM (ECO). Enzyme was added at a rate of 8.3 kg per ton of MLF product by manufacturer and conferred activity of 3,500 BXU/g of Xylanase and 105 ECU/g of Cellulase. Diets were fed from 45 days prepartum to 56 days in milk (DIM). Data were analyzed using the MIXED procedure of SAS. Prepartum dry matter intake (DMI) was similar (14.2 vs. 15.0 kg/d) for CON and ECO treatments, respectively. Enzyme supplementation did not affect intake during prepartum period and positive energy balance (EB) was kept during the period, with no differences observed on nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), glucose and haptoglobin serum concentrations. Ruminal pH was not affected by treatments and no changes were observed on VFA concentrations. Enzyme supplementation tended to increase colostrum

yield by 32 % although no changes were observed on total colostrum solids. Postpartum DMI was similar between treatments, and NEFA, BHBA and glucose concentrations were also similar between treatments. Postpartum ruminal fluid pH tended to be greater for ECO supplemented cows (6.71) compared to CON (6.53). However, ammonia concentration was similar between treatments, and we did not observe any effects of enzyme supplementation on any VFA measured from ruminal fluid during postpartum. Intake of NDF was also similar between treatments and no changes were observed in NDFd (%) or DMd (%) between CON and ECO. Biological cows' response when fed enzyme supplementation was small, although positive. Future research is required to elucidate how different doses and length of feeding enzymes may affect growth and performance of animals in first lactation.

Key Words: digestibility, fibrolytic enzymes, primiparous.

Introduction

Increases in feed prices, especially grains, and declining enzyme costs have prompted interest in using enzyme additives in dairy cattle diets to increase feed efficiency and improve income over feed cost (IOFC) (Beauchemin et al., 2008). Enzyme feed additives with fibrolytic properties offer a choice of optimizing fiber digestibility and improving ruminal energy utilization (Chung et al., 2012). Multiparous and primiparous cows fed low-concentrate diets treated with fibrolytic enzymes had better performance than cows fed untreated high-concentrated diets (Ariola et al., 2011), which leads to the conclusion that greater forage proportions can be fed to dairy cows without jeopardizing milk production, lowering the cost of diets and reducing risk of rumen acidosis.

However, efficacy of fibrolytic enzymes in improving animal performance while increasing feed conversion has been variable. A meta-analysis of 20 dairy cow studies with 30 experiments (Adesogan et al., 2014) revealed that only a few exogenous fibrolytic enzymes (EFE) improved lactational performance and the response was inconsistent. The variability of responses to EFE can be attributed to the duration of feeding period, stage of lactation, and inappropriate choice of enzymes with lack of sufficient potency and specificity for improving digestibility under ruminal conditions (Adesogan et al. 2014).

Enzyme additives increase the rate of fiber digestion, which can provide more digestible energy to the animal for growth or milk production (Beauchemin et al., 2008). Dairy animals usually calve for the first time at about 24 months of age as this maximizes economic benefit (Hoffman and Funk, 1992). Animals are not yet physically mature at

this age; they require nutrients for their own continued growth in addition to that of their developing calf (Coffey, 2006). Supplementing primiparous cows with fibrolytic enzymes before calving can boost energy supply for growth during prepartum resulting in greater periparturient energy balance in addition to increasing their adaptation to enzyme additives before lactating starts which may guarantee greater enzyme response during their first lactation.

Enzyme additives are usually powdered products that need to be diluted into water daily, added along with other ingredients and then fed to cows (Yang et al., 2000), which might be inconvenient due the additional on-farm labor and uncertainty if the right dosage is being delivery to diets. This is an important concern that should be raised since factors such as level of enzyme provided and method of application can account for inconsistent responses (Zin and Salinas, 1999). Technologies that increase the efficacy of fibrolytic enzymes ensuring delivery of accurate dosage can play an important role in enhancing cows' performance responses to exogenous enzymes. Fibrolytic enzyme preparation ready to be used, delivered through a molasses-based liquid feed (MLF) might help to enhance binding of the enzyme with the feed substrate, and also it may increase the acceptance of this new technology by producers as it reduces on-farm labor and decrease errors of enzyme preparation. According to Morgavi et al. (2000) applying a solution of enzymes to the feed allows the enzyme to bind to substrate, which increases the resistance of the enzymes to proteolysis within the rumen. Moreover, MLF is an ingredient that provides rapidly degradable carbohydrate sources and improves particle adhesion of diet decreasing sorting (Litherland et. al, 2013). Molasses, made up of 70% sucrose sugar, has more favorable effects on the ruminal environment, especially for fiber

digestion, compared with starch, and feeding this ingredient has been associated with increased molar yields of acetate and butyrate (Broderick and Radloff, 2004; Hall and Weimer, 2007; Oelker et al., 2009). Surprisingly, when dietary sucrose increased from 2.8 to 5.7%, ruminal pH increased which can lead to increases in fiber-digesting microorganisms (Penner et al., 2009). The objective of this study was to determine the effects of a developmental exogenous fibrolytic enzyme product on primiparous cows' performance during prepartum to 56 DIM. We hypothesized that the developmental enzyme product would increase fiber digestibility, efficiency of nutrient use of pre- and postpartum diets and assure greater performance compared to Control diets.

Materials and Methods

Cows and Treatments

Thirty-six (n=18/treatment) Holstein and Holstein cross (Holstein × Montbéliarde × Swedish Red) nulliparous cows at 60 days (15 days pretreatment period) prior to expected calving date were blocked by body weight, BCS, expected calving date, and randomly assigned to one of two treatments and followed until 56 DIM. Dietary treatments included: 1) control diet pre- and postpartum + 0.5 ml of untreated MLF to each kg of DM (CON); 2) control diet pre- and postpartum + 0.5 ml of enzyme treated MLF to each kg of DM (ECO). Enzyme was added at a rate of 8.3 kg per ton of MLF product by manufacturer and conferred activity of 3,500 BXU/g of Xylanase and 105 ECU/g of Cellulase. Diets were fed from 45 days prepartum to 56 days in milk (DIM). The enzyme product was a proprietary blend (AB Vista, Marlborough, UK) derived from a strain of *Trichoderma reesi*. To enhance enzyme activity MFL (enzyme-treated or

untreated) was applied to dry ingredients; Yang et al. (2000) suggested that the effects of enzymes might be greater when applied to dry feeds. Feed preparation and feeding proceeded as follows. Corn gluten pellets, dry cow, protein mix and MFL (enzyme-treated or untreated) were added into a mixer wagon of a Data Ranger (American Calan, Inc., Northwood, NH). After sufficient time to ensure complete blending of dry ingredients and MFL (enzyme-treated or untreated), alfalfa hay, corn silage and wheat straw (only prepartum diet) were also added into the mix, mixed for 5 to 7 min then TMR was fed (Table 20).Total mixed ration was formulated to meet or exceed NRC (2001) requirements, and to provide 0.5ml of treated/untreated enzyme QLF product per each kg of TMR DM. Diets (Table 20) were designed to have similar nutrient composition differing only on enzyme activity. All diets were formulated using CNCPS dairy software (Version 6.1; Cornell University, Ithaca, NY). Diets were fed at ad libitum rate (to ensure 10% feed refusals).

Animal Housing and Management

This experiment was performed from September 2013 to April 2014 at the University of Minnesota Dairy Teaching and Research Center. All experimental procedures were conducted under an approved protocol by the Institutional Animal Care and Use Committee of the University of Minnesota. Throughout the experiment, animals were housed in individual tie-stalls with rubber-filled mattresses and bedded with sawdust in a mechanically ventilated barn. Water was available ad libitum in each stall. Animals were fed once daily during the dry period (1200 h) and twice daily after calving (0300 and 1100 h). Animals were milked twice daily (0200 and 1400 h).

Enzyme activity

Molasses-based liquid feed treated and untreated samples were collected monthly and analyzed for xylanase activity (EC 3.2.18) using the assay of Bailey et al. (1992). Assay conditions were 39°C and pH 6.0 to reflect ruminal conditions.

Sample Collection and Preparation

Feed Collection and Analysis. Individual ingredients used on dry and lactation diets (Table 20) were sampled weekly, frozen at −20°C and composited monthly on a wet weight basis, dried in a 60°C forced air oven for 48 h (or until static weight was achieved) and then ground through a 1-mm screen in a Wiley mill (Thomas-Wiley, Philadelphia, PA). Dried samples were analyzed at Dairyland Laboratories (St. Cloud, MN) using wet chemistry methods. Monthly averages of the nutrient composition of individual ingredients were used in the CNCPS dairy model to calculate the nutrient composition of the diets. Organic matter concentration of feed and feces was calculated as the difference between DM content and ash content. Ash content was determined using method 942.05 (AOAC International, 2000). Crude protein was determined using method 990.03 (AOAC International, 2000). Heat-stable, α-amylase treated, sodium sulfite NDF for feed ingredients and fecal samples was determined using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY) based on procedures described by Van Soest et al. (1991). Acid detergent fiber was determined using method 973.18 (AOAC International, 2000). Lignin was determined using method 973.18. Levels of ADF-insoluble CP was determined by method 973.18 (AOAC International, 2000) and ether extract was determined by method 920.39 (AOAC International, 2000).

DMI and Nutrient Intake. Daily individual cow DMI was measured from 42 d prior to calving to 56 DIM. Feed offered and refused were measured daily and recorded electronically. Dry matter intake was calculated by correcting as-fed offered minus refusals and multiplied by weekly diet DM. Energy balance both pre- and postpartum was calculated for each animal using equations for primiparous cows described by NRC (2001). Net energy intake was determined by multiplying DMI by the calculated mean NEL density of the diet. The NEL 3 × maintenance was predicted from total digestible nutrients according to the NRC (2001). Maintenance NEL (Mcal) was calculated as BW0.75 × 0.080. Pregnancy requirements for NEL (Mcal) were calculated as [(0.00318 × day of gestation – 0.0352) × (calf birth weight/45)]/0.218. Requirements of NEL for milk production were calculated as (0.0929 × fat %) + (0.0547 × protein %) + (0.0395 × lactose %) (NRC, 2001). Since animals were primiparous, retained energy (RE) required during pregnancy was calculated using equation 11–2 in NRC (2001). The equation for calculation of RE was as follows: RE = 0.0635 × [0.891 × (0.96 × current BW)]. The NEL value for forages was adjusted with the Van Soest variable discount method (Dairy One, 1999). Weekly means for individual animals were used as model inputs for prepartum DMI, and BW were used for prepartum EB calculations. Postpartum EB was calculated using inputs of weekly averages of DMI, BW, milk yield and components, BW and BCS. Individual cows were weighed weekly at 1600 h from wk – 6 through 8 relative to calving. The BCS was measured weekly on 0.25-unit increments (Ferguson et al., 1994) and averages of 3 trained investigators from wk – 6 through 16 relative to calving were reported.

Milk Yield and Composition. Cows were milked twice daily (0200 and 1400 h), individual milk yields were recorded at each AM and PM milking from d 1 through 56 DIM. Milk samples were taken weekly from individual cows, preserved (800 Broad Spectrum Microtabs II; D and F Control Systems Inc., San Ramon, CA) until next day analysis using mid-infrared procedures (AOAC International, 1995) for fat, protein, lactose, SCC, and MUN. Total solids of colostrum samples were measured using a digital MISCO PA201 brix refractometer (MISCO Inc., Cleveland, OH).

Apparent Total-Tract NDF Digestibility. To determine apparent total-tract NDF and DM digestibility during d – 21, 2, 14 and 56 relative to calving, TMR samples were collected once daily on the same day as 2 fecal grab samples were collected per cow at 0800 h and 1600 h. The TMR were composited by treatment and fecal samples were composited by cow on a wet weight basis, dried at 600C for 48 hr, and ground through a 1-mm screen in a Wiley mill. Analysis of TMR and fecal samples included DM and NDF digestibility using acid-insoluble ash as an internal marker as described by Block et al. (1981).

Blood Collection and Analysis. Blood samples were collected weekly on d - 28, -21, -14, -7, 7, 14, 21, 28 relative to calving at 0800 h. Approximately 10 mL of blood was collected from the coccygeal vein into an evacuated serum tube (serum separator, Becton Dickinson Vacutainer systems, Franklin Lakes, NJ), centrifuged at 2,000 x g for 20 min immediately after sample collection, and frozen at - 200C until analysis. Serum NEFA concentrations were determined using a NEFA C kit (Wako Diagnostic, Richmond, VA). Serum glucose concentrations were quantified by enzymatic reaction (Stanbio Laboratory, Boerne, TX). Serum haptoglobin concentrations were determined by a

colorimetric procedure as described by Hulbert et al. (2011). Absorbance for NEFA, glucose, calcium, and haptoglobin assays were quantified using a microplate spectrophotometer (Eon TM, BioTek Instruments Inc., Winooski, VT). Serum BHBA concentrations were quantified using the Precision Xtra® ketone monitoring system direct electrochemical test (Abbot Laboratories Inc., Abbott Park, IL).

Rumen Fluid. Rumen fluid samples were collected on d -14, 2, 14, and 56 relative to calving at 0800 h via oral-esophageal tube technique (OST) at 200 cm insertion depth (Shen et. al., 2012) described with details by Duffield et al. (2004). Immediately after sampling, pH was measured using a pH meter 345 (pH meter 345 TM, Corning Inc., New York City, NY), samples were then placed in liquid N and transferred to - 200C freezer on the same day until further analysis. Rumen fluid samples were analyzed for volatile fatty acids (VFA) and ammonia N by Dairyland Laboratories Inc. (St. Cloud, MN). Volatile fatty acids were quantified using high liquid chromatography (HPLC) technique, and ammonia N determined by distillation.

Statistical Analysis

Data were analyzed using SAS (version 9.2 SAS Institute, Cary, NC). Prepartum and postpartum data were analyzed separately. Prepartum data were analyzed as a completely randomized design using MIXED models procedures of SAS. Model included effects of breed, treatment, time and interaction of treatment by time.

Pre-and postpartum periods repeated measures over time were modeled with autoregressive [AR (1)], and denominator degrees of freedom were estimated using Kenwards-Rogers method. Single measurements were modeled with autoregressive [AR

(1)], and denominator degrees of freedom were estimated using Satterthwaite methods. Least squares means for breed, treatment, time and all interactions were separated by use of PDIFF statement when the overall F-test was $P \leq 0.05$. Trends were indicated when $P \leq 0.10$.

Results and Discussion

DMI and Nutrient Intake, BW, and Body Condition

Results for this section are presented in Table 21. Prepartum DMI (14.2 vs. 15.0) was not affected ($P = 0.17$) by enzyme application. Intake of DM (% of BW) was also similar between treatments and averaged 2.53 and 2.63 for CON and ECO, respectively. Nulliparous animals maintained a similar and constant intake during the prepartum phase enough to keep themselves in a similar ($P = 0.58$) and positive energy balance throughout this period with energy balance (% of requirements) averaging 118 % for both treatments. Body weight and BCS although similar between treatments, increased ($P = 0.001$ and P <.0001; respectively) as calving approached.

Postpartum DMI was similar ($P = 0.57$) between treatments; however, it increased ($P < .0001$) overtime from calving to 56 DIM for both CON and ECO treatments. Intake of DM (% of BW) was similar ($P = 0.38$) between CON and ECO, and averaged 4.02 % and 4.14 %, respectively. Energy balance (% of requirements) was 12 and 10 % greater than normal requirements for CON and ECO, respectively, resulting in a positive energy balance for the 56 d of experiment and body weight gains for both groups.

Although energy demand increases in late gestation and early lactation, feed intake typically decreases (Grummer, 1995). Rapid growth of the fetus can account for

the decrease in prepartum intakes due to extra abdominal compression and reduction in the rumen capacity, and after calving DMI continually increase and peak around 9 – 13 weeks of lactation (Kertz et al., 1991). The period from parturition until peak milk production is the most critical phase for a dairy cow (Schingoethe et al, 1993). Although enzyme additives can improve efficiency of nutrient use, we observed no advantages in nutrient intake and BW or BCS from supplementing enzyme. Our results are in agreement with Zheng et al. (2000) who found no changes in DMI and body condition scores when cows were fed enzyme during both pre- and postpartum phase. Also, Ariola et al. (2011) observed no effects of fibrolytic enzymes on DMI of neither multiparous nor primiparous cows when supplemented from early lactation to 84 DMI, and also no effects on BW, BCS or BW change.

Blood Metabolites

Results for this section are presented in Table 22 and Figures 10 and 11. Prepartum glucose and haptoglobin concentrations were similar ($P = 0.39$ and $P = 0.87$) and constant for CON and ECO treatments during this period. Concentrations of NEFA and BHBA ($P = 0.66$ and $P = 0.21$) were also not affected by prepartum enzyme supplementation. However, both serum metabolites increased (NEFA: $P < .0001$ and BHBA: $P = 0.02$) as nulliparous approached calving.

Postpartum enzyme supplementation did not affect glucose, NEFA, BHBA or haptoglobin concentrations during the first 28 DIM, and whereas glucose and BHBA were constant during early postpartum, NEFA ($P <.001$) and haptoglobin ($P <.001$) concentrations were greater at 7 DIM.

We hypothesized that the developmental enzyme product would increase fiber digestibility and efficiency of nutrient use leaving cows in a greater positive energy balance compared to Control fed animals. During peripartum period, cows consume less energy than they require, leading to a negative energy balance (NEB), fat mobilization and losses in body weight. According to Bertics et al. (1992) declines of DMI can be as low as 30% at week one before calving and can lead to a maximum NEB at the first week after calving (Grummer, 1995). Blood concentration of NEFA can be used as a marker of energy deficit at prepartum and as an index of lipid mobilization during postpartum (Duffield, 2000). However, primiparous and multiparous cows have different blood metabolite patterns (Meikle et al., 2004; Santos et al., 2001).

Rumen Fluid and Apparent Total-Tract Digestibility

Results for this section are presented in Table 23 and 24. Prepartum ruminal pH and ammonia concentration were not affected by enzyme supplementation ($P = 0.64$ and $P = 0.14$). No changes were observed in any ruminal VFA measured such as acetate, butyrate, propionate as well as the sum of other VFA found in smaller concentrations in ruminal fluid such as isobutyrate, isovalerate and valerate. Prepartum DMI on d – 21, same day that DM and NDF digestibilities were measured, was increased ($P = 0.04$) by enzyme supplementation which resulted in increases ($P = 0.02$) in NDF intake, and NDFd (kg/d). However, NDFd (%) and DMd (%) were not different; CON treatment had similar averages (60.0 vs. 57.9, $P = 0.48$) and (64.9 vs. 61.3, $P = 0.15$) compared to ECO treatment. Decreases in digestibilities might be due to increases in rate of passage as a consequence of greater DMI for ECO treated cows.

Postpartum ruminal fluid pH tended ($P = 0.08$) to be greater for ECO supplemented cows (6.71) compared to CON (6.53). However, ammonia concentration was similar between treatments, and we did not observe any effects of enzyme supplementation on any VFA measured from ruminal fluid during postpartum. DMI on d 2, 14 and 56 postpartum, same days digestibilities were measured, was not affected ($P = 0.78$) by enzyme supplementation. Intake of NDF was also similar between treatments and no changes were observed in NDFd (%) or DMd (%) between CON and ECO.

We hypothesized that fibrolytic enzyme supplementation would increase NDF digestibility in the digestive tract, and the improvements in ruminal fiber digestibility would allow cows to consume more feed by reducing physical fill. Arriola et al. (2011) observed no changes in DMI when fibrolytic enzymes were supplemented to multiparous and primiparous cows but it tended to decrease intake of CP, NDF and ADF. On the same study fibrolytic enzyme increased total VFA concentration by 10 percentage units, and increased apparent total tract digestibility of DM by 4 and NDF by 3 percentage units. Recently, Chung et al. (2013) reported no changes in ruminal fermentation or rumen pH; however, improvements in feed conversion efficiency and improved fiber digestion were associated to a shift in ruminal bacterial communities as ruminal fibrolytic bacteria such as *Fibrobacter succinogenes*, and non-fibrolytic bacteria, *Ruminobacter amylophilus* and *Selenomonas ruminantium* populations increased linearly with increasing levels of enzyme in the diet. It is important to consider that dietary changes influence populations of ruminal microbes, which can be highly specific to the ruminant host, and if correctly manipulated can improve the nutritional management of ruminants (Mullins et al., 2013). There is some evidence that rumen microbial populations adapt to dietary changes

(Ramirez et al., 2012). However, some studies have also demonstrated that rumen microbiome can be remarkably resistant to change (Weimer et al., 2010; Mhammet et al., 2012).

Milk Yield and Components and Feed Efficiency

Results for this section are presented in Table 25 and Figures 12 and 13. Milk yield and 3.5% FCM similar ($P = 0.15$ and $P = 0.27$) between treatments. No changes ($P = 0.43$) were observed on feed efficiency between treatments. Percent milk fat and milk fat yield (kg/d) were also similar between treatments, and no changes were observed in percent milk protein and milk protein yield (kg/d) or milk lactose (%) and yield (kg/d). However, MUN (mg/dl) was lower ($P = 0.01$) for CON (16.67) compared to ECO (17.81).

Arriola et al. (2011) observed only numerical increases in milk production when fibrolytic enzymes were fed to cows, but because there was no similar response in DMI, feed efficiency was greater for treated than control diets. The extent to which adding enzymes to the diet increases fiber digestion capacity of the rumen depends upon the amount of enzyme added to the diet and the activity of the exogenous enzymes under ruminal conditions. Yang et al. (1999) found significant increases in OM and NDF digestibility along with increases in 3.5% FCM yield when amount of enzyme was doubled compared to control. In a meta-analysis Eun and Beauchemin (2007) reported a range in degradability of various fibrolytic enzyme products showing the importance of product formulation, and also demonstrating that enzyme additives can have detrimental effects on fiber digestion when enzyme activity and dose rates are not optimized.

Holtshausen et al. (2011) fed the same exogenous enzyme used in the current study; however, enzyme was applied differently and diets were based on barley silage. Their study reported improved feed efficiency when enzyme was provided at 1.0 mL/kg of TMR DM. We hypothesized that starting enzyme supplementation at 0.5 ml/kg of TMR DM during prepartum would increase the adaptation to the product and result in greater lactation performance. Lactation stage is likely to affect EFE response, e.g., Schingoethe et al. (1999) reported 10% increase in milk production in cows fed EFE at less than 100 DIM but no effect when cows were fed the same enzyme after 100 DIM. However, our results are in agreement with Zheng et al. (2000) who found no additional advantage to starting enzyme supplementation during the prepartum period, and best performances were observed when cows started receiving enzyme supplementation right after calving.

Calf weight, colostrum yield and total solids

Calf birth was similar between treatments (Table 26). Colostrum is an essential nourishment for newborn mammals (Levieux, 1984), which is critical for disease prevention in calves and hence their growth and development (Collier et al., 2012). Enzyme supplementation tended to increase (P = 0.09) colostrum yield by 32% although no changes (P = 0.41) were observed on total colostrum solids (CON= 26.4 % and ECO = 25.1 %, respectively). According to Bielmann et al. (2010) a cut-off point of 22% Brix score is an indication of good quality colostrum.

Conclusions

Adding exogenous fibrolytic enzyme to dry ingredients using a molasses liquid feed (MLF) resulted in increases in DMI on d - 21 prepartum which resulted in increases

in NDF and NDFd intake, but without changes on rumen parameters. Blood metabolites during the parturition were unaffected by enzyme supplementation. During postpartum exogenous fibrolytic enzymes tended to increase NDF intake, and increased ruminal pH. However, there were no changes in milk yield, 3.5% FCM and feed efficiency.

Although small, cows' biological' response appeared to be positive when enzyme was added to the diet. The lack of response in NDFd and DMd was unexpected and it is suggested that future research should investigate the effects of this novel exogenous fibrolytic enzyme using greater doses of the product at different stages of lactation**.**

Conclusions and Implications

Exogenous fibrolytic enzyme and DFMs additives are emerging technologies that show promise in terms of increasing diet formulation flexibility. They may improve fiber digestibility making possible greater inclusions of forages in the ration without negatively impacting animal performance. Improvements in forage cell wall digestion by EFE and *Bacillus pumilus* might potentially increase forage digestibility and feed utilization eliciting better performance of dairy cows at minimal costs leading to more profitable livestock enterprises. Moreover, supplementation of the *Bacillus pumilus* can be used as a feeding strategy to diminish the use of antibiotic fed to dairy cows, as these live microbials are likely to promote gut health.

Bacillus pumilus 8G-134 supplementation increases diet energy availability and promote better energy balance when fed to multiparous cows. This novel DFMs supplementation might be an advantageous feeding strategy when fed to early lactation cows due to a reduction in NEFA and tendency to a reduction in BHBA, which demonstrates a decrease in body lipid mobilization due to low DMI and high energetic demands leading to high incidences of metabolic diseases during this critical time of cow's life. Increases in DMI as DIM increased and consequently a reduction of rumen feed retention time might have diminished the benefits of *Bacillus pumilus* 8G-134 on fiber digestion and no changes in % NDFd were seen at mid-lactation of multiparous cows. Further research is needed to evaluate the use of BP as a feeding strategy during peripartum period and also the potential benefits of adjusting *Bacillus pumilus* 8G-134 dose supplementation according to increases in DMI as cows progress through lactation.

Supplementation of low and high starch primiparous cow diets with *Bacillus pumilus* 8G-134 in early lactation resulted in increases of NFDd for both diets. Moreover, supplementation of *Bacillus pumilus* 8G-134 throughout 112 DIM improved DMI and increased yields of 3.5% FCM, milk fat and protein. These results show that this novel DFM can be used to enhance performance of first lactation cows.

Adding exogenous fibrolytic enzyme to dry ingredients using a molasses liquid feed resulted in increases in DMI on d - 21 prepartum which resulted in increases in NDF and NDFd intake, but without changes on rumen parameters. Blood metabolites during the parturition were unaffected by enzyme supplementation. During postpartum exogenous fibrolytic enzymes tended to increase NDF intake, and increased ruminal pH. However, there were no changes in milk yield, 3.5% FCM and feed efficiency was unaffected. Although small, cows' biological response appeared to be positive when enzyme was added to the diet. The lack of response in NDFd and DMd was unexpected and it is suggested that future research should investigate the effects of this novel exogenous fibrolytic enzyme using greater doses of the product in different stages of lactation**.**

Literature Cited

Adesogan A.T., Z. X. Ma, J. J. Romero, K. G. Arriola. 2014. Ruminant Nutrition Symposium: Improving cell wall digestion and animal performance with fibrolytic enzymes. J. Anim. Sci. 92:1317-1330.

Akin, D. E., and W. S. Borneman. 1990. Role of rumen fungi in fiber degradation. J. Dairy Sci. 73:3023–3032.

Aldrich, J. M., L. D. Muller, G. A. Varga, and L. C. Griel, Jr. 1993. Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. J. Dairy Sci. 76:1091–1105.

Allen M. S. and B.J. Bradford. 2009. Strategies to optimize feed intake in lactating cows. WCDS Advances in Dairy Technology.21: 161-172.

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

Annison, E. F., R. Bickerstaffe, and J. L. Linzell. 1974. Glucose and fatty acid metabolism in cows producing milk of low fat content. J. Agric. Sci. 82: 87–95.

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD.

Armentano, L., and M. Pereira. 1997. Measuring the effectiveness of fiber by animal response trials. J. Dairy Sci. 80:1416 - 1425.

Arriola, K. G., S. C. Kim, C. R. Staples, and A. T. Adesogan. 2011. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. J. Dairy Sci. 94:832-841.

Badhan, A.K., B.S. Chadha, J. Kaur, H.S. Saini, M.K. Bhat. 2007. Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora sp*. IMI 387099. Bioresource Technology 98: 504 - 510.

Balcázar,J.L., Blas, I.D., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., Múzquiz, J.L. 2006. The role of probiotics in aquaculture.Vet.Microbiol. 114: 173–186.

Battan, B., J. Sharma , S. S. Dhiman, R. C. Kuhad. 2007. Enhanced production of cellulase-free thermostable xylanase by *Bacillus pumilus* ASH and its potential application in paper industry. Enzyme and Microbial Technology 41:733 - 739.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: Homeostasis and homeorhesis revisited. Pages 311–328 in Ruminant Physiology. P. B. Cronje, ed. CABI Publishing, Cambridge.

Beauchemin, K. A., L. Eriksen, P. Nørgaard, and L. M. Rode. 2008. Salivary secretion during meals in lactating dairy cattle. J. Dairy Sci. 91:2077-2081.

Beauchemin, K. A., M. Rode, and W. Z. Yang. 1997. Effects of nonstructural carbohydrates and source of cereal grain in high concentrate diets of dairy cows. J Dairy Sci 80:1640–1650.

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.

Beharka A.A, Nagaraja T.G. 1993. Effect of *Aspergillus oryzae* fermentation extract (Amaferm) on in vitro fiber degradation. J Dairy Sci. 76: 812-818.

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

Benini S., G. Degrassi, I. Krastanova, D. Lamba, V. Venturi. 2001. Purification, crystallization and preliminary X-ray analysis of an acetylxylan esterase from *Bacillus pumilus.* Acta Cryst. D57:1906-1907.

Block, E., L. H. Kilmer, and L. D. Muller. 1981. Acid insoluble ash as a marker of digestibility for sheep fed corn plants or hay for lactating dairy cattle fed hay ad libitum. J. Anim. Sci. 52:1164–1169.

Broderick, G. A., and W. J. Radloff. 2004. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. J. Dairy Sci. 87:2997–3009.

Bozic,M., J. Newton, C. S. Thraen, and B. W. Gould. 2012. Mean reversion in income over feed cost margins: Evidence and implications for managing margin risk by US dairy producers. J. Dairy Sci. 95: 7417-7417.

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

Carpita, N. & McCann. 2002. M. in Biochemistry & Molecular Biology of Plants Ch. 2 (eds Buchanan, B., Gruissem, W. & Jones, R. L.) (John Wiley & Sons, New Jersey).

Casula, G.; Cutting, S.M. 2002. *Bacillus* probiotics: Spore germination in the gastrointestinal tract. *Appl. Environ. Microbiol.* 68, 2344 - 2352.

Chamberlain, D. G., S. Robertson and J. J. Choung. 1993. Sugars versus starch as supplements to grass silage: Effects of ruminal fermentation and the supply of microbial protein to the small. intestine, estimated from the urinary excretion of purine derivatives, in sheep. J. Sci. Food Agric. 63:189-194.

Chen X.A, N. Ishida,N. Todaka, R. Nakamura , J. Maruyama. 2010. Promotion of efficient saccharification of crystalline cellulose by *Aspergillus fumigatus* Swo1. Appl. Environ. Microbiol. 76:2556–61.

Chung Y.H., L. Holtshausen, T. W. Alexander, M. Oba, and K. A. Beauchemin. 2013. A fibrolytic enzyme additive for lactating dairy cow diets: ruminal fermentation, pH, bacterial populations and enteric methane emissions. J. Dairy Sci. 95(3):1419-27.Coffey MP, Hickey J, Brotherstone S. 2006. Genetic aspects of growth of Holstein–Friesian dairy cows from birth to maturity. J Dairy Sci. 89:322–9.

Cutting, S.M. 2011. *Bacillus* probiotics. *Food Microbiol.* 2011. *2*8(2): 214 – 220.

Davies, R. W. 1994. Heterologous gene expression and protein secretion in *Aspergillus*. Prog. Ind. Microbiol. 29:527–560.

Degrassi, G., P. Polverino De Laureto, and C. V. Bruschi. 1995. Purification and characterization of ferulate and p-coumarate decarboxylase from *Bacillus pumilus.* Appl. Environ. Microbiol. 61(1):326.

Ding, S. Y. and M.E.Himmel. 2006. The maize primary cell wall microfibril: a new model derived from direct visualization. J. Agric. Food Chem. 54, 597–606.

Dornez, E., K. Gebrueres, J.A. Delcour, and C.M. Courtin.2009. Grain-associated xylanases: occurrence, variability, and implications for cereal processing. Trends in Food Science & Technology 20, 495–510.Douglas, G. N., T. R. Overton, H. G. Bateman II, H. M. Dann, and J. K. Drackley. 2006. Prepartal Plane of Nutrition,

Regardless of Dietary Energy Source, Affects Periparturient Metabolism and Dry Matter Intake in Holstein Cows. J. Dairy Sci. 89:2141–2157.
Drackley, J. K. 1999. Biological of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82: 2259 - 2273.
Duffield T. 2000. Subclinical ketosis in lactating dairy cattle. Met Disord Rum 16:231–53.
Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci. 87:59–66.
Edens, F. 2003. An alternative for antibiotic se in poultry: Probiotics. Revista Brasileira de Ciência Avícola 5:75–97.
EFSA. 2008. Report of the task force on zoonoses data collection on the analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, Part A. *EFSA J. 135*, 1–111.
Enemark, J. M. D. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. Vet. J. 176:32–43.
FAO (Food and Agriculture Organization of the United Nations). 2013. Mitigation of greenhouse gas emissions in livestock production. A review of technical Options for non-CO_2 emissions.
Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2013. Effect of ce- real grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. J. Dairy Sci. 96:533–550.
Ferraretto, L. F., R. D. Shaver, M. Espineira, H. Gencoglu , and S. J. Bertics. 2011. Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. J. Dairy Sci. 94:1490 - 1499.
Festucci-Buselli, R. A., Otoni, W. C. & Joshi, C. P. 2007. Structure, and functions of cellulose synthase complexes in higher plants. Braz. J. Plant Physiol. 19, 1–13.
Firkins, J. L., M. L. Eastridge, N. R. St-Pierre, and S. M. Noftsger. 2001. Effects of grain variability and processing on starch utiliza- tion by lactating dairy cattle. J. Anim. Sci. 79(E. Suppl.):E218– E238.
Gäbel, G., J. R. Aschenbach, and F. Müll. 2002. Transfer of energy substrates across the ruminal epithelium: Implications and limitations. Anim. Health Res. Rev. 3:15–30.
Gaggìa,F., Mattarelli, P.; Biavati, B. 2010. Probiotics and prebiotics in animal feeding for safe food production.Int.J Food Microbiol. 141: S15–S28.3.
Galloway, D. L. Sr., A. L. Goetsch, L. A. Forster Jr., W. Sun, and Z. B. Johnson. 1991. Feed intake and digestion by Holstein steers fed warm or cool season grass hays with corn, dried molasses or wheat middlings. J. Dairy Sci. 74:1038–1046.
Gao, J., H. J. Zhang, S. G. Wu, S. H. Yu, I. Yoon, D. Moore, Y. P. Gao, H. J. Yan, and G. H. Qi. 2009. Effect of *Saccharomyces cerevisiae* fermentation product on immune functions of broilers challenged with Eimeria tenella. Poult. Sci. 88:2141–2151.
Garrett, E. F., K. V. Nordlund, W. J. Goodger, and G. R. Oetzel. 1997. A cross-sectional field study investigating the effect of periparturient dietary management on ruminal pH in early lactation dairy cows. J. Dairy Sci. 80(Suppl. 1):169. (Abstr.)

Gencoglu, H., R. D. Shaver , W. Steinberg , J. Ensink , L. F. Ferraretto , S. J. Bertics , J. C. Lopes ,and M. S. Akins. 2010. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. J. Dairy Sci. 93: 723 - 732.

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. 80:1977–1985.

Glass, N. L., M. Schmoll, J. H. D. Cate, and S. Coradetti. 2013. Plant cell wall deconstruction by ascomycete fungi. Annu. Rev. Microbiol. 67:477–498.

Grant R.. 2005. Optimizing Starch Concentrations in Dairy Rations. Tri-State Dairy Nutrition Conference pages 73 – 79.

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

Hall, M. B. 2002 Characteristics of manure: What do they mean? Pages 141–147 in Tri-State Dairy Nutr. Conf. Proc., Fort Wayne, IN. Michigan St. Univ., Ohio St. Univ., and Purdue Univ.

Hall, M. B., and P. J. Weimer. 2007. Sucrose concentration alters fermentation kinetics, products, and carbon fates during in vitro fermentation with mixed ruminal microbes. J. Anim. Sci. 85:1467–1478.

Hammon, H. M., Metges, C. C., Schulz, A., Junghans, P., Steinhoff, J., Schneider, F., Pfuhl, R., Bruckmaier, R. M., Weikard, R., and Ku¨hn, C. 2010 Differences in milk production, glucose metabolism, and carcass composition of two Charolais 3 Holstein F2 families derived from reciprocal paternal and maternal grandsire crosses. J. Dairy Sci. 93, 3007–3018.

Harrison, L.E., Q.M. Wang, G.P. Studzinski. 1999. Butyrate-induced G2/M block in Caco-2 colon cancer cells is associated with decreased p34cdc2 activity. P.S.E.B.M. 222(2):150-6.

Heinrichs, J. 2013. DSE 2013-186. The Penn State Particle Separator. http://extension.psu.edu/animals/dairy/nutrition/forages/forage-quality-physical/separator.

Hoffman P.C., D.A. Funk. 1992. Applied dynamics of dairy replacement growth and management. J Dairy Sci. 76:3179–87.

Huhtanen, P., M. Rinne, and J. Nousiainen. 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. J. Dairy Sci. 92: 5031–5042.

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake effects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. J. Dairy Sci. 94:1385–1400.

Johnson, L., J. H. Harrison, C. Hunt, K. Shinners, C. G. Doggett, and D. Sapienza. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: A contemporary review. J. Dairy Sci. 82:2813-2825.

Jung, H. J. G., and D. A. Deetz. 1993. Cell wall lignification and degradability. In: H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph, editors, Forage cell wall structure and digestibility. ASA, CSSA and SSSA, Madison, WI. p. 315–346.

Jung, H., and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73:2774 - 2790.

Kanehisa, M. and Goto, S. 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 28, 27-30.

Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. 2014. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res. 42, D199–D205.

Kaufman, W., H. Hagemeister and G. Dirksen. 1980. Adaptation to changes in dietary composition, level and frequency of feeding. In: Digestive Physiology and Metabolism in ruminants. P. 587. Y. Ruckebusch and P. Thivend, eds. AVI Publishing Co., Westport, CT.

Kertz A.F, Reutzel L.F, Thomson G.M. 1991 Dry matter intake from parturition to midlactation. J Dairy Sci. 74(7): 2290-5.

Knapp J. R., G. L. Laur, P. A .Vadas, W. P.Weiss, and J. M.Tricarico. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J Dairy Sci. 97(6):3231-61.

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.

Kononoff, P. J., A. J. Heinrichs, and D. A. Buckmaster. 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. J. Dairy Sci. 86:1858–1863.

Komatsu, T., Itoh, F., Kushibiki, S., and Hodate, K. 2005. Changes in gene expression of glucose transporters in lactating and nonlactating cows. J. Anim. Sci. 83, 557–564.

Krause, K. M., and G. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Technol. 126:215–236.

Kritas, S.K; A. Govaris, G. Christodoulopoulos, and A.R Burriel. 2006: Effect *of Bacillus licheniformis* and *Bacillus subtilis* supplementation of ewe's feed on sheep milk production and young lamb mortality. Journal of Veterinary Medicine. A Physiol. Pathol. Clin. Med. 53, 170–173.

Kubicek C.P, M. Mikus, A. Schuster, M. Schmoll, B. Seiboth. 2009. Metabolic engineering strategies for the improvement of cellulase production by Hypocrea jecorina. Biotechnol. Biofuels 2:19.

Kumar R., S. Singh, and O.V. Singh. 2008. Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. J. Ind. Microbiol. Biotechnol. 35 (5): 377–91.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922–928.

Litherland, N. B., D.N.L. da Silva, W.P. Hansen, L. Davis, S. Emanuele, H. Blalock. 2013. Effects of prepartum controlled-energy wheat straw and grass hay diets supplemented with starch or sugar on periparturient dairy cow performance and lipid metabolism. J. Dairy Sci. 96: 3050-3063.

Liu, X., L.J. Han, Z.L. Yang. 2011. Transfer of near infrared spectrometric models for silage crude protein detection between different instruments. J. Dairy Sci. 94 :5599–5610.

Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J.Dairy Sci. 85:1165–1175.

Magalhaes V. J. A., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. P. Santos. 2008. Effect of Feeding Yeast Culture on Performance, Health, and Immunocompetence of Dairy Calves. J. Dairy Sci. 91:1497–1509.

Martin S. A., H. M. Sullivan, and J. D. Evans. 2000. Effects of sugars and malate on ruminal microorganisms. J. Dairy Sci. 83:2574-2579.

Martin, C., E. Devillard, and B. Michalet-Doreau. 1999. Influence of sampling site on concentrations and carbohydrate-degrading enzyme activities of protozoa and bacteria in the rumen. J. Animal. Sci. 77:979–987.

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

McCarthy, R. D., Jr., T. H. Klusmeyer, J. L. Vicini, J. H. Clark, and D. R. Nelson. 1989. Effects of source of protein and carbo- hydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72: 2002–2016.

Meikle A, M .Kulcsar, Y.Chilliard, H. Febel, C. Delavaud, D. Cavestany. 2004. Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction. 127: 727–37.

Meissner, H. H.; D.V. Paulsmeier, K.J. Leeuw, and C.M. Coetzer. 1996. Ruminal and post-ruminal digestion of dietary protein and starch in steers: 2 multivariate model prediction of non-ammonia nitrogen and starch passage and digestibility. South African J. of Ani. Sci. 26: 66 – 74.

Mertens, D. R. 2002. Measuring fiber and its effectiveness in ruminant diets. Page 40-66 in Proc. Plains Nutr. Cncl. Spring Conf. San Antonio, TX.

Mertens, D.R. 1989. Fiber analysis and its use in ration formulation. Proc. 24th Pacific NW Anim. Nutr. Conf. (R.G. Bull, B.J. Hawk and K.K. Dickinson, eds.). p. 1-10.

Mertens, D.R. 1993. Importance of the detergent system of feed analyses for improving animal nutrition. Proc. Cornell Nutr. Conf. p. 25-36.

Mertens, D.R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci 80:1463–1481.

Metchnikoff, E. and P. Chalmers .1910. The Prolongation of Life: Optimistic Studies. G. P. Putman's Sons, New York.

Minor, D. J., S. L. Trower, B. D. Strang, R. D. Shaver, and R. R. Grummer. 1997. Effects of nonfiber carbohydrate and niacin on periparturient metabolic status and lactation of dairy cows. J Dairy Sci 81:189 - 200.

Mirzaei Alamouti H. R, H. Amanlou1, K. Rezayazdi and A. Towhidi. 2009. Effects of Prepartum Dietary Carbohydrate Source on Metabolism and Performance of Primiparous Holstein Cows during the Periparturient Period. Asian-Aust. J. Anim. Sci. 22(11): 1513 – 1520.

Moe, P. W., and H. F. Tyrrell. 1972. Metabolizable energy requirements of pregnant dairy cows. J. Dairy Sci. 55:480 – 483.

Morgavi, D. P., C. J. Newbold, D. E. Beever, and R. J. Wallace. 2000. Stability and stabilization of potential feed additive enzymes in rumen fluid. Enz. Microb. Technol. 26:171–177

Mosier, N. et al. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Biores. Tech. 96, 673–686.

Mullins C. R., L. K.Mamedova, A. J. Carpenter, Y.Ying, M. S. Allen, I. Yoon, and B. J. Bradford. 2013. Analysis of rumen microbial populations in lactating dairy cattle fed diets varying in carbohydrate profiles and Saccharomyces cerevisiae fermentation product. J. Dairy Sci. 96: 5872 – 5881.

Nafikov R. A. and D.C. Beitz. 2007. Carbohydrate and Lipid Metabolism in Farm Animals. J. Nutr. 137: 702–705.

Nagaraja, T. G., and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. J. Dairy Sci. 90(E. Suppl.):E17–E38

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

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.

Nocek, J. E., W. P. Kautz, J. A. Z. Leedle, and J. G. Allman. 2002. Ruminal supplementation of direct-fed microbials on diurnal ph variation and in situ digestion in dairy cattle. J. Dairy Sci. 85:429 - 433.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington DC.

O'Hara, A. M., and F. Shanahan. 2006. The gut flora as a forgotten organ. EMBO Rep. 7:688–693.

Oba, M., and M.S. Allen. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82:589-596.

Oelker, E. R., C. Reveneau, and J. L. Firkins. 2009. Interaction of molasses and monensin in alfalfa hay-or corn silage-based diets on rumen fermentation, total tract digestibility, and milk production by Holstein cows. J. Dairy Sci. 92:270-285.

Oetzel G. and McGuirk S. 2007. Fact sheet – Cowside blood BHBA testing with hand-held "ketometer". Univeristy of Wisconsin-Madison.

Overton, T. R., and M. R. Waldron. 2004. Nutritional management of transition dairy cows: Strategies to optimize metabolic health. J. Dairy Sci. 87:105 - 119

Paloheimo, M., J. Piironen, and J. Vehmaanperä. 2011. Xylanases and cellulases as feed additives. In: M. R. Bedford and G. G. Partridge, editors, Enzymes in farm animal nutrition. 2nd ed. CABI (CAB International), London, UK. p. 12–53.

Peng,H., J. Q. Wang, H. Y. Kang, S. H. Dong, P. Sun, D. P. Bu and L. Y. Zhou. 2012. Effect of feeding *Bacillus subtilis natto* fermentation product on milk production and composition, blood metabolites and rumen fermentation in early lactation dairy cows. Journal of Animal Physiology and Animal Nutrition 96: 506 - 512.

Penner, G. B. and M. Oba. 2009. Increasing dietary sugar concentration may improve dry matter intake, ruminal fermentation, and productivity of dairy cows in the postpartum phase of the transition period. J. Dairy Sci. 92:3341-3353.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Sub-acute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176:21–31.

Poorna, C. A., P. Prema. 2006. Production of cellulase-free endoxylanase from novel alkalophilic thermotolerent *Bacillus pumilus* by solid-state fermentation and its application in wastepaper recycling. Bioresource Technology 98: 485–490.

Popper Z.A, G. Michel, C. Herve, D.S Domozych, W.G Willats. 2011. Evolution and diversity of plant cell walls: from algae to flowering plants. Annu. Rev. Plant Biol. 62:567–90.

Prieto M. L., Laurie O'Sullivan, Shiau Pin Tan, Peter McLoughlin , Helen Hughes, Montserrat Gutierrez Jonathan A. Lane , Rita M. Hickey, Peadar G. Lawlor and Gillian E. Gardiner. 2014. In Vitro Assessment of Marine *Bacillus* for Use as Livestock Probiotics. Mar. Drugs 12 (5), 2422 – 2445.

Prieto, M.L.; O' Sullivan, L., Tan, S.P., McLoughlin, P., Hughes, H.; O'Connor, P.M, Cotter, P.D., Lawlor, P.G., Gardiner, G.E. 2012. Assessment of the bacteriocinogenic potential of marine bacteria reveals lichenicidin production by seaweed-derived Bacillus spp.Mar. Drugs.10:2280 – 2299.

Rabelo, E., R. L. Rezende, S.J Bertics, and R. R. Grummer. 2003. Effects of transition diets varying in energy density on lactation performance and ruminal parameters of dairy cows. J. Dairy. Sci. 86: 916 - 925.

Ramirez, H. A. R., K. Nestor, L. O. Tedeschi, T. R. Callaway, S. E. Dowd, S. C. Fernando, and P. J. Kononoff. 2012. The effect of brown midrib corn silage and dried distillers' grains with solubles on milk production, nitrogen utilization and microbial community structure in dairy cows. Can. J. Anim. Sci. 92:365–380.

Ramsing E. M., PAS, J. A. Davidson , P. D. French , PAS, I. Yoon ,PAS, M. Keller, and H. Peters-Fleckenstein. 2009. Effects of yeast culture on peripartum intake and milk production of primiparous and multiparous holstein cows. The Professional Animal Scientist 25: 487–495.

Russel, J. B. and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci 79:1503-1509.

Samanya, M; Yamauchi, K, 2002: Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. *natto*. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 133, 95–104.

Santos J.E, DePeters EJ, Jardon PW, Huber JT. 2001. Effect of prepartum dietary protein level on performance of primigravid and multiparous Holstein dairy cows. J Dairy Sci 84:213–24.

Schingoethe, D. J., F. M. Byers, and G. T. Schelling. 1993. Nutrient needs during critical periods of the life cycle. Pages 421–445 in The Ruminant Animal Digestive Physiology and Nutrition. D. C. Church, ed. Wareland Press, Inc., Prospect Heights, IL.

Shen, Y. B., X. S. Piao, S. W. Kim, L. Wang, P. Liu, I. Yoon, and Y. G. Zhen. 2009. Effect of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J. Anim. Sci. 87:2614–2624.

Spurlock, D. M., J. C. M. Dekkers, R. Fernando, D. A. Koltes, and A. Wolc. 2012. Genetic parameters for energy balance, feed efficiency, and related traits in Holstein cattle. J. Dairy Sci. 95:5393 - 5402.

Staples C. R., Feeding dairy cows when corn prices are high. 2007. Pages 7 – 21. Proceedings 44th Florida Dairy Production Conf., Gainesville, FL.

Sticklen, M.B. 2008. Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol. Nature Reviews Genetics 9, 433–443.
Sullivan, H. M., and S. A. Martin. 1999. Effects of a *Saccharomyces cerevisiae* culture on in vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 82:2011–2016.
Sun P, J. Q Wang, L.F. Deng. 2013. Effects of *Bacillus subtilis natto* on milk production, rumen fermentation and ruminal microbiome of dairy cows. Animal. 7(2):216-222.
Sunna, A. and Antranikian, G. 1997. Xylanolytic enzymes from fungi and bacteria. Critical Reviews in Biotechnology 17, 39–67.
Tafaj, M., B. Junck, A. Maulbetsch, H. Steingass, H. P. Piepho and W. Drochner. 2004. Digesta characteristics of dorsal, middle and ventral rumen of cows fed with different hay qualities and concentrates levels. Arch. Anim. Nutr. 58:325-342.
Tas, B.M., Taweel, H.Z., Smit, H.J., Elgersma, A., Dijkstra, J. & Tamminga, S. 2005. Effects of perennial ryegrass cultivars on intake, digestibility, and milk yield in dairy cows. J. Dairy Sci. 88: 3240–3248.
Undersander, D., Mertens, D.R. and Thiex, N. 1993. Forage Analyses Procedures. National Forage Testing Assoc., Omaha, NE. 154 pp.
van Knegsel, A.T.M, van den Brand, H., Dijkstra, J., Tamminga, S., Kemp, B. 2005. Effects of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reproduction, nutrition, development 45: 665 – 688.
Van Soest, P. J., and R. H. Wine. 1967. The use of detergents in analysis of fibrous feeds: IV. Determination of plant cellwall constituents. J. AOAC 50:50.
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. 74:3583 - 3597.
Van Vuuren , A. M., Hindle, V. A., Klop A., and Cone, J. W. 2010. Effect of maize starch concentration in the diet on starch and cell wall digestion in the dairy cow. Journal of Animal Physiology and Animal Nutrition. 94: 319 - 329.
Vicini, J. L., H. G. Bateman, M. K. Bhat, J. H. Clark, R. A. Erdman,R. H. Phipps, M. E. Van Amburgh, G. F. Hartnell, R. L. Hintz,and D. L. Hard. 2003. Effect of feeding supplemental fibrolytic enzymes or soluble sugars with malic acid on milk production. J Dairy Sci. 86(2):576-85.
Voelker Linton, J.A. and M.S. Allen. 2007. Nutrient demand affects ruminal digestion responses to a change in dietary forage concentration. J. Dairy Sci. 90:4770-4779.
Weidner, S. J., and R. J. Grant. 1994. Altered ruminal mat consistency by high percentages of soyabean hulls to lactating cows. J. Dairy Sci. 77:522–532.
Woodward, J. 1984. Xylanases: functions, properties and applications. Topics in Enzyme and
Fermentation Biotechnology 8, 9–30.
Wong, K. K.Y, L.U.L, Tan, J.N., Saddler. 1988. Multiplicity of β-1,4 xylanase in microorganisms: functions and applications. Microbiol Rev 52:305 - 17.
Yang, W. Z., and K. A. Beauchemin. 2006. Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. J. Dairy Sci. 89:217–228.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. J. Dairy Sci. 83:2512–2520.
Yoon, I. K., and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants:A review. Asian-australas. J. Anim. Sci. 8:533–555
Zaworski, E. M., C. M. Shriver-Munsch, N. A. Fadden,W. K. Sanchez,I.Yoon, and G.Bobe. 2014. Effects of feeding various dosages of Saccharomyces cerevisiae fermentation product in transition dairy cows. J Dairy Sci. 97(5):3081-98.
Zebeli, Q., and Metzler-Zebeli, B.U. 2012. Interplay between rumen digestive disorders and diet induced inflammation in dairy cattle. Research in Veterinary Science. 93: 1099 - 1108.
Zebeli, Q., J. Dijkstra, M. Tafaj, H. Steingass, B. N. Ametaj, and W. Drochner. 2008. Modeling dietary fiber adequacy in dairy cows based on responses of ruminal pH and milk fat production to diet composition. J. Dairy Sci. 91:2046–2066.
Zinn, R. A., and J. Salinas. 1999. Influence of fibrozyme on digestive function and growth performance of feedlot steers fed a 78% concentrate growing diet. Pages 313–319 in Proc. Alltech's Fifteenth Annual Symposium, Nottingham University Press, Loughborough, UK.

Table 1. Ingredient and nutrient composition of multiparous cow far-off diets, low or high starch close-up diets, and low or high starch postpartum diets with or without $5x10^9$ CFU/head/day of *Bacillus pumilus* 8G-134 topdressing

	Diets[1]				
	Prepartum			Postpartum	
Item	Far off	LS	HS	LS	HS
Ingredient, % of DM					
Wheat straw, chopped	13.3	16.7	16.7	0.0	0.0
Grass hay, chopped	15.3	18.3	18.3	0.0	0.0
Alfalfa hay, chopped	15.0	7.0	7.0	17.0	17.0
Corn silage	38.2	30.0	14.1	50.2	32.8
Corn gluten pellets	5.7	8.3	8.3	10.8	10.8
Dry corn (ground)	0.8	0.0	16.3	3.6	21.0
Dry cow protein mix [2]	11.7	11.7	11.7	0.0	0.0
Biochlor[3]	0.0	8.0	7.6	0.0	0.0
Lactation protein mix [4]	0.0	0.0	0.0	16.4	16.4
Molasses-based LF[5]	0.0	0.0	0.0	2.0	2.0
Nutrient, % of DM					
DM	49.3	51.6	52.9	47.7	50.8
Forage	81.8	72.0	56.0	67.2	49.8
CP	14.9	17.7	17.6	17.6	17.7
NEL, Mcal/kg	1.3	1.3	1.4	1.5	1.6
ADF	27.9	27.8	24.1	20.4	16.5
NDF	41.2	41.8	36.4	31.6	25.8
Lignin	3.9	3.9	3.3	4.3	3.4
NFC	32.5	29.0	35.0	39.5	45.8
Sugar	4.2	4.2	4.5	4.7	5.1
Starch	13.9	12.1	18.7	20.1	27.1
Total ether extract	2.9	2.9	3.0	3.0	3.1
Ash	8.5	8.6	8.0	8.3	7.6
DCAD, mEq/100g	18.7	-12.0	-12.5	27.6	25.6

[1]Diets fed from d 42 prepartum through d 112 postpartum; Far off diet fed from d 42 to 22 prepartum; Low (LS) and high starch (HS) prepartum diet fed from d 21 prepartum to calving; Low (LS) and high starch (HS) postpartum diet fed from d 1 to 112 with and without topdressing $5x10^9$ CFU/head/day of *Bacillus pumilus* 8G-134.

[2]Dry cow protein mix = 43.0% CP, 13.14% ash, 11.25% sugar, 3.88% fat.

[3]Biochlor = BioChlor (Church & Dwight Co. Inc., Princeton, NJ).

[4]Lactation protein mix = 40.7% CP, 14.65% ash, 9.34% sugar, 3.57% fat.

[5]Molasses-based LF = Quality Liquid Feeds, Dodgeville, WI; 61.13% DM, 39.83% sugar, 34.68% ash, 5.9% Ca, 3.78% K.

Table 2. Least squares means of prepartum (wk - 3 through calving) dry matter intake, energy balance, body weight, and body condition score of multiparous cows

	Treatments[1]			*P*-value
Variables	LS	HS	SEM	S[2]
DMI,[3] kg/d	11.88	12.82	0.87	0.32
DMI,[3] % of BW	1.71	1.81	0.12	0.52
EB,[3,4] Mcal/d	1.14	3.62	1.17	0.11
EB,[3,5] % Req	108.42	125.02	8.05	0.12
BW,[6] Kg	712.63	730.82	15.16	0.36
BCS[6,7]	3.39	3.41	0.09	0.86

[1]Low starch prepartum (LS – 12% starch) and high starch prepartum (HS – 19% starch) diet fed from d preprepartum to calving.
[2]Starch effect.
[3]Measured daily from d - 21 through 0 relative to calving.
[4]Energy intake – energy requirements.
[5]Energy intake/energy requirements (Req.) × 100. Prepartum Req.= NE_M + NE_P, where NE_M = net energy for maintenance, and NE_P = net energy requirements for pregnancy.
[6]Measured weekly from d − 21 through 0 relative to calving.
[7]BCS = 1–5 scale, 0.25 unit increments; 1=extremely thin, 5=extremely fat.

Table 3. Least squares means of prepartum (wk - 3 through calving) serum metabolites for multiparous cows

	Treatments[1]			*P*-value
Variables	LS	HS	SEM	S[2]
NEFA,[3] µEq/l	155.13	207.14	28.48	0.19
BHBA,[3] mg/dl	5.51	6.77	0.78	0.11
Glucose,[3] mg/dl	85.18	83.03	3.2	0.34
Haptoglobin,[3] OD x100	5.85	5.85	0.07	0.97
Calcium,[4] mg/dl	10.63	11.26	0.39	0.22

[1]Low starch prepartum (LS – 12% starch) and high starch prepartum (HS – 19% starch) diet fed from d 21 prepartum to calving.
[2]Starch effect.
[3]Blood collected on d -21, -14, and -7 relative to calving.
[4]Blood collected on d -7 relative to calving.

Table 4. Least squares means of prepartum (wk - 2 through calving) rumen fluid and apparent total-tract digestibility parameters of multiparous cows

	Treatments[1]			*P*-value
Variables	LS	HS	SEM	S[1]
Rumen fluid[2]				
pH	7.60	7.59	0.14	0.97
Ammonia, g/dl	12.63	12.75	0.99	0.93
Volatile fatty acids, %				
Acetate	72.72	70.10	1.03	0.06
Propionate	16.37	17.56	0.90	0.32
Butyrate	10.91	12.36	0.44	0.02
A:P	4.34	4.17	0.52	0.52
Apparent total-tract[3]				
DMI, kg/d week - 2	11.89	13.14	0.83	0.28
NDF intake, kg/d week - 2	4.41	4.86	0.41	0.43
NDFd, %	45.68	44.78	2.30	0.77
DMd, %	58.67	54.32	1.74	0.07
NDFd, kg/d	2.03	2.18	0.21	0.60
DMd, kg/d	6.20	7.05	0.61	0.31

[1]Starch effect.
[2]Rumen fluid collected via oral-esophageal tube technique at 0800 h on d - 14 relative to calving.
[3]Fecal grab samples collected at 0800 h and 1600 h on d - 14 relative to calving. Samples analyzed using acid insoluble ash as an internal maker.

Table 5. Least squares means of early lactation (wk 1 and 2) and mid-lactation (wk 12) rumen fluid and apparent total-tract digestibility parameters for multiparous cows

	Treatments[1]							
	LS		HS			*P*-value		
Variables	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
Early lactation								
Rumen fluid[5]								
pH	7.00	6.99	6.95	7.35	0.15	0.27	0.16	0.14
Ammonia, mg/dl	9.14	9.19	8.45	9.80	0.90	0.98	0.37	0.42
Volatile fatty acids, %								
Acetate	61.54	61.37	57.58	58.41	1.80	0.04	0.84	0.76
Propionate	25.25	25.31	28.16	25.98	1.30	0.13	0.36	0.34
Butyrate	13.45	13.44	14.31	13.51	0.49	0.30	0.37	0.37
A:P	2.57	2.51	2.13	2.34	0.19	0.08	0.65	0.44
Apparent total-tract[6]								
DMI, kg/d wk 1,2	15.99	16.83	15.88	18.30	1.14	0.54	0.15	0.48
NDF intake, kg/d (wk 1,2)	4.99	5.04	4.13	4.67	0.38	0.09	0.40	0.50
NDFd, %	45.65	47.31	52.99	53.39	4.71	0.14	0.82	0.89
DMd, %	66.23	67.75	70.39	68.65	2.91	0.36	0.97	0.55
NDFd, kg/d	2.32	2.39	2.18	2.51	0.33	0.98	0.51	0.67
DMd, kg/d	10.53	10.81	11.18	12.37	1.08	0.28	0.46	0.65
Mid-lactation								
Rumen fluid[7]								
pH	6.92	7.09	6.94	6.89	0.14	0.46	0.60	0.37
Ammonia, mg/dl	11.41	11.01	10.80	9.78	0.89	0.28	0.39	0.71
Volatile fatty acids, %								
Acetate (A)	64.00	62.28	60.94	62.40	1.06	0.18	0.90	0.15
Propionate (P)	23.90	25.24	27.95	25.32	1.36	0.12	0.63	0.13
Butyrate	12.10	12.46	11.11	12.28	0.48	0.23	0.12	0.41
A:P	2.74	2.62	2.20	2.56	0.19	0.11	0.54	0.20
Apparent total-tract[8]								
DMI, kg/d wk 12	26.79	23.37	27.23	26.25	1.22	0.16	0.06	0.29
NDF intake, kg/d (wk 12)	9.29	8.23	7.47	6.60	1.56	0.35	0.55	0.95
NDFd, %	52.26	54.08	43.51	44.83	7.18	0.26	0.84	0.98
DMd, %	71.01	71.72	63.64	61.40	5.21	0.07	0.87	0.75
NDFd, kg/d	5.21	4.67	4.71	3.03	2.17	0.63	0.62	0.78
DMd, kg/d	19.90	15.98	18.06	15.67	1.66	0.47	0.05	0.61

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (HSBP) Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving $5x10^9$CFU/head/day of *Bacillus pumilus* 8G-134 (BP).

[2]Starch effect.

[3]*Bacillus pumilus* 8G-134 effect.

[4]Interaction of starch and *Bacillus pumilus* 8G-134.

[5]Rumen fluid collected via oral-esophageal tube technique at 0800 h on d 7 and 14 after calving.

[6]Fecal grab samples collected at 0800 h and 1600 h on d 7 and 14 after calving. Samples analyzed using acid insoluble ash as an internal maker

[7]Rumen fluid collected via oral-esophageal tube technique at 0800 h on d 84 after calving.

[8]Fecal grab samples collected at 0800 h and 1600 h on d 84 after calving. Samples analyzed using acid insoluble ash as an internal maker.

Table 6. Least squares means of early lactation (1 wk through wk 4) serum metabolites for multiparous cows

	Treatments[1]							
	LS		HS			*P*-value		
Variables	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
NEFA,[5] µEq/L	363.95	311.66	475.85	314.84	68.80	0.29	0.05	0.33
BHBA,[5] mg/dl	12.76	10.27	14.52	8.02	2.57	0.92	0.07	0.41
Glucose,[5] mg/dl	73.52	76.05	75.82	76.39	2.37	0.56	0.49	0.66
Haptoglobin,[5] OD x100	6.83	7.13	6.93	6.39	0.30	0.22	0.66	0.09
Calcium,[6] mg/dl	10.15	10.12	10.04	9.73	0.25	0.31	0.47	0.57

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving $5x10^9$CFU/head/day of *Bacillus pumilus* 8G-134 (BP).

[2]Starch effect.

[3]*Bacillus pumilus* 8G-134 effect.

[4]Interaction of starch and *Bacillus pumilus* 8G-134.

[5]Blood collected on d 7, 14, 21 and 28 after calving.

[6]Blood collected within 24 h after calving.

Table 7. Least squares means of postpartum early lactation (1 wk through wk 4) dry matter intake, energy balance, body weight, body condition score, body weight loss, body condition score loss, milk yield, and milk components of multiparous cow

	Treatments[1]							
	LS		HS			*P*-value		
Variables	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
DMI,[5] kg/d	18.34	18.68	17.18	19.86	0.80	0.16	0.25	0.98
DMI,[5] % of BW	2.91	2.86	3.24	3.47	0.09	0.45	0.29	0.33
EB,[5,6] Mcal/d	-10.66	-10.18	-9.29	-7.52	1.83	0.25	0.51	0.71
EB,[5,7] % Req	71.13	74.63	75.50	82.47	5.05	0.21	0.27	0.72
BW,[6] Kg	633.00	645.42	637.88	627.72	19.53	0.73	0.95	0.54
BCS[6]	2.84	3.03	2.95	3.01	0.12	0.68	0.28	0.55
BW change,[6,7] kg	-20.09	-35.31	-53.37	-30.33	-14.15	0.29	0.77	0.16
BCS change [7,8]	-0.01	-0.20	-0.38	-0.15	-0.10	0.09	0.94	0.02
Milk,[6] kg/d	37.25	37.71	38.26	40.57	1.81	0.26	0.42	0.59
3.5% FCM,[6,9] kg/d	39.98	39.87	40.72	42.25	1.78	0.35	0.67	0.62
3.5% ECM,[5,10] kg/d	39.59	39.51	40.11	42.01	1.80	0.37	0.59	0.56
Milk fat,[6] %	4.01	3.81	3.97	3.77	0.17	0.83	0.24	0.99
Milk fat,[6] kg/d	1.47	1.45	1.49	1.52	0.08	0.56	0.94	0.72
Milk protein,[6] %	3.36	3.33	3.23	3.38	0.08	0.66	0.42	0.27
Milk protein,[6] kg/d	1.22	1.22	1.21	1.32	0.05	0.38	0.30	0.32
Milk lactose,[6] %	4.83	4.81	4.73	4.84	0.07	0.62	0.46	0.36
Milk lactose,[6] kg/d	1.80	1.82	1.82	1.97	0.09	0.35	0.33	0.46
MUN,[6] mg/dl	15.76	14.54	14.25	13.05	0.61	0.01	0.04	0.96
Linear SCC,[6] x1000 cell/ml	1.08	1.20	1.74	1.02	0.30	0.38	0.29	0.15
F:P ratio[6,9]	1.23	1.18	1.23	1.17	0.06	0.91	0.37	0.88

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving $5x10^9$CFU/head/day of *Bacillus pumilus* 8G-134 (BP).

[2]Starch effect.

[3]*Bacillus pumilus* 8G-134 effect.

[4]Interaction of starch and *Bacillus pumilus* 8G-134.

[5]Measured daily from d 1 through 28 relative to calving.

[6]Measured weekly from d 0 to 28 relative to calving.

[7]Week 4 minus week 1.

[8]BCS = 1–5 scale, 0.25 unit increments.

[9]3.5% FCM = 0.4324 × (kg of milk) + 16.2162 × (kg of fat).

[10]3.5% ECM = (12.82 x kg fat) + (7.13 x kg protein) + (0.323 x kg of milk).

[11]Milk fat divided by milk protein.

Table 8. Least squares means of entire lactation trial period (1 wk through wk 16) of dry matter intake, 3.5 % fat corrected milk, feed efficiency, milk yield and components for multiparous cows

	Treatments[1]							
	LS		HS			*P*-value		
Variables	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
DMI,[5] kg/d	24.22	22.11	23.71	23.88	1.28	0.86	0.57	0.08
DMI,[5] % of BW	3.85	3.53	3.76	3.85	0.14	0.39	0.40	0.14
EB,[5,6] Mcal/d	- 4.80	- 6.39	- 3.01	- 2.47	1.43	0.04	0.69	0.43
EB,[5,7] % Req	88.08	84.21	92.66	95.08	3.60	0.03	0.83	0.36
BW,[6] Kg	633.73	630.19	631.82	625.52	20.88	0.87	0.76	0.92
BCS[6]	2.78	2.97	2.79	2.99	0.12	0.87	0.08	0.98
BW change,[7] kg	-4.01	-49.20	-34.87	-12.54	-20.40	0.96	0.44	0.06
BCS change [7]	-0.07	-0.20	-0.24	-0.10	-0.16	0.96	0.81	0.06
Milk,[6] kg/d	43.38	42.45	45.86	45.14	2.08	0.11	0.61	0.95
3.5% FCM,[6,8] kg/d	43.49	42.03	43.73	44.39	2.50	0.44	0.81	0.52
Milk fat,[6] %	3.66	3.55	3.35	3.53	0.26	0.18	0.79	0.23
Milk fat,[6] kg/d	1.56	1.50	1.51	1.56	0.12	0.99	0.95	0.39
Milk protein,[6]%	3.13	3.00	3.05	3.10	0.07	0.89	0.54	0.15
Milk protein,[6] kg/d	1.34	1.26	1.39	1.38	0.06	0.05	0.28	0.01
Milk lactose,[6] %	4.87	4.83	4.83	4.82	0.05	0.56	0.65	0.83
Milk lactose,[6] kg/d	2.11	2.06	2.22	2.18	0.08	0.13	0.53	0.92
MUN,[6] mg/dl	17.08	16.36	15.52	15.05	0.87	0.01	0.14	0.75
Linear SCC,[6] x 1000 cell/ml	1.85	1.51	2.00	1.59	0.34	0.72	0.23	0.91
Feed efficiency [9]	1.83	1.97	1.85	1.86	0.12	0.97	0.78	0.17

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving $5x10^9$CFU/head/day of *Bacillus pumilus* 8G-134 (BP).

[2]Starch effect.

[3]*Bacillus pumilus* 8G-134 effect.

[4] Interaction of starch and *Bacillus pumilus* 8G-134.

[5]Measured daily from d 1 through d 112 postpartum.

[6]Measured weekly from d 1 through d 112 postpartum.

[7]Week 16 minus week.

[8]3.5% FCM = 0.4324 × (kg of milk) + 16.2162 × (kg of fat).

[9]3.5% FCM divided by DMI.

Table 9. Health events recorded d −42 prepartum through d 112 postpartum for multiparous cows

	Treatments[1]			
	LS		HS	
Item	CO	BP	CO	BP
Twins	1	1	2	1
Dystocia[2]	2	0	2	0
Retained placenta[3]	2	0	3	1
Metritis[4]	5	2	7	2
Ketosis[5]	0	2	9	0
Displaced abomasum	0	0	1	1

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* 8G-134 postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving $5x10^9$CFU/head/day of *Bacillus pumilus* 8G-134 (BP).

[2]Dystocia was scored on a 1 to 5 scale (1 = easy, 5 = mechanically assisted pull); score of 4 or higher recorded.

[3]Retained fetal membranes for more than 24 h.

[4]Presence of fever (>39.5 ^{0}C) and red/brown watery fetid vaginal discharge.

[5]Serum BHBA levels above 29mg/dl between calving to 28 DIM.

Table 10. Ingredient and nutrient composition of far-off diets, low or high starch close-up diets, and low or high starch postpartum diets fed with or without 5 x 10^9 CFU/head/day of *Bacillus pumilus* topdressing to primiparous cows

	Diets[1]				
	Prepartum			Postpartum	
Item	Far off	LS	HS	LS	HS
Ingredient, % of DM					
Wheat straw, chopped	13.3	16.7	16.7	0.0	0.0
Grass hay, chopped	15.3	18.3	18.3	0.0	0.0
Alfalfa hay, chopped	15.0	7.0	7.0	17.0	17.0
Corn silage	38.2	30.0	14.1	50.2	32.8
Corn gluten pellets	5.7	8.3	8.3	10.8	10.8
Dry corn (ground)	0.8	0.0	16.3	3.6	21.0
Dry cow protein mix [2]	11.7	11.7	11.7	0.0	0.0
Biochlor[3]	0.0	8.0	7.6	0.0	0.0
Lactation protein mix [4]	0.0	0.0	0.0	16.4	16.4
Molasses-based LF[5]	0.0	0.0	0.0	2.0	2.0
Nutrient, % of DM					
DM	49.3	51.6	52.9	47.7	50.8
Forage	81.8	72.0	56.0	67.2	49.8
CP	14.9	17.7	17.6	17.6	17.7
NEL, Mcal/kg	1.3	1.3	1.4	1.5	1.6
ADF	27.9	27.8	24.1	20.4	16.5
NDF	41.2	41.8	36.4	31.6	25.8
Lignin	3.9	3.9	3.3	4.3	3.4
NFC	32.5	29.0	35.0	39.5	45.8
Sugar	4.2	4.2	4.5	4.7	5.1
Starch	13.9	12.1	18.7	20.1	27.1
Total ether extract	2.9	2.9	3.0	3.0	3.1
Ash	8.5	8.6	8.0	8.3	7.6
DCAD, mEq/100g	18.7	-12.0	-12.5	27.6	25.6

[1]Diets fed from d 42 prepartum through d 112 postpartum; Far off diet fed from d 42 to 22 prepartum; Low (LS) and high starch (HS) prepartum diet fed from d 21 prepartum to calving; Low (LS) and high starch (HS) postpartum diet fed from d1to 112 with and without topdressing 5^9 CFU/head/day of *Bacillus pumilus*.

[2]Dry cow protein mix = 43.0% CP, 13.14% ash, 11.25% sugar, 3.88% fat.

[3]Biochlor = BioChlor (Church & Dwight Co. Inc., Princeton, NJ).

[4]Lactation protein mix = 40.7% CP, 14.65% ash, 9.34% sugar, 3.57% fat.

[5]Molasses-based LF = Quality Liquid Feeds, Dodgeville, WI; 61.13% DM, 39.83% sugar, 34.68% ash, 5.9% Ca, 3.78% K.

Table 11. Least squares means of prepartum (wk - 3 through calving) dry matter intake, energy balance, body weight, and body condition score of primiparous cows

	Treatments[1]			*P*-value
Item	LS	HS	SEM	S[2]
DMI,[3] kg/d	11.59	11.54	0.55	0.93
DMI,[3] % of BW	1.81	1.83	0.07	0.80
EB,[3,4] Mcal/d	-1.26	-0.29	0.62	0.26
EB,[3,5] % Req	92.40	98.49	3.82	0.25
BW,[6] Kg	636.39	618.09	12.48	0.29
BCS[6,7]	3.23	3.25	0.04	0.73

[1]Low starch prepartum (LS – 12% starch) and high starch prepartum (HS – 19% starch) diet fed from d 21 prepartum to calving.
[2]Starch effect.
[3]Measured daily from d - 21 through 0 relative to calving.
[4]Energy intake – energy requirements.
[5]Energy intake/energy requirements (Req.) × 100. Prepartum Req.= $NE_M + NE_P$, where NE_M = net energy for maintenance, and NE_P = net energy requirements for pregnancy.
[6]Measured weekly from d − 21 through 0 relative to calving.
[7]BCS = 1–5 scale, 0.25 unit increments.

Table 12. Least squares means of prepartum (wk - 3 through calving) serum metabolites from primiparous cows

	Treatments[1]			*P*-value
Variables	LS	HS	SEM	S[2]
NEFA,[3] µEq/l	160.52	168.11	30.7	0.79
BHBA,[3] mg/dl	5.39	6.12	1.81	0.78
Glucose,[3] mg/dl	87.75	84.04	1.73	0.16
Haptoglobin,[3] OD x100	6.15	6.25	0.21	0.73
Calcium,[4] mg/dl	10.63	11.26	0.37	0.22

[1]Low starch prepartum (LS – 12% starch) and high starch prepartum (HS – 19% starch) diet fed from d 21 prepartum to calving.
[2]Starch effect.
[3]Blood collected on d -21, -14, and -7 relative to calving.
[4]Blood collected on d -7 relative to calving.

Table 13. Least squares means of prepartum (wk - 2 through calving) rumen fluid and apparent total-tract digestibility parameters of primiparous cows

	Treatments[1]			*P*-value
Variables	LS	HS	SEM	S[1]
Rumen fluid[2]				
pH	7.80	7.73	0.08	0.55
Ammonia, g/dl	10.85	12.01	0.85	0.34
Volatile fatty acids, %				
Acetate	72.18	71.21	0.51	0.19
Propionate	16.59	16.40	0.37	0.72
Butyrate	11.23	12.40	0.26	0.01
A:P	4.37	4.41	0.12	0.83
Apparent total-tract[3]				
DMI, kg/d week - 2	11.14	11.03	0.49	0.87
NDF intake, kg/d week - 2	4.95	4.49	0.23	0.18
NDFd, %	43.52	44.88	2.90	0.73
DMd, %	52.75	55.59	3.30	0.53
NDFd, kg/d	2.18	2.06	0.20	0.66
DMd, kg/d	5.74	6.20	0.41	0.47

[1]Starch effect.
[2]Rumen fluid collected via oral-esophageal tube technique at 0800 h on d - 14 relative to calving.
[3]Fecal grab samples collected at 0800 h and 1600 h on d - 14 relative to calving. Samples analyzed using acid insoluble ash as an internal maker.

Table 14. Least squares means of early lactation (wk 1 and 2) and mid-lactation (wk 12), rumen fluid and apparent total-tract digestibility parameters from primiparous cows

	Treatments[1]							
	LS		HS			*P*-value		
Variables	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
Early lactation								
Rumen fluid[5]								
pH	7.07	7.23	7.18	7.20	0.14	0.77	0.56	0.67
Ammonia, mg/dl	10.23	10.99	9.06	7.96	0.75	0.01	0.81	0.21
Volatile fatty acids, %								
Acetate	60.23	58.52	63.88	59.93	1.51	0.05	0.18	0.32
Propionate	26.20	22.48	27.58	26.33		0.01	0.01	0.22
Butyrate	13.56	14.29	14.42	13.66	0.58	0.85	0.98	0.20
A:P	2.32	2.84	2.16	2.28	0.14	0.01	0.02	0.16
Apparent total-tract[6]								
DMI, kg/d wk 1,2	14.49	15.68	12.66	14.01	2.76	0.56	0.66	0.98
NDF intake, kg/d								
(wk 1,2)	4.89	5.26	4.14	4.80	0.94	0.55	0.60	0.88
NDFd, %	48.07	52.47	49.10	57.25	3.28	0.37	0.05	0.56
DMd, %	67.90	69.08	66.69	71.00	2.24	0.87	0.22	0.48
NDFd, kg/d	2.35	2.58	2.00	2.51	0.24	0.33	0.09	0.53
DMd, kg/d	9.77	10.20	8.01	9.11	0.57	0.02	0.19	0.55
Mid-lactation								
Rumen fluid[7]								
pH	7.14	7.24	7.01	7.11	0.10	0.23	0.38	0.99
Ammonia, mg/dl	11.13	10.67	11.69	12.02	0.84	0.26	0.94	0.65
Volatile fatty acids, %								
Acetate (A)	63.07	64.12	60.25	60.99	0.92	0.01	0.33	0.86
Propionate (P)	23.34	21.43	25.41	25.85	1.09	0.01	0.50	0.28
Butyrate	12.19	12.36	11.73	11.53	0.42	0.13	0.96	0.66
A:P	2.75	3.03	2.48	2.41	0.18	0.01	0.49	0.27
Apparent total-tract[8]								
DMI, kg/d wk 12	20.46	23.54	21.42	22.80	0.83	0.88	0.004	0.26
NDF intake, kg/d								
(wk 12)	6.72	7.76	7.17	7.48	0.32	0.74	0.17	0.01
NDFd, %	54.09	57.30	44.72	47.32	3.88	0.06	0.56	0.95
DMd, %	72.92	72.99	62.44	64.65	3.99	0.008	0.75	0.73
NDFd, kg/d	3.74	4.41	3.23	3.67	0.36	0.09	0.76	0.14
DMd, kg/d	14.91	17.08	13.30	14.74	0.88	0.007	0.33	0.02

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving 5^9CFU/head/day of *Bacillus pumilus* (BP).
[2]Starch effect.
[3]*Bacillus pumilus* effect.
[4]Interaction of starch and *Bacillus pumilus*.
[5] Rumen fluid collected via oral-esophageal tube technique at 0800 h on d 7 and 14 after calving.
[6]Fecal grab samples collected at 0800 h and 1600 h on d 7 and 14 after calving. Samples analyzed using acid insoluble ash as an internal maker
[7]Rumen fluid collected via oral-esophageal tube technique at 0800 h on d 84 after calving.
[8]Fecal grab samples collected at 0800 h and 1600 h on d 84 after calving. Samples analyzed using acid insoluble ash as an internal maker.

Table 15. Least squares means of early lactation (1 wk through wk 4) serum metabolites for primiparous cows

	Treatments[1]							
	LS		HS			*P*-value		
Variables	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
NEFA,[5] µEq/L	245.51	228.51	258.67	239.91	30.57	0.68	0.55	0.97
BHBA,[5] mg/dl	9.62	7.69	8.27	6.77	1.01	0.21	0.06	0.80
Glucose,[5] mg/dl	80.97	78.44	81.83	81.96	1.58	0.14	0.42	0.37
Haptoglobin,[5] OD x100	7.19	7.18	7.89	7.49	0.41	0.28	0.66	0.67
Calcium,[6] mg/dl	10.05	9.95	9.69	10.33	0.44	0.98	0.54	0.40

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving 5^9CFU/head/day of *Bacillus pumilus* (BP).
[2]Starch effect.
[3]*Bacillus pumilus* effect.
[4]Interaction of starch and *Bacillus pumilus*.
[5]Blood collected on d 7, 14, 21 and 28 after calving.
[6]Blood collected within 24 h after calving.

Table 16. Least squares means of postpartum early lactation (1 wk through wk 4) dry matter intake, energy balance, body weight, body condition score, body weight loss, body condition score loss, milk yield, and milk components of primiparous cows

	Treatments[1]					*P*-value		
	LS		HS					
Item	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
DMI,[5] kg/d	15.27	16.15	14.23	15.08	0.90	0.16	0.25	0.98
DMI,[5] % of BW	2.76	2.89	2.64	2.80	0.15	0.38	0.21	0.92
EB,[5,6] Mcal/d	-6.88	-6.11	-4.89	-6.09	1.04	0.32	0.83	0.34
EB, [5,7] % Req	77.11	79.49	82.43	79.83	3.40	0.40	0.97	0.46
BW,[8] Kg	550.62	558.10	537.34	539.07	18.72	0.32	0.78	0.86
BCS[8]	2.94	2.98	3.00	2.92	0.04	0.86	0.74	0.33
BW change,[6,7] kg	- 9.81	- 22.13	- 9.28	- 16.97	- 8.45	0.73	0.23	0.78
BCS change[7,8]	- 0.27	- 0.14	- 0.07	- 0.22	- 0.08	0.44	0.88	0.09
Milk,[5] kg/d	27.96	28.30	25.92	29.52	1.21	0.73	0.10	0.18
3.5% FCM,[5,9] kg/d	28.07	28.57	25.75	29.60	1.24	0.60	0.08	0.17
3.5% ECM,[5,10]kg/d	28.33	28.80	26.03	29.99	1.24	0.66	0.08	0.16
Milk fat,[6] %	3.46	3.61	3.45	3.59	0.15	0.88	0.28	0.99
Milk fat,[6]kg/d	0.99	1.02	0.91	1.05	0.05	0.62	0.12	0.23
Milk protein,[6] %	3.22	3.32	3.30	3.21	0.08	0.85	0.93	0.19
Milk protein,[6] kg/d	0.89	0.94	0.85	0.95	0.03	0.60	0.04	0.52
Milk lactose,[6] %	4.97	4.92	4.98	5.00	0.04	0.23	0.71	0.42
Milk lactose,[6] kg/d	1.41	1.41	1.31	1.50	0.06	0.98	0.13	0.15
MUN,[6] mg/dl	14.42	14.16	12.68	13.23	0.60	0.02	0.80	0.48
SCC,[6]	100.95	84.50	145.73	119.39	37.6	0.28	0.56	0.89
F:P ratio,[6,9]	1.12	1.09	1.07	1.12	0.05	0.83	0.86	0.35

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving 5^9CFU/head/day of *Bacillus pumilus* (BP).
[2]Starch effect.
[3]*Bacillus pumilus* effect.
[4]Interaction of starch and *Bacillus pumilus*.
[5]Measured daily from d 1 through 28 relative to calving.
[6]Measured weekly from d 0 to 28 relative to calving.
[7]Week 4 minus week 1.
[8]BCS = 1–5 scale, 0.25 unit increments.
[9]3.5% FCM = 0.4324 × (kg of milk) + 16.2162 × (kg of fat).
[10]3.5% ECM = (12.82 x kg fat) + (7.13 x kg protein) + (0.323 x kg of milk).

Table 17. Least squares means of entire lactation trial period (1 wk through wk 16) of dry matter intake, 3.5 % fat corrected milk, feed efficiency, milk yield and components for primiparous cows

	Treatments[1]							
	LS		HS			*P*-value		
Variables	CO	BP	CO	BP	SEM	S[2]	BP[3]	S*BP[4]
DMI,[5] kg/d	18.97	20.58	18.85	20.30	0.94	0.73	0.01	0.89
DMI,[5] % of BW	3.43	3.62	3.38	3.68	0.11	0.94	0.02	0.61
EB,[5,6] Mcal/d	- 3.67	- 2.60	- 1.30	- 0.80	0.91	0.01	0.35	0.74
EB,[5,7] % Req	88.54	91.84	95.89	97.19	2.73	0.02	0.35	0.68
BW,[6] Kg	557.58	570.90	558.87	554.24	18.20	0.64	0.79	0.58
BCS[6]	2.92	3.02	3.10	2.91	0.08	0.67	0.58	0.08
BW change,[7] kg	- 27.46	- 17.93	- 46.60	- 31.64	11.00	0.10	0.21	0.78
BCS change [7]	0.20	0.21	0.41	0.18	0.14	0.47	0.38	0.35
Milk,[6] kg/d	32.00	32.86	31.67	33.86	1.32	0.78	0.20	0.58
3.5% FCM,[6,7] kg/d	31.63	33.00	30.87	33.62	1.71	0.95	0.06	0.51
Milk fat,%	3.40	3.50	3.33	3.50	0.09	0.71	0.16	0.70
Milk fat, kg/d	1.10	1.15	1.06	1.18	0.07	0.87	0.04	0.42
Milk protein,%	3.05	3.15	3.18	3.16	0.07	0.30	0.56	0.36
Milk protein, kg/d	0.97	1.03	1.00	1.07	0.04	0.30	0.06	0.92
Milk lactose,%	5.02	4.99	5.06	5.06	0.03	0.05	0.65	0.71
Milk lactose, kg/d	1.62	1.66	1.62	1.73	0.07	0.51	0.22	0.53
MUN, mg/dl	16.70	16.39	14.75	15.63	0.42	0.05	0.49	0.17
SCC[8]	60.01	53.61	96.01	104.25	23.19	0.06	0.97	0.74
Feed efficiency[9]	1.74	1.64	1.69	1.71	0.06	0.84	0.57	0.35

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving 5^9CFU/head/day of *Bacillus pumilus* (BP).
[2]Starch effect.
[3]*Bacillus pumilus* effect.
[4] Interaction of starch and *Bacillus pumilus*.
[5]Interaction of starch, *Bacillus pumilus* and week.
[6]Measured from d 1 through d 112 postpartum.
[7]3.5% FCM = 0.4324 × (kg of milk) + 16.2162 × (kg of fat).
[8]Somatic cell count × 1,000 cells/mL.
[9]3.5% FCM divided by DMI.

Table 18. Least squares means of colostrum yield and calf weight from primiparous cows fed low or high starch diet concentration during 21 d prepartum

	Treatments[1]			
Variables	LS	HS	SEM	*P*-value[2]
Colostrum[3]				
Yield, kg	4.51	4.11	0.80	0.16
Calf weight, kg	40.86	40.48	0.97	0.79

[1]Diets fed from d 21 prepartum through calving. Prepartum diets: LS (12 % starch), and HS (19 % starch).
[2]Starch effect.
[3]Colostrum measured within 12 hrs after calving.

Table 19. Health events recorded d −42 prepartum through d 112 postpartum for primiparous cows fed low or high starch diet concentration during 21 d prepartum and fed low or high starch diet postpartum with or without additional 5 x 10^9 CFU/head/day of *Bacilus pumillus*

	Treatments[1]			
	LS		HS	
Item	CO	BP	CO	BP
Twins	1	0	0	0
Dystocia[2]	1	2	3	1
Retained placenta[3]	2	1	3	1
Metritis[4]	3	2	6	4
Ketosis[5]	0	1	0	1
Displaced abomasum	0	0	0	0

[1]Low starch pre- and postpartum + carrier postpartum (LSCO); Low starch pre- and postpartum + *Bacillus pumilus* postpartum (LSBP); High starch pre- and postpartum + carrier postpartum (HSCO); High starch pre- and postpartum + *Bacillus pumilus* postpartum (HSBP).Prepartum diets: LS (12 % starch), and HS (19 % starch). Postpartum diets: LS (20 % starch), and HS (27 % starch). The amount of 28 g/d of powder product was topdressing on TMR proving 5^9CFU/head/day of *Bacillus pumilus* (BP).
[2]Dystocia was scored on a 1 to 5 scale (1 = easy, 5 = mechanically assisted pull); score of 4 or higher recorded.
[3]Retained fetal membranes for more than 24 h.
[4]Presence of fever (>39.5 ^{0}C) and red/brown watery fetid vaginal discharge.
[5]Serum BHBA levels above 29mg/dl between calving to 28 DIM.

Table 20. Ingredient and nutrient composition for prepartum and postpartum diets fed to primiparous cows from d - 45 prepartum through 56 postpartum

	Diets[1]			
Item	Prepartum		Postpartum	
Ingredients, % Diet DM	CON	ECO	CON	ECO
Alfalfa hay	9.87	9.87	17.49	17.49
Wheat straw	19.73	19.73	-	-
Corn silage	44.39	44.39	30.89	30.89
Corn gluten pellets	3.95	3.95	4.12	4.12
Dry corn (ground)	1.64	1.64	21.63	21.63
Dry cow protein mix[2]	16.44	16.44	-	-
Lactation protein mix[3]	-	-	14.92	14.92
Molasses-based LF[4]	3.98	-	3.97	-
Enzyme treated molasses-based LF[5]	-	3.98	-	3.97
Nutrient composition				
DM %	52.01	52.01	55.04	55.04
CP, % DM	14.83	14.83	16.95	16.96
RUP, %CP	34.38	34.58	42.20	42.37
RDP, %CP	65.62	65.42	57.80	57.63
ADF, % DM	28.41	28.41	19.33	19.33
NDF, % DM	43.27	43.28	29.59	29.59
Lignin, % DM	3.98	3.98	3.45	3.45
Lignin, % NDF	9.19	9.19	11.65	11.65
Starch, % DM	15.72	15.72	25.91	25.90
Sugar, %DM	5.31	5.21	6.01	5.91
Ash, % DM	9.90	9.91	8.14	8.15
Ca, % DM	0.95	0.97	0.93	0.94
P, % DM	0.41	0.41	0.43	0.43
Mg, % DM	0.30	0.30	0.26	0.26
K, % DM	1.49	1.49	1.52	1.53
S, % DM	0.26	0.26	0.27	0.27
Na, % DM	0.39	0.39	0.42	0.42
Cl, % DM	0.62	0.60	0.57	0.55
DCAD, meq/kg	240.00	219.00	244.00	249.00
NE_L, Mcal/kg	1.23	1.23	1.54	1.54

[1]Diets from d -45 prepartum through 56 d postpartum.
[2]Dry cow protein mix = 42.07% CP, 15.10% ash, 9.72% sugar, 4.66% fat.
[3]Lactation protein mix = 40.2% CP, 16.52% ash, 9.19% sugar, 3.13% fat.
[4]Molasses-based LF = Quality Liquid Feeds, Dodgeville, WI; 61.29% DM, 42.42% sugar, 34.44% ash, 5.60% Ca, 3.61% K.
[5]Enzyme treated molasses-based LF = Quality Liquid Feeds, Dodgeville, WI; 61.30% DM, 39.83% sugar, 34.68% ash, 5.89% Ca, 3.78% K. Inclusion of 8.03 kg/ton with Xylanase activity of 3492 BXU/g from Econase RDE, Feedworks USA, Cincinnati, OH.

Table 21. Least squares means of prepartum and postpartum dry matter intake, energy balance, body weight, body condition score, body weight and body condition score change of primiparous cows

	Treatments[1]			*P* - value		
Variables	CON	ECO	SEM	Trt	time	Trt x time
Prepartum						
DMI,[2] kg/d	14.16	15.04	0.44	0.17	0.32	0.12
DMI,[2] % of BW	2.53	2.63	0.08	0.36	0.70	0.12
EB,[2,3] Mcal/d	2.62	3.05	0.61	0.58	0.12	0.56
EB,[2,4] % Req	118.12	118.59	4.64	0.94	0.39	0.04
BW,[5] Kg	558.70	546.98	13.57	0.54	<.0001	0.44
BW change,[6] kg	45.05	65.79	23.88	0.94	-	-
BCS,[7]	2.95	2.94	0.07	0.87	0.001	0.41
BCS change,[8]	0.30	0.25	0.09	0.73	-	-
Postpartum						
DMI,[2] kg/d	21.75	22.21	0.57	0.57	<.0001	0.87
DMI,[2] % of BW	4.02	4.14	0.10	0.38	<.0001	0.85
EB,[2,3] Mcal/d	3.38	3.05	0.84	0.77	<.0001	0.53
EB,[2,4] % Req	111.80	110.41	2.93	0.72	0.007	0.59
BW,[5] Kg	540.82	533.34	12.86	0.68	<.0001	0.63
BW change,[6] kg	7.13	9.50	6.00	0.78	-	-
BCS,[7]	2.82	2.81	0.07	0.94	0.02	0.12
BCS change,[8]	- 0.04	0.07	0.05	0.12	-	-

[1]Treatments (Trt); CON = no enzyme; ECO = enzyme-treated diet (0.5ml/kg of TMR DM) from d - 45 through d 56 relative to calving.
[2]Prepartum (measured daily from d - 42 through 0 relative to calving); Postpartum (measured daily from d - 42 through 0 relative to calving).
[3]Energy intake – energy requirements.
[4]Energy intake/energy requirements (Req.) × 100. Prepartum Req.= NE_M + NE_P + NEg and Postpartum Req. = NE_M + NE_L + NEg. Where NE_M = net energy for maintenance, NE_P = net energy requirements for pregnancy, NEg = net energy for growth, and NE_L = net energy requirements for lactation.
[5]Body weight measured weekly. Prepartum (d - 42 to d - 7 relative to calving); Postpartum (d 7 to d 56 relative to calving).
[6]Body weight change; Prepartum (week - 6 BW minus week -1 BW); Postpartum (week 8 BW minus week 1 BW).
[7]Body condition score measured weekly. Prepartum (d - 42 to d - 7 relative to calving); Postpartum (d 7 to d 56 relative to calving).
[8]Body condition score; Prepartum (week - 6 BCS minus week -1 BCS); Postpartum (week 8 BCS minus week 1 BCS).

Table 22. Least squares means of prepartum and postpartum blood metabolites of primiparous cows

	Treatments[1]			*P* – value		
Variables	CON	ECO	SEM	Trt	time	Trt x time
Prepartum[2]						
Glucose, mg/dl	75.99	72.98	2.65	0.39	0.67	0.33
NEFA, µEq/l	116.96	104.83	20.73	0.66	<0.001	0.50
BHBA, mg/dl	4.17	4.82	0.36	0.21	0.02	0.10
Haptoglobin, OD x100	5.89	5.91	0.10	0.87	0.80	0.26
Postpartum[3]						
Glucose, mg/dl	69.05	66.86	1.81	0.37	0.38	0.93
NEFA, µEq/l	181.04	161.05	17.76	0.41	<0.001	0.35
BHBA, mg/dl	5.82	5.58	0.39	0.66	0.19	0.86
Haptoglobin, OD x100	6.27	5.93	0.16	0.16	<0.001	0.52

[1]Treatments (Trt); CON = no enzyme; ECO = enzyme-treated diet (0.5ml/kg of TMR DM) prepartum from d -45 through calving and (1ml/kg of TMR DM) postpartum from calving through d 56 relative to calving.

[2]Prepartum: Blood collected on d -28, -21, -14, and -7 relative to calving.

[3]Postpartum: Blood collected on d 7, 14, 21 and 28 relative to calving.

Table 23. Least squares means of prepartum and postpartum rumen fluid parameters of primiparous cows

	Treatments[1]			*P* – value		
Variables	CON	ECO	SEM	Trt	time	Trt x time
Prepartum[2]						
pH	7.11	7.06	0.07	0.64	-	-
Ammonia, g/dl	9.82	11.92	1.03	0.14	-	-
Volatile fatty acids, mM					-	-
Acetate (A)	52.51	48.90	4.64	0.36	-	-
Propionate (P)	15.55	14.60	0.93	0.46	-	-
Butyrate	9.02	10.05	1.24	0.55	-	-
Others	2.19	2.31	0.21	0.68	-	-
A:P	3.38	3.39	0.05	0.98	-	-
Postpartum[3]						
pH	6.53	6.71	0.07	0.08	0.16	0.88
Ammonia, g/dl	15.63	15.52	0.52	0.88	0.03	0.46
Volatile fatty acids, mM						
Acetate (A)	66.68	63.15	2.04	0.23	0.67	0.41
Propionate (P)	30.51	29.60	1.66	0.70	<.0001	0.49
Butyrate	14.09	13.27	0.50	0.25	0.85	0.58
Others[4]	3.15	2.92	0.17	0.36	0.28	0.91
A:P	2.38	2.35	0.10	0.79	<.0001	0.74

[1]Treatments (Trt); CON = no enzyme; ECO = enzyme-treated diet (0.5ml/kg of TMR DM) from d - 45 through d 56 relative to calving.
[2]Prepartum sampling: rumen fluid collected via oral-esophageal tube technique at 1630 h on d - 21 relative to calving.
[3]Postpartum sampling: rumen fluid collected via oral-esophageal tube technique at 1630 h on d 2, 14 and 56 relative to calving.
[4]Others = branched chain fatty acid: Isobutyrate,Isovalerate, and valerate.

Table 24. Least squares means of prepartum and postpartum apparent total-tract digestibility parameters of primiparous cows

	Treatments[1]			*P* - value		
Variables	CON	ECO	SEM	Trt	time	Trt x time
Prepartum[2]						
DMI, kg/d[3]	13.75	15.44	0.58	0.04	-	-
NDF intake, kg/d	5.85	7.08	0.26	0.02	-	-
Apparent total-tract						
NDFd, %	60.00	57.94	2.09	0.48	-	-
DMd, %	64.93	61.30	1.78	0.15	-	-
NDFd, kg/d	3.45	4.02	0.19	0.04	-	-
DMd, kg/d	8.85	9.25	0.37	0.44	-	-
Postpartum[3]						
DMI, kg/d	20.65	20.89	0.62	0.78	<.001	0.25
NDF intake, kg/d	6.76	6.97	0.62	0.11	<.001	0.80
Apparent total-tract						
NDFd, %	53.71	52.12	2.03	0.58	<.001	0.15
DMd, %	63.37	62.52	1.50	0.69	<.001	0.94
NDFd, kg/d	3.34	3.37	0.16	0.90	0.08	0.33
DMd, kg/d	12.64	12.37	0.39	0.63	0.01	0.64

[1]Treatments (Trt); CON = no enzyme; ECO = enzyme-treated diet (0.5ml/kg of TMR DM) from d - 45 through d 56 relative to calving.

[2]Prepartum sampling: fecal grab samples collected at 0800 h and 1600 h on d - 21 relative to calving. Samples analyzed using acid insoluble ash as an internal maker.

[3]Postpartum sampling: fecal grab samples collected at 0800 h and 1600 h on d 2, 14 and 56 relative to calving. Samples analyzed using acid insoluble ash as an internal maker.

Table 25. Least squares means of dairy efficiency, milk yield, and milk components of primiparous cows

	Treatments[1]			*P* - value		
Variables	CON	ECO	SEM	Trt	time	Trt x time
Milk, kg/d	28.32	30.13	0.86	0.15	<.0001	0.62
3.5% FCM,[2] kg/d	28.45	29.93	0.93	0.27	<.0001	0.80
Dairy Efficiency[3]	1.32	1.37	0.04	0.43	0.36	0.47
3.5% ECM,[4] kg/d	28.36	29.77	0.88	0.27	<.0001	0.74
Milk fat, %	3.56	3.49	0.09	0.59	<.001	0.85
Milk fat, kg/d	1.00	1.04	0.04	0.43	<.0001	0.89
Milk protein, %	3.21	3.15	0.05	0.41	<.0001	1.00
Milk protein, kg/d	0.90	0.94	0.03	0.31	<.0001	0.50
Milk lactose, %	4.97	5.00	0.03	0.48	<.0001	0.86
Milk lactose, kg/d	1.41	1.51	0.05	0.15	<.0001	0.53
MUN, mg/dl	16.67	17.81	0.31	0.01	<.0001	0.88
SCC[5]	78.74	85.69	18.47	0.79	<.0001	0.52
F:P ratio[6]	1.12	1.11	0.03	0.95	0.86	0.86

[1]Treatments (Trt); CON = no enzyme; ECO = enzyme-treated diet (0.5ml/kg of TMR DM) from d - 45 through d 56 relative to calving.
[2]3.5% FCM, kg/d = (0.4324 x kg of milk) + kg of milk fat).
[3]Dairy Efficiency = 3.5% FCM divided by DMI.
[4]3.5% ECM, kg/d = (12.82 x kg of milk fat) + (7.13 x kg of milk protein) + (0.323 x kg of milk).
[5]SCC = Somatic cell count × 1,000 cells/mL.
[6]F:P ratio = milk fat divided by milk protein.

Table 26. Least squares means of calf weight, colostrum yield and specific gravity of primiparous cows

	Treatments[1]			*P* - value
Variables	CON	ECO	SEM	Trt
Calf weight,[2] kg	38.6	36.7	1.21	0.25
Colostrum				
Yield,[2] kg	3.91	5.18	0.52	0.09
Total solids,[2,3] %	26.39	25.10	1.18	0.41

[1]Treatments (Trt); CON = no enzyme; ECO = enzyme-treated diet (0.5ml/kg of TMR DM) from d - 45 through d 56 relative to calving.
[2]Measured within 12 hrs after calving.
[3]Measured using brix refractometer (MISCO PA201 digital brix. MISCO Inc., Cleveland, OH).

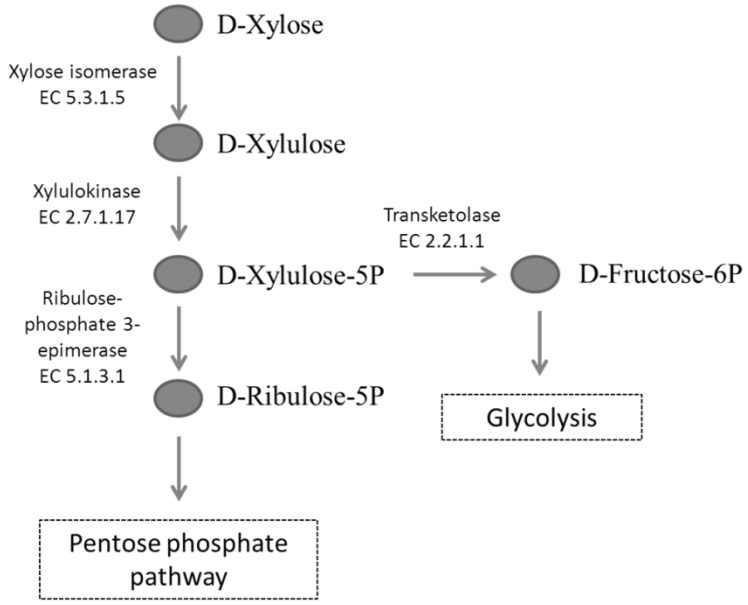

Figure 1. Proposed metabolic pathway for the utilization of xylose by *Bacillus pumilus* 8G-134. A KEGG1,2 map construction of the suspected route of xylose metabolism via pentose interconversion by BP 8G-134 based on genome sequencing analysis. Kanehisaet al.,2014. Kanehisa et al., 2000.

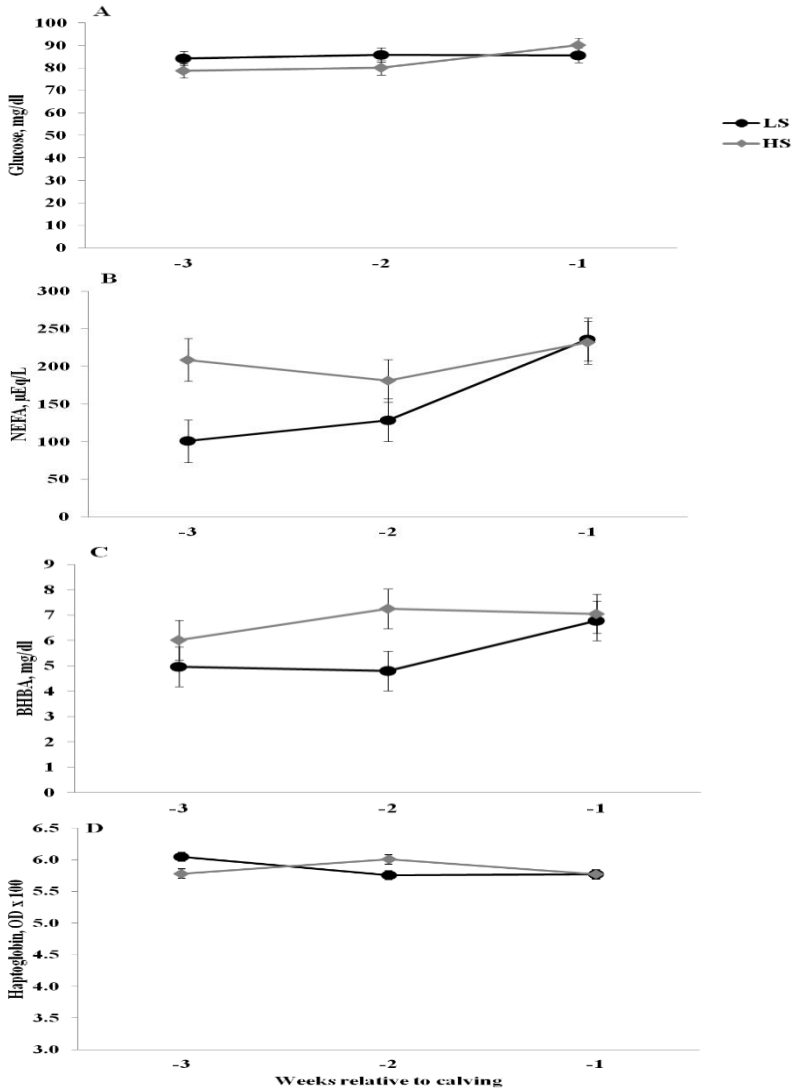

Figure 2. Association between dietary starch concentration and serum metabolites parameters during prepartum.(A) Glucose: Starch (P = 0.34), and interation between starch and day relative to calving (P = 0.14) were not associated with glucose concentreation, SEM = 3.20 .(B) NEFA: Starch (P = 0.19), and interation between starch and day relative to calving (P = 0.33) were not associated with glucose concentreation, SEM = 28.48 .(C) BHBA: Starch (P = 0.11), and interation between starch and day relative to calving (P = 0.11) were not associated with glucose concentreation, SEM = 0.78 . (D) Haptoglobin: Starch (P = 0.97) were not associated with haptoglobin levels, but high starch was lower on w -3 and greated on w -2 relative to calving (P = 0.05) compare to low starch, SEM = 0.07.

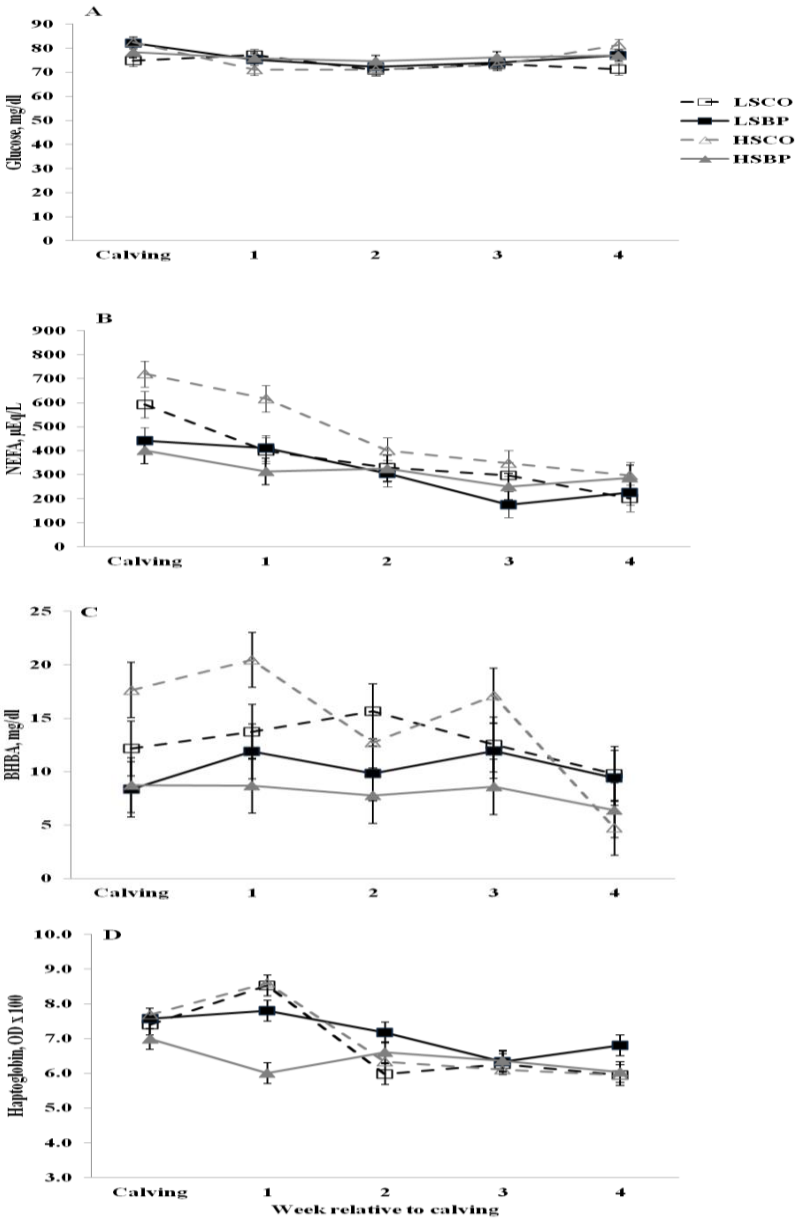

Figure 3. Association between dietary starch and *Bacillus pumilus* 8G-134 concentration on postpartum serum metabolites.(A) Glucose: S[1](*P* = 0.56);BP[2](P = 0.49); S*BP[3](*P* = 0.66); S*B*W[4](*P* = 0.97); SEM = 2.37; (B) NEFA: S[1](*P* = 0.29); BP[2](*P* = 0.05); S*BP[3](*P* = 0.33); S*B*W[4](*P* = 0.33); SEM= 68.8;(C) BHBA:S [1](P = 0.92); BP[2](*P* = 0.07); S*BP[3](*P* = 0.41); S*B*W[4](*P* = 0.50); SEM= 2.57. (D) Haptoglobin: S[1](*P* = 0.22); BP[2](*P* = 0.66); S*BP[3](*P* = 0.09); S*B*W[4](*P* = 0.46). Interactions: [1]Starch; [2]*Bacillus pumillus* 8G-134 ; [3]Starch and *Bacillus pumilus* 8G-134; [4]Starch, *Bacillus pumilus* 8G-134 and week.

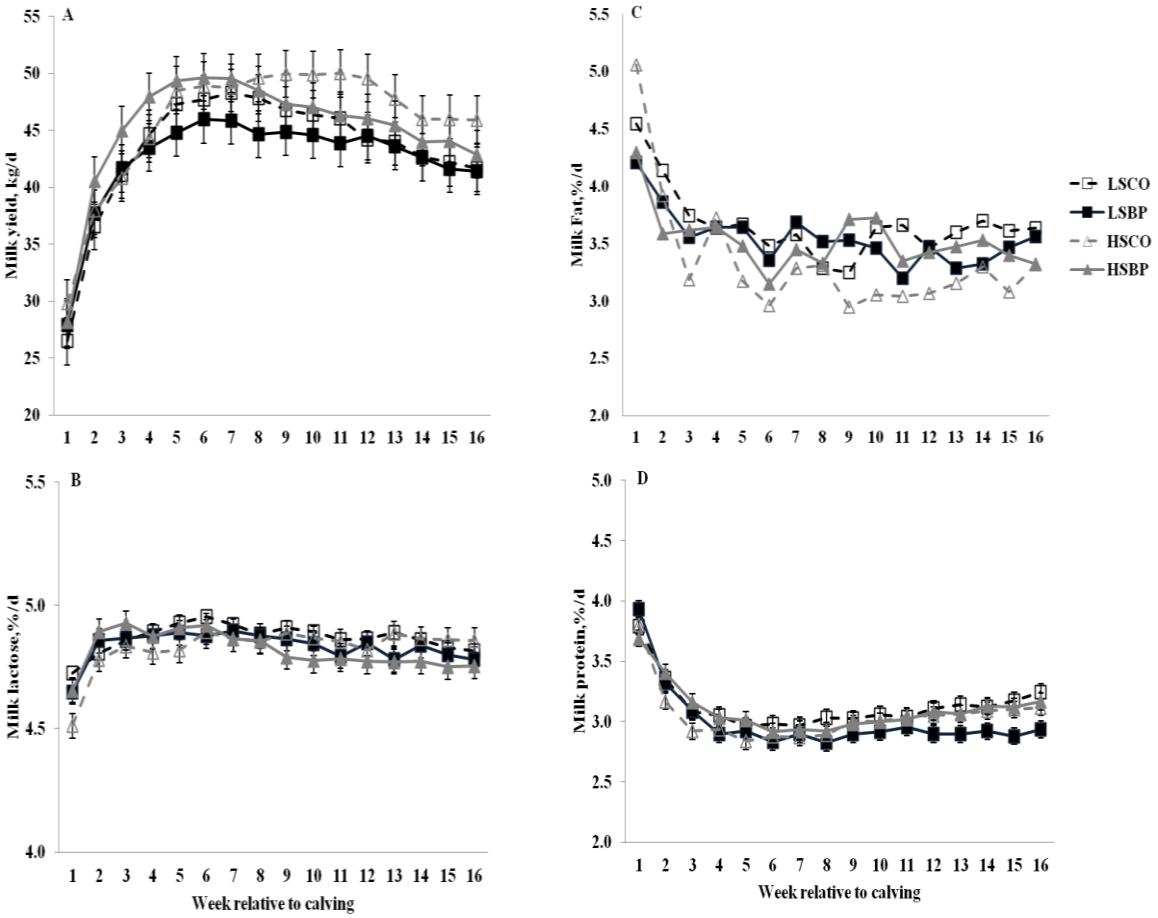

Figure 4. Association between dietary starch and *Bacillus pumilus* 8G-134 concentration on milk yield and components from 1 through 112 days in milk. (A) Milk yield: S^1(*P* = 0.26);BP^2(*P* = 0.42); $S*BP^3$(*P* = 0.59); $S*B*W^4$(*P* = 0.15); SEM = 1.81. (B) Milk lactose: S^1(*P* = 0.56);BP^2(*P* = 0.65); $S*BP^3$(*P* = 0.83); $S*B*W^4$(*P* = 0.17); SEM = 0.05 .(C) Milk fat: S^1(*P* = 0.18);BP^2 *P* = (0.79);$S*BP^3$(*P* = 0.23); $S*B*W^4$(*P* = 0.40); SEM = 0.26. (D) Milk protein: S^1(*P* = 0.89);BP^2(*P* = 0.54); $S*BP^3$(*P* = 0.15); $S*B*W^4$(*P* = 0.01); SEM= 0.07. Interactions: [1]Starch; [2]*Bacillus pumilus* 8G-134; [3]Starch and *Bacillus pumilus* 8G-134; [4]Starch, *Bacillus pumilus* 8G-134 and week.

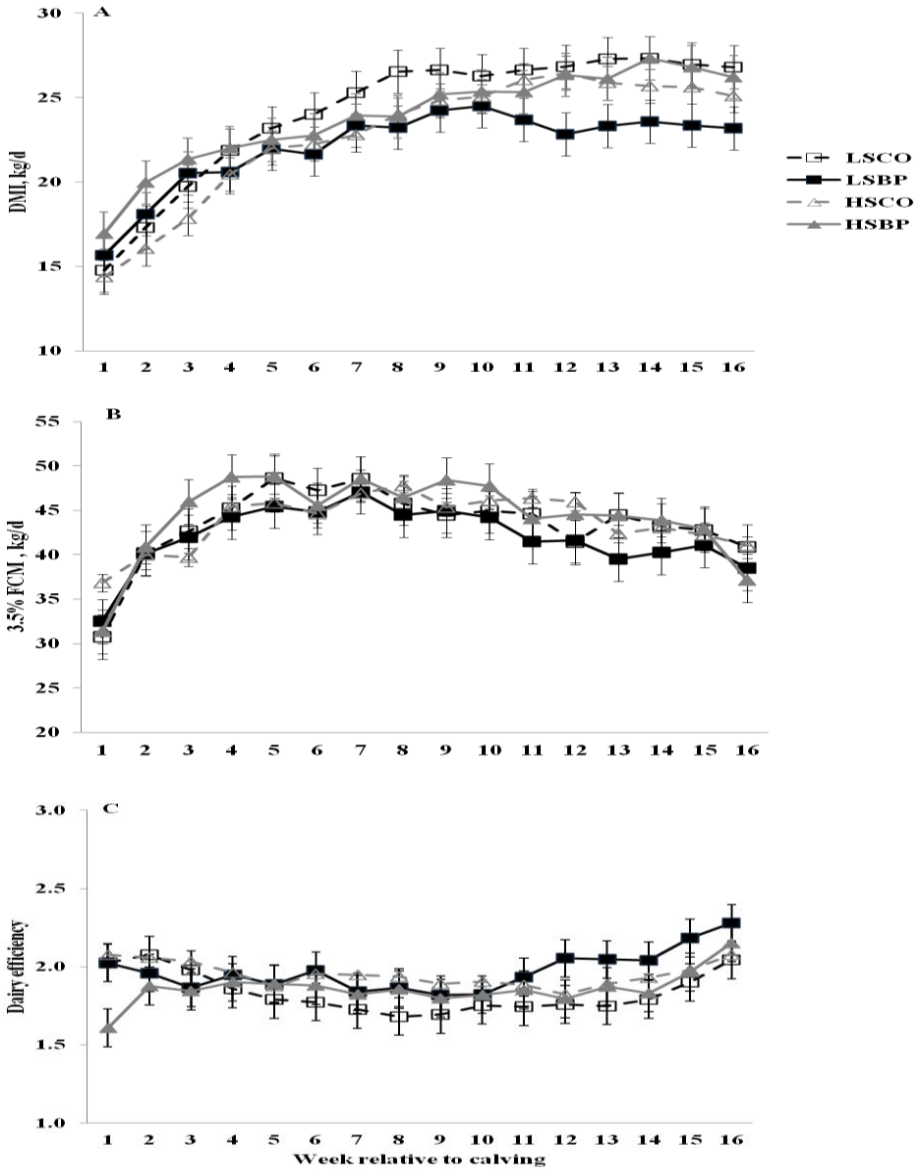

Figure 5. Association between dietary starch and *Bacillus pumilus* concentration on dry matter intake, 3.5% fat corrected milk ,and feed efficiency from 1 through 112 days in milk. (A) DMI: S[1](*P* = 0.86);BP[2](*P* = 0.57); S*BP[3](*P* = 0.08); S*B*W[4](*P* = 0.43); SEM = 1.28. (B) 3.5% FCM: S[1](*P* = 0.44);BP[2](*P* = 0.81); S*BP[3](*P* = 0.52); S*B*W[4](*P* = 0.71); SEM = 2.50. (C) Dairy efficiency: S[1](*P* = 0.97);BP[2](*P* = 0.78); S*BP[3](*P* = 0.17); S*B*W[4](*P* = 0.37); SEM = 0.12. Interactions: [1]Starch; [2]*Bacillus pumilus*; [3]Starch and *Bacillus pumilus*; [4]Starch, *Bacillus pumilus* and week.

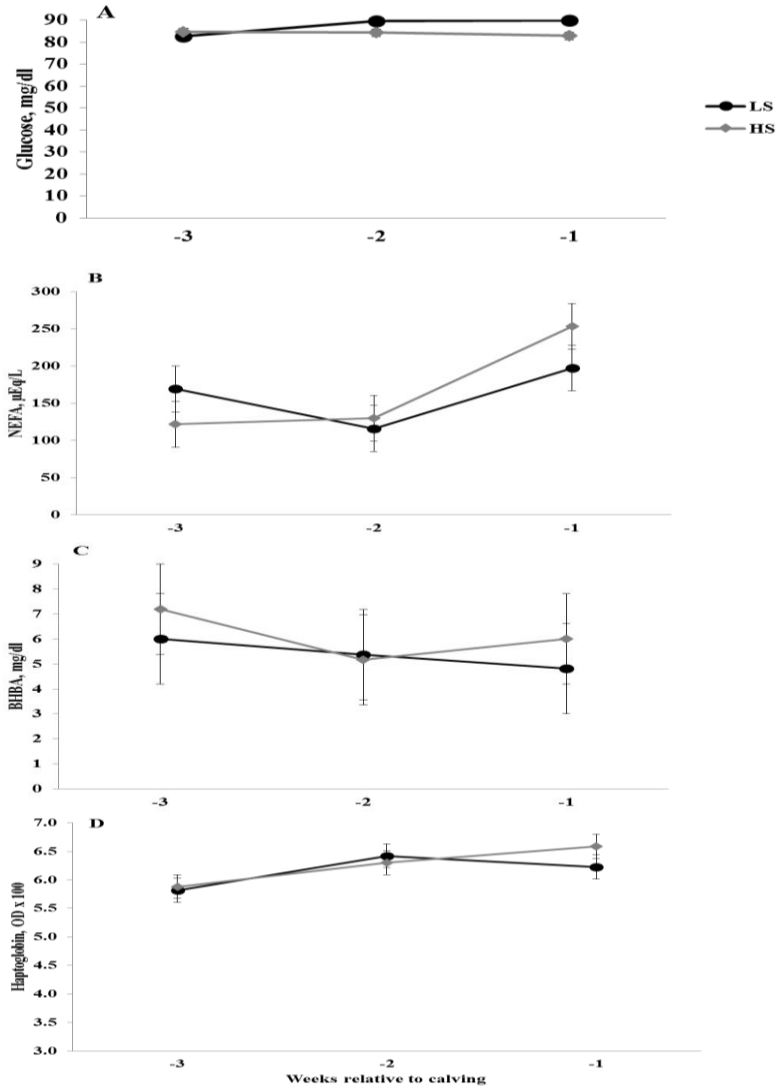

Figure 6. Effect of dietary starch concentration on serum metabolites parameters during prepartum.(A) Glucose: Starch (P = 0.16), and interation between starch and day relative to calving (P = 0.25) were not associated with glucose concentration, SEM = 1.73 .(B) NEFA: Starch (P = 0.79), and interation between starch and day relative to calving (P = 0.16) were not associated with starch concentration, SEM = 28.48 .(C) BHBA: Starch (P = 0.79), and interation between starch and day relative to calving (P = 0.16) were not associated with starch concentration, SEM = 1.78. (D) Haptoglobin: Starch (P = 0.72) were not associated with haptoglobin levels, and its levels were similar during the three weeks before calving, SEM = 0.21.

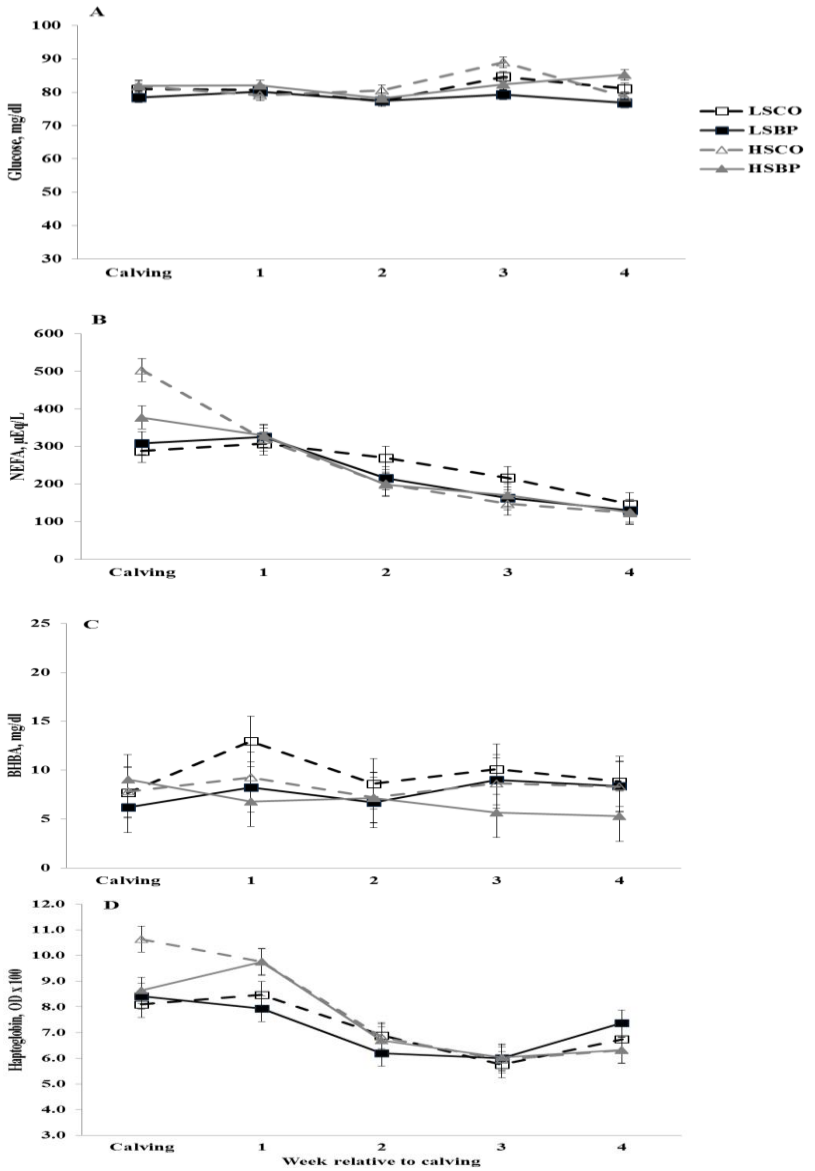

Figure 7. Association between dietary starch and *Bacillus pumilus* 8G-134 concentration on postpartum serum metabolites. (A) Glucose: S[1](*P* = 0.14);BP[2](P = 0.42); S*BP[3](*P* = 0.37); SEM = 1.58; (B) NEFA: S[1](*P* = 0.68); BP[2](*P* = 0.55); S*BP[3](*P* = 0.97); SEM= 30.6;(C) BHBA:S [1](*P* = 0.21); BP[2](*P* = 0.06); S*BP[3](*P* = 0.80); SEM= 1.01. (D) Haptoglobin: S[1](*P* = 0.27); BP[2](*P* = 0.66); S*BP[3](*P* = 0.67); S*B*W[4](*P* = 0.70). Interactions: [1]Starch; [2]*Bacillus pumilu*8G-134; [3]Starch and *Bacillus pumilus* 8G-134; [4]Starch, *Bacillus pumilus* 8G-134 and week.

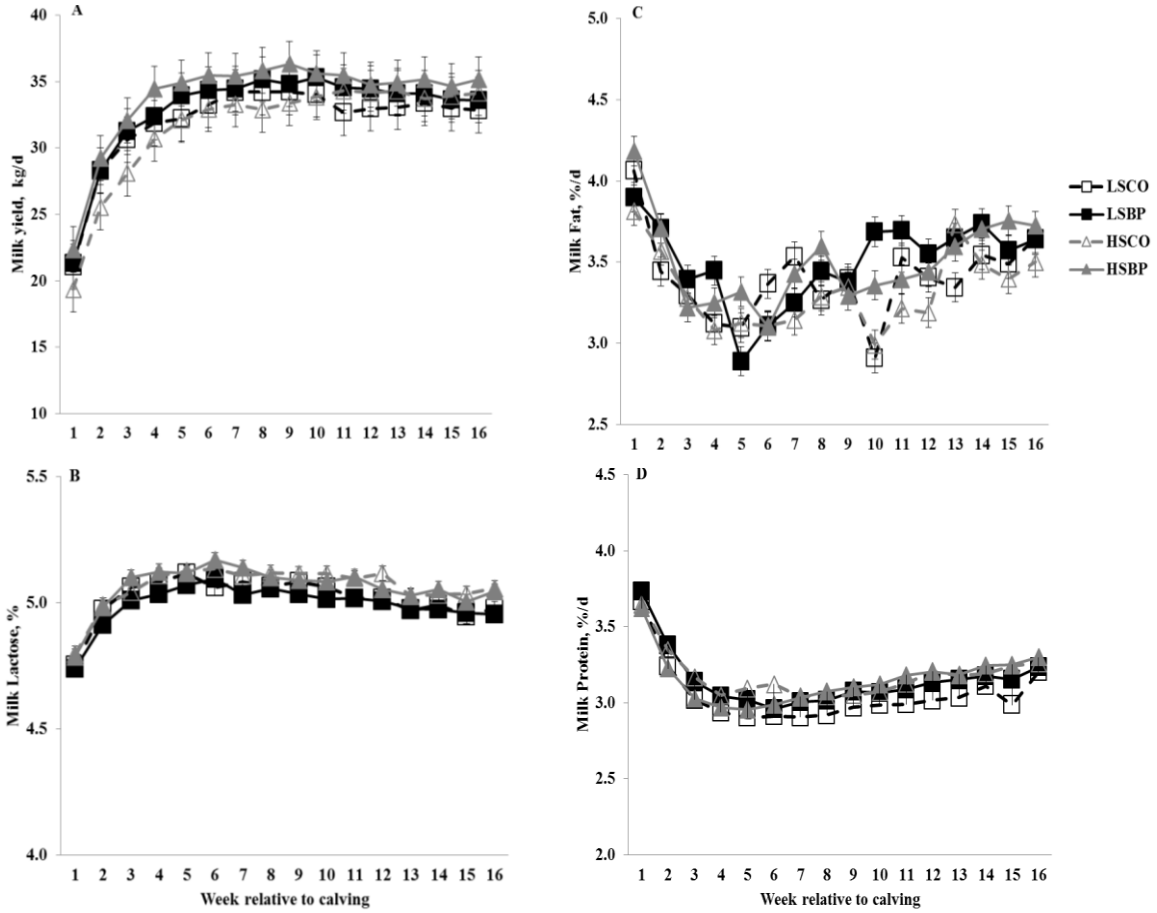

Figure 8. Association between dietary starch and *Bacillus pumilus* 8G-134 concentration on milk yield and components from 1 through 112 days in milk. (A) Milk yield: S^1(*P* = 0.78);BP^2(*P* = 0.20); $S*BP^3$(*P* = 0.58); $S*B*W^4$(*P* = 0.09); SEM = 1.32. (B) Milk lactose: S^1(*P* = 0.05);BP^2(*P* = 0.65); $S*BP^3$(*P* = 0.71); $S*B*W^4$(*P* = 0.99); SEM = 0.03 .(C) Milk fat: S^1(*P* = 0.70);BP^2 *P* = (0.16);$S*BP^3$(*P* = 0.69); $S*B*W^4$(*P* = 0.81); SEM = 0.09. (D) Milk protein: S^1(*P* = 0.30);BP^2(*P* = 0.56); $S*BP^3$(*P* = 0.35); $S*B*W^4$(*P* = 0.45); SEM= 0.07. Interactions: [1]Starch; [2]*Bacillus pumilus* 8G-134; [3]Starch and *Bacillus pumilus* 8G-134; [4]Starch, *Bacillus pumilus* 8G-134 and week.

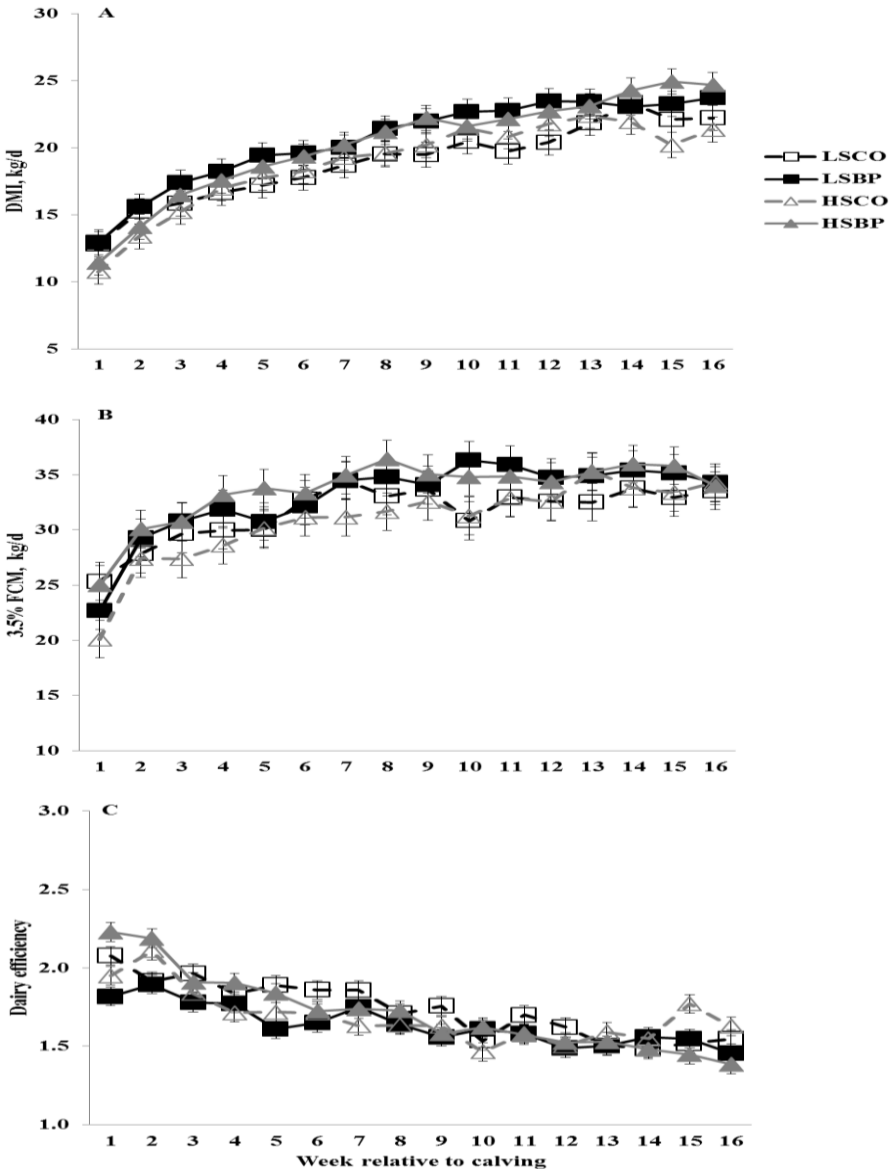

Figure 9. Association between dietary starch and *Bacillus pumilus* concentration on dry matter intake, 3.5% fat corrected milk, and feed efficiency from 1 through 112 days in milk. (A) dry matter intake: S^1(*P* = 0.73);BP^2(*P* = 0.01); $S*BP^3$(*P* = 0.89); $S*B*W^4$(*P* = 0.03); SEM = 0.37. (B) 3.5% FCM: S^1(*P* = 0.94);BP^2(*P* = 0.06); $S*BP^3$(*P* = 0.52); $S*B*W^4$(*P* = 0.39); SEM = 1.71. (C) Dairy efficiency: S^1(*P* = 0.84);BP^2(*P* = 0.57); $S*BP^3$(*P* = 0.35); $S*B*W^4$(*P* = 0.36); SEM = 0.06. Interactions: [1]Starch; [2]*Bacillus pumilus*; [3]Starch and *Bacillus pumilus*; [4]Starch, *Bacillus pumilus* and week.

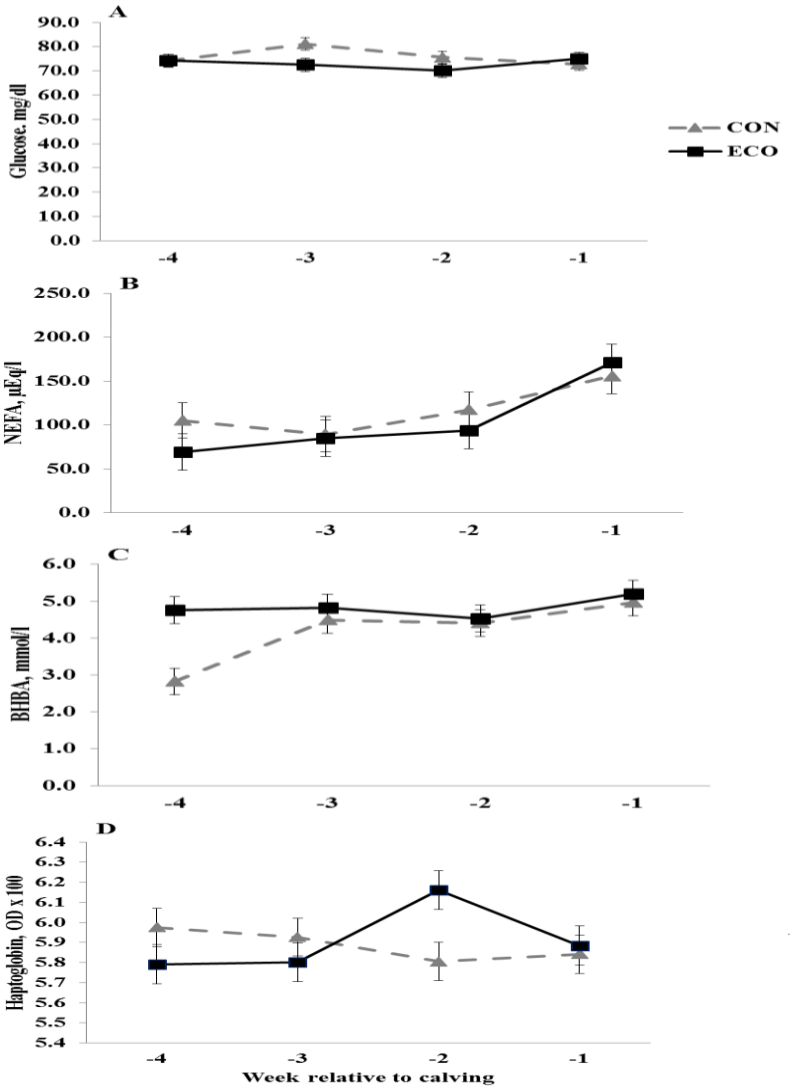

Figure 10. Effect of exogenous fibrolytic enzyme on serum metabolites parameters prepartum. (A) Glucose: treatment (P = 0.39), and interation between treatment and day relative to calving (P = 0.33), SEM = 2.65.(B) NEFA: treatment (P = 0.66), and interation between treatment and day relative to calving (P = 0.49), SEM = 20.73 .(C) BHBA: Treatment (P = 0.21), and interation between treatment and day relative to calving (P = 0.09), SEM = 0.36. (D) Haptoglobin: Treatment (P = 0.86), interation between treatment and day relative to calving (P = 0.25), SEM = 0.10.

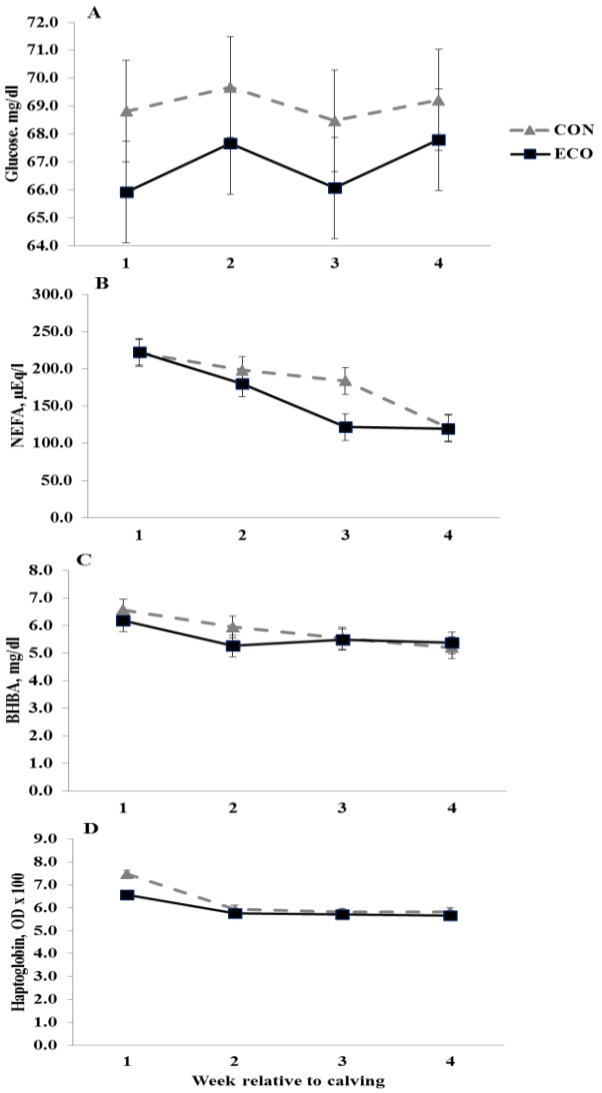

Figure 11. Effect exogenous fibrolytic enzyme on serum metabolites parameters during postpartum. (A) Glucose: Treatment (P = 0.37), and interation between treatment and day relative to calving (P = 0.93), SEM = 1.81.(B) NEFA: Treatment (P = 0.40), and interation between treatment and day relative to calving (P = 0.35), SEM = 17.76 .(C) BHBA: Treatment (P = 0.39), and interation between treatment and day relative to calving (P = 0.86), SEM = 0.39. (D) Haptoglobin: Treatment (P = 0.15) were not, SEM = 0.16.

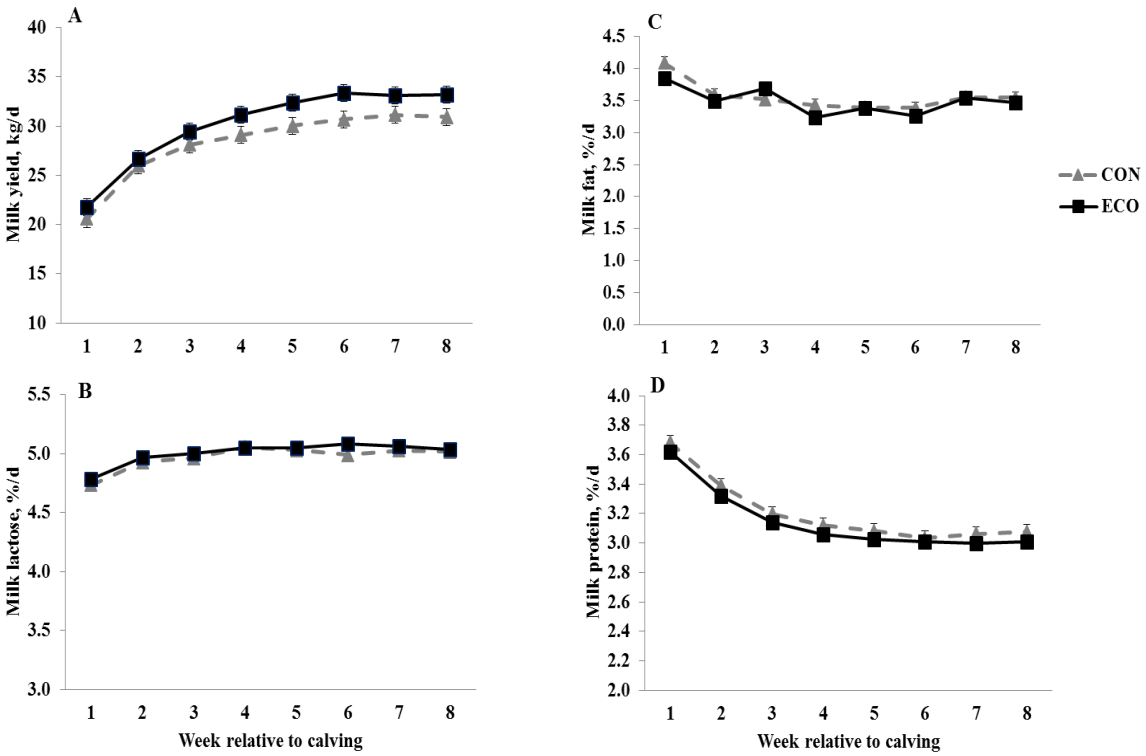

Figure 12. Effect of exogenous fibrolytic enzyme on milk yield and components from 1 through 56 days in milk. (A) Milk yield: Trt[1](*P* =0.15); Trt*W[2] (*P* =0.62); W[4](*P* <.0001); SEM =.0.86 (B) Milk lactose: Trt[1](*P* =0.48); Trt*W[2] (*P* =0.86); W[4](*P* <.0001); SEM =.0.03 (C) Milk fat: Trt[1](*P* =0.59); Trt*W[2] (*P* =0.84); W[4](*P* =0.0002); SEM =.0.09 (D) Milk protein: Trt[1](*P* =0.41); Trt*W[2] (*P* =0.99); W[4](*P* <.0001); SEM =.0.05 Interactions: [1]Treatment; [2]Treatment*Week; [3]Week.

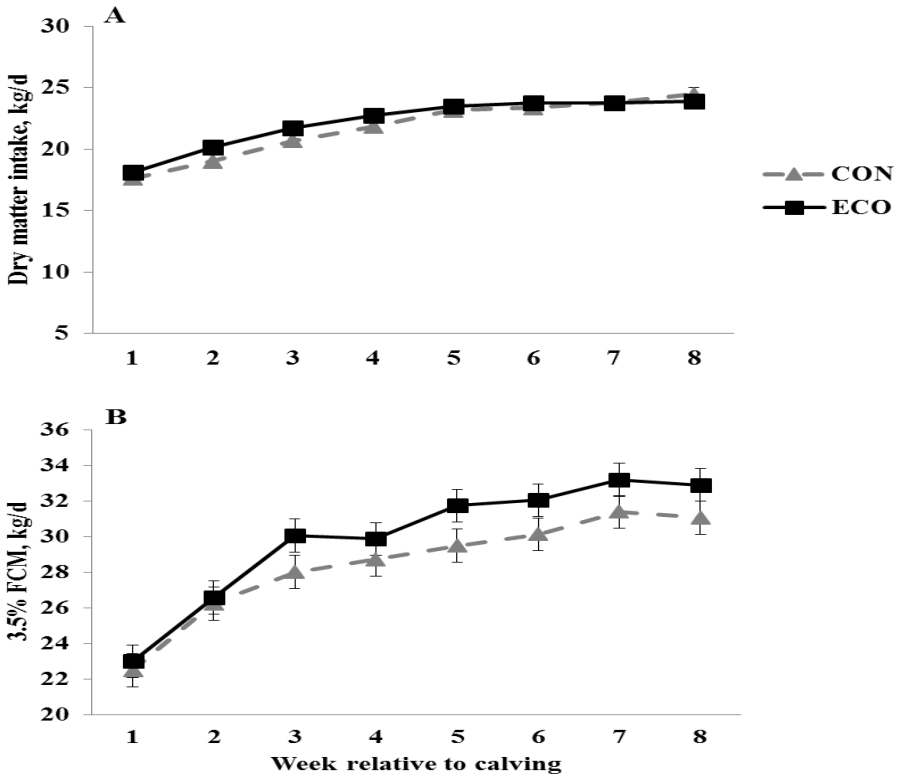

Figure 13. Effect of exogenous fibrolytic enzyme on dry matter intake, 3.5% fat corrected milk, and feed efficiency from 1 through 56 days in milk. (A) DMI: Trt[1](*P* =0.57); Trt*W[2] (*P* =0.87); W[4](*P* <.0001); SEM =.0.57. (B) 3.5% FCM: Trt[1](*P* =0.26); Trt*W[2] (*P* =0.79); W[4](*P* <.0001); SEM =.0.92.