EFFECTS OF FORAGE PRESERVATION METHODS, DIETARY STARCH AMOUNT AND SUPPLEMENTATION WITH *PROPIONIBACTERIUM FREUDENREICHII* STRAIN P169 ON TRANSITION COW HEALTH AND PRODUCTION, AND KETOSIS PREDICTION TOOLS.

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Dedication

I dedicate this thesis to my family. Thank you for your love, support and encouragement.

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List of Abbreviations

ADF = Acid detergent fiber

ATP = Adenosine triphosphate

BCS = Body condition score

BHBA = Beta-hydroxybutyrate

BW = Body weight

CFU = Colony forming units

CSG = Colostrum specific gravity

CY = Colostrum yield

DFM = Direct fed microbial

DHIA = Dairy herd information association

DIM = Days in milk

DM = Dry matter

DMI = Dry matter intake

DS = Days ensiled

ECM = Energy corrected milk

FCM = Fat corrected milk

HOT = Hepatic oxidation theory

HS = High starch

IgG = Immunoglobulin G

IVSD = *In vitro* starch digestibility

LS = Low starch

ME = Metabolizable energy

ME-305d = Mature equivalent 305 day milk yield

MP = Metabolizable protein

NDF = Neutral detergent fiber

NDFd = Neutral detergent fiber digestibility

NE_i = Net energy intake

NE_l = Net energy lactation

NEFA = Non-esterified fatty acid

P169 = *Propionibacterium freudenreichii* strain P169

PVC = Polyvinyl chloride

RFS = Rumen fill score

ROC = Receiver operating characteristic

SCC = Somatic cell count

TCA = Tricarboxylic acid

TCI = Transition cow index

TG = Triaclyglycerol

TMR = Total mixed ration

VFA = Volatile fatty acid

GENERAL INTRODUCTION

The transition period for dairy cows is a critical and challenging time as cows are at the greatest risk for metabolic diseases, and are trying to meet the energy demands for high milk production. In order to achieve high milk yield and reduce metabolic disorders, quality forages are required in the ration to promote DMI and deliver highly digestible nutrients to the transition dairy cow.

Preservation of silage quality is a large nutritional and economical factor contributing to the success of dairy operations. By achieving proper silage fermentation, there is a reduction in DM loss and spoilage through the removal of oxygen (Kung, 2001). Affordable and reliable technology that consistently increases the rate and quality of the ensiling process is needed to ensure high quality forage to support high milk production. Volatility in the market value of corn and milk has resulted in an increased sense of urgency to focus on increasing the quality, value, and digestibility of forages to achieve profitable margins.

Packing density is important when preserving silage to allow for proper anaerobic conditions through the exclusion of oxygen. In 2001, Kung and Nylon showed the importance of packing density on the rate of fermentation through the increase in yeast counts with loosely packed silage. Loosely packed silage has potential for greater shrink and DM loss as demonstrated by Ruppel (1992) who measured a decrease in DM loss in alfalfa silage when packing density was increased.

Loosely packed silage would also be predisposed to poor aerobic stability due to a more aerobic environment and potential for increased yeast growth. Previous research indicated that inoculation with *Lactobacillus buchneri* increased aerobic stability through

an increase in acetic acid concentration (Ranjit and Kung, 2000; Muck, 2004). Acetic acid acts as a natural yeast and mold inhibitor (Narendranath et al., 2001) which provides greater aerobic stability at feed out. Inoculation with propionate producing bacteria did not increase aerobic stability (Higginbotham et al., 1998). However, it is possible that with greater lactic acid production and a reduction in acetic acid, which is a natural yeast and mold inhibitor (Narendranath et al., 2001), aerobic stability may be reduced when homofermentative lactic acid producing inoculants are applied. In a review by Kung (2001) lactic acid producing bacterial inoculants resulted in increased aerobic stability only 33 percent of the time. Therefore, there has been many conflicting results for aerobic stability with homofermentative inoculants due to reduction of acetic acid which is a yeast inhibitor and thus diminishes aerobic stability (Muck, 2012). The mixed results with homo-lactic inoculants indicates the need for a more reliable and consistent inoculant resulting in greater aerobic stability of silage.

The practical application of inoculants to enhance fermentation and reduce DM loss under variable on-farm situations has been studied in previous years. Previous research (Aksu et al., 2004; Arriola et al., 2011) has shown that addition of inoculants containing homo- and heterofermentative bacteria can decrease pH and increase lactic acid production during fermentation. A rapid decline in pH helps preserve the silage quickly reducing the potential for clostridial fermentation and yeast and mold growth. By encouraging proper fermentation, nutrient retention and digestibly increase and the resulting silage is potentially more aerobically stable.

Corn silage is a combination of a fibrous grass and a starchy grain. Additionally, due to increases in dairy feed ingredient costs, nutritionists are formulating diets that

contain a greater proportion of diet DM as corn silage (Allen, 2001) which results in a need to place emphasis on both corn silage fiber and starch digestibility. The starch molecule in corn is bound by a prolamin protein matrix (Mu-Forster and Wasserman, 1998). This prolamin protein matrix is hydrophobic and resists solubilization in water and rumen fluid (Lawton, 2002). However, prolamin is soluble in lactic and acetic acid which are produced during fermentation (Lawton, 2002). Previous studies have shown that starch digestibility increases with increased silage storage time (Jurjanz and Monteils, 2005; Hoffman et al., 2011) as the result of fermentation and bacterial and enzymatic proteolytic activity. When protease was applied to whole plant corn silage, *in vitro* starch digestibility (**IVSD**) was increased after 45 days ensiled (Windle et al., 2014). Recent research indicates that an inoculant containing *Lactobacillus casei* and *Lactobacillus buchneri* increased *in vitro* neutral detergent fiber digestibility (**NDFd**) (Kang et al., 2009). Reich and Kung, (2010) also reported an increase in *in vitro* NDFd with the application of inoculant containing *Lactobacillus buchneri* 40788 and *Lactobacillus plantarum*. However, variable results have been obtained with application of inoculants on corn silage regarding NDF and IVSD digestibility. Therefore, identification of consistent and reliable inoculants is needed to enhance corn silage fermentation, increase aerobic stability, and increase digestibility of starch and fiber at various packing densities to be applicable on farm.

Along with the use of inoculants to improve corn silage quality, utilizing direct fed microbials (**DFM**, *Propionibacterium freudenreichii* strain P169 (**P169**), (DuPont Industrial and Biological Sciences, Waukesha, WI)) during the transition period in attempt to reduce the duration and severity of negative energy balance and increase

propionate production for increased gluconeogenesis has been a key focus for many research projects in recent years. Previous research has reported a decrease of 1.0 kg/d of DMI (Weiss et al., 2008) when lactating cows were fed P169. Despite the decrease in DMI when P169 was fed, milk response ranged from no response (Francisco et al., 2002; Weiss et al., 2008) to an increase of 3.2 kg/d (Stein et al., 2006). It is not known if cow response to P169 is dependent upon the dietary starch concentration or if the response can be altered by supplementing cows with P169 during the transition period. Increasing dietary starch in the ration is one way to increase propionate production in the rumen. The P169 microbe was selected for its ability to produce propionate from lactate in the rumen, thus potentially increasing gluconeogenesis in early lactation dairy cows. Since ruminants rely heavily on gluconeogenesis for glucose production, it is important for early lactation cows to be provided with sufficient precursors so they can maximize gluconeogenesis to reduce the severity and duration of negative energy balance. Propionate is one of the major precursors for gluconeogenesis in dairy cows. Thus, focusing on increasing propionate production in early lactation may be beneficial for both increased milk production and improved cow health.

Previous studies have demonstrated that over-feeding energy to dry cows resulted in a reduction in gluconeogenesis, decreased DMI and an increase in rate of postpartum lipolysis (Holtenius et al., 1996; Douglas et al., 2007; Janovick et al., 2011). Postpartum DMI decreased by 1.5 kg/d with increasing dietary starch concentration (21.0% vs. 25.5%) (Nelson et al., 2011) and by 2.4 kg/d (21.8% vs. 27.1%) (Gencoglu et al., 2010). Moderate dietary starch concentration (23.2%) increased milk yield by 5.7 kg/d over high starch (25.5%) but was similar to low starch (21.0%) (Nelson et al., 2011). High dietary

starch (27.1%) had similar milk yield to low starch (21.8%), however, 3.5% FCM was reduced by 2.9 kg/d for high starch compared to low starch (Gencoglu et al., 2010).

There are also concerns with feeding high starch diets to fresh cows due to an increased flux of propionate (Oba and Allen, 2003a). It has been suggested that excessive amounts of propionate delivered to the liver would increase the amount of propionate being oxidized in the TCA cycle which could decrease the firing rate of the hepatic vagus nerve resulting in increased satiety (Allen et al., 2009). Supplementation with P169 may amplify this pathway and cause a greater reduction in DMI. Conversely, lower amounts of propionate in circulation could result in a reduction in the stimulation of the hepatic vagus nerve increasing its firing rate resulting in greater DMI (Allen et al., 2009). Recent research has focused on the implications of feeding groupings of cows based on stage of lactation (Allen, 2012). Based on the hepatic oxidation theory (**HOT**, Allen et al., 2009), feeding a single lactation TMR, which is formulated for peak milk production, may result in over-feeding starch in early lactation leading to a reduction in DMI. As a result, milk production might be compromised and risk of metabolic disorders may increase. Therefore, a successful fresh cow diet should maximize DMI emphasizing fiber digestibility which results in increased energy balance and reduction in lipolysis (Litherland et al., 2013).

There have been previous studies evaluating the effects of feeding diets varying in starch concentration in early lactation. Replacing corn with soy hulls resulted in a tendency for a decrease in milk yield driven mostly by soy hull feeding rates of 30 and 40% of diet DM (Ipharraguerre et al., 2002). Decreased dietary starch concentration (32.9 to 17.6%) by replacing shell corn and soybean meal with wheat middlings, dried

brewers grains, and soy hulls decreased DMI and increased milk fat content (Batajoo and Shaver, 1994). Another study (Dann et al., 2008) found no differences in DMI or milk yield when feeding dietary starch concentrations of 17.7, 21.0, and 24.6 % of diet DM when corn grain was replaced with dried beet pulp, wheat middlings, and distillers grains. Replacing high moisture corn with pelleted beet pulp (34.6, 30.5, 26.5 and 18.4% starch) linearly decreased DMI but 3.5% FCM tended to increase quadratically with cows fed the 30.5% starch diet having the greatest milk yield (Voelker and Allen, 2003). Dry cracked corn was replaced by dried citrus pulp to decrease dietary starch from 31.0 to 20.0% of diet DM. Feeding the low starch diet reduced DMI, milk yield, fat yield, and true protein content (Broderick et al. 2002). Based on these previous studies, replacing starch with non-forage fiber did not necessarily decrease milk production.

Prepartum feeding strategies for optimal postpartum performance and health have not yet been clearly defined. Prepartum treatment with propionibacteria has resulted in some positive improvements in postpartum hormone (Francisco et al., 2002; Lehloenya et al., 2008) and metabolite profiles (Stein et al., 2006) as well as increased postpartum energetic efficiency (Weiss et al., 2008). However, supplementation of P169 with varying dietary starch concentrations is not definitive and it is not known if this feeding strategy will reduce the incidence of subclinical ketosis.

Due to the increase demand for energy in early lactation, high producing dairy cows mobilize body fat to meet their energy demands. Around the time of calving, there is an increase in somatotropin which reflects a reduction in DMI (Bell, 1995; Grum et al., 1996). Lower DMI in early lactation leads to a reduction in circulating insulin and an increase in glucagon (Ingvartsen and Anderson, 2000; Ingvartsen, 2006). Also, adipose

tissue becomes less sensitive to insulin (Hayirli, 2006; Locher et al., 2011). In early lactation, an increase in glucagon, catecholamines, and adrenocorticotropic hormone stimulate hormone-sensitive lipase (Vaughan et al., 1964; Slavin et al., 1994; Holtenius et al., 1996) which shifts metabolism in adipose tissue from lipogenesis to lipolysis to provide non-esterified fatty acid (**NEFA**) as a energy source (McNamara, 1991). The greater amount of NEFA being mobilized results in greater uptake of NEFA by the liver (Emery et al., 1992). The liver can oxidize NEFA to produce ATP and ketones, or reconvert NEFA to produce triglycerides and store them in the liver (Drackley, 1999; Ingvartsen, 2006). If cows are unable to adapt to negative energy balance successfully, excess body reserves are mobilized resulting in an increase in NEFA which leads to an increase in circulating ketones primarily beta-hydroxybutyrate (**BHBA**) due to oxidation in the liver. The resulting excess production of ketone bodies leads to cows experiencing clinical or subclinical ketosis in early lactation. The major ketone body that is measured in dairy cows to determine subclinical ketosis is beta-hydroxybutyrate (**BHBA**) concentration.

Concentrations of BHBA above 1.2 mmol/L is commonly used as a cut point to classify cows as experiencing symptoms of subclinical ketosis (Iwersen et al., 2009; Konkol et al., 2009, McArt et al., 2011). Subclinical ketosis affects 26.4-55.7% (McArt et al., 2011) of cows and is estimated to cost $78 per case (Geishauser et al., 2001). Various studies have looked at the effects of elevated beta-hydroxybutyrate (**BHBA**) concentration. Elevated BHBA concentration above 1.0 mmol/L increased the likelihood of a displaced abomasum (Geishauser et al., 1997). In addition, BHBA concentration above 1.4 mmol/L indicated an increased risk for metritis (Dohoo and Martin, 1984),

severity of mastitis (Kremer et al., 1993), and days from calving to conception (Cook et al., 2001) and a decrease in both daily milk yield (1.4 kg/d) in early lactation (Dohoo and Martin, 1984) and annual milk yield (393 kg) for multiparous cows (Ospina et al., 2010).

The current tools to measure subclinical ketosis are blood BHBA (Precision Xtra Meter; Abbott Laboratories, Inc., North Chicago, IL), urine acetone and acetoacetate (uristick, Bayer Corp., Pittsburgh, PA), milk acetone and acetoacetate (afimilk, Afimilk USA, Inc., Massillon, OH), and fat:protein ratio in an early lactation milk sample. These tests range in accuracy depending on cut points (sensitivity 27% - 91%; specificity 94% - 99%), but have been used successfully to identify cows with subclinical ketosis. However, these tools identify cows already experiencing the negative effects of subclinical ketosis. In order for a subclinical ketosis model to be most beneficial, it must be easy to use, economically feasible, and able to predict the susceptibility of cows to ketosis. Therefore, a tool is needed to predict cows that are at risk for subclinical ketosis to provide dairy producers the opportunity to implement preventative treatment(s) and reduce the symptoms of subclinical ketosis.

Effects of various silage inoculants on fermentation characteristics of corn silage during short and long term storage in mini-silos.

SUMMARY

Affordable and reliable bacterial silage inoculants that consistently increase the rate and quality of fermentation of corn silage by reducing dry matter (**DM**) loss and improving aerobic stability are needed. Objectives were to determine the effects of bacterial inoculants on pH, yeast and mold counts, *in vitro* starch digestibility, and aerobic stability of whole plant corn silage. We hypothesized that compared with non-

inoculated corn silage; bacterial inoculant would reduce DM loss, decrease pH, and increase lactic acid concentration of ensiled whole corn plant silage. Eighty mini silos were used to evaluate corn silage fermentation characteristics after short (30 d) and long-term (120 d) fermentation with four commercially available homo- or heterofermentative inoculants. Corn silage (DM 40.2%) containing 35.8% starch and 36.6% neutral detergent fiber was harvested from one field using a pull-type John Deere chopper equipped with a kernel processor. Freshly chopped whole corn plant served as the control (CON) and treatments were CON treated with bacterial inoculants 1) Promote HN-3 (PHN; Cargill, Minneapolis, MN), 2) Promote LC-1000 (PLC; Cargill, Minneapolis, MN), or 3) Vigorsile EBL II (VEBL; Provimi, Brookville, OH). Treatments were added before packing and silos were stored at room temperature. Silos were opened after 30, 60, 90, or 120 d of storage. Silage temperature was measured immediately and samples were frozen until further analysis using near-infrared spectroscopy and wet chemistry methods. Data were analyzed using PROC MIXED with 5 reps per treatment and silo as experimental unit. Final DM recovery was greatest with VEBL inoculation than CON, PHN, and PLC while PLC had lower DM recovery than CON and PHN. Aerobic stability was defined as time until temperature rose above 2°C of ambient air temperature and tended to increase with inoculant after 48 hrs compared to CON. Averaged across all time points, addition of PLC and VEBL decreased pH compared with CON. Averaged across all time points, lactic acid concentration in silage was greater for PLC compared with CON, PHN, and VEBL. *In vitro* NDFd was similar among treatments. Corn silage inoculated with PLC had greater 7h in-vitro starch digestibility (**IVSD**) compared with CON, PHN, and VEBL after 90 and 120 d of storage.

The increase in IVSD was likely due to an increase in lactic acid concentration. Treatment had no effect on mold count; however, inoculants had lower yeast count than CON silage. In conclusion, corn silage inoculated with VEBL increased DM recovery. However, PLC increased lactic acid concentration and IVSD.

INTRODUCTION

Preservation of silage quality is a large nutritional and economical factor contributing to the success of dairy operations. By achieving proper silage fermentation, there is a reduction in DM loss and spoilage through the removal of oxygen (Kung, 2001). Affordable and reliable technology that consistently increases the rate and quality of the ensiling process is needed to ensure high quality forage to support high milk production. Volatility of the corn market and meeting energy demands in high producing dairy cows has resulted in an increased sense of urgency to focus on increasing the quality, value, and digestibility of forages.

Greater aerobic stability at feed out has been the focus of improving silage quality in recent years. Previous research indicated that inoculation with *Lactobacillus buchneri* bacteria increased aerobic stability through an increase in acetic acid concentration (Muck, 1996; Ranjit and Kung, 2000; Muck, 2004). Various other studies resulted in mixed results, as inoculation with propionate producing bacteria did not improve aerobic stability (Higginbotham et al., 1998), and in a review by Kung (2001), lactic acid producing bacterial inoculants resulted in improved aerobic stability only 33 percent of the time. This is likely due to a reduction in acetic acid concentration as a result of the increase in lactic acid concentration.

Corn silage is a combination of a fibrous grass and a starchy grain. Additionally, due to increases in dairy feed ingredient costs, nutritionists are formulating diets that

contain a greater proportion of diet DM as corn silage (Allen, 2001). With the greater use of corn silage in diets, there is a greater emphasis on factors that affect digestibility of corn silage fiber and starch. Recent research indicates that an inoculant containing *Lactobacillus casei* and *Lactobacillus buchneri* increased *in vitro* NDF digestibility (NDFd) (Kang et al., 2009). Reich and Kung, (2010) also reported increased *in vitro* NDFd with the application of inoculant containing *Lactobacillus buchneri* 40788 and *Lactobacillus plantarum*.

The starch molecule in corn is bound by a prolamin protein matrix (Mu-Forster and Wasserman, 1998). This prolamin protein matrix is hydrophobic and resists solubilization in water and rumen fluid (Lawton, 2002). However, prolamin is soluble in lactic and acetic acids which are produced during fermentation (Lawton, 2002). Previous studies have shown that starch digestibility increases with increased storage time (Jurjanz and Monteils, 2005; Hoffman et al., 2011) as the result of fermentation and bacterial and enzymatic proteolytic activity. Objectives of this research were to determine if varying bacterial inoculant strain increases silage fermentation, silage quality, and nutrient digestibility.

MATERIALS AND METHODS

Whole corn plant harvest and inoculation:

A corn hybrid (Pioneer 36V51, Pioneer Hi-Bred International, Des Moines, IA) was grown at the University of Minnesota, St. Paul, MN. Corn was harvested at the 2/3 milk line stage and 40% DM and chopped to a 3/4 inch theoretical cut length with a two row JD 3975 chopper equipped with a kernel processor. Chopped forages (90.7 kg) were treated with 475 mL of water (**CON**), Promote HN-3 (**PHN;** Cargill, Minneapolis, MN),

Promote LC-1000 (**PLC;** Cargill, Minneapolis, MN), or Vigorsile EBL II (**VEBL;** Provimi, Brookville, OH). Inoculant was applied using a spray bottle with continuous mixing. Application of PHN delivered 10^5 cfu/g of a mixture of *Pediococcus* species and *Lactobacillus plantarum*. Application of PLC delivered 10^5 cfu/g of a mixture of *Pediococcus acidilactici*, *Pediococcus pentosaceous* and *Lactobacillus plantarum*. Application of VEBL delivered 2×10^5 cfu/g of a mixture of *Propionibacterium freudenreichii*, *Lactobacillus plantarum*, and *Pediococcus acidilactici*. Bacteria concentrations added to silages were based on suppliers recommendations and guaranteed concentrations of bacteria. Fresh samples were collected and refrigerated (2°C) at the time of inoculation to conduct microbiology analysis to determine amount of inoculant on the corn silage (Cargill laboratory, Elk River, MN).

After inoculation, silage was packed with a hand powered mechanical jack in 10.2 × 45.7 cm polyvinyl chloride (PVC) drain tile mini silos to achieve packing density of 205.6 kg of DM/m^3. Mini silos were sealed with rubber lids with a one way gas release valve and secured with metal hose clamps. Five replicates of each treatment for each time point of 30, 60, 90, and 120 d ensiling were prepared giving a total of 80 mini silos. Weights of the empty and full silos were recorded and silos were stored indoors at room temperature of approximately 21°C and away from direct sunlight. Silos were opened at their respective time points of 30, 60, 90, or 120 d and at each silo opening, final weights were recorded to determine DM recovery. Silos were then emptied and contents divided into three aliquots and frozen (-20°C) for future analysis. After 120 d ensiling, one non-frozen sample was analyzed for yeast and mold counts using plating technique (Rtech laboratories, St. Paul, MN). Aerobic stability of corn silage was determined for each

mini silo by placing 0.5 kg of silage into a 1.9 L polystyrene bucket covered with 2 layers of cheesecloth. Temperature was measured by inserting a digital thermometer in the center of silage every 24 hours until temperature increased 2°C above ambient air temperature (21°C).

Laboratory analysis:

Frozen samples were submitted to a commercial laboratory (Dairyland Laboratories Inc., St. Cloud, MN) for wet chemistry analysis using the following analytical methods. Samples were dried for 3 hours at 105°C (Shreve et al., 2006) to determine DM. Corn silage pH was determined utilizing Analytical Methods Guide, Orion Research, May 1977. Volatile fatty acids were determined in aqueous acid extracts by high performance liquid chromatography (Canale et al., 1984). Acid detergent fiber (**ADF**) was measured using AOAC Official Method 973.18 (Goering and Van Soest, 1970; AOAC, 1996) and modified by using sea sand for a filtering aid as needed, and neutral detergent fiber (**NDF**) was measured using AOAC Official Method 2002.04 (AOAC, 2005) and modified by using sea sand as a filtering aid and Whatman GF/C filter paper for residue collection. *In vitro* NDFd was determined by the method of (Goering and Van Soest, 1970) with the modification that a composite inoculum containing strained ruminal fluid and blended ruminal solids was used for incubation. The residue after incubation was analyzed for NDF using the procedure above. Starch was determined using an enzymatic method described by Knudsen (1997). *In vitro* starch digestibility was determined using the technique of Richards et al. (1995) with the modification that a composite inoculum containing strained ruminal fluid and blended

ruminal solids was used for incubation. Then residue after incubation was analyzed for starch using the procedure described above.

Statistical analysis:

This experiment was a completely randomized design and data were analyzed using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Individual silo served as the experimental unit. Before analyses, data were tested for normality using Proc Univariate. Measurements over time were processed using the REPEATED statement in the MIXED procedure with 3 covariance structures: compound symmetry, auto-regressive order 1, and heterogeneous auto-regressive order 1. The covariance structure that resulted in the lowest Akaike information criterion was used (Littell et al., 1996). The model included treatment, time, and treatment by time interactions. Data not analyzed over time were subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996). Least squares means for treatment, time, and treatment by time interactions were separated using the PDIFF statement. Significant differences were declared at $P < 0.05$ and trends discussed when $0.05 > P < 0.10$.

RESULTS

Physical and chemical composition

Nutrient and fermentation characteristics of whole plant corn silage vary greatly based on variety, moisture content, and growing season. Nutrient and initial characteristics of whole corn plant before ensiling are in Table 1.1. Recovery of corn silage DM increased ($P < 0.01$) with EBL inoculation compared with CON, PHN, and PLC (Table 1.2). Also, PLC inoculation of corn silage decreased ($P < 0.01$) DM

recovery compared to CON and PHN. Corn silage pH decreased ($P < 0.01$) over time and was affected ($P < 0.01$) by treatment (Figure 1.1). Average pH across all time points ranged from 3.64 to 3.73 which was similar to the pH reported by Kung et al. (2000) at 3.72 after 60 d of ensiling. After 60 and 90 days ensiled (**DS)** all inoculated corn silage had a lower ($P < 0.01$) pH than CON (Figure 1.1). The pH of PLC silage was lower ($P < 0.01$) at 60 and 90 DS than that of PHN. Corn silage treated with PLC had a lower ($P < 0.01$) pH than VEBL treated silage after 90 DS.

The change in pH can be attributed to increased production of lactic acid ($P < 0.01$) with length of time ensiled (Figure 1.2). The PLC treated silage had the greatest ($P < 0.01$) overall lactic acid concentration compared to CON, PHN and VEBL treated silages (Table 1.3). Acetic acid concentration was affected ($P = 0.02$) by the interaction of treatment and time. Silage treated with VEBL tended ($P = 0.08$) to have a lower acetic acid concentration at 30 DS than PHN and PLC (Figure 1.3). However, after 120 DS, VEBL had a greater ($P < 0.05$) acetic acid concentration than CON, PHN or PLC (Figure 1.3).

Nutrient composition

Averaged across all time points, control and treated silages were similar in ADF and NDF content (Table 1.2). *In vitro* NDFd was similar among treatments (Figure 1.4). The 7 h IVSD of CON and treated silages (Figure 1.5) indicate starch digestibility increased with time ensiled for all treatments, but increases for PLC were greater than PHN, VEBL, and CON (Table 1.2). A general linear trend in increased starch digestibility was found for all treatments with 120 d IVSD values greater ($P < 0.01$) than 30 d values.

Fermentation Indices of Corn silage ensiled for 120 d

Temperature (aerobic stability) of exposed silage to air was similar among treatments through 48h, increased ($P < 0.01$) by 72 h and decreased through 96 h (Figure 1.6). Temperature tended to be lower ($P = 0.09$) at 72h for PHN, PLC, and VEBL compared to CON. After 96h, PLC and VEBL had lower temperature than CON ($P < 0.01$) and PHN ($P < 0.05$). After 120 DS, mold counts were similar (non-detectable) among treatments (Table 1.3). Yeast counts were not detected in PHN, PLC, and VEBL compared to CON which had a low count of 35.4 cfu/g (Table 1.3).

DISCUSSION

The purpose of these inoculants was to deliver additional lactic acid producing bacteria to the freshly chopped whole corn plant to increase anaerobic fermentation. The PHN and PLC inoculants contained homofermentative bacteria and the VEBL inoculant contained heterofermentative bacteria. Homofermentative inoculants function to reduce pH quickly because of their ability to produce two molecules of lactic acid from one molecule of glucose (Contreras-Govea and Muck, 2006). Heterofermentative inoculants are less efficient because they produce one molecule of lactic acid, one molecule of acetic acid or ethanol, and one molecule of carbon dioxide from one molecule of glucose (Contreras-Govea and Muck, 2006). Our initial corn silage characteristics were similar to previous research, after 6 h of fermentation, Kung et al. (2000) measured a pH of 5.69, lactate concentration of 0.69 % of DM, and an acetate concentration of 0.17 % of DM. Initial pH of fresh corn silage was 6.52 (Kung et al., 2000). Fresh corn silage *in vitro* NDFd was measured at 73.4% of NDF (Hunt et al., 1993). Fresh corn silage *in vitro* starch digestibility ranged from 54% of starch (Windle et al., 2014) to 62% of starch

(Ferraretto et al., 2014). The difference in reported fresh corn silage values for IVSD to our values may be attributed to variation in laboratory analysis procedures, corn hybrid, and corn maturity. Our results indicate there was an increase in lactic acid production when whole plant corn silage was treated with PLC. The high lactic content of PLC silage is consistent with it having the lowest mean pH and the lowest pH after 90 and 120 h of ensiling. Although the heterofermentative VEBL inoculant reduced silage pH below that of CON overall (Table 1.3) and at 60 and 90 DS (Figure 1.1), there was no difference in overall lactate content (Table 1.3) or at any of the ensiling endpoints (Figure 1.2). Previous research (Aksu et al., 2004; Arriola et al., 2011) also has shown that addition of inoculants containing homo- and heterofermentative bacteria can decrease pH and increase lactic acid production during fermentation.

Dry matter content of the fermented silage across all treatments was lower than the fresh silage (37 vs. 40%). The reduction in actual dry matter of the silage can be attributed to fermentation. This reduction is similar to that observed by Cleale et al. (1990) and Kleinschmit et al. (2006a). However, dry matter recovery of corn silage was increased with homofermentative inoculation of *Pediococcus acidilactici* and *Lactobacillus xylosus* than untreated silage (90.4% vs. 87.9%) (Cleale et al., 1990). However, Kleinschmit et al. (2006a) measured similar DM recovery with inoculation with *Lactobacillus buchneri* (heterolactic) and *Pediococcus pentosaceus* (homolactic) compared to control. In a meta-analysis, Kleinschmit and Kung (2006b) reported that relative to untreated silage, DM recovery decreased when silage was inoculated with *Lactobacillus buchneri* (heterolactic). In the present study, DM recovery was improved

with VEBL (combination of hetero- and homo-fermentative bacteria) treatment compared to CON.

Typical homofermentative inoculants produce higher concentrations of lactic acid and thus increase the ratio of lactic to acetic acid being produced (Muck, 2004; Contreras-Govea and Muck, 2006). Acetic acid is a natural yeast and mold inhibitor (Narendranath et al., 2001) and less acetic acid production can reduce aerobic stability. However, aerobic stability of silages treated with inoculants and the impact of reduced acetic acid has been variable (Muck, 2012). In 2000, Ranjit and Kung demonstrated the benefits of inoculants on aerobic stability due to lower pH as time exposed to air increased. It was expected that aerobic stability would increase with the addition of the heterofermentative VEBL. Although, acetic acid concentration increased after 120 d of ensiling, aerobic stability of VEBLwas similar to PHN and PLC.

Interestingly, our research showed that the inoculants only increased aerobic stability sometime after 48 hours. The greater aerobic stability after 48 h may be due to a greater proliferation of yeast (not measured) in the CON silage as it was the only silage to have a measurable yeast count at the time of opening. The homo- and heterofermentative inoculants used in this study performed similarly. Previous research has found increased *in vitro* NDFd when *Lactobacillus buchneri* inoculants have been applied (Aksu et al., 2004; Reich and Kung, 2010). Contrary to the previous research, NDFd in our study was not increased by inoculants. For all silage treatments, NDFd was lower 120 d after ensiling than pre-ensiling. The inoculants used in previous studies (Aksu et al., 2004; Reich and Kung, 2010) contained *L. buchneri* which was not included in our inoculants and could account for the differences in NDFd between inoculant studies.

Our PLC treatment contained no proteases, but produced silage with the highest lactic acid content and had greater IVSD than CON. Corn is high in prolamins-zein which are hydrophobic and are not solubilized in water or broken down in rumen fluid (Lawton, 2002). These prolamins-zein make up 50-60 percent of the protein in corn (Hamaker et al., 1995) and can be broken down during the fermentation process because they are soluble in lactic and acetic acid (Lawton, 2002). However, according to (Hoffman et al., 2011), it is more likely that this increase in IVSD is attributed to proteolytic activity instead of solely solubilization in lactic and acetic acid. In a recent study, Windle et al. (2014) reported an increase in IVSD when corn silage was inoculated with an exogenous protease. This supports findings by Hoffman et al. (2011) that IVSD is not improved solely because of increased storage time and exposure to lactic and acetic acid.

CONCLUSIONS

The inoculants did not alter silage ADF, NDF, or NDFd. The PLC inoculant decreased pH, increased lactic acid concentration, increased IVSD, and reduced DM recovery relative to untreated silage. Although it is not clear if the increased IVSD was sufficient to offset the reduction in DM recovery. Although IVSD was not altered, DM recovery and aerobic stability of corn silage treated with VEBL were greater than untreated silage. Concluding statement: The bacterial inoculants PLC and VEBL used in this study appear to be useful tools for modifying fermentation towards greater preservation and the production of higher quality end products from corn silage.

Table 1.1. Characteristics of whole plant, freshly chopped corn silage shortly after inoculation, but before ensiling.

	Treatments[1]			
Variable	CON	PHN	PLC	VEBL
DM, %	40.17	39.83	40.02	39.91
pH	5.41	5.16	5.20	5.41
Starch, % of DM	35.24	34.72	34.93	35.76
Sugar, % of DM	0.09	0.10	0.09	0.09
Ash, % of DM	3.29	4.09	3.10	2.97
Lactate, % of DM	0.57	0.24	0.36	0.43
Acetate, % of DM	0.27	0.16	0.28	0.19
ADF, % of DM	20.56	22.22	23.11	22.73
NDF, % of DM	36.58	38.35	40.02	39.98
NDFd[2], % of NDF	55.82	56.19	54.15	53.78
IVSD[3], % of Starch	83.84	83.20	81.65	81.52

[1] Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**).
[2]Neutral detergent fiber digestibility, 30-h.
[3]*In vitro* starch digestibility, 7-h.

Table 1.2. Effect of ensiling treatment on nutrient analysis[A] of corn silage.

	Treatments[1]					*P*-value		
Variable	CON	PHN	PLC	VEBL	SEM[2]	Trt[3]	T[4]	Trt*T[5]
DM, %	37.56[b]	37.15[bc]	36.70[c]	38.30[a]	0.17	<0.01	0.02	<0.01
DM Recovery,%	92.41[b]	92.16[b]	90.78[c]	94.82[a]	0.22	<0.01	0.94	0.27
ADF, % DM	22.77	22.64	22.46	22.58	0.25	0.85	0.36	0.26
NDF, % DM	37.44	37.31	37.09	37.23	0.40	0.94	0.28	0.47
NDFd[6], % NDF	48.71	48.70	48.95	48.48	0.40	0.87	0.71	0.21
IVSD[7], % Starch	86.98[b]	87.37[b]	88.04[a]	87.10[b]	0.17	<0.01	<0.01	0.03

[A] Averaged across all time points (30, 60, 90, 120 days) of ensiling.

[a-c] Means within a row with different superscripts differ ($P < 0.05$).

[1] Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**).

[2] Largest standard error.

[3] Effect of inoculant.

[4] Effect of time ensiled.

[5] Interaction of treatment with time.

[6] *In vitro* neutral detergent fiber digestibility, 30-h.

[7] *In vitro* starch digestibility, 7-h.

Table 1.3. Effect of treatment on fermentation characteristics of corn silage for averages across all time points.

	Treatments[1]					*P*-value		
Variable	CON	PHN	PLC	VEBL	SEM[2]	Trt[3]	T[4]	Trt*T[5]
pH	3.73[a]	3.70[ab]	3.64[c]	3.69[b]	0.01	<0.01	<0.01	0.20
Lactate, % of DM	5.04[b]	5.24[b]	5.92[a]	5.20[b]	0.13	<0.01	<0.01	0.37
Acetate, % of DM	0.84	0.80	0.78	0.85	0.04	0.54	0.16	0.02
Temperature[6], °C	20.74	20.51	20.32	20.50	0.22	0.63	<0.01	0.04
Mold count, CFU/g	ND	ND	ND	ND	---	---	---	---
Yeast count, CFU/g	35.40	ND	ND	ND	---	---	---	---

[a-c] Means within a row with different superscripts differ ($P < 0.05$).

ND = Non-detectable amounts.

[1]Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**).

[2] Largest standard error.

[3]The effect of inoculant.

[4] Time effect, at 30, 60, 90, 120 days ensiled.

[5] Interaction of treatment * time.

[6] Measured every 24 h by placing thermometer into center of 0.5 kg of silage in a 2 qt. polystyrene bucket covered with 2 layers of cheesecloth.

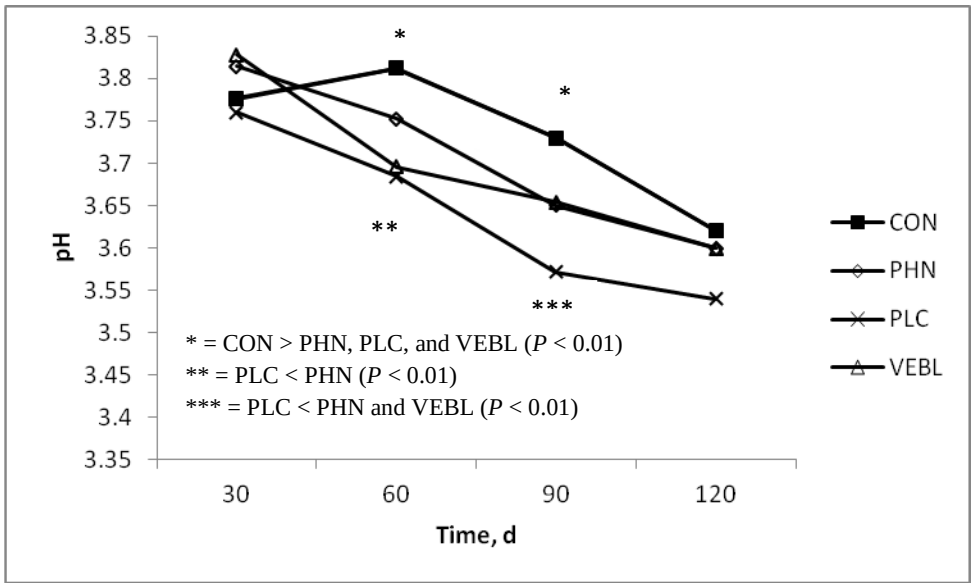

Figure 1.1. Effect of treatment on corn silage pH after 30, 60, 90, and 120 days ensiling. Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**). Largest SEM = 0.01, Treatment: $P < 0.01$, Time: $P < 0.01$, Treatment × Time: $P = 0.20$. For clarity, SEM bars have been omitted.

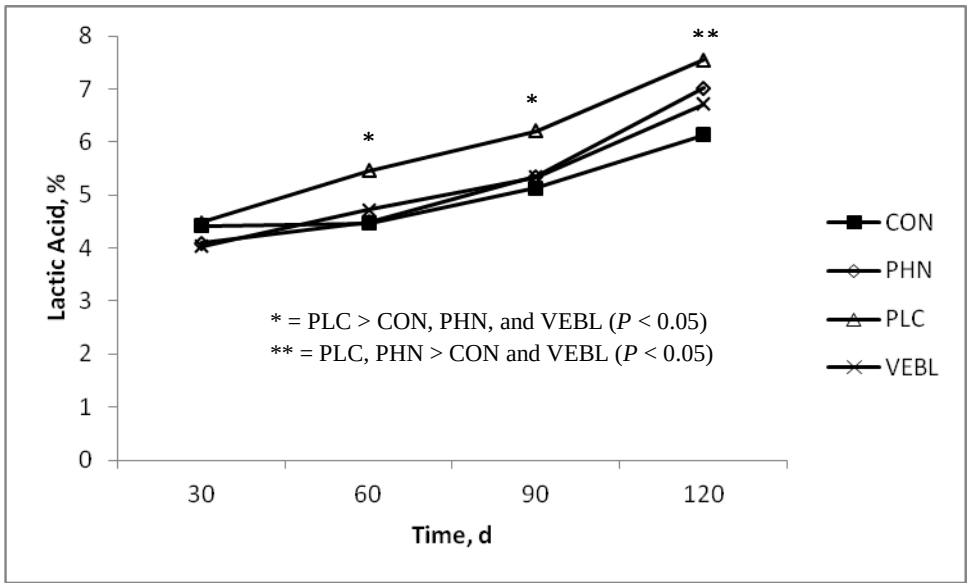

Figure 1.2. Effect of treatment on corn silage lactic acid percent after 30, 60, 90, and 120 days ensiling. Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**). Largest SEM = 0.13, Treatment: $P < 0.01$, Time: $P < 0.01$, Treatment × Time: $P = 0.37$. For clarity, SEM bars have been omitted.

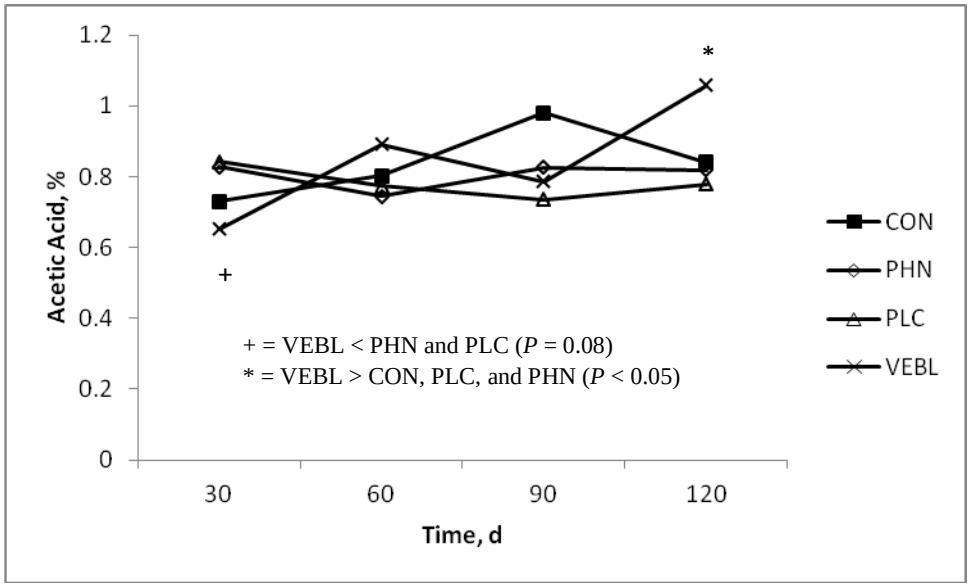

Figure 1.3. Effect of treatment on corn silage acetic acid percent after 30, 60, 90, and 120 days ensiling. Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**). Largest SEM = 0.04, Treatment: $P = 0.54$, Time: $P = 0.16$, Treatment × Time: $P = 0.02$. For clarity, SEM bars have been omitted.

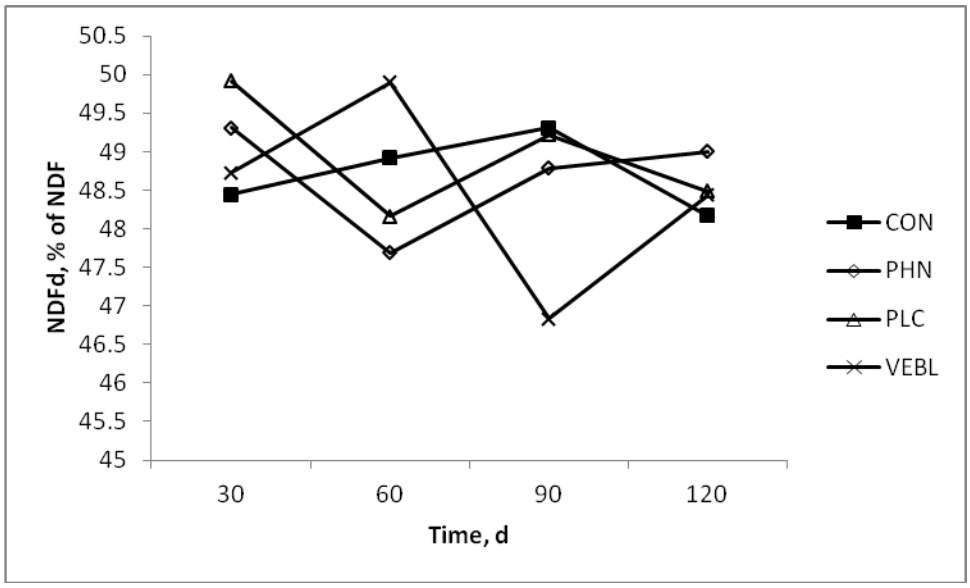

Figure 1.4. Effect of treatment on corn silage 30 hour neutral detergent fiber digestibility (NDFd) measured after 30, 60, 90, and 120 days of ensiling. Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**). Largest SEM = 0.40, Treatment: $P = 0.87$, Time: $P = 0.71$, Treatment × Time: $P = 0.21$. For clarity, SEM bars have been omitted.

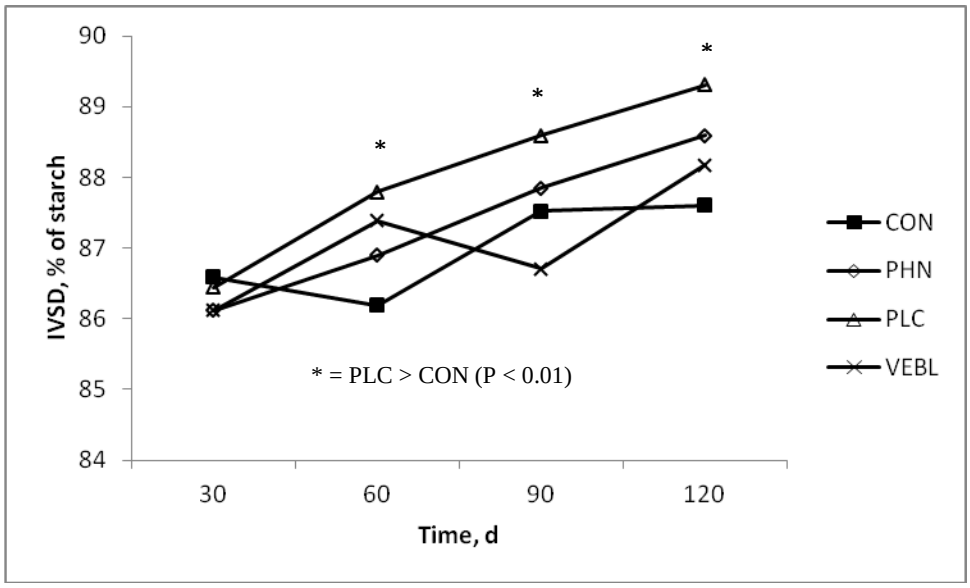

Figure 1.5. Effect of treatment on corn silage on 7 hour *in vitro* starch digestibility (IVSD) measured after 30, 60, 90, and 120 days ensiling. Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**). Largest SEM = 0.17, Treatment: $P < 0.01$, Time: $P < 0.01$, Treatment × Time: $P = 0.03$. For clarity, SEM bars have been omitted.

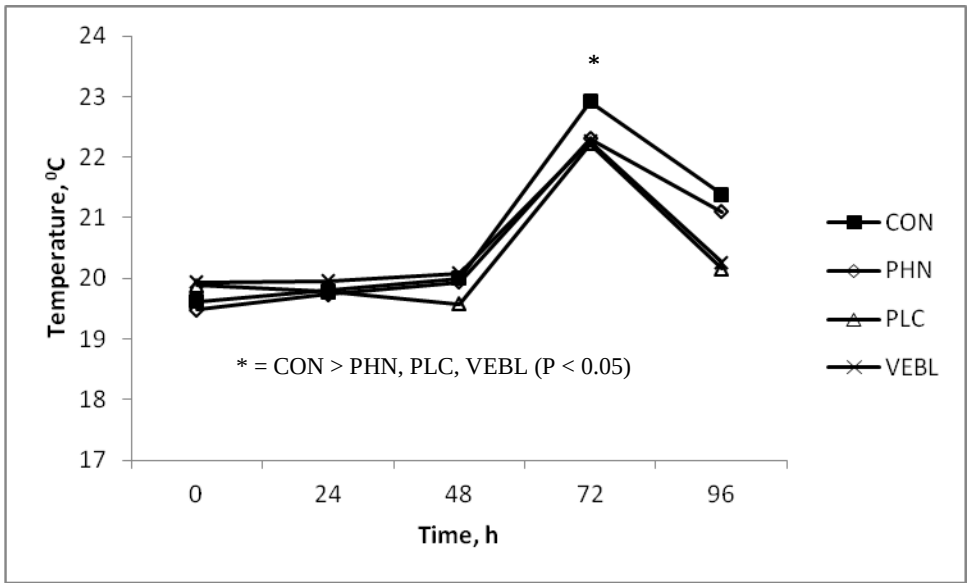

Figure 1.6. Effect of treatment on temperature (measured every 24 h) of exposed corn silage after 120 days of ensiling. All silages had similar aerobic stability, defined as the interval from opening to the point at which silage temperature increased more than 2°C, through 48 h. Although all silages were deemed unstable at 72 h, temperature of all inoculated silages were less than that of CON. Corn silage treated with water (**CON**), Promote HN-3 (**PHN**), Promote LC-1000 (**PLC**), or Vigorsile EBL II (**VEBL**). Largest SEM = 0.22, Treatment: $P = 0.63$, Time: $P < 0.01$, Treatment × Time: $P = 0.04$. For clarity, SEM bars have been omitted.

Effects of various silage inoculants and packing density on fermentation characteristics of corn silage after 30 and 90 day storage in mini-silos.

SUMMARY

Affordable and reliable inoculants that consistently increase the rate and quality of fermentation of corn silage by reducing dry matter loss and increasing aerobic stability are needed. Objectives were to determine the effect of various inoculants at low (135.7 kg of DM/m^3) and high (205.6 kg of DM/m^3) packing density on silage pH, yeast and mold counts, and aerobic stability. We hypothesized that inoculants would decrease pH and increase lactic and acetic acid concentration resulting in increased aerobic stability. Forty-eight PVC mini silos were used to evaluate corn silage fermentation characteristics after short (30 d) and long (90 d) term fermentation with four commercially available homo- or heterofermentative inoculants. Corn silage (DM 36.4%) containing 39.0% starch and 36.3% neutral detergent fiber was harvested from one field using a chopper equipped with a kernel processor. Freshly chopped silage was treated with water control (CON), or treated with Promote AP (**AP**), Promote EBL (**EBL**), or Promote BP (**BP**) before packing at low or high packing density and stored at room temperature. Immediately after opening the silos, samples were frozen until further analysis by near-infrared spectroscopy and wet chemistry. Data were analyzed using PROC MIXED with 6 reps (silos) per treatment. After 90 d ensiled, corn silage inoculated with BP had higher pH, lower lactate, higher acetate concentration, and increased aerobic stability than CON, AP, and EBL. Digestibility of NDF and mold count was not affected by treatment. However, corn silage inoculated with BP tended to have greater yeast count than CON, AP, and EBL. Overall, aerobic stability, yeast and mold count, pH, and acetic acid

concentration were similar for low and high packing density silages. High packing density silage tended to have greater lactic acid concentration compared to low packing density. In conclusion, inoculants did not increase NDFd and corn silage inoculated with BP had increased acetic acid concentration and greater aerobic stability.

INTRODUCTION

Preservation of silage quality is a large nutritional and economical factor contributing to the success of dairy operations. Removal of oxygen from the silage, helps achieve a proper fermentation, and reduce DM loss and spoilage (Kung, 2001). Affordable and reliable technologies that consistently increase the rate and quality of the ensiling process are needed to ensure availability of high quality forage to support high milk production. Volatility in the market value of corn has resulted in an increased sense of urgency to focus on increasing the quality, value, and digestibility of forages. Kung and Nylon (2001) showed that loosely packed silage had greater yeast counts and Ruppel (1992) demonstrated that loosely packed silage has greater potential for shrink and DM loss. Loosely packed silage would also be predisposed to poor aerobic stability due to a more aerobic environment. Previous research indicated that silage inoculation with *Lactobacillus buchneri* increased aerobic stability through an increase in acetic acid concentration (Ranjit and Kung, 2000; Muck, 2004). Inoculation with propionate producing bacteria did not increase aerobic stability (Higginbotham et al., 1998). In a review by Kung (2001) lactic acid producing bacterial inoculants increased aerobic stability only 33 percent of the time. The mixed result with homofermentative inoculants indicates the need for a more reliable and consistent inoculant that helps produce silages with greater aerobic stability.

Corn silage is a combination of a fibrous grass and a starchy grain. Due to increases in dairy feed ingredient costs, nutritionists are formulating diets that contain a greater proportion of the diet DM as corn silage (Allen, 2001) which results in a need for greater emphasis on efforts to increase digestibility of both fiber and starch from corn silage. Recent research indicates that an inoculant containing *Lactobacillus casei* and *Lactobacillus buchneri* increased *in vitro* NDFd (Kang et al., 2009). Reich and Kung, (2010) also reported an increase in *in vitro* NDFd with the application of inoculant containing *Lactobacillus buchneri* 40788 and *Lactobacillus plantarum*. Objectives of this research were to determine the impact of packing density and inoculants that contained *L. buchneri* or *L. plantarum* with other bacterial species on fermentation, aerobic stability, and nutrient digestibility of corn silage.

MATERIALS AND METHODS

Corn silage production:

A corn hybrid (Pioneer 36V51, Pioneer Hi-Bred International, Des Moines, IA) was grown at the University of Minnesota-twin cities research field at the St. Paul, MN campus. Corn was harvested at the 2/3 milk line stage and 36% DM and chopped with a two row JD 3975 chopper equipped with a kernel processor to a 3/4 inch theoretical cut length. The chopped whole corn plant silage (75 kg/treatment) was inoculated with 475 mL of water (**CON**), Promote® AP (**AP;** Provimi North America Inc., Brookville, OH), Promote® EBL (**EBL;** Provimi North America Inc., Brookville, OH), or Promote® BP (**BP;** Provimi North America Inc., Brookville, OH). Application of AP delivered 10^5 cfu/g of a mixture of *Pediococcus acidilactici, Pediococcus pentosaceus,* and *Lactobacillus plantarum*. Application of EBL delivered 2×10^5 cfu/g of a mixture of

Propionibacterium freudenreichii, *Lactobacillus plantarum*, and *Pediococcus acidilactici*. Application of BP delivered 5×10^5 cfu/g of a mixture of *Lactobacillus buchneri, Pediococcus acidilactici,* and *Propionibacterium freudenreichii.* Inoculant was applied by dissolving in water in a spray bottle and applied with continuous mixing immediately before packing.

The 4 x 2 factorial arrangement of inoculant and corn silage packing density (low, L, 135.7 kg of DM/m^3 or high, H, 205.6 kg of DM/m^3) treatments resulted in 8 treatments: **CON-H**, **CON-L**, **AP-H**, **AP-L**, **EBL-H**, **EBL-L**, **BP-H**, and **BP-L**.

Immediately after inoculation, samples were collected and stored in the refrigerator at (2°C) for subsequent microbiology analysis (Cargill laboratory, Elk River, MN) to determine the amount of inoculant on the corn silage. Inoculated, chopped whole corn plant was packed with a hand powered mechanical jack into 10.2 × 45.7 cm polyvinyl chloride (PVC) drain tile mini silos at 135.7 or 205.6 kg of DM/m^3. Mini silos were sealed with rubber lids with a one way gas release valve and secured with metal hose clamps. Six replicates of each treatment at 2 ensiling durations (30 or 90 d) were prepared giving a total of 96 mini silos.

Weights of the empty and full silos were recorded and silos were stored away from direct sunlight at a room temperature of 21°C. When opened, final weights were recorded to determine DM recovery. Silos were then emptied and divided into three aliquots which were stored (-20°C) for future analysis. After 90 d ensiling, one sample from each silo was analyzed for yeast and mold counts (Cargill, Elk River, MN). Aerobic stability was assessed by placing 0.5 kg of silage back into its opened PVC mini silo, covering it with 2 layers of cheesecloth and placing a temperature data logger (UA-

001-08 HOBO® Pendant® temperature data logger, Onset Computer Corp., Bourne, MA) into the center of the silage. Mini silos were then placed for 7 days in growth chambers set to maintain temperatures of 37°C for 16 hours and 35°C for 8 hours at 80 percent humidity to simulate a hot summer day. Temperature was recorded every twenty minutes. Aerobic stability was assessed by determining the interval required to increase silage temperature 2°C above growth chamber air temperature.

Laboratory analysis:

Frozen samples were submitted to a commercial laboratory (Cargill, Elk River, MN) for analysis. Samples were dried for 3 hours at 105°C (Shreve et al., 2006) to determine DM. Corn silage pH was determined using methods by Erdman (1988), and volatile fatty acid content was determined using modified procedures by Siegfried et al. (1984). Acid detergent fiber (**ADF**) was measured using AOAC Official Method 989.03 (AOAC, 1995). Neutral detergent fiber (**NDF**) was measured using AOAC Official Method 989.03 (AOAC, 1995). *In vitro* NDFd was determined by the method of (Goering and Van Soest, 1970) with the modification by Cargill, Elk River, MN. Starch was determined using procedures described by Holm et al. (1986) with slight modification by Cargill, Elk River, MN.

Statistical analysis:

This experiment used a 2 x 4 x 2 factorial arrangement of treatments (silage packing density, inoculant, and time) and effects were assessed using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Individual silo was the experimental unit. Before analyses, data were tested for normality and distribution using Proc Univariate. The statistical model included density, inoculant, and density × inoculant interactions.

Data was analyzed separately for each time point. Data was subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996). Least squares means for density, inoculant, and density × inoculant interactions were separated using the PDIFF statement. Significant effects were declared at $P < 0.05$ and trends discussed when $0.05 > P < 0.10$.

RESULTS

Physical and chemical composition

Initial corn silage microbial populations are displayed in Table 2.1. Initial corn silage characteristics are displayed in Table 2.2. After 30 d ensiling, there was an interaction of inoculant and density as CON-H had lower ($P < 0.05$) pH than treatments CON-L, EBL-H, EBL-L, and BP-L (Table 2.3). Treatments AP-H, AP-L, and BP-H had lower ($P < 0.05$) pH than treatments CON-L and BP-L (Table 2.3). Even though there was a change in pH, lactic acid concentration was similar among treatments (Table 2.3). However, there was an interaction of inoculant × density ($P = 0.02$) for acetic acid concentration as treatment CON-L had greater ($P < 0.05$) acetic acid concentration than treatment EBL-H and EBL-L (Table 2.3). Also, there was an interaction of inoculant × density ($P < 0.01$) for propionic acid concentration as treatment CON-L had greater ($P < 0.05$) propionic acid concentration than treatments AP-H, EBL-H, and EBL-L (Table 2.3). Overall VFA concentrations after 30 d ensiling were similar among treatments (Table 2.3).

After 90 d ensiling, inoculant had an effect on pH as CON, AP, and EBL had lower ($P < 0.01$) pH than BP and averaged 3.86, 3.85, 3.83, and 4.05 ± 0.04 respectively. Silages with high packing density had greater ($P = 0.01$) lactic acid concentrations than

those with low packing density (8.67 and 8.06 ± 0.23%, respectively). Lactic acid concentration of CON was greater ($P < 0.05$) than EBL and BP and averaged 9.21, 8.32, and 7.14 ± 0.25% respectively, and BP had lower ($P < 0.05$) lactic acid concentration than AP and EBL and averaged 7.14, 8.79, and 8.32 ± 0.25% respectively. Acetic acid concentration in corn silage was increased ($P < 0.01$) with BP inoculant compared to CON, AP, and EBL and averaged 2.58, 1.90, 1.72, and 1.58 ± 0.15% respectively. Propionic acid concentration was lower ($P < 0.01$) for corn silage inoculated with EBL compared to CON and BP and averaged 0.33, 0.41, and 0.44 ± 0.02% respectively. Total VFA production was greater ($P < 0.01$) in CON corn silage compared to EBL and BP and averaged 11.53, 10.23, and 10.16 ± 0.26% respectively. Also, high packing density increased ($P < 0.01$) total VFA production compared to low packing density and averaged 11.05 and 10.36 ± 0.20% respectively.

Nutrient composition

Dry matter was similar among treatments after 30 d ensiled (Table 2.4). The interaction of inoculant × density tended ($P = 0.06$) to affect acid detergent fiber (**ADF**) as CON-H had greater ($P < 0.05$) ADF than treatments AP-L and BP-H after 30 d ensiled. In addition, inoculant × density tended ($P = 0.05$) to affect neutral detergent fiber (**NDF**) as treatment CON-H had greater ($P < 0.05$) NDF than treatments AP-L and BP-H. Also, in *vitro* (**NDFd**) measured at 30 h, tended to be greater ($P = 0.10$) for treatment CON-L compared to treatments CON-H, AP-H, and BP-L after 30 d ensiled.

After 90 d ensiled, DM tended ($P = 0.06$) to be lower for BP compared to CON, AP, and EBL and averaged 35.35, 36.80, 36.72, and 37.07 ± 0.66% respectively. Corn silage inoculated with BP had greater ($P = 0.03$) ADF and tended ($P = 0.08$) to have

greater NDF compared to EBL, but all treatments were similar to CON corn silage. Corn silage NDFd was similar among treatments after 90 d ensiling.

Fermentation Indices of Corn silage ensiled for 90 d

Corn silage aerobic stability was increased ($P < 0.01$) with treatment EBL-L, BP-H, and BP-L compared to treatments CON-H, CON-L, AP-H, AP-L, and EBL-H (Table 2.5). Corn silage aerobic stability was increased ($P < 0.01$) with BP compared to CON, AP, and EBL and averaged 168.0, 118.6, 97.8, and 136.0 ± 7.9 hrs respectively. Also, corn silage inoculated with EBL had greater ($P < 0.05$) aerobic stability than AP. Mold count was similar among treatments (Table 2.5). However, corn silage inoculated with BP had greater ($P < 0.01$) yeast count than CON, AP, and EBL and averaged 1189.4, 47.0, 30.0, and 0.3 ± 355.5 cfu/g.

DISCUSSION

The AP inoculant provided homofermentative bacteria while the EBL and BP inoculants provided heterofermentative bacteria. The purpose of these inoculants was to deliver additional lactic acid producing bacteria to the fresh corn silage to increase fermentation. Homofermentative inoculants function to reduce pH quickly because of their ability to produce two molecules of lactic acid from one molecule of glucose (Contreras-Govea and Muck, 2006). Heterofermentative inoculants are less efficient because they produce one molecule of lactic acid, one molecule of acetic acid or ethanol, and one molecule of carbon dioxide from one molecule of glucose (Contreras-Govea and Muck, 2006). In the present study all inoculants were effective at dropping the pH below 4.0 after 30 d ensiled. However, after 90 d ensiled corn silage inoculated with BP had a slightly higher pH than the other treatments. The increased pH for BP was similar to the

results by Kleinschmit and Kung (2006b). This is likely due to greater acetic acid concentration in corn silage inoculated with BP. Since acetic acid (pKa 4.76 at 25°C) (Sergeant and Dempsey, 1979) is not as strong of an acid as lactic acid (pKa 3.86 at 25°C) (Sergeant and Dempsey, 1979), the corn silage inoculated with BP would be expected to have a higher pH.

A meta-analysis by Kleinschmit and Kung (2006b) reported inoculants containing *L. buchneri* had higher pH, lower lactic acid concentration, and greater acetic acid concentration compared to untreated silage. In addition, the current results are similar to previous research that found that *L. buchneri* inoculants produce greater acetic acid concentrations (Ranjit and Kung, 2000; Muck, 2004). Acetic acid is a natural yeast and mold inhibitor (Narendranath et al., 2001) and thus provides greater aerobic stability. In the present study, corn silage inoculated with BP had greater yeast count than the other treatments. However, the number of colony forming units is still well below 100,000 cfu/g which is the level of yeast count that begins to be detrimental to aerobic stability (Seglar, 2003). Corn silage inoculated with BP had increased aerobic stability compared to the other treatments as a result of having greater acetic acid concentration regardless of packing density which was similar to the previous research trials utilizing *L. buchneri* (Ranjit and Kung, 2000; Muck, 2004).

Compared with low packing density, our high packing density silages had increased lactic acid concentrations. This result would be expected as lactic acid producing bacteria are aerotolerant anaerobes and function more efficiently under anaerobic conditions (Axelsson, 2004). Previous research done by Kung and Nylon (2001) demonstrated that loosely packed alfalfa silage had a slower rate of fermentation

measured by a slower rate of decline in pH. In addition, Kung and Nylon (2001) measured an increase in yeast counts in loosely packed silage. However, in the present study packing density did not affect yeast and mold counts. The low yeast and mold count suggest our method of packing in the PVC mini silos, even at low packing density, was successful at removing oxygen quickly and promoting anaerobic conditions. Aerobic stability in the present study was not affected by packing density and this is likely due to having very low yeast and mold counts.

CONCLUSIONS

The BP inoculant increased acetic acid concentration, decreased lactic acid concentration, and increased aerobic stability. The NDFd was similar among treatments. High packing density increased lactic acid concentration, but had similar yeast and mold counts and aerobic stability compared to low packing density. In conclusion, corn silage inoculated with BP performed consistently at low and high packing densities to improve aerobic stability and corn silage fermentation.

Table 2.1. Bacterial counts of fresh corn silage (one sample per treatment) after inoculation but before packing.

Treatment[1]	CFU/g 1×10^5
CON-H	2.90
CON-L	3.68
AP-H	2.00
AP-L	1.99
EBL-H	2.03
EBL-L	2.87
BP-H	3.62
BP-L	2.87

[1] Corn silage was treated with water (**CON**) and packed in mini-silos at high (205.6 kg of DM/m3, **CON-H**) or low (135.7 kg of DM/m3, **CON-L**) density, with Promote AP at high (**AP-H**) or low (**AP-L**) density, with Promote EBL at high (**EBL-H**) or low (**EBL-L**) density, or with Promote BP at high (**BP-H**) or low (**BP-L**) density.

Table 2.2. Characteristics of freshly chopped corn silage before inoculation.

Variable	CON
DM, %	36.4
pH	5.9
Starch, % of DM	39.0
Lactate, % of DM	0.3
Acetate, % of DM	0.0
ADF, % of DM	19.3
NDF, % of DM	36.3
NDFd[1], % of NDF	47.2

[1]*In vitro* neutral detergent fiber digestibility, 30-h.

Table 2.3. Fermentation characteristics of corn silage ensiled with inoculants and packed at low and high densities for 30 or 90 d.

Treatment[1]	Variable				
	pH	Lactate, % of DM	Acetate, % of DM	Propionate, % of DM	Total VFA, % of DM
Day 30					
CON-H	3.89[c]	8.20	1.58[ab]	0.43[ab]	10.21
CON-L	3.94[a]	7.26	2.00[a]	0.48[a]	9.74
AP-H	3.90[bc]	8.05	1.51[ab]	0.39[b]	9.95
AP-L	3.91[bc]	7.50	1.65[ab]	0.42[ab]	9.57
EBL-H	3.93[ab]	8.16	1.34[b]	0.41[b]	9.90
EBL-L	3.93[ab]	8.20	1.30[b]	0.40[b]	9.90
BP-H	3.91[bc]	7.63	1.74[ab]	0.42[ab]	9.80
BP-L	3.94[a]	8.42	1.77[ab]	0.45[ab]	10.65
SEM[2]	0.01	0.31	0.14	0.02	0.24
P-value					
Inoculant[3]	0.49	0.49	<0.01	<0.01	0.34
Density[4]	<0.01	0.48	0.21	0.06	0.99
Inoculant* density[5]	0.05	0.13	0.02	<0.01	0.11
Day 90					
CON-H	3.79	9.77	1.96	0.43	12.16
CON-L	3.93	8.65	1.85	0.40	10.90
AP-H	3.84	8.74	1.69	0.37	10.80
AP-L	3.86	8.84	1.74	0.40	10.97
EBL-H	3.87	8.47	1.78	0.34	10.59
EBL-L	3.80	8.17	1.39	0.31	9.87
BP-H	4.02	7.69	2.53	0.43	10.64
BP-L	4.09	6.60	2.63	0.44	9.67
SEM[2]	0.05	0.33	0.15	0.02	0.34
P-value					
Inoculant[3]	<0.01	<0.01	<0.01	<0.01	<0.01
Density[4]	0.32	0.01	0.40	0.87	<0.01
Inoculant* density[5]	0.22	0.19	0.35	0.53	0.19

[a-c] Means within a column with different superscripts differ ($P < 0.05$).

[1] Corn silage was treated with water (**CON**) and packed in mini-silos at high (205.6 kg of DM/m3, **CON-H**) or low (135.7 kg of DM/m3, **CON-L**) density, with Promote AP at high (**AP-H**) or low (**AP-L**) density, with Promote EBL at high (**EBL-H**) or low (**EBL-L**) density, or with Promote BP at high (**BP-H**) or low (**BP-L**) density.

[2] Largest standard error.

[3] Effect of inoculant.

[4] Effect of packing density.

[5] Interaction of inoculant and packing density.

Table 2.4. Nutrient characteristics of corn silage ensiled with inoculants and packed at low and high densities for 30 or 90 d.

Treatment[1]	Variable			
	DM, %	ADF, % DM	NDF, % DM	NDFd[2], % NDF
Day 30				
CON-H	36.98	22.67	43.11[a]	45.95
CON-L	36.76	22.62	42.88[a]	47.54
AP-H	37.39	21.73	40.84[ab]	46.57
AP-L	37.23	20.60	39.22[b]	46.85
EBL-H	37.30	21.58	41.22[ab]	45.90
EBL-L	37.63	21.40	40.75[ab]	46.14
BP-H	37.25	21.17	39.65[b]	46.73
BP-L	37.11	22.99	42.95[a]	45.57
SEM[3]	0.41	0.57	1.00	0.47
P-value				
Inoculant[4]	0.49	0.07	0.05	0.34
Density[5]	0.87	0.80	0.75	0.50
Inoculant* density[6]	0.89	0.06	0.05	0.10
Day 90				
CON-H	37.24	22.71	43.05	45.15
CON-L	36.36	21.25	39.68	46.54
AP-H	37.02	20.77	38.50	46.54
AP-L	36.42	21.84	40.38	45.76
EBL-H	36.43	20.60	39.14	45.04
EBL-L	37.70	19.92	37.58	45.08
BP-H	34.93	22.49	41.75	46.07
BP-L	35.77	23.09	41.64	46.16
SEM[3]	0.66	0.83	1.46	0.86
P-value				
Inoculant[4]	0.06	0.03	0.08	0.56
Density[5]	0.74	0.85	0.45	0.76
Inoculant* density[6]	0.29	0.41	0.33	0.65

[a-b] Means within a column with different superscripts differ ($P < 0.05$).

[1] Corn silage was treated with water (**CON**) and packed in mini-silos at high (205.6 kg of DM/m3, **CON-H**) or low (135.7 kg of DM/m3, **CON-L**) density, with Promote AP at high (**AP-H**) or low (**AP-L**) density, with Promote EBL at high (**EBL-H**) or low (**EBL-L**) density, or with Promote BP at high (**BP-H**) or low (**BP-L**) density.

[2] Neutral detergent fiber digestibility, 30-h.

[3] Largest standard error.

[4] Effect of inoculant.

[5] Effect of packing density.

[6] Interaction of inoculant and packing density.

Table 2.5. Aerobic stability, mold count, and yeast count of corn silage after 90 d ensiled.

Treatment[1]	Variable		
	Aerobic stability[2], hrs	Mold count, CFU/g	Yeast count, CFU/g
CON-H	115.90[bc]	0.67	25.00
CON-L	120.75[b]	27.67	69.00
AP-H	90.75[c]	14.00	13.83
AP-L	104.75[bc]	14.67	16.17
EBL-H	118.75[bc]	0.00	0.00
EBL-L	153.33[a]	0.67	0.50
BP-H	168.00[a]	0.00	1414.00
BP-L	168.00[a]	0.00	964.83
SEM[3]	11.46	10.95	355.51
P-value			
Inoculant[4]	<0.01	0.35	<0.01
Density[5]	0.23	0.37	0.75
Inoculant * density[6]	<0.01	0.52	0.07

[a-c] Means within a column with different superscripts differ ($P < 0.05$).

[1] Corn silage was treated with water (**CON**) and packed in mini-silos at high (205.6 kg of DM/m3, **CON-H**) or low (135.7 kg of DM/m3, **CON-L**) density, with Promote AP at high (**AP-H**) or low (**AP-L**) density, with Promote EBL at high (**EBL-H**) or low (**EBL-L**) density, or with Promote BP at high (**BP-H**) or low (**BP-L**) density.

[2] Interval from silo opening until silage temperature increased to more than 2°C above that of ambient temperature.

[3] Largest standard error.

[4]Effect of inoculant.

[5]Effect of packing density.

[6] Interaction of inoculant and packing density.

Effects of varying periparturient dietary starch amount and supplementation with *Propionibacterium* on performance, metabolism, and health of multiparous dairy cows.

SUMMARY

Multiparous dairy cows (n = 17/treatment) were used in a 2 × 2 factorial arrangement of dietary starch (low or high) and direct fed microbial (**DFM**) to determine if daily supplementation with *Propionibacterium freudenreichii* strain P169, **(**DuPont Industrial and Biological Sciences, Waukesha, WI) altered response to periparturient diets that differed in starch concentration. Cows were assigned to one of four treatments 42 d prepartum. Factors were low starch (LS; 15.5% prepartum and 20.1% postpartum) or high starch (HS; 26.7% prepartum and 29.7% postpartum) and DFM (20 g/d of control, DFM carrier or carrier plus P169 providing approximately 6×10^{11} cfu/head/day *P. freudenreichii* at the time of feeding. Cows were housed in a tie-stall barn, fed *ad libitum* once daily and milked twice daily. Pre- and postpartum dietary starch was increased by replacing corn silage and soy hulls with ground corn which reduced dietary NDF. Control and DFM was top-dressed at feeding using a calibrated scoop starting 21 d pre- through 56 d postpartum. Factors combined to form four treatments; 1) low starch + carrier (**LSC**), 2) low starch + P169 (**LSM**), 3) high starch + carrier (**HSC**), 4) high starch + P169 (**HSM**). Data were analyzed using PROC MIXED in SAS as a 2 × 2 factorial arrangement with model including starch, DFM, week, and breed. We hypothesized that DFM would increase milk yield in low and high starch diets. Prepartum DMI was lower for DFM compared with Control but was not altered by STARCH. Postpartum DMI tended to be lowest for LSM. Interaction of high starch and DFM increased milk yield on weeks 5, 6, 7, and 8 over LSM and HSC. Fat corrected

milk yield was similar among treatments. Feed efficiency was increased by DFM. Serum NEFA and BHBA increased at calving and were similar among treatments. Postpartum liver triaclyglycerol and glycogen concentration were similar among treatments. Feeding behavior was monitored once pre- and postpartum before d -21 and after d 21 using 10 minute visual scans for a 24h period. Time spent ruminating postpartum increased with HS and cows fed HS spent 2.0 ± 0.9 h/d more time standing than LS. In summary, cows fed HSM produced greater milk yield than LSM and HSC but was similar to LSC, milk yield was similar for LSC, LSM and HSC, and feed efficiency was increased by DFM for both LS and HS.

INTRODUCTION

The transition period is a critical time three weeks before and three weeks after parturition (Grummer et al., 1995; Drackley, 1999). In early lactation cows enter a period of negative energy balance due to high energy demands for milk production and an inability to meet these demands through dry matter intake. This is typically the most stressful period for a dairy cow, so a majority of the metabolic and infectious diseases such as milk fever, ketosis, retained fetal membranes, metritis, mastitis, and displaced abomasum happen during this interval (Drackley, 1999). Effects of these metabolic and infectious diseases reduce milk production and overall profitability of individual dairy cows (Wallace et al., 1996). However, an increase in the supply of glucose in early lactation reduces non-esterified fatty acids (**NEFA**) and beta-hydroxybutrate (**BHBA**) concentrations and associated metabolic disorders such as fatty liver and ketosis (Burhans and Bell, 1998).

Glucose is the primary nutrient required by the mammary gland for metabolism (Bell, 1995). Due to a negative energy balance in early lactation, metabolism shifts from lipogenesis to lipolysis to provide NEFA as a glucogenic precursor from adipose tissue (McNamara, 1991). Ruminants rely heavily on gluconeogenesis for glucose production. Therefore, an increase in glucogenic precursors can increase glucose production. Propionate is the single most important precursor for gluconeogenesis (Drackley et al., 2001). Propionate also inhibits hepatic lipid oxidation, thus it is antiketogenic (Drackley, 1999). Since propionate is the major glucogenic precursor and NEFA are the primary metabolic fuels extensively utilized by the liver in early lactation (Emery et al., 1992), early lactation diets that promote propionate production will increase milk production, reduce lipid oxidation, and reduce severity of negative energy balance.

Utilizing direct fed microbials (**DFM**, *Propionibacterium freudenreichii* strain P169 (**P169**), (DuPont Industrial and Biological Sciences, Waukesha, WI) during the transition period to reduce the duration and severity of negative energy balance and increase propionate production for increased gluconeogenesis has been a key focus for many research projects in recent years. The use of *Propionibacterium* strain P169 has been chosen for its ability to convert lactate into propionate. Previous research measured an increase in propionate production in the rumen when P169 was supplemented at a rate of 6×10^{11} cfu/d (Stein et al., 2006; Weiss et al., 2008). Theoretically, the increase in propionate production would lead to an increase in gluconeogenesis as evidenced by an increase in lactose concentration (Stein et al., 2006). However, plasma glucose concentration was similar to control when P169 was supplemented at a rate of 6×10^{10} cfu/d (Francisco et al., 2002) or 6×10^{11} cfu/d (Weiss et al., 2008). Previous research has

reported a decrease of 1.0 kg/d of DMI when P169 was fed at a rate of 6×10^{11} cfu/d (Weiss et al., 2008) and 3.6 g/kg of body weight when lactating cows were fed P169 at approximately 6×10^{10} cfu/d (Francisco et al., 2002) (feeding rate reported by Stein et al., 2006). Despite the decrease in DMI when P169 was fed in these studies, milk yield was similar among treatments (Francisco et al., 2002; Weiss et al., 2008). However, supplementation of P169 increased milk yield by 3.2 kg/d in a study by Stein et al. (2006). It is not known if cow response to P169 is dependent upon the dietary starch concentration or if the response can be altered by supplementing cows with P169 during the transition period.

Previous studies have demonstrated that over-feeding energy to dry cows results in a reduction in gluconeogenesis, decreased DMI and an increase in rate of postpartum lipolysis (Holtenius et al., 1996; Douglas et al., 2007; Janovick et al., 2011). Postpartum DMI decreased by 1.5 kg/d with increasing dietary starch concentration (21.0% vs. 25.5%) (Nelson et al., 2011) and by 2.4 kg/d (21.8% vs. 27.1%) (Gencoglu et al., 2010). Moderate dietary starch concentration (23.2%) increased milk yield by 5.7 kg/d over high starch (25.5%) but was similar to low starch (21.0%) (Nelson et al., 2011). High dietary starch (27.1%) had similar milk yield to low starch (21.8%), however, 3.5% FCM was reduced by 2.9 kg/d for high starch compared to low starch (Gencoglu et al., 2010).

There are also concerns with feeding high starch diets to fresh cows due to an increased flux of propionate (Oba and Allen, 2003a). It has been suggested that excessive amounts of propionate delivered to the liver would increase the amount of propionate being oxidized in the TCA cycle which could decrease the firing rate of the hepatic vagus nerve resulting in increased satiety (Allen et al., 2009). Conversely, lower

amounts of propionate in circulation may result in a reduction in the stimulation of the hepatic vagus nerve increasing its firing rate resulting in greater DMI (Allen et al., 2009).

Recent research has focused on the implications of feeding groupings of cows based on stage of lactation (Allen, 2012). Based on Allen et al. (2009) hepatic oxidation theory (**HOT**), feeding a single lactation TMR, which is formulated for peak milk production, may result in over-feeding starch in early lactation leading to a reduction in DMI. As a result, milk production might be compromised and risk of metabolic disorders might increase. A successful fresh cow diet should maximize DMI emphasizing fiber digestibility which might result in increased energy balance and reduction in lipolysis (Litherland et al., 2013).

There have been previous studies evaluating the effects of feeding diets varying in starch concentration in early lactation. Replacing corn with soy hulls resulted in a tendency for a decrease in milk yield driven mostly by soy hull feeding rates of 30 and 40% of diet DM (Ipharraguerre et al., 2002). Decreased dietary starch concentration (32.9 to 17.6%) by replacing shell corn and soybean meal with wheat middlings, dried brewers grains, and soy hulls decreased DMI and increased milk fat content (Batajoo and Shaver, 1994). Another study (Dann et al., 2008) found no differences in DMI or milk yield when feeding dietary starch concentrations of 17.7, 21.0, and 24.6 % of diet DM when corn grain was replaced with dried beet pulp, wheat middlings, and distillers grains. Replacing high moisture corn with pelleted beet pulp (34.6, 30.5, 26.5 and 18.4% starch) linearly decreased DMI but 3.5% FCM tended to increase quadratically with 30.5% starch being the greatest (Voelker and Allen 2003). Dry cracked corn was replaced by dried citrus pulp to decrease dietary starch from 31.0 to 20.0% of diet DM. Feeding the

low starch diet reduced DMI, milk yield, fat yield, and true protein content (Broderick et al. 2002). Thus, replacing starch with non-forage fiber did not necessarily decrease milk production.

Prepartum feeding strategies for optimal postpartum performance and health have not yet been clearly defined. Prepartum treatment with propionibacteria has resulted in some positive improvements in postpartum hormone (Francisco et al., 2002; Aleman et al., 2007; Lehloenya et al., 2008) and metabolite profiles (Stein et al., 2006) as well as increased postpartum energetic efficiency (Weiss et al., 2008). Objectives of this study were to determine the effects of periparturient starch concentration and P169 supplementation on periparturient DMI, milk production, lipid metabolism, and health. We hypothesized that supplementation with P169 would increase milk yield for both low and high starch treatments.

MATERIALS AND METHODS

Animal Housing and Management

This experiment was conducted from September 2011 to March 2012 at the University of Minnesota Dairy Teaching and Research Center, St. Paul, MN. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Sixty- eight multiparous Holstein and Holstein-cross (Holstein × Montbeliard × Swedish Red) dairy cows were assigned to one of four dietary treatments (as designed to provide a 2 x 2 factorial design) 42 d prior to projected calving date and removed from the study after 56 days in milk (**DIM**). Cows were housed in individual tie stalls with rubber mattresses and bedded with sawdust in a mechanically cross-ventilated barn. Cows were moved to a calving area once calving appeared

imminent and were returned to their tie-stalls after calving. Cows were milked twice daily (0200 and 1400 h) in a milking parlor and cows were fed once daily during the dry period (1300 h) and once daily after calving (1100 h).

Assignment to treatment and treatment description

Prepartum treatments were balanced by breed, lactation number (mean ± SEM = 3.2 ± 0.3 lactations), previous lactation 305-d mature equivalent for milk production (11,020 ± 455 kg), and BCS at dry-off (3.58 ± 0.2 units) (Table 3.1). Ingredients and nutrient compositions of the prepartum diets is shown in Table 3.2 with treatments including: 1) low starch + control (direct fed microbial (**DFM**) carrier (sucrose)) (**LSC**); 2) low starch + DFM (*Propionibacterium freudenreichii* strain P169 (**P169**), DuPont Industrial and Biological Sciences, Waukesha, WI) (**LSM)**; 3) high starch + control (**HSC**); 4) high starch + DFM (**HSM**). Prepartum treatments were continued after parturition through 56 DIM. Diets were formulated using the Cornell-Net-Carbohydrate-Protein-System (**CNCPS**, version 6.1; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) to supply adequate ME and MP for a 650 kg dry cow with a 3.5 body condition score at 280 d in gestation prepartum and postpartum a 650 kg cow producing 40 kg of milk with fat concentration of 3.7%. Diets (Table 3.2) were fed at an *ad libitum* rate (to ensure 10 % feed refusals) and were formulated to provide varying amounts of dietary starch by varying the amount of corn silage, dry corn, and soybean hulls to obtain prepartum dietary starch differences of (low, 15.5 ± 0.8% and high, 26.7 ± 0.8%) and postpartum (low, 20.1 ± 0.7% and high, 29.7 ± 0.8%). Diets were formulated to test the effects of interactions of dietary starch concentration with the addition of P169,

20 g/cow/d delivering 6×10^{11} cfu, which was top dressed daily at the time of feeding starting 21days before expected calving and continued through 56 DIM. Alfalfa hay and wheat straw were chopped in a vertical mixer for forty minutes to reduce particle size to a uniform consistency between forages. Tap water was added to both diets to maintain equal DM among treatments. Individual ingredient DM was maintained weekly by drying in a 60°C oven for 24h and adjusting ingredient as-fed amounts. Low and high starch rations were designed to provide similar macro and micro-mineral amounts (Table 3.2). Individual diet ingredients were sampled weekly, frozen at -20°C and composited monthly on a wet weight basis. Wet samples were analyzed at Dairyland Laboratories (St. Cloud, MN) using wet chemistry methods. Monthly averages of the nutrient composition of individual ingredients were analyzed and used in the CNCPS dairy model to calculate monthly diet nutrient composition and the average of these diets is reported in (Table 3.2). Ash content was determined using method 942.05 (AOAC International, 1996). Crude protein was determined using method 990.03 (AOAC International, 2000). Neutral detergent fiber for feed ingredients was determined using method 2002.04 (AOAC International, 2005). Acid detergent fiber was determined using method 973.18 (AOAC International, 1996). Lignin was determined using method 973.18 (AOAC International, 1996). Concentrations of minerals in feed were determined by method 953.01 (AOAC International, 1995). Water soluble carbohydrate was determined with extraction procedure adopted from Derias (1961). Detection procedure utilized from Neutral Detergent- Soluble Carbohydrates Nutritional Relevance and Analysis (Hall, 2000). Starch was determined using an enzymatic method described by Knudsen (1997). Mean particle size of the coarsely ground corn was determined by shaking in a sieve

(ASTM sieve, Gilson Company, Inc., Worthington, OH) (utilizing screen sizes of 4.75 mm, 2.36 mm, 0.85 mm, 0.60 mm, and a pan) and calculation of weighted average was conducted utilizing ASAE method S319.2 (ASAE, 1993).

Nutrient Intake

Individual cow DMI was measured daily from dry-off to 56 DIM. Feed offered and refused were recorded electronically at 0930 h. Daily DMI was calculated by correcting as-fed offered minus refusals and multiplied by weekly diet DM for each treatment. Energy balance, both pre- and postpartum, was calculated using equations described by National Research Council (NRC) (2001). Briefly, net energy intake (NE_I) was determined by multiplying DMI by the calculated mean NE_L density of the diet using CPM Dairy (Version 3.0.8; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY). Weekly means for prepartum DMI and BW in addition to number of days dry and calf body weight were used as model inputs for prepartum energy balance calculations. Postpartum energy balance was calculated using inputs of weekly averages of DMI, BW, milk yield, and milk composition.

Body Weight, Body Condition Score and Rumen Fill Score

Body weight was measured, body condition score (**BCS**) assigned using 0.25-unit increments (Ferguson et al., 1994), and rumen fill score (**RFS**) assigned on a scale from 1-5 according to Zaaijer and Noordhuizen (2003) for each cow weekly from dry-off to fifty-six DIM. Two trained individual scorers assigned BCS and RFS weekly before morning feeding and averages of the two scores are reported.

Colostrum Yield and Composition

Colostrum was harvested after calving and individual cows were milked last at the next milking session after calving. Colostrum yield was recorded and two samples from the first milking were collected and frozen at -20°C. One frozen sample was sent to Colorado State Veterinary Diagnostic Laboratories (Colorado State University, Fort Collins, CO, 80523) where total IgG concentration was determined using the Bovine IgG Vet-RID (radial immune-diffusion) kit (Triple J Farms, Bellingham, WA, 98226). Due to high concentrations of IgG, the sample was first diluted 10-fold with distilled water then 5 μl of dilluted colostrum sample was analyzed according to kit instructions. After the samples were placed on the plates they were left at room temperature for a minimum of 18 h, and then the precipitation ring diameters measured and IgG values calculated. Three standards with known values (196, 1,402, and 2,748 mg/dl) were also tested in each run. The diameters of the known standards were then used to calculate the tested colostrum samples using a programmable calculator. The other sample was thawed and analyzed at room temperature for specific gravity using a brix refractometer (Reichert Rhino VET 360, Reichert, Depew, NY).

Milk Yield and Composition

Milk weights were recorded from 1 to 56 DIM and composite samples were obtained from consecutive a.m. and p.m. milking's weekly from 4 to 56 DIM. Milk samples were preserved (800 Broad Spectrum Microtabs II; D and F Control Systems, Inc. San Ramon, CA) and were analyzed for fat, protein, lactose, milk urea N, and SCC using mid-infrared procedures (AOAC, 1995) at a commercial laboratory (DHIA, Zumbrota, MN).

Concentrations of Serum NEFA, BHBA, and total blood calcium

Blood was sampled from the coccygeal vein or artery on d -43, -28, -14, -7, -3 and -1 relative to expected parturition and at +1, +7, +14, +21, and +28 d and 12 h and 24 h after parturition for total blood calcium analysis. Samples were collected before feeding (0800) into evacuated serum tubes (SST; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing clot activator. Serum was obtained by centrifugation for 20 minutes at 1,300 × *g*. Aliquots of serum were frozen at -20°C until later analysis for contents of NEFA (NEFA-HR(2) kit; Wako chemicals USA, Inc., Richmond VA), BHBA (Precision Xtra Meter; Abbott Laboratories, Inc., North Chicago, IL), and total blood calcium (Calcium Arsenazo III kit, Pointe Scientific, Inc., Canton, MI)

Liver composition

Liver was sampled via puncture biopsy (Hughes, 1962; Veenhuizen et al., 1991) from cows under local anesthesia at approximately 0700 h on d -14 (to determine effects of dry period treatments), +7 and +14 (to determine effect of dry period treatments on the immediate lipid accumulation following parturition). Before biopsy, local anesthesia was infiltrated in the intercostal space posterior to the tenth rib followed by aseptic preparation of the surgical site with Vedadine® (Vedco Inc., St. Joseph, MO). Liver cores were frozen immediately in liquid N, transferred to a -80°C freezer and later analyzed for contents of total lipids (Hara and Radin, 1978), triaclyglycerol (Fletcher, 1968; Foster and Dunn, 1973), and glycogen (Lo et al., 1970).

Prepartum diet sorting analysis and feeding behavior

Orts were collected at 0700 h and TMR collected at 1100 h from individual feed bunks for three consecutive day's prepartum (d -21, -20 and -19) and were combined together to form a composite sample. Particle size distribution was determined by

shaking procedure described by Kononoff et al. (2003) in a Penn State Particle Separator (**PSPS**) and TMR and orts were dried at 60^0C for 48 hours. Intake of particles on an as-fed basis was calculated for each screen for each cow as difference between diets offered and refused. Sorting was calculated using guidelines described by Leonardi and Armentano (2003); as the actual intake of each pan expressed as a percentage of the predicted intake, where predicted intake is percent of TMR delivered in each pan. Selective particle consumption was defined using guidelines described by Leonardi et al. (2005); values equal to 100 percent represent no sorting, < 100 percent show selective refusals, and > 100 percent indicate preferential consumption. Feeding behavior of eating, ruminating, and standing were monitored once prepartum between d -28 to d -21 relative to expected calving and postpartum between d 21 to 35 relative to actual calving using 10 minute scans for a continuous 24h period. Number of events were recorded and multiplied by 10 minutes to determine the amount of time a day cows spent eating, ruminating, and standing according to Endres et al. (2005).

Statistical analysis

Statistical analysis was completed using SAS® version 9.3 (SAS Institute Inc., Cary, NC). Data were analyzed as a 2 × 2 factorial design with factors being starch amount (STARCH) and DFM. Before analyses, data were tested for normality and distribution using Proc Univariate. One cow was removed from LSC due to leg injury. Twinning and resulting leg injury led to the loss of one cow from LSP. Also, two cows were culled from the herd for injury during calving from LSP. Two cows were removed due to lameness from LSP. One cow was fed a high starch diet and therefore moved treatments from LSP onto HSP. Two cows were removed from HSC due to

complications with milk fever, and one cow was removed due to severe lameness. One cow was removed due to severe lameness and three cows were removed from HSP due to complications with milk fever. The resulting cow numbers per treatment are outlined in Table 3.3. Data with daily measurements were averaged weekly and multiple measurements over time were processed using the REPEATED statement in the MIXED procedure with 3 covariance structures: compound symmetry, auto-regressive order 1, and heterogeneous auto-regressive order 1. The covariance structure that resulted in the lowest Akaike information criterion was used (Littell et al., 1996). The model included breed, STARCH, DFM, time, and interactions between STARCH, DFM, and time. Data not analyzed over time were subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996). Least squares means for STARCH, DFM, time, and all interactions were separated using the PDIFF statement. Prepartum and postpartum data sets were analyzed separately. Significant data were declared at $P < 0.05$ and trends are discussed when $P < 0.10$.

RESULTS

Prepartum DMI, energy balance, rumen fill, body condition, body weight, and diet sorting

There were no differences for STARCH ($P = 0.45$) or DFM ($P = 0.12$) on prepartum DMI, however, low starch (**LS**) with DFM supplementation tended ($P = 0.08$) to decrease prepartum DMI (Table 3.3). DMI expressed as a percent of BW was similar among treatments. Prepartum DMI decreased ($P < 0.01$) as calving date approached (Figure 3.1). At week minus five, LSM had lower ($P = 0.05$) DMI compared to LSC and tended ($P = 0.09$) to be lower than HSM. At week minus four, LSM tended to have

lower DMI ($P = 0.10$) than LSC. At week minus two, LSM tended ($P < 0.10$) to have lower DMI than HSC and HSM, and was lower ($P < 0.01$) than LSC. The week prior to calving, LSM had lower ($P = 0.02$) DMI compared to LSC and HSM. The reduction in DMI for LSM resulted in a lower ($P < 0.01$) energy balance than LSC and HSM; however, all cows remained in a positive energy balance prepartum. Increase in the dietary concentration of STARCH or the addition of DFM did not affect rumen fill score (Table 3.3). Body condition score and body weight were similar among treatments (Table 3.3). Selection against particles in the top box of the Penn State shaker box increased ($P < 0.01$) for HSM cows compared to LSM and HSC but not for LSC (Table 3.4).

Postpartum DMI, energy balance, rumen fill, body condition, and body weight

Postpartum LS with DFM supplementation tended ($P = 0.06$) to reduce DMI (Figure 3.1). At week two through eight, DMI for LSC was greater ($P < 0.05$) than LSM. At week two ($P = 0.07$), and three ($P = 0.09$), LSM tended to have lower DMI than HSC. At week two, LSM had lower ($P = 0.05$) DMI than HSM and tended ($P = 0.09$) to have lower DMI at week three. At week eight, HSM had greater ($P < 0.05$) DMI than LSM, LSC had greater ($P < 0.05$) DMI than LSM, but HSC and HSM had similar DMI. Increasing the dietary STARCH concentration increased ($P = 0.03$) energy balance (Table 3.3) as DMI was similar for LS and high starch (**HS**), but HS diet had greater energy density. Rumen fill score, body condition score, and body weight were similar among treatments (Table 3.3).

Milk, milk component yield, and feed efficiency

Milk yield was increased by HS and supplementation with DFM ($P = 0.04$) (Figure 3.2). Milk yield tended ($P = 0.08$) to be greater for LSC at week two and four than LSM. Milk yield on week two tended ($P = 0.07$) to be greater for HSM than LSM, and HSM had greater milk yield ($P < 0.05$) at weeks three through six than LSM. Milk production for HSM tended ($P < 0.10$) to be greater at weeks four through seven than LSC and greater at week eight ($P = 0.03$). Yield of 3.5% FCM was similar among treatments. Compared with Control, supplementation with DFM increased ($P = 0.05$) feed efficiency. STARCH and DFM did not alter milk fat percent or true protein concentration (Table 3.5). The interaction of HS and DFM tended ($P = 0.07$) to increase true protein yield due to an increase in milk production by HSM vs. LSM (Table 3.5).

Prepartum liver total lipid, liver triaclyglycerol, liver glycogen and serum NEFA and BHBA

Increasing dietary STARCH concentration tended ($P = 0.09$) to decrease blood serum NEFA (Figure 3.3) and the addition of DFM also tended ($P = 0.06$) to increase blood serum NEFA (Table3. 6). Serum concentration of BHBA was similar among treatments (Figure 3.4). Liver total lipid and triaclyglycerol concentration were similar among treatments (Table 3.6). However, liver glycogen content was increased ($P = 0.01$) with greater dietary STARCH as LSC had lower ($P = 0.03$) liver glycogen than HSC and lower ($P = 0.01$) glycogen than HSM.

Postpartum liver total lipid, triaclyglycerol, glycogen, and serum NEFA, BHBA, and blood calcium homeostasis

Neither STARCH or DFM altered serum NEFA (Figure 3.3) or serum BHBA concentration (Figure 3.4) (Table 3.6). Liver total lipid (Figure 3.5), triaclyglycerol

(Figure 3.6), and glycogen concentration (Figure 3.7) were also similar among treatments (Table 3.6). Serum total calcium at 12 h was similar among treatments (Table 3.6), however, at 24 h after parturition LSC tended ($P = 0.08$) to have greater blood total calcium than HSC.

Calf birth weight, colostrum yield, and quality

Calf birth body weight, colostrum yield, colostrum IgG concentration, and colostrum specific gravity were similar among treatments (Table 3.7).

Feeding behavior pre- and postpartum

Prepartum feeding behavior indicated that cows fed LSC spent the greatest ($P = 0.03$) amount of time eating (Table 3.8). Time spent ruminating was similar ($P = 0.19$) among treatments. Cows fed HS tended ($P = 0.06$) to spend more time standing than those fed LS (Table 3.8). Cows fed LSM postpartum had decreased ($P = 0.05$) eating time compared to LSC (Table 3.8). Compared with LS, HS tended ($P = 0.08$) to increase time spent ruminating and increased ($P = 0.02$) standing time (Table 3.8).

DISCUSSION

DMI, rumen fill, energy balance, body weight, and body condition

Dry matter intake was measured to study the effects of varying starch concentration and supplementation of DFM during the periparturient period. It has been suggested (Allen, 2012) that starch concentration should be adjusted according to stage of lactation. Starch digestibility is also a key factor in dairy rations not just starch amount, as starch is not a nutrient required by dairy cattle (Chase, 2007). According to Allen et al. (2009) lowering dietary starch concentration should result in increased DMI early in lactation due to the reduction of the amount and rate of propionate absorption in the

rumen resulting in less hepatic oxidation of propionate signaling satiety. Feeding a low starch diet 22.1 % vs. 27.0% increased DMI (Ferraretto et al., 2011). However in the current study, LSC had numerically the greatest DMI but was not statistically greater than HSC or HSM.

The supplementation of DFM decreased DMI early in lactation by 1 kg/d (Weiss et al., 2008) and 3.6 g/kg of BW (Franscisco et al., 2002). Our data shows that supplementation with DFM in HS diets did not affect DMI. However, when DFM was added to LS diets DMI was reduced by 1.6 kg/d prepartum and 3.4 kg/d postpartum. Reasons for decreased DMI beginning five weeks prepartum for LSM are not clear. At the start of the trial, DMI was similar among treatments (Figure 3.1). An increase in propionate yield was measured when cows were fed a high starch diet compared to a low starch diet (Oba and Allen, 2003b). Also, Oba and Allen (2003a) determined that increased amount of propionate flux around meal time, decreases DMI. The reduction in DMI might be due to the flux of propionate produced in the rumen at the time of feeding with the supplementation of DFM. Previous studies (Stein et al., 2006; Weiss et al., 2008) feeding P169 measured an increase in propionate production in the rumen. Also utilizing another *Propionibacteria* strain, Raeth-Knight et al. (2007) measured a tendency for increased ruminal propionate production. We hypothesize this effect was not observed in HS diets as these cows might have adapted to greater amounts of propionate delivered to the liver on a more consistent basis throughout the day. This more constant supply or elevated supply of propionate might have allowed the HS cows to adapt to higher propionate amounts compared to the LS diet. Additionally, cow response to high starch diets is often variable due to the rate and site of digestion of various starch sources

in the rumen (Owens et al., 1986; Huntington, 1997; Oba and Allen, 2003b). Changes in proportions of rumen VFAs are influenced by the rumen availability of starch in the supplemental corn and resultant associative effects. In the present study coarsely ground corn was the major starch source which would have a slower rate of fermentation than high moisture corn reducing the propionate flux at feeding.

Milk, milk component yield, and feed efficiency

Due to the increase in propionate production supplied in the rumen as measured by Stein et al. (2006) and Weiss et al. (2008), an increase in milk yield and feed efficiency could be expected due to an increase in substrate supply for hepatic gluconeogenesis. A recent study by Weiss et al. (2008) measuring the effects of P169 with a high starch (31.1%) diet, however, reported no differences in milk production. Our study demonstrated that HS and DFM has potential to increase milk yield. Stein et al. (2006) reported an increase of 4% fat corrected milk yield of 3.2 and 2.9 kg/d respectively over control with P169 supplementation. Cow response in this study supports that of Francisco et al. (2002) and Weiss et al. (2008) as 3.5% FCM and ECM were similar among treatments. Since 3.5% FCM was similar and DMI was lower with DFM supplementation, we observed an increase in feed efficiency of 14.4% for LSM compared to LSC and an increase of 6.2% for HSM compared to HSC.

High starch diets with dry ground corn might have the potential to increase milk production, however, Ipharraguerre et al. (2002) found that decreasing dietary starch by replacing corn grain with soyhulls resulted in similar 3.5% FCM yield. In another study, Stone et al. (1996) replaced high moisture corn with soyhulls (Starch % of DM: 16.0% vs 25.0%) and reported similar milk and 3.5% FCM yield. Replacing coarsely ground dry

corn with soyhulls in the present study, resulted in similar 3.5% FCM and ECM yield indicating similar milk production in early lactation can be achieved with low starch diets.

Liver total lipid, triaclyglycerol, glycogen, and serum NEFA, BHBA, and blood calcium homeostasis

In order to investigate lipid metabolism in the liver, liver total lipid, triaclyglycerol, and glycogen concentration in biopsies of liver were measured. Janovick et al. (2011) reported an increase in postpartum liver total lipid and triaclyglycerol concentration in cows overfed energy prepartum. In this study, however, liver total lipid and triaclyglycerol concentration were similar among treatments. Differences in the degree of liver lipid accumulation between the two studies can likely be attributed to differences in the amount of over-feeding as Janovick et al. (2011) fed cows at 150 percent of energy requirements compared to 131 and 141 percent of requirements for LS and HS in the current study. In the present study, liver glycogen concentration was increased prepartum in cows fed high starch diets. This might be attributed to a combination of greater amounts of starch being delivered to the small intestine due to the lower rumen digestibility of dry ground corn as described by Ying and Allen, (2005), and an increase in propionate production in the rumen (Oba and Allen, 2003b). These results are similar to Grum et al. (1996) as high grain diet increased liver glycogen concentration prepartum compared to control. Despite prepartum differences, postpartum liver glycogen concentration was similar among treatments. In the current study, Serum NEFA concentration was similar among treatments. This is in contrast to Francisco et al. (2002) as they measured an increase of NEFA wk 1 postpartum and Weiss et al. (2008)

who measured an increase of NEFA on d 6 postpartum with P169 supplementation. The contrast in results may be attributed to the slow take off in milk production and similar DMI among treatments wk 1 postpartum in the present study. Concentration of BHBA was similar among treatments which are in agreement with Weiss et al. (2008). Previous studies involving supplementation with P169 have not reported serum calcium concentration shortly after parturition. In the current study even though there is a trend for HSC to have lower serum calcium concentration 24 h after parturition compared to LSC, the lack of additional sample points and previous research make it difficult to identify the mode of action for this result.

Feeding behavior pre- and postpartum

Prepartum feeding behavior was altered as LSC cows spent the greatest amount of time eating. The physical form of the diet with the addition of soyhulls compared to the high starch diet likely contributed to more time spent eating. Reduction in DMI for LSM pre- and postpartum compared to LSC resulted in less time spent eating. Time allotted to postpartum rumination was also altered by HS as cows tended to spend more time a day ruminating, which is in contrast to Oba and Allen (2003b) as they reported a decrease for ruminating time for high starch (31.1%) diet compared to low starch (21.0%). Their study, however, used high moisture corn and finely ground corn to achieve varying dietary starch concentration compared to coarsely ground dry corn which would have a slower rate of rumen fermentation in the present study. Supplementation with DFM did not affect feeding behavior pre- or postpartum. It is worth noting that standing time prepartum was increased by 1h and postpartum was increased by 2 h/d with HS compared with LS diets. Increased standing time during the transition period increases maintenance

energy required (Susenbeth et al., 2004), predisposes these cows to increased incidence of lameness later in lactation (Blowey, 2005), and might be an indication for subacute ruminal acidosis.

Colostrum yield and quality

Knowledge of factors influencing colostrum yield and quality is limited. Cows with a 40 d dry period produced 2.2 kg less colostrum than cows with a 60 d dry period (Grusenmeyer et al., 2006). Beef cows supplemented over winter had greater colostrum yield than cows that were not supplemented (Petrie et al., 1984) indicating increased energy intake increases colostrum yield. However, in the current study increased energy intake in the HS diet did not alter colostrum yield or quality. The supplementation of DFM also had no effect on colostrum yield or quality. Results from the present study suggest that energy intake above requirements does not affect colostrum yield or quality in dairy cows.

CONCLUSIONS

Low starch and supplementation with DFM tended to reduce DMI both pre- and postpartum. The reduction in DMI resulted in LSM cows having a lower energy balance prepartum compared to LSC and HSM. Postpartum HS diets did however reduce the severity of negative energy balance. Total milk yield was increased by HS and DFM resulting in greater milk yield for HSM compared to LSM and HSC but was similar to LSC; however, 3.5 % FCM and ECM yield were similar among treatments. Feed efficiency was increased by supplementation with DFM. Prepartum serum NEFA tended to be increased by DFM and increased STARCH tended to decrease prepartum serum NEFA but BHBA were similar among treatments. Liver total lipid, triaclyglycerol, and

glycogen concentration were similar among treatments prepartum. Postpartum STARCH concentration and DFM did not alter liver lipid metabolism. In summary, cows fed HSM had greater milk yield than LSM and HSC, milk yield was similar for LSC, LSM and HSC, and feed efficiency was increased by DFM.

Table 3.1. Description of multiparous dairy cows at the start of the prepartum phase 42 d before expected parturition fed low or high starch and carrier or direct fed microbial (DFM, *Propionibacterium freudenreichii* strain P169) both pre- and postpartum.

	Treatments[1]				
Item	LSC	LSM	HSC	HSM	SEM[2]
N	17	17	17	17	---
305ME[3] previous lactation, kg	10,990.0	11,041.0	11,135.0	10,917.0	455.0
Body weight, kg	686.2	686.0	694.7	683.4	26.8
Body condition score[4] d (-42)	3.6	3.5	3.6	3.6	0.2
Parity	3.1	3.1	3.2	3.2	0.3
Days dry	47.3	45.2	46.9	42.2	2.0

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).

[2] Largest standard error.

[3] Complete 305 d mature equivalent milk production.

[4] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.

Table 3.2. Diet ingredients and nutrient composition of diets fed pre- and postpartum (CNCPS 6.1).

	Diets[1]			
	Prepartum		Postpartum	
	Low Starch	High Starch	Low Starch	High Starch
Ingredients, % Diet DM				
Alfalfa hay, chopped	11.6	11.6	10.8	11.8
Wheat straw, chopped	23.2	23.2	0.0	0.0
Corn silage, processed	44.7	29.8	38.0	38.0
Protein mix[2,3]	13.9	13.9	19.8	21.3
Corn, coarsely ground 1.60 mm[4]	0.0	21.5	9.2	22.4
Soyhulls, loose	6.6	0.0	15.7	0.0
Cottonseed, fuzzy	0.0	0.0	2.9	2.9
Liquid supplement[5]	0.0	0.0	3.5	3.5
Water, tap kg/d per cow	1.3	4.5	4.0	4.3
Nutrient composition				
DM %	50.1	50.0	50.0	50.0
CP, % DM	13.1	13.2	16.5	16.8
RUP[6], %CP	30.6	32.3	37.2	37.3
RDP[6], %CP	69.4	67.7	62.8	62.7
ADF, % DM	33.5	26.9	24.9	17.9
NDF, % DM	47.6	39.2	36.5	27.7
Starch, % DM	15.5	26.7	20.1	29.7
Water soluble carbohydrates, % DM	4.0	4.3	6.9	7.2
Lignin, % DM	4.3	3.8	3.4	2.8
Soluble fiber[7], % DM	4.3	3.8	5.7	5.1
Ash, % DM	9.2	9.0	9.0	9.0
Ca[7], % DM	0.51	0.43	0.83	0.75
P, % DM	0.34	0.36	0.40	0.44
Mg[7], % DM	0.29	0.27	0.31	0.29
K, % DM	1.42	1.28	1.70	1.62
S, % DM	0.18	0.18	0.21	0.22
Na, % DM	0.15	0.15	0.40	0.41
Cl, % DM	0.37	0.37	0.59	0.60
Iron, ppm	205.65	161.68	215.46	160.88
Zinc, ppm	28.48	28.24	97.60	96.70
Copper, ppm	10.05	8.89	25.32	23.59
Manganese, ppm	40.00	35.94	70.51	70.45
Selenium, ppm	0.37	0.37	0.36	0.37
Cobalt, ppm	0.28	0.28	0.99	0.99
Iodine, ppm	0.58	0.58	1.04	1.05
Vit-A,[8]IU/kg	6769.8	6769.8	6104.1	6080.5
Vit-D,[8] IU/kg	1354.8	1354.8	1221.8	1217.1
Vit-E,[8] IU/kg	67.7	67.7	24.5	24.4
DCAD1,[9] meq/kg	209.0	172.0	310.0	289.0
NE_L[10], Mcal/kg	1.44	1.55	1.71	1.85
ME[8], Mcal/kg	2.05	2.17	2.33	2.45

[1]Diets fed from 42 d prepartum through 56 d postpartum.
[2] Prepartum protein mix, ingredients: 42.2% soybean meal (48% CP), 21.5 % soy pass, 29.8 corn dried distillers grains with solubles, 5.7% Munson Lakes (Howard Lake, MN) dry cow supreme premix, 0.9% MgO; composition: 43.2% CP, 7.5% ADF, 17.5% NDF, 13.4% Ash, 4.2% EE, 1.2% Ca, 1.2% P, 1.1% Mg, 2.1% K, 0.6% S, 0.8% Na, 0.9% Cl.
[3] Postpartum protein mix, ingredients: 33.1% corn dried distillers grains with solubles, 27.8% soybean meal (48% CP), 14.9% soy pass, 2.9% sodium bicarbonate, 0.8% salt, 2.5% calcium carbonate, 0.3% MgO, 3.1% potassium carbonate, 2.3% blood meal, 11.1% canola meal, 0.3% alimet® (Novus International, St. Charles, MO), 0.8% megalac® (Church & Dwight Co. Inc., Princeton, NJ); composition: 34.5% CP, 15.5% ADF, 27.5% NDF, 17.1% Ash, 6.0% EE, 1.6% Ca, 0.9% P, 0.6% Mg, 2.0% K, 0.5% S, 2.2% Na, 1.5% Cl.
[4] Calculated value using equations from ASAE standard S319.2.
[5] Molasses-based liquid feed, Quality Liquid Feeds, Dodgeville, WI; 64.3% DM, 23.7% ash, 4.9% Ca, 3.4% K.
[6] Formulated value from Cornell-Net-Carbohydrate-Protein-System (CNCPS, version 6.1).
[7] Actual value lower than formulated value.
[8] Calculated value from Cornell-Net-Carbohydrate-Protein-System (CNCPS, version 6.1).
[9] Dietary cation anion difference equation 1 Calculated value Cornell-Net-Carbohydrate-Protein-System (CNCPS, version 6.1) = [(Na + K) – (Cl + S)]
[10] Calculated value from Cornell-Penn-Miner (CPM 3.1).

Table 3.3. Dry matter intake, energy balance, body condition score, rumen fill score, and body weight of cows fed low or high starch and carrier or DFM from day -42 to 56 days in milk.

	Treatments[1]								
	Low Starch		High Starch			*P*-value			
Variable	Control	DFM	Control	DFM	SEM[2]	S[3]	P[4]	S × P[5]	Wk
Prepartum									
Number	16	11	14	13					
DMI, kg/d	13.0	11.4	12.6	12.7	0.5	0.45	0.12	0.08	<0.01
DMI, % of BW	1.9	1.6	1.8	1.9	0.1	0.38	0.38	0.11	<0.01
Energy balance[6], Mcal/d	5.5[a]	2.0[b]	4.4[ab]	5.8[a]	0.9	0.12	0.23	<0.01	<0.01
Energy balance[7], % Req.	136.5[a]	115.1[b]	131.2[ab]	141.4[a]	6.7	0.08	0.35	0.01	<0.01
Rumen fill score[8]	3.0	2.8	3.0	2.8	0.2	0.99	0.28	0.73	<0.01
Body condition score[9]	3.7	3.7	3.9	3.8	0.1	0.37	0.53	0.59	<0.01
Body condition change[10]	0.3	0.2	0.4	0.3	0.1	0.03	0.20	0.78	----
Body weight, kg	712.4	689.9	723.5	693.4	28.7	0.78	0.31	0.88	<0.01
Body weight change, kg[11]	62.2	52.1	44.5	47.2	8.4	0.12	0.60	0.37	----
Postpartum									
DMI, kg/d	22.6	19.2	21.3	21.4	1.0	0.62	0.08	0.06	<0.01
DMI, % of BW	3.7	3.3	3.6	3.5	0.2	0.88	0.21	0.44	<0.01
Energy balance[6], Mcal/d	-3.9	-7.8	-1.6	-2.4	2.0	0.03	0.19	0.39	<0.01
Energy balance[7], % Req.	92.3	80.2	95.9	93.2	4.4	0.03	0.06	0.23	<0.01
Rumen fill score[8]	2.5	2.6	2.7	2.8	0.2	0.24	0.41	0.99	<0.01
Body condition score[9]	3.5	3.2	3.4	3.4	0.1	0.50	0.15	0.32	<0.01
Body condition change[12]	-0.3[b]	-0.9[a]	-0.6[ab]	-0.4[b]	0.2	0.37	0.21	0.03	----
Body weight, kg	630.5	592.0	614.8	620.3	22.7	0.75	0.41	0.27	<0.01
Body weight change, kg[13]	-39.1	-61.3	-41.5	-32.1	11.9	0.21	0.54	0.14	----

[a-b] Means within a row with different superscripts differ ($P < 0.05$).

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).

[2] Largest standard error.

[3] Low starch vs. high starch.

[4] DFM carrier vs. DFM.

[5] Interaction of starch concentration and DFM supplementation.

[6] Energy balance = energy intake – energy requirements.

[7] Energy balance % requirement = (energy intake /energy requirements)*100.

[8] Rumen fill score on a 1-5 scale, 1 = empty rectangle and 5 = extended over last rib.

[9] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.

[10] Body condition change week -1 minus week -6.

[11] Body weight change week -1 minus week -6.

[12] Body condition change week 8 minus week 1.

[13] Body weight change week 8 minus week 1.

Table 3.4. Prepartum selective particle consumption (composite of day -21, -20, and -19 (N = 50)) at each of the four screens of the Penn State shaker box expressed as a percentage of expected consumption.

	Treatments[1]							
	Low Starch		High Starch			*P*-value		
Variable	Control	DFM	Control	DFM	SEM[2]	S[3]	P[4]	S × P[5]
Top, %	97.0[ab]	103.0[a]	99.0[a]	93.0[b]	2.0	0.07	0.91	<0.01
Middle, %	99.0	97.0	99.0	99.0	1.0	0.07	0.31	0.17
Bottom, %	102.0	103.0	102.0	102.0	1.0	0.59	0.91	0.98
Pan, %	97.0	88.0	88.0	85.0	6.0	0.30	0.29	0.63

[a-b] Means within a row with different superscripts differ ($P < 0.05$).

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).

[2] Largest standard error.

[3] Low starch vs. high starch.

[4] DFM carrier vs. DFM.

[5] Interaction of starch concentration and DFM supplementation.

Table 3.5. Cow feed efficiency, milk production, and milk quality postpartum day 1 to 56.

	Treatments[1]								
	Low Starch		High Starch			*P*-value			
Variable	Control	DFM	Control	DFM	SEM[2]	S[3]	P[4]	S × P[5]	Wk
Number	16	11	14	13					
Milk[6], kg/d	45.5[ab]	43.3[b]	43.8[b]	47.2[a]	1.5	0.43	0.66	0.04	<0.01
3.5% FCM[7], kg/d	47.7	45.4	45.8	46.3	1.6	0.71	0.53	0.34	<0.01
ECM[8], kg/d	47.5	44.7	46.1	46.9	1.5	0.78	0.41	0.16	<0.01
Feed efficiency[9]	2.15	2.46	2.09	2.22	0.12	0.16	0.05	0.38	<0.01
Fat, %	3.84	3.89	3.82	3.41	0.18	0.12	0.26	0.15	<0.01
Fat, kg/d	1.71	1.64	1.65	1.59	0.08	0.41	0.34	0.95	<0.01
True protein,%	3.07	3.01	3.17	3.05	0.07	0.25	0.16	0.67	<0.01
True protein, kg/d	1.36	1.29	1.34	1.41	0.05	0.20	0.97	0.07	<0.01
Fat:protein	1.24	1.30	1.20	1.11	0.05	0.02	0.71	0.10	0.16
Lactose, %	4.83	4.84	4.88	4.92	0.05	0.13	0.63	0.74	<0.01
MUN[10], mg/dL	12.82	12.29	11.98	12.87	0.52	0.77	0.69	0.13	<0.01
Total solids,%	12.67	12.69	12.83	12.31	0.21	0.56	0.17	0.14	<0.01
SCC[11], cells/mL	322.17	209.86	394.04	68.99	181.65	0.83	0.18	0.51	0.13

[a-b] Means within a row with different superscripts differ ($P < 0.05$).

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).

[2] Largest standard error.

[3] Low starch vs. high starch.

[4] DFM carrier vs. DFM.

[5] Interaction of starch concentration and DFM supplementation.

[6] Milk yield through 56 d postpartum.

[7] 3.5% Fat corrected milk = 0.4324 × (milk kg) + 16.2162 × (fat kg), through 56 d postpartum.

[8] Energy corrected milk = (0.327 × (milk kg)) + (12.95 × (fat kg)) + (7.65 × (protein kg)), through 56 d postpartum.

[9] Feed efficiency = 3.5% FCM divided by DMI.

[10] Milk urea nitrogen.

[11] Somatic cell count × 1000's cells/mL.

Table 3.6. Cow liver lipid metabolism d 14 prepartum and d 7 and 14 postpartum, blood serum non-esterified fatty acids (NEFA) and beta-hydroxybutrate (BHBA) and blood total calcium levels at 12h and 24 h postpartum.

	Treatments[1]					*P*-value			
	Low Starch		High Starch						
	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]	day
Prepartum									
Liver tissue									
Total lipid[6], % wet wt	4.5	4.1	4.2	5.1	0.7	0.54	0.66	0.33	--
Triaclyglycerol[7], % wet wt	1.2	0.7	0.6	0.6	0.5	0.41	0.56	0.51	--
Glycogen[6], % wet wt	4.4	5.0	5.7	6.3	0.6	0.01	0.25	0.90	--
Triaclyglycerol:glycogen	0.15	0.16	0.11	0.11	0.02	0.03	0.65	0.87	--
Serum									
NEFA[8], µEq/L	226.9	310.1	199.2	232.0	34.8	0.09	0.06	0.41	<0.01
BHBA[9], µmol/L	513.5	518.7	585.7	641.3	77.8	0.15	0.65	0.71	0.04
Postpartum									
Liver tissue									
Total lipid[10],% wet wt	9.8	10.1	10.1	9.1	1.4	0.79	0.78	0.61	0.06
Triaclyglycerol[11],% wet wt	6.1	5.8	5.0	5.3	1.1	0.41	0.99	0.77	0.04
Glycogen[10], % wet wt	2.4	1.9	2.3	2.7	0.4	0.29	0.90	0.21	<0.01
Triaclyglycerol:glycogen	3.2	4.3	4.4	3.2	1.2	0.95	0.91	0.23	0.76
Serum									
NEFA[12], µEq/L	558.5	603.6	513.5	490.3	58.7	0.13	0.83	0.51	<0.01
BHBA[13], µmol/L	865.7	871.1	763.2	765.5	85.0	0.17	0.96	0.98	<0.01
Calcium[14], total 12 h, mg/dL	8.3	8.4	8.5	9.1	0.7	0.46	0.62	0.70	----
Calcium[15], total, 24 h mg/dL	9.5	8.8	7.9	9.1	0.6	0.22	0.73	0.08	----

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.
[6] Percent of wet weight from liver samples from day -14 relative to parturition.
[7] Percent of liver total lipid from day -14 relative to parturition.
[8] NEFA analysis of serum from days -43, -28, -21, -14, -7, -3 and -1 relative to parturition.
[9] BHBA analysis of serum from days -7, -3 and -1 relative to parturition.
[10] Percent of wet weight from liver samples from day 7 and 14 relative to parturition.
[11] Percent of liver total lipid from day 7 and 14 relative to parturition.
[12] NEFA analysis of serum from days 0.5, 1, 7, 14, 21 and 28 relative to parturition.
[13] BHBA analysis of serum from days 1, 7, 14, 21 and 28 relative to parturition.
[14] Serum total calcium at 12 hours after parturition.
[15] Serum total calcium at 24 hours after parturition.

Table 3.7. Calf weight and colostrum quantity and quality for cows.

	Treatments[1]							
	Low Starch		High Starch			*P*-value		
Variable	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]
Calf weight, kg	47.7	47.7	48.7	48.8	3.5	0.74	0.99	0.98
Colostrum								
Yield[6], kg	9.3	7.4	6.3	8.2	1.4	0.40	0.96	0.15
Specific gravity[7], g/dL	27.6	27.6	25.0	27.7	1.7	0.45	0.42	0.41
IgG[8], mg/dL	4290.3	5217.9	4126.3	4797.6	591.1	0.61	0.16	0.82

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.
[6] Colostrum measured within 12 hours after parturition.
[7] Colostrum specific gravity measured using Brix refractometer.
[8] Colostrum IgG concentration measured at Colorado State Veterinary Diagnostic Lab, Fort Collins, CO.

Table 3.8. Cow (n=68) feeding behavior using ten minute scans prepartum on d -21, -20 and -19 and postpartum on d 21, 22, 23.

	Treatments[1]							
	Low Starch		High Starch			*P*-value		
Variable	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]
				Prepartum				
Eating, h/d	3.8[a]	2.9[b]	3.0[b]	3.0[b]	0.2	0.17	0.04	0.03
Ruminating, h/d	8.2	9.0	8.2	8.0	0.4	0.17	0.46	0.19
Standing, h/d	9.5	10.0	10.9	11.2	0.7	0.06	0.51	0.92
				Postpartum				
Eating, h/d	3.8[a]	2.9[b]	3.2[ab]	3.4[ab]	0.3	0.92	0.24	0.05
Ruminating, h/d	8.0	8.0	8.7	8.8	0.5	0.08	0.99	0.91
Standing, h/d	11.7	11.3	13.1	13.3	0.8	0.02	0.83	0.67

[a-b] Means within a row with different superscripts differ ($P < 0.05$).

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).

[2] Largest standard error.

[3] Low starch vs. high starch.

[4] DFM carrier vs. DFM.

[5] Interaction of starch concentration and DFM supplementation.

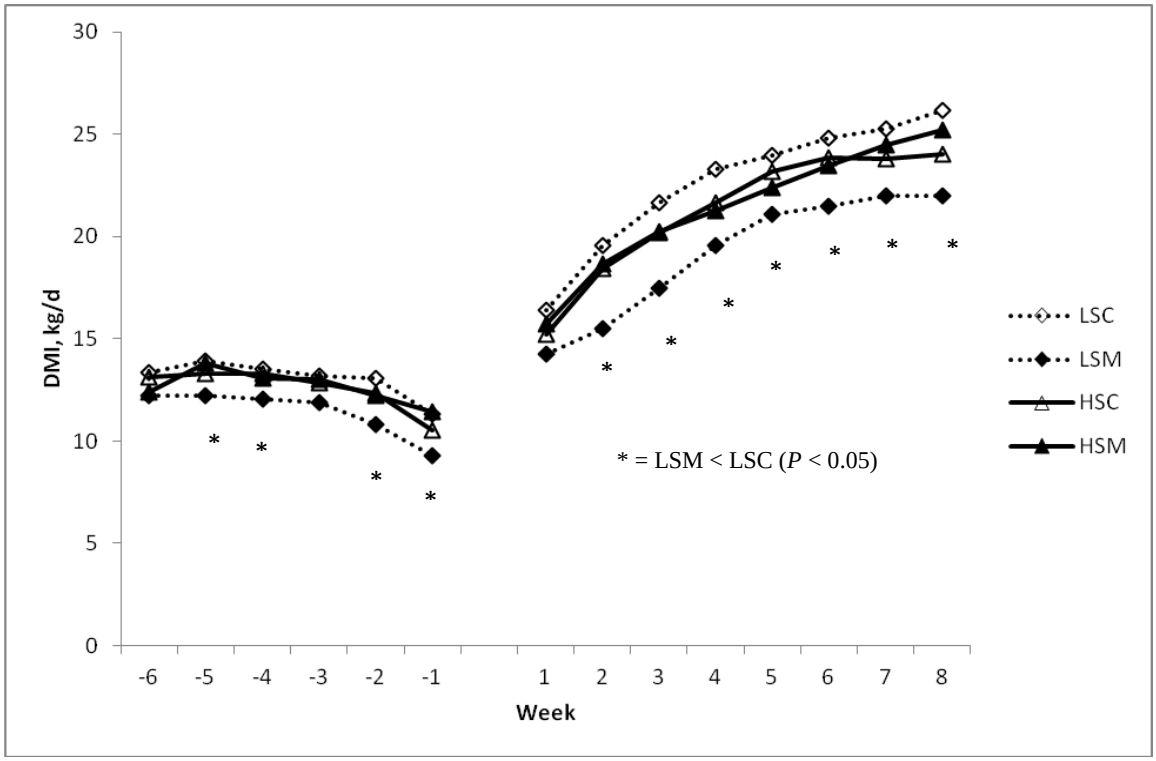

Figure 3.1. Effect of treatment on pre- and postpartum DMI, kg/d by week from -42 d to 56 d. Treatment from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.45$; DFM, $P = 0.12$; Starch × DFM, $P = 0.08$; Week, $P < 0.01$. Largest SEM = 0.5 kg/d. Postpartum: Starch, $P = 0.62$; DFM, $P = 0.08$; Starch × DFM, $P = 0.06$; Week, $P < 0.01$. Largest SEM = 1.0 kg/d. For clarity, SEM bars have been omitted.

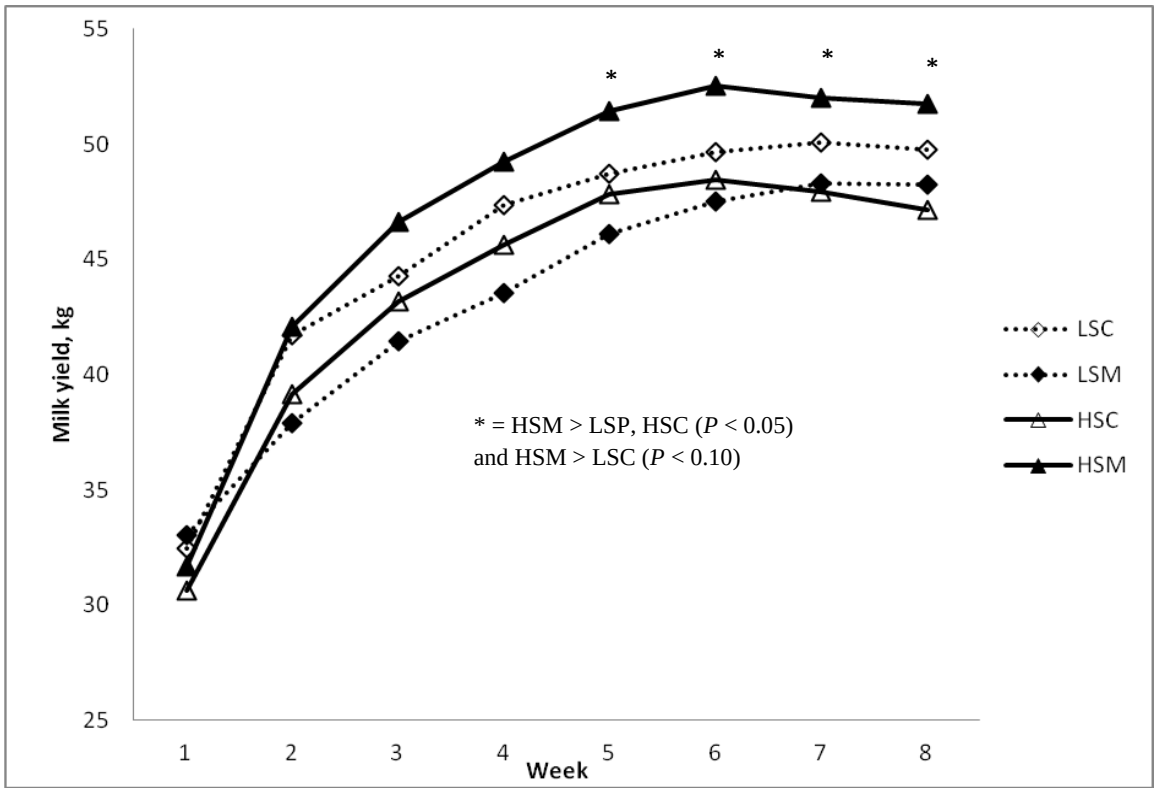

Figure 3.2. Effect of treatment on milk yield, kg by week. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Starch, $P = 0.43$; DFM, $P = 0.66$; Starch × DFM, $P = 0.04$; Week, $P < 0.01$. Largest SEM = 1.5 kg. For clarity, SEM bars have been omitted.

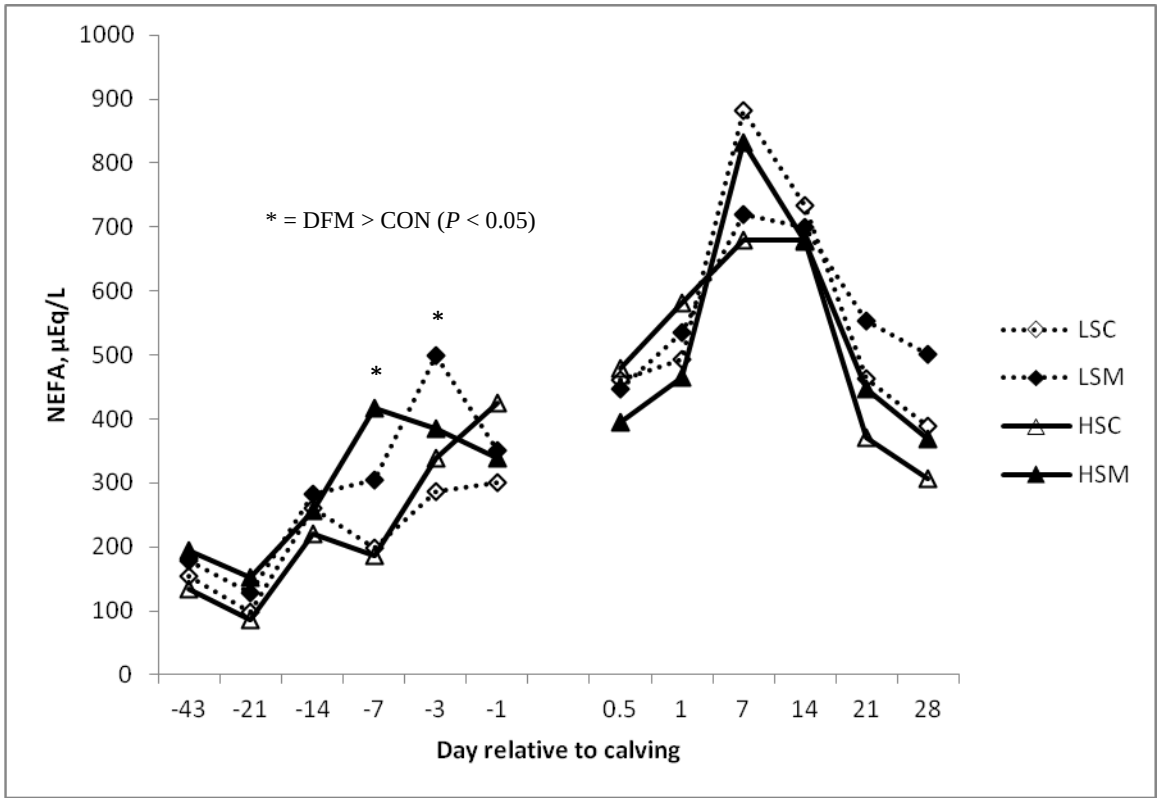

Figure 3.3. Effect of treatment on blood serum NEFA, µEq/L pre- and postpartum. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.09$; DFM, $P = 0.06$; Starch × DFM, $P = 0.41$; Day, $P < 0.01$. Largest SEM = 34.8 µEq/L. Postpartum: Starch, $P = 0.13$; DFM, $P = 0.83$; Starch × DFM, $P = 0.51$; Day, $P < 0.01$. Largest SEM = 58.7 µEq/L. For clarity, SEM bars have been omitted.

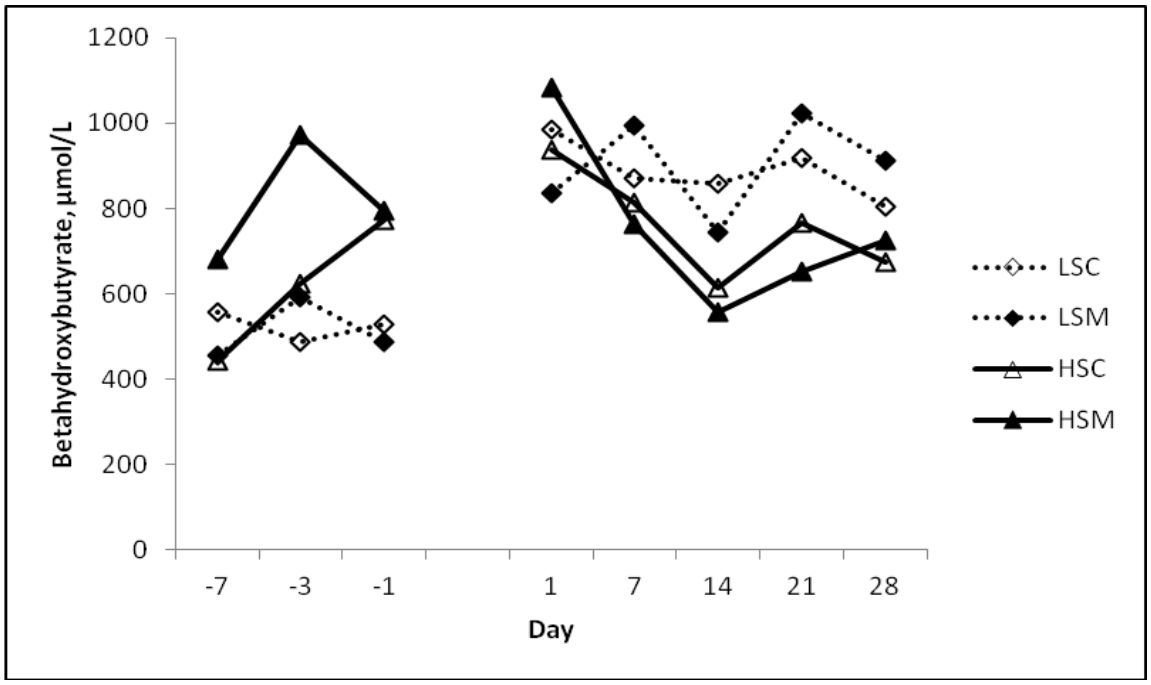

Figure 3.4. Effect of treatment on blood serum BHBA, µmol/L pre- and postpartum. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.15$; DFM, $P = 0.65$; Starch × DFM, $P = 0.71$; Day, $P = 0.04$. Largest SEM = 77.8 µmol/L. Postpartum: Starch, $P = 0.17$; DFM, $P = 0.96$; Starch × DFM, $P = 0.98$; Day, $P < 0.01$. Largest SEM = 85.0 µmol/L. For clarity, SEM bars have been omitted.

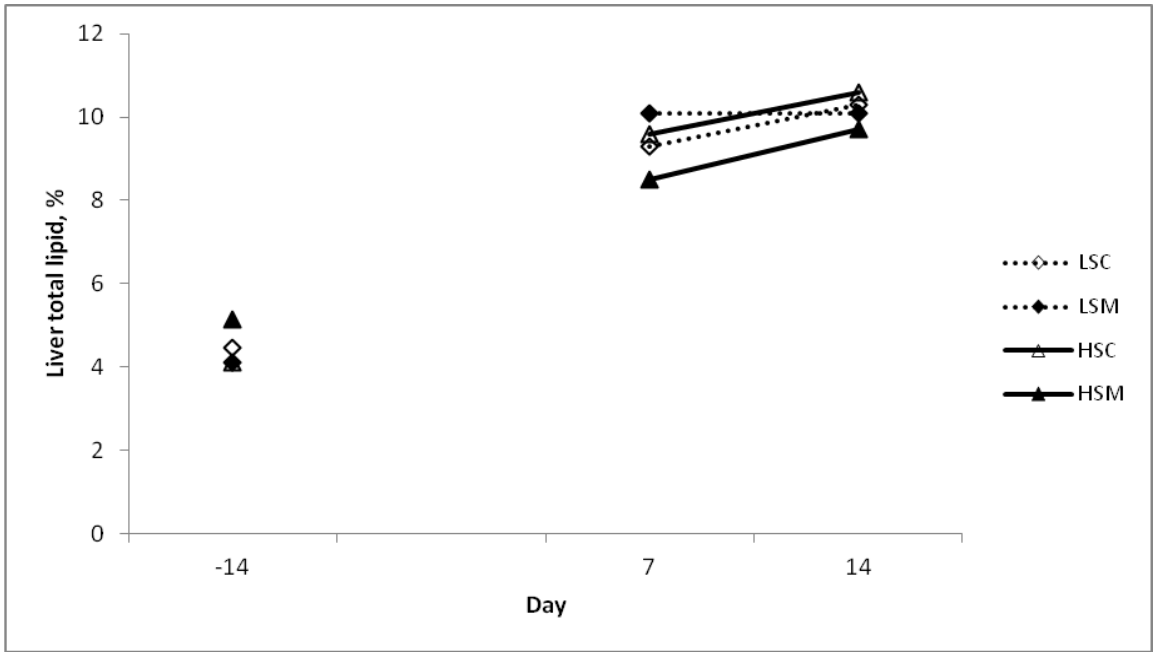

Figure 3.5. Effect of treatment on liver total lipid percent d -14, 7 and 14. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, P = 0.54; DFM, P = 0.66; Starch × DFM, P = 0.33. Largest SEM = 0.7 % of wet wt. Postpartum: Starch, P = 0.79, DFM, P = 0.78; Starch × DFM, P = 0.61; Day, P = 0.06. Largest SEM = 1.4 % of wet wt. For clarity, SEM bars have been omitted.

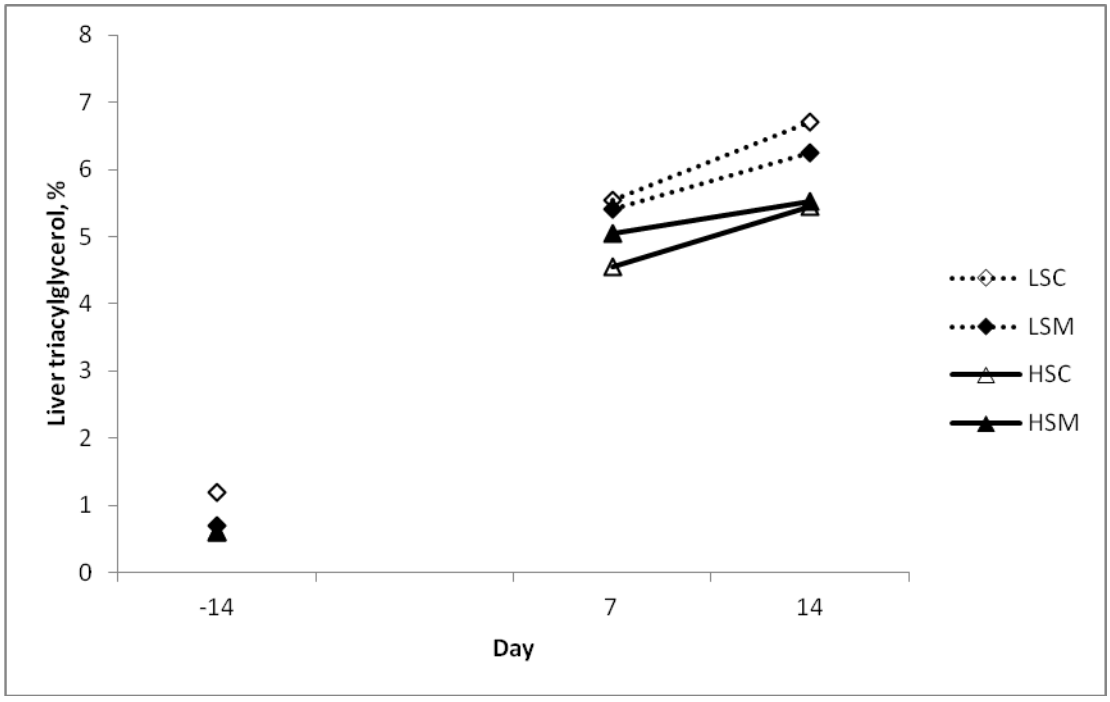

Figure 3.6. Effects of treatment on liver triaclyglycerol percent d -14, 7 and 14. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, P = 0.41; DFM, P = 0.56; Starch × DFM, P = 0.51. Largest SEM = 0.5 % wet wt. Postpartum: Starch, P = 0.41; DFM, P = 0.99; Starch × DFM, P = 0.77; Day, P = 0.04. Largest SEM = 1.1 % wet wt. For clarity, SEM bars have been omitted.

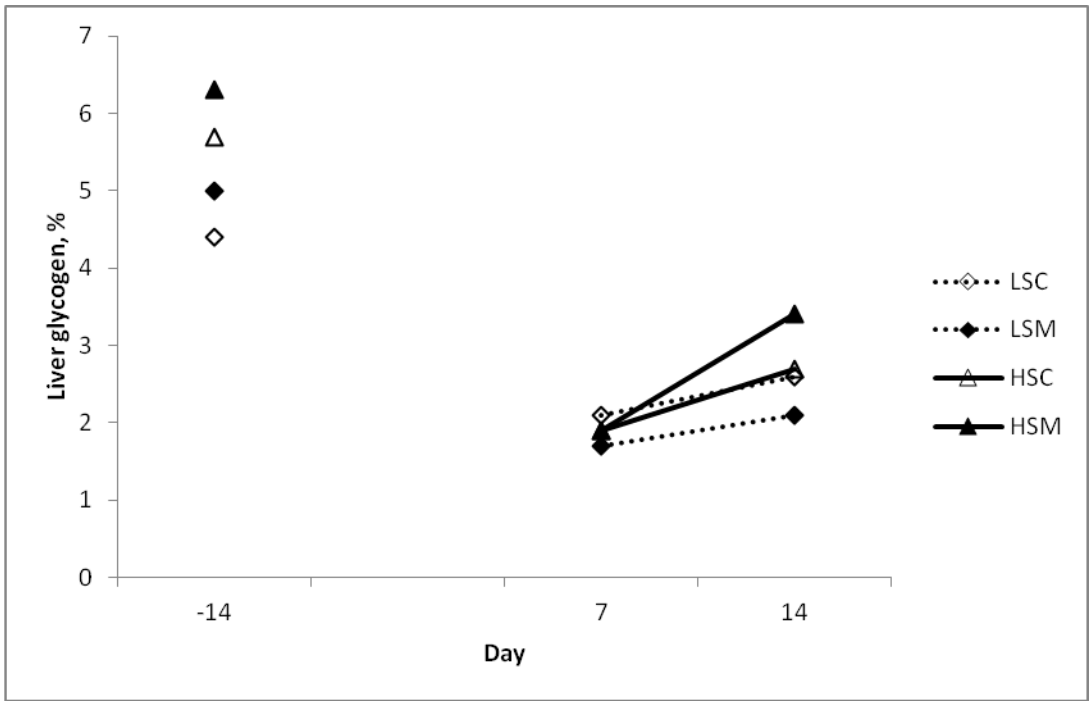

Figure 3.7. Effects of treatment on liver glycogen percent d -14, 7 and 14. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.01$; DFM, $P = 0.25$; Starch × DFM, $P = 0.90$. Largest SEM = 0.6 % wet wt. Postpartum: Starch, $P = 0.29$; DFM, $P = 0.90$; Starch × DFM, $P = 0.21$; Day: $P < 0.01$. Largest SEM = 0.4 % wet wt. For clarity, SEM bars have been omitted.

Effects of varying periparturient dietary starch amount and supplementation with *Propionibacterium* on primiparous dairy cow performance, metabolism, and health.

SUMMARY

Primiparous dairy cows (n = 8/treatment) were used in a 2 × 2 factorial design to determine if daily supplementation with *Propionibacterium freudenreichii* strain P169, **(**DuPont Industrial and Biological Sciences, Waukesha, WI) direct fed microbial (**DFM**) altered response to periparturient diets varying in starch concentration. Cows were assigned to one of four treatments 42 d prepartum. Factors were STARCH; low starch (LS) prepartum (13.1% CP; 15.5% starch; 47.6% NDF) and postpartum (16.5% CP; 20.1% starch; 36.5% NDF) or high starch (HS) prepartum (13.2% CP; 26.7% starch; 39.2% NDF) and postpartum (16.8% CP; 29.7% starch; 27.7% NDF) and DFM; Control (DFM carrier) or 20 g/d of P169 providing approximately 60 billion CFU/head/day *Propionibacterium* at the time of feeding. Cows were housed in a tie-stall barn and fed at an *ad libitum* rate once daily and milked twice daily. Pre- and postpartum dietary starch was increased by replacing corn silage and soy hulls with ground corn. Control and DFM was top-dressed at feeding using a calibrated scoop starting 21 d pre- through 56 d postpartum. Factors combined to form four treatments; 1) low starch + carrier (**LSC**), 2) low starch + P169 (**LSM**), 3) high starch + carrier (**HSC**), 4) high starch + P169 (**HSM**). Data were analyzed using PROC MIXED in SAS as a 2 × 2 factorial arrangement with model including starch, DFM, week, and breed. We hypothesized that DFM would increase milk yield in low and high starch diets. Pre- and postpartum DMI was similar among treatments. Milk yield and feed efficiency were similar among treatments. However, high starch tended to increase 3.5% fat corrected milk (**FCM**) and energy

corrected milk (**ECM**) yield. Serum NEFA and BHBA increased at calving and were increased by HS on d 1. The average of d 7 and d 14 liver total lipid and triaclyglycerol concentration increased with increasing dietary starch concentration. Feeding behavior was monitored once pre- and postpartum before d -21 and after d 21 using 10 minute scans for 24h period. Prepartum low starch increased time spent feeding. Postpartum time spent feeding, ruminating, and standing were similar among treatments. In summary, milk yield and feed efficiency were similar among treatments. Feeding high starch diets tended to increase 3.5% FCM and ECM. High starch increased postpartum liver total lipid and triaclyglycerol concentration in primiparous cows.

INTRODUCTION

The primiparous cow must successfully adapt to numerous physiological and behavioral changes related to calving and the initiation of lactation. Primiparous cows must adapt to changes in housing, feeding, management, and to milking. Strategies that reduce stress around the time of calving should optimize the chance for success. Our goal for a successful primiparous heifer program should be to reduce the number of metabolic, psychological, and pathogenic hurdles during the transition period. Calving and initiation of lactation are two unavoidable hurdles. Transitioning primiparous cows often receive less attention than second and greater lactation cows. Perhaps primiparous cows have a lower status on the priority list due to seemingly fewer health problems such as milk fever and displaced abomasum during the transition period when compared to their older counterparts. Fresh primiparous cows have a greater prevalence of mastitis than older cows, despite lower reported incidence of mastitis later in lactation (Miltenburg et al., 1996; Barkema et al., 1998; Nyman et al., 2007).

Often primiparous cows are moved from the heifer grower and placed into the far-off dry cow group 60 days prior to calving. The objective of this pen movement is to adapt first calf heifers to new surroundings, expose them to social interactions with older cows, and to feed an appropriate diet. Moderate energy or controlled energy diets are diets based on wheat straw and silage that are offered at an ad libitum rate, but do not allow cows to greatly over-consume energy (Dann et al., 2006; Janovick-Guretzky et al., 2006). Previous research has demonstrated that over-feeding energy to dry cow's results in a reduction in gluconeogenesis, decreased dry matter intake (**DMI**) and an increase in lipolysis (Holtenius et al., 1996; Douglas et al., 2007; Janovick et al., 2011). Work examining the role of energy intake during the dry period has been limited in primiparous cows. Researchers in Wisconsin found that primiparous cows fed a more moderate energy diet prepartum had higher DMI postpartum than heifers fed higher energy (Grummer et al., 1995).

Metabolic differences between primiparous and multiparous cows prepartum are numerous. Primiparous cows have not yet reached mature body weight so changes associated with growth place demands on anabolic pathways. Previous research conducted with multiparous cows reported postpartum DMI decreased by 1.5 kg/d with increasing dietary starch concentration (21% vs. 25.5%) (Nelson et al., 2011) and by 2.4 kg/d (21.8% vs. 27.1%) (Gencoglu et al., 2010). Moderate dietary starch concentration (23.2%) increased milk yield by 5.7 kg/d over high starch (25.5%) and was similar to low starch (21.0%) (Nelson et al., 2011). High dietary starch (27.1%) had similar milk yield to low starch (21.8%), however, 3.5% FCM was decreased by 2.9 kg/d for high starch compared to low starch (Gencoglu et al., 2010).

Utilizing direct fed microbials (**DFM**, *Propionibacterium* strain P169 (**P169**), (DuPont Industrial and Biological Sciences, Waukesha, WI) during the transition period to reduce the duration and severity of negative energy balance and increase propionate production for increased gluconeogenesis has been a key focus for many research projects in recent years. The use of *Propionibacterium* strain P169 has been chosen for its ability to convert lactate into propionate. Previous research measured an increase in propionate production in the rumen when P169 was supplemented at a rate of 6×10^{11} cfu/d (Stein et al., 2006; Weiss et al., 2008). The increase in propionate production would lead to an increase in gluconeogenesis. Previous research has reported a decrease of 1.0 kg/d of DMI (Weiss et al., 2008) and 3.6 g/kg of body weight (Francisco et al., 2002) when lactating cows were fed P169. Despite the decrease in DMI when P169 was fed in these studies, milk yield was similar among treatments (Francisco et al., 2002; Weiss et al., 2008). However, supplementation of P169 increased milk yield by 3.2 kg/d in a study by Stein et al. (2006). It is not known if cow response to P169 is dependent upon the dietary starch concentration or if the response can be altered by supplementing cows with P169 during the transition period.

There are concerns with feeding high starch diets to fresh cows due to increased flux of propionate delivered in circulation to the liver according to Oba and Allen (2003a). Also, supplementation with P169 may increase the flux of propionate delivered to the liver. Allen et al. (2009) suggested excessive amounts of propionate delivered to the liver increased amount oxidized in the TCA cycle decreasing the firing rate of the hepatic vagus nerve resulting in increased satiety. Conversely, lower amounts of

propionate in circulation may result in a reduction in the stimulation of the hepatic vagus nerve increasing its firing rate resulting in greater DMI.

Recent research has focused on the implications of feeding a single lactation TMR to reduce labor costs and mixing time. Utilizing the single lactation TMR however, may result in over-feeding starch leading to a reduction in DMI and thus milk production and increase the incidences of metabolic disorders. A successful fresh cow diet should maximize DMI emphasizing fiber digestibility which may result in increased energy balance and reduction in lipolysis (Litherland et al., 2013). High starch diets may have a greater negative impact on primiparous cows as they typically consume more DM as a percent of BW compared to multiparous cows. According to the NRC (2001) primiparous cows consume less feed and in a different pattern (peaking later) than multiparous cows. Additionally, it is believed that primiparous cows are usually more timid and occupy a lower rank in the social herd hierarchy (Wierenga, 1990). Also, multiparous cows have a greater demand for glucose as milk production is greater than primiparous cows resulting in greater propionate demand.

There have been previous studies evaluating the effects of feeding low starch diets to lactating dairy cows. Replacing corn with soy hulls resulted in a tendency for a decrease in milk yield driven mostly by soy hull feeding rates of 30 and 40% of diet DM (Ipharraguerre et al., 2002). Decreased dietary starch concentration (32.9 to 17.6%) by replacing shell corn and soybean meal with wheat middlings, dried brewers grains, and soy hulls decreased DMI and increased milk fat content (Batajoo and Shaver, 1994). Another study (Dann et al., 2008) found no differences in DMI or milk yield when feeding dietary starch concentrations of 17.7, 21.0, and 24.6 % of diet DM when corn

grain was replaced with dried beet pulp, wheat middlings, and distillers grains. Replacing high moisture corn with pelleted beet pulp (34.6, 30.5, 26.5 and 18.4% starch) linearly decreased DMI but 3.5% FCM tended to have a quadratic response with 30.5% starch being the greatest (Voelker and Allen 2003). Dry cracked corn was replaced by dried citrus pulp to decrease dietary starch from 31.0 to 20.0% of diet DM. Feeding the low starch diet reduced DMI, milk yield, fat yield, and true protein content (Broderick et al. 2002).

Prepartum feeding strategies for optimal postpartum performance and health have not yet been clearly defined. Prepartum treatment with propionibacteria has resulted in some positive improvements in postpartum hormone (Francisco et al., 2002; Aleman et al., 2007; Lehloenya et al., 2008) and metabolite profiles (Stein et al., 2006) as well as improved postpartum energetic efficiency (Weiss et al., 2008) . Objectives of this study were to determine the effects of periparturient starch concentration and P169 supplementation on periparturient DMI, milk production, lipid metabolism, and health. We hypothesized that supplementation with P169 would increase milk yield for both low and high starch treatments and that LSM and HSC would have similar milk yield.

MATERIALS AND METHODS

Animal Housing and Management

This experiment was conducted from September 2011 to March 2012 at the University of Minnesota Dairy Teaching and Research Center, St. Paul, MN. All procedures were approved by the U of MN Institutional Animal Care and Use Committee. Thirty primiparous Holstein and Holstein-cross (Holstein × Montbeliard × Swedish Red) dairy cows were assigned to one of four dietary treatments (as designed to

provide a 2 x 2 factorial design) 42 d prior to projected calving date and removed from the study at 56 days in milk (**DIM**). Cows were housed in individual tie stalls with rubber mattresses and bedded with sawdust in a mechanically cross-ventilated barn. Cows were moved to a calving area once calving appeared imminent and were returned to their tie-stalls after calving. Cows were milked twice daily (0200 and 1400 h) and fed once daily during the dry period (1300 h) and once daily after calving at (1100 h).

Assignment to treatment and treatment description

Prior to the start of the trial, treatment groups (Table 4.1) were balanced by breed, dam's best 305 ME milk production to estimate milking potential (11,366 ± 739 kg), body weight (552 ± 18 kg), and body condition score at -42 days (3.2 ± 0.1 points). As planned, the number of days on prepartum dietary treatment (43.8 ± 3 days) was similar (P = 0.77) among treatments (Table 4.1). Prepartum treatments (Table 4.2) included: 1) low starch + control (direct fed microbial (**DFM**) carrier (sucrose)) (**LSC**), 2) low starch + DFM (**P169**, Dupont Bioscience, Waukesha, WI) (**LSM)**, 3) high starch + control (**HSC**), 4) high starch + DFM (**HSM**). Prepartum treatments were continued after parturition. Diets were formulated using the Cornell-Net-Carbohydrate-Protein-System (CNCPS, version 6.1; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY). Diets (Table 4.2) were fed at an *ad libitum* rate (to ensure 10 % feed refusals) and were formulated to provide varying amounts of dietary starch by varying the amount of corn silage, dry corn, and soybean hulls to obtain prepartum dietary starch differences of (low, 15.5 ± 0.8% and high, 26.7 ± 0.8%) and postpartum (low, 20.1 ± 0.7% and high, 29.7 ± 0.8%). Diets were formulated to test the effects of interactions of dietary starch

concentration with the addition of DFM P169, 20 g/cow/d delivering 60 billion cfu. Alfalfa hay and wheat straw were chopped in a vertical mixer for forty minutes to reduce particle size to a uniform consistency between forages. Tap water was added to both diets to maintain equal DM among treatments. Individual ingredient DM was maintained weekly by adjusting ingredient as-fed amounts based on DM measured after drying overnight at 100°C. Low and high starch diets were designed to provide similar macro- and micro-mineral ingredients to provide a TMR with similar mineral and vitamin composition (Table 4.2). Individual diet ingredients were sampled weekly, frozen at -20°C and composited monthly on a wet weight basis. Samples were analyzed at Dairyland Laboratories (St. Cloud, MN) using wet chemistry methods. Monthly averages of the nutrient composition of individual ingredients were analyzed and entered in the CNCPS dairy model to calculate monthly diet nutrient composition (Table 4.2). Ash content was determined using method 942.05 (AOAC International, 1996). Crude protein was determined using method 990.03 (AOAC International, 2000). Neutral detergent fiber for feed ingredients was determined using method 2002.04 (AOAC International, 2005). Acid detergent fiber was determined using method 973.18 (AOAC International, 1996). Lignin was determined using method 973.18 (AOAC International, 1996). Concentrations of minerals in feed were determined by method 953.01 (AOAC International, 1995). Water soluble carbohydrate was determined with extraction procedure adopted from Derias (1961). Detection procedure utilized from Neutral Detergent- Soluble Carbohydrates Nutritional Relevance and Analysis (Hall, 2000). Total sugar (expressed as invert) was determined by method 968.28 (AOAC International, 2000). Starch was determined using an enzymatic method described by

Knudsen (1997). Mean particle size of the coarsely ground corn was determined by shaking in a sieve (ASTM sieve, Gilson Company, Inc., Worthington, OH) (utilizing screen sizes of 4.75 mm, 2.36 mm, 0.85 mm, 0.60 mm, and a pan) and calculation of weighted average was conducted utilizing ASAE method S319.2 (ASAE, 1993).

Nutrient Intake

Individual cow DMI was measured daily from dry-off to 56 DIM. Feed offered and refused were recorded electronically at 0930 h. Daily DMI was calculated by correcting as-fed offered minus refusals and multiplied by weekly diet DM. Energy balance both pre- and postpartum was calculated using equations described by NRC (2001). Net energy intake (NE_I) was determined by multiplying DMI by the calculated mean NE_L density of the diet using CPM Dairy (Version 3.0.8; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY). Weekly means for prepartum DMI and BW in addition to day's dry and calf body weight were used as model inputs for prepartum energy balance calculations. Postpartum energy balance was calculated using inputs of weekly averages of DMI, BW, milk yield, and milk composition.

Prepartum diet sorting analysis and feeding behavior

Orts were collected at 0700 h from individual feed bunks for three consecutive day's prepartum (d -21, -20 and -19) and a composite of the TMR was collected and particle size distribution was determined by shaking procedure described by Kononoff et al. (2003) in a Penn State Particle Separator (**PSPS**) and TMR and orts were dried at 60^0C for 48 hours. Intake of particles on an as-fed basis was calculated for each screen for each cow as difference between diets offered and refused. Selective particle

consumption (sorting) was calculated using guidelines described by Leonardi and Armentano (2003); as the actual intake of each pan expressed as a percentage of the predicted intake, where predicted intake is percent of TMR delivered in each pan. Selective particle consumption was defined using guidelines described by Leonardi et al. (2005); values equal to 100 represent no sorting, < 100 show selective refusals, and > 100 indicate preferential consumption. Feeding behavior of eating, ruminating, and standing were monitored once prepartum between d -28 to d -21 relative to expected calving and postpartum between d 21 to 35 relative to actual calving using 10 minute scans for a continuous 24h period. Number of events were recorded and multiplied by 10 minutes to determine the amount of time a day cows spent eating, ruminating, and standing according to Endres et al. (2005).

Body Weight, Body Condition Score and Rumen Fill Score

Body weight was measured, body condition score (**BCS**) assigned using 0.25-unit increments (Ferguson et al., 1994), and rumen fill score (**RFS**) assigned on a scale from 1-5 according to Zaaijer and Noordhuizen (2003) for each cow weekly from dry-off to fifty-six DIM. Two trained individual scorers assigned BCS and RFS weekly before morning feeding and averages of the two scores are reported.

Colostrum Yield and Composition

Colostrum was harvested after calving and individual cows were milked last at the next milking session after calving. Colostrum yield was recorded and two samples from the first milking were collected and frozen at -20°C. One frozen sample was sent to Colorado State Veterinary Diagnostic Laboratories (Colorado State University, Fort Collins, CO) where total IgG concentration was determined using the Bovine IgG Vet-

RID (radial immunodiffusion) kit (Triple J Farms, Bellingham, WA, 98226). Due to high concentrations of IgG, the sample was first diluted 10-fold with distilled water then 5 μl of colostrum was analyzed according to kit instructions. After plating colostrum remained at room temperature for a minimum of 18 h, and then the precipitation ring diameters measured and IgG values calculated. Three standards with known IgG concentration (196; 1,402; and 2,748 mg/dl) were analyzed in each run. The diameters of the known standards were then used to calculate the tested colostrum samples. The second colostrum sample was thawed and analyzed at room temperature for specific gravity using a brix refractometer (Reichert Rhino VET 360, Reichert, Depew, NY) and dried in a 60°C oven for 72 h to determine DM content.

Milk Yield and Composition

Milk weights were recorded for individual cows from 1 to 56 DIM and composite samples were obtained from consecutive a.m. and p.m. milking's weekly from 4 to 56 DIM. Milk samples were preserved (800 Broad Spectrum Microtabs II; D and F Control Systems, Inc. San Ramon, CA) and were analyzed for fat, protein, lactose, milk urea N, and SCC using mid-infrared procedures (AOAC, 1995) at a commercial laboratory (DHIA, Zumbrota, MN).

Concentrations of Serum NEFA, BHBA, and total blood calcium

Blood was sampled from the coccygeal vein or artery on d -43, -28, -14, -7, -3 and -1 relative to expected parturition and at +1, +7, +14, +21, and +28 d and 12 h and 24 h after parturition for total blood calcium analysis relative to actual parturition. Samples were collected before feeding (0800) into evacuated serum tubes (SST; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing clot activator. Serum was obtained

by centrifugation for 20 minutes at 1,300 × *g*. Aliquots of serum were frozen at -20°C until later analysis for contents of NEFA (NEFA-HR(2) kit; Wako chemicals USA, Inc., Richmond VA), BHBA (Precision Xtra Meter; Abbott Laboratories, Inc., North Chicago, IL), and total blood calcium (Calcium Arsenazo III kit, Pointe Scientific, Inc., Canton, MI)

Liver composition

Liver was sampled via puncture biopsy (Hughes, 1962; Veenhuizen et al., 1991) from cows under local anesthesia at approximately 0700 h on d -14 (to determine effects of dry period treatments), +7 and +14 (to determine effect of pre- and postpartum nutrition on lipid accumulation following parturition). Before biopsy, local anesthesia was infiltrated in the intercostal space posterior to the tenth rib followed by aseptic preparation of the surgical site with Vedadine®(Vedco Inc., St. Joseph, MO). Liver cores were frozen immediately in liquid N, transferred to a -80°C freezer and later analyzed for contents of total lipids (Hara and Radin, 1978), triaclyglycerol (Fletcher, 1968; Foster and Dunn, 1973), and glycogen (Lo et al., 1970).

STATISTICAL ANALYSIS

Statistical analysis was completed using SAS® version 9.3 (SAS Institute Inc., Cary, NC). Data were analyzed as a 2 x 2 factorial design with factors being Starch amount and DFM. All cows completed the study. Before analyses, data were tested for normality using Proc Univariate. Data with daily measurements were averaged weekly and multiple measurements over time were processed using the REPEATED statement in the MIXED procedure with 3 covariance structures: compound symmetry, auto-regressive order 1, and heterogeneous auto-regressive order 1. The covariance structure

that resulted in the lowest Akaike information criterion was used (Littell et al., 1996). The model included breed, STARCH, DFM, time, and interactions between STARCH, DFM, and time. Data not analyzed over time were subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996). Least squares means for starch, DFM, time, and all interactions were separated using the PDIFF statement. Prepartum and postpartum data were analyzed separately. Significant data were declared at $P < 0.05$ and trends are discussed when $P < 0.15$.

RESULTS

Prepartum DMI, rumen fill, energy balance, diet sorting, body weight, and body condition

There were no significant effects of STARCH ($P = 0.43$), DFM ($P = 0.83$), or interaction of STARCH and DFM ($P = 0.84$) on DMI (Table 4.3), and DMI decreased ($P < 0.01$) as calving approached (Figure 4.1). High starch tended ($P = 0.07$) to increase prepartum rumen fill score (Table 4.3). High starch increased ($P = 0.04$) prepartum energy balance. Selective feed particle consumption prepartum was similar among treatments (Table 4.4). Starch amount and DFM had no effect on body weight or body condition score.

Postpartum DMI, rumen fill, energy balance, body weight, and body condition

Dietary starch concentration and DFM did not affect postpartum DMI (Figure 4.1). Energy balance was similar among treatments (Table 4.3). Body weight and body condition score were similar among treatments. Compared with Control, DFM supplementation increased rumen fill score on weeks six ($P = 0.01$) and seven ($P = 0.03$).

Milk, milk component yield, and feed efficiency

Milk yield (Figure 4.2) was similar among treatments. However, cows fed high starch tended ($P = 0.11$) to produce more 3.5 % fat corrected milk (FCM) and energy corrected milk (ECM) ($P = 0.11$) (Table 4.5). STARCH and DFM did not alter feed efficiency. Milk fat and true protein concentration were also similar among treatments (Table 4.5). Fat yield, however, tended ($P = 0.14$) to be greater for high starch compared to low starch. Cows fed high starch with DFM supplementation tended ($P = 0.14$) to have greater fat:protein ratio compared to LSM and HSC.

Prepartum liver total lipid, triaclyglycerol, glycogen and serum NEFA and BHBA

STARCH and DFM had no effect on serum concentration of NEFA or BHBA (Table 4.6). Liver total lipid, triaclyglycerol, and glycogen concentration were all similar among treatments (Table 4.6).

Postpartum liver total lipid, triaclyglycerol, glycogen, and serum NEFA, BHBA, and blood calcium homeostasis

Serum NEFA (Figure 4.3) and BHBA (Figure 4.4) concentration were similar among treatments. High starch increased ($P = 0.02$) liver total lipid (Figure 4.5) and increased ($P = 0.03$) liver triaclyglycerol concentration (Figure 4.6). Liver glycogen concentration (Figure 4.7) tended ($P = 0.08$) to increase with greater dietary starch concentration. Blood serum total calcium at 12 h after parturition was decreased ($P = 0.05$) with the supplementation of DFM, but by 24 h after calving total blood calcium was similar among treatments (Table 4.6).

Cow health events

Cows fed HS had a numerical increase in the number of observed incidences of metritis two, zero, two, and five and mastitis zero, one, two, and one for LSC, LSM, HSC, and HSM respectively (Table 4.7).

Calf birth weight, colostrum yield, and quality

Calf birth body weight was similar among treatments and averaged 41.6 ± 2.3 kg. Increasing dietary starch concentration tended ($P = 0.09$) to increase colostrum yield, decreased ($P < 0.01$) colostrum IgG concentration, and decreased ($P = 0.02$) specific gravity (Table 4.8).

Feeding behavior pre- and postpartum

Prepartum feeding behavior indicated that feeding high starch decreased ($P = 0.05$) time spent eating compared with low starch diets (Table 4.9). Time spent ruminating and standing was similar among treatments. Postpartum time spent eating, ruminating, and standing were not altered by STARCH or DFM (Table 4.9).

DISCUSSION

DMI, rumen fill, energy balance, body weight, and body condition

Dry matter intake was measured to study the interactions of varying starch concentration and supplementation of DFM during the periparturient period. It has been suggested (Allen, 2012) that starch concentration should be adjusted according to stage of lactation. Additionally, starch digestibility is also a key factor in dairy rations not just starch amount, as starch is not a nutrient required by dairy cattle (Chase, 2007). It is important to note that in the present study, the major starch source was from coarsely ground dry corn which has a slower rate and site of fermentation and digestion than finely ground high moisture corn (Ying and Allen, 2005). According to Allen et al.

(2009) compared with diets containing high amounts of rapidly fermentable starch, lower starch diets should increase DMI early in lactation due to the reduction of the amount and rate of propionate production in the rumen resulting in less hepatic oxidation of propionate signaling satiety. Ferraretto et al. (2011) found that replacing dry ground shelled corn with wheat middlings and cottonseed to produce a low starch diet (22.1 %) vs. a high starch diet (27.0%) increased DMI. Our data is in contrast to these studies as DMI was similar among treatments.

The supplementation of P169 decreased DMI by 1 kg/d (Weiss et al., 2008) and 3.6 g/kg of BW (Franscisco et al., 2002). Our data shows that supplementation with P169 did not alter DMI or DMI expressed as a percent of BW. Oba and Allen (2003b) measured an increase in propionate yield when cows were fed a high starch diet compared to a low starch diet. Also, Oba and Allen (2003a) determined that increased amount of propionate infusion around meal time, decreases DMI in early and mid-lactation dairy cows. In the present study however, DMI was not reduced with the increase in starch concentration. We hypothesized that this effect is due to reduced DMI by primiparous cows compared to multiparous cows thus a reduction in total propionate yield due to lower starch intake. Also, we fed coarsely ground dry corn that has a slower rate of fermentation in the rumen compared to other starch sources such as high moisture corn or finely ground corn (Owens et al., 1986; Huntington, 1997; Oba and Allen, 2003b). Rumen digestibility of coarsely ground dry corn (1863 μm) in a high starch diet (32%) was reported to be 46.9% as measured by Oba and Allen (2003b). Prepartum energy balance was greater for cows fed high starch diets, as high starch diet had greater energy density which was similar to results by Rabelo et al. (2005).

Milk, milk component yield, and feed efficiency

Due to the increase in propionate production supplied in the rumen as reported by Stein et al. (2006), an increase in milk yield and feed efficiency would be expected due to an increase in substrate supply for hepatic gluconeogenesis. A study by Weiss et al. (2008) measuring the effects of P169 with a high starch (31.1%) diet reported no differences in milk production. However, Weiss et al. (2008) reported an increase in rumen propionate concentration with P169 supplementation at 100 days in milk. Our study also demonstrated that supplementation with P169 had no effect on milk yield. Stein et al. (2006) reported an increase of 4% fat corrected milk yield of 3.2 and 2.9 kg/d over control with P169 supplementation at two increasing concentrations. Our data, however, supports Francisco et al. (2002) and Weiss et al. (2008) as 3.5% fat corrected and energy corrected milk yield were similar among treatments with P169 supplementation.

In early lactation, rapidly fermentable high starch diets may have the potential to increase milk production due to an increase in propionate production (Norzière et al., 2014), and supplementation of P169 also increases propionate production in the rumen (Stein et al., 2006; Weiss et al., 2008) to deliver glucogenic precursors to the liver. However, Ipharraguerre et al. (2002) found that decreasing dietary starch by replacing corn grain with soyhulls resulted in similar 3.5% FCM yield. In another study Stone (1996), replaced high moisture corn with soyhulls (Starch % of DM: 16.0% vs 25.0%) and reported no effect on milk and 3.5% FCM yield. Replacing coarsely ground dry corn with soyhulls in the present study resulted in a tendency for decreased 3.5% FCM and energy corrected milk yield.

Liver total lipid, triaclyglycerol, glycogen, and serum NEFA, BHBA, and blood calcium homeostasis

In order to investigate periparturient hepatic lipid metabolism in the liver, liver total lipid, triaclyglycerol, and glycogen concentration in biopsies of liver were measured. Hepatic lipid metabolism is early lactation is important to determining transition cow health and success (Drackley, 1999; Janovick et al., 2011). An increase in lipolysis leads to an increase in circulating non-esterified fatty acids which results in an increase risk for developing fatty liver and ketosis (Emery et al., 1992; Drackley, 1999; Ingvartsen, 2006). Over feeding energy prepartum has led to an increase in liver total lipid and triaclyglycerol concentration and decrease in glycogen postpartum (Dann et al., 2006; Douglas et al., 2007; Janovick et al., 2011). Our results are similar to Janovick et al. (2011) as high starch increased liver total lipid and triaclyglycerol concentration postpartum when cows were overfed energy prepartum.

Ruminants rely heavily on gluconeogenesis to supple glucose to meet energy requirements (Aschenbach et al., 2010). Liver glycogen concentration is related to the amount of gluconeogenesis relative to glycolysis occurring in the liver. Liver glycogen concentration was increased with low starch and P169 supplementation prepartum, and low compared to high starch tended to increase liver glycogen concentration postpartum.

Oba and Allen (2003b) measured an increase in propionate yield when cows were fed a high starch diet compared to a low starch diet. Additionally, the cow response to high starch diets is variable due to the rate and site of digestion of various starch sources in the rumen (Owens et al., 1986; Huntington, 1997; Oba and Allen. 2003b). Changes in proportions of rumen VFA's are influenced by the microbial population and rumen

availability of starch in the supplemental corn. Previous studies (Oba and Allen, 2003b) measured an increase in propionate production in the rumen in high starch diets. Higher propionate concentrations in the rumen would result in an increase in the amount of propionate being delivered to the liver by portal circulation.

Feeding behavior pre- and postpartum

Feeding behavior was altered by low starch, but not by P169, as cows tended to spend more time eating prepartum. This is likely attributed to a reduction in palatability with the addition of soyhulls. Ruminating time was similar among treatments, which is in contrast to Oba and Allen (2003b) as they reported a decrease in ruminating time for high starch (31.1%) compared to low starch (21.0%). Their study, however, used high moisture corn and finely ground corn to alter dietary starch concentration compared to coarsely ground dry corn which would have a slower rate of rumen fermentation as described by Ying and Allen (2005).

Colostrum yield and quality

Knowledge of factors influencing colostrum yield and quality is limited. Beef cows supplemented with additional forage over winter had greater colostrum yield than cows that were not supplemented (Petrie et al., 1984). In the current study, increased energy intake in the high starch diet increased colostrum yield but reduced colostrum quality as measured by specific gravity. However, this change in colostrum yield and colostrum specific gravity is likely due to a dilution factor.

CONCLUSIONS

DMI was not affected with the addition of STARCH or DFM pre- or postpartum. Milk yield and feed efficiency were similar among treatments. However, high starch

tended to increase 3.5 % fat corrected milk and energy corrected milk yield. Prepartum liver total lipid and triaclyglycerol concentration were similar among treatments and liver glycogen concentration increased with low starch and supplementation with DFM. STARCH and DFM did not alter serum NEFA or BHBA concentrations prepartum. However, postpartum high starch increased liver total lipid and triaclyglycerol concentration. Postpartum blood serum NEFA and BHBA were similar among treatments. In summary, milk yield and feed efficiency were similar among treatments. High starch tended to increase 3.5% FCM and ECM. High starch increased postpartum liver total lipid and triaclyglycerol content.

Table 4.1. Description of primiparous dairy cows at the start of the prepartum phase 42 d before expected parturition fed low or high starch and carrier or direct fed microbial (DFM, *Propionibacterium freudenreichii* strain P169) both pre- and postpartum.

	Treatments[1]				
Variable	LSC	LSM	HSC	HSM	SEM[2]
n	7	7	8	8	---
305ME[3], dam's best lactation, kg	11,543.0	11,430.0	11,040.0	11,451.0	739.0
Body weight, kg	540.0	553.1	579.6	537.5	20.7
Body condition score[4] d (-42)	3.1	3.2	3.2	3.2	0.1
Days on prepartum diet	44.6	45.7	41.4	43.5	3.0

[1] Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM).
[2] Largest standard error.
[3] Complete 305 d mature equivalent milk production.
[4] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.

Table 4.2. Diet ingredients and nutrient composition of diets fed pre- and postpartum (CNCPS 6.1).

	Diets[1]			
	Prepartum		Postpartum	
	Low Starch	High Starch	Low Starch	High Starch
Ingredients, % Diet DM				
Alfalfa hay, chopped	11.6	11.6	10.8	11.8
Wheat straw, chopped	23.2	23.2	0.0	0.0
Corn silage, processed	44.7	29.8	38.0	38.0
Protein mix[2,3]	13.9	13.9	19.8	21.3
Corn, coarsely ground 1.60 mm[4]	0.0	21.5	9.2	22.4
Soyhulls, loose	6.6	0.0	15.7	0.0
Cottonseed, fuzzy	0.0	0.0	2.9	2.9
Liquid supplement[5]	0.0	0.0	3.5	3.5
Water, tap kg/d per cow	1.3	4.5	4.0	4.3
Nutrient composition				
DM %	50.1	50.0	50.0	50.0
CP, % DM	13.1	13.2	16.5	16.8
RUP[6], %CP	30.6	32.3	37.2	37.3
RDP[6], %CP	69.4	67.7	62.8	62.7
ADF, % DM	33.5	26.9	24.9	17.9
NDF, % DM	47.6	39.2	36.5	27.7
Starch, % DM	15.5	26.7	20.1	29.7
Water soluble carbohydrates, % DM	4.0	4.3	6.9	7.2
Lignin, % DM	4.3	3.8	3.4	2.8
Soluble fiber[7], % DM	4.3	3.8	5.7	5.1
Ash, % DM	9.2	9.0	9.0	9.0
Ca[7], % DM	0.51	0.43	0.83	0.75
P, % DM	0.34	0.36	0.40	0.44
Mg[7], % DM	0.29	0.27	0.31	0.29
K, % DM	1.42	1.28	1.70	1.62
S, % DM	0.18	0.18	0.21	0.22
Na, % DM	0.15	0.15	0.40	0.41
Cl, % DM	0.37	0.37	0.59	0.60
Iron, ppm	205.65	161.68	215.46	160.88
Zinc, ppm	28.48	28.24	97.60	96.70
Copper, ppm	10.05	8.89	25.32	23.59
Manganese, ppm	40.00	35.94	70.51	70.45
Selenium, ppm	0.37	0.37	0.36	0.37
Cobalt, ppm	0.28	0.28	0.99	0.99
Iodine, ppm	0.58	0.58	1.04	1.05
Vit-A,[8]IU/kg	6769.8	6769.8	6104.1	6080.5
Vit-D,[8] IU/kg	1354.8	1354.8	1221.8	1217.1
Vit-E,[8] IU/kg	67.7	67.7	24.5	24.4
DCAD1,[9] meq/kg	209.0	172.0	310.0	289.0
NE_L[10], Mcal/kg	1.44	1.55	1.71	1.85
ME[8], Mcal/kg	2.05	2.17	2.33	2.45

[1]Diets fed from 42 d prepartum through 56 d postpartum.
[2] Prepartum protein mix, ingredients: 42.2% soybean meal (48% CP), 21.5 % soy pass, 29.8 corn dried distillers grains with solubles, 5.7% Munson Lakes (Howard Lake, MN) dry cow supreme premix, 0.9% MgO; composition: 43.2% CP, 7.5% ADF, 17.5% NDF, 13.4% Ash, 4.2% EE, 1.2% Ca, 1.2% P, 1.1% Mg, 2.1% K, 0.6% S, 0.8% Na, 0.9% Cl.
[3] Postpartum protein mix, ingredients: 33.1% corn dried distillers grains with solubles, 27.8% soybean meal (48% CP), 14.9% soy pass, 2.9% sodium bicarbonate, 0.8% salt, 2.5% calcium carbonate, 0.3% MgO, 3.1% potassium carbonate, 2.3% blood meal, 11.1% canola meal, 0.3% alimet® (Novus International, St. Charles, MO), 0.8% megalac® (Church & Dwight Co. Inc., Princeton, NJ); composition: 34.5% CP, 15.5% ADF, 27.5% NDF, 17.1% Ash, 6.0% EE, 1.6% Ca, 0.9% P, 0.6% Mg, 2.0% K, 0.5% S, 2.2% Na, 1.5% Cl.
[4] Calculated value using equations from ASAE standard S319.2.
[5] Molasses-based liquid feed, Quality Liquid Feeds, Dodgeville, WI; 64.3% DM, 23.7% ash, 4.9% Ca, 3.4% K.
[6] Formulated value from Cornell-Net-Carbohydrate-Protein-System (CNCPS, version 6.1).
[7] Actual value lower than formulated value.
[8] Calculated value from Cornell-Net-Carbohydrate-Protein-System (CNCPS, version 6.1).
[9] Dietary cation anion difference equation 1 Calculated value Cornell-Net-Carbohydrate-Protein-System (CNCPS, version 6.1) = [(Na + K) – (Cl + S)]
[10] Calculated value from Cornell-Penn-Miner (CPM 3.1).

Table 4.3. Cows fed low or high starch and carrier or DFM pre- and postpartum DMI, energy balance, body condition score, rumen fill score and body weight from day -42 to 56.

	Treatments[1]								
	Low Starch		High Starch			*P*-value			
Variable	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]	Wk
Prepartum									
n	7	7	8	8					
DMI, kg/d	11.6	11.6	12.2	12.0	0.6	0.43	0.83	0.84	<0.01
DMI, % of BW	2.1	2.1	2.1	2.1	0.1	0.73	0.91	0.96	<0.01
Energy balance[6], Mcal/d	2.4	2.2	4.0	3.7	0.7	0.04	0.76	0.89	<0.01
Energy balance[7], % Req.	115.7	114.8	125.4	124.2	4.7	0.04	0.81	0.97	<0.01
Body condition score[8]	3.2	3.3	3.4	3.3	0.1	0.21	0.84	0.36	<0.01
Body condition change[9]	0.2	0.3	0.2	0.3	0.1	0.78	0.41	0.82	---
Rumen fill score[10]	3.2	3.6	3.8	3.8	0.2	0.07	0.31	0.37	0.17
Body weight, kg	560.6	574.9	586.1	575.0	20.7	0.50	0.93	0.50	<0.01
Body weight change, kg[11]	42.4	58.7	44.3	58.4	7.5	0.91	0.04	0.88	---
Postpartum									
DMI, kg/d	20.2	19.9	19.1	20.7	1.2	0.91	0.57	0.44	<0.01
DMI, % of BW	3.8	3.7	3.6	3.9	0.2	0.83	0.48	0.25	<0.01
Energy balance[6], Mcal/d	4.4	4.7	3.1	6.7	1.7	0.84	0.25	0.32	<0.01
Energy balance[7], % Req.	115.5	116.4	109.4	122.9	6.2	0.98	0.24	0.31	0.15
Body condition score[8]	3.2	3.3	3.3	3.3	0.1	0.47	0.89	0.42	0.01
Body condition change[12]	0.1	-0.1	-0.2	-0.1	0.1	0.19	0.87	0.21	---
Rumen fill score[10]	2.9	3.3	2.9	3.2	0.1	0.75	0.04	0.67	0.04
Body weight, kg	517.3	532.3	534.1	529.4	18.2	0.70	0.77	0.58	0.02
Body weight change, kg[13]	-1.6	-6.9	-11.9	-6.6	10.3	0.57	0.99	0.60	---

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + P169 pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + P169 pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.
[6] Energy balance = energy intake – energy requirements.
[7] Energy balance % requirement = (energy intake /energy requirements)*100.
[8] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.
[9] Body condition change week -1 minus week -6.
[10] Rumen fill score on a 1-5 scale, 1 = empty rectangle and 5 = extended over last rib.
[11] Body weight change week -1 minus week -6.
[12] Body condition change week 8 minus week 1.
[13] Body weight change week 8 minus week 1.

Table 4.4. Prepartum selective particle consumption (composite of day -21, -20 and -19 (N = 30)) at each of the four screens of the Penn State shaker box expressed as a percentage of expected consumption.

	Treatments[1]							
	Low Starch		High Starch			*P*-value		
Variable	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]
Top	100.0	95.0	100.0	95.0	0.04	0.91	0.21	0.98
Middle	98.0	98.0	101.0	99.0	0.01	0.15	0.60	0.56
Bottom	101.0	104.0	101.0	99.0	0.02	0.19	0.91	0.22
Pan	101.0	102.0	94.0	90.0	0.10	0.16	0.78	0.77

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + P169 pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + P169 pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.

Table 4.5. Primiparous cow feed efficiency, milk production, and milk quality postpartum day 1 to 56.

Variable	Treatments[1]				SEM[2]	*P*-value			
	Low Starch		High Starch						
	Control	P169	Control	P169		S[3]	P[4]	S × P[5]	Wk
n	7	7	8	8					
Milk[6], kg/d	29.2	29.2	32.8	30.8	1.9	0.17	0.58	0.62	<0.01
3.5% FCM[7], kg/d	29.3	28.4	32.0	31.1	1.7	0.11	0.59	0.99	<0.01
ECM[8], kg/d	30.0	29.4	33.0	31.7	1.7	0.11	0.55	0.84	<0.01
Feed efficiency[9]	1.46	1.48	1.65	1.50	0.09	0.24	0.47	0.34	<0.01
Fat, %	3.57	3.34	3.48	3.60	0.16	0.59	0.76	0.28	0.07
Fat, kg/d	1.04	0.96	1.10	1.10	0.07	0.14	0.56	0.56	<0.01
True protein,%	3.25	3.23	3.28	3.22	0.08	0.88	0.59	0.74	<0.01
True protein, kg/d	0.94	0.93	1.06	0.97	0.06	0.17	0.41	0.52	<0.01
Fat:protein	1.10	1.04	1.05	1.13	0.05	0.71	0.86	0.14	0.11
Lactose, %	4.99	4.96	4.87	5.00	0.06	0.50	0.40	0.16	<0.01
MUN[10], mg/dL	11.82	12.05	12.37	12.28	0.39	0.30	0.85	0.67	<0.01
Total solids,%	12.72	12.43	12.53	12.70	0.22	0.87	0.77	0.28	<0.01
SCC[11], cells/mL	227.68	127.22	203.44	120.91	83.16	0.85	0.27	0.91	0.01

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + P169 pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + P169 pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.
[6] Milk yield through 56 d postpartum.
[7] 3.5% Fat corrected milk = 0.4324 × (milk kg) + 16.2162 × (fat kg), through 56 d postpartum.
[8] Energy corrected milk = (0.327 × (milk kg)) + (12.95 × (fat kg)) + (7.65 × (protein kg)), through 56 d postpartum.
[9] Feed efficiency = 3.5% FCM divided by DMI.
[10] Milk urea nitrogen.
[11] Somatic cell count (1000's of cells)

Table 4.6. Primiparous cow liver lipid metabolism d 14 prepartum and d 7 and 14 postpartum, blood serum non-esterified fatty acids and beta-hydroxybutrate and blood total calcium levels at 12h and 24 h after parturition.

	Treatments[1]					*P*-value			
	Low Starch		High Starch						
	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]	day
Prepartum									
Liver tissue									
Total lipid[6], %	4.0	3.9	3.9	4.3	0.2	0.44	0.31	0.18	--
Triaclyglycerol[7], %	0.5	0.7	0.6	0.5	0.2	0.52	0.61	0.21	--
Glycogen[6],%	5.4	6.6	5.7	5.5	0.5	0.28	0.19	0.08	--
Triaclyglycerol:glycogen	0.10	0.11	0.10	0.09	0.03	0.72	0.81	0.64	--
Serum									
NEFA[8], µEq/L	164.7	177.5	146.6	165.0	22.6	0.48	0.47	0.90	<0.01
BHBA[9], µmol/L	359.4	377.4	391.3	390.2	40.8	0.53	0.81	0.79	0.13
Postpartum									
Liver tissue									
Total lipid[10],%	4.7	5.1	7.9	6.3	0.9	0.02	0.45	0.22	0.07
Triaclyglycerol[11],%	0.8	0.9	1.1	2.4	0.4	0.03	0.10	0.17	0.09
Glycogen[10],%	4.0	4.4	3.5	3.3	0.5	0.08	0.88	0.52	0.11
Triaclyglycerol:glycogen	0.3	0.4	0.3	0.7	0.2	0.17	0.09	0.29	0.53
Serum									
NEFA[12], µEq/L	248.3	245.6	271.7	276.6	38.8	0.47	0.98	0.92	<0.01
BHBA[13], µmol/L	533.0	451.6	536.5	557.5	61.6	0.37	0.62	0.40	<0.01
Calcium[14], total 12 h, mg/dL	10.1	7.0	9.4	8.6	1.0	0.65	0.05	0.24	----
Calcium[15], total 24 h, mg/dL	8.0	7.8	8.5	8.4	0.9	0.53	0.82	0.98	----

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + P169 pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + P169 pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.
[6] Percent of wet weight from liver samples from day -14 relative to parturition.
[7] Percent of liver total lipid from day -14 relative to parturition.
[8] Non-esterified fatty acid analysis of serum from days -43, -28, -21, -14, -7, -3 and -1 relative to parturition.
[9] Beta-hydroxybutyrate analysis of serum from days -7, -3 and -1 relative to parturition.
[10] Percent of wet weight from liver samples from day 7 and 14 relative to parturition.
[11] Percent of liver total lipid from day 7 and 14 relative to parturition.
[12] Non-esterified fatty acid analysis of serum from days 0.5, 1, 7, 14, 21 and 28 relative to parturition.
[13] Beta-hydroxybutyrate analysis of serum from days 1, 7, 14, 21 and 28 relative to parturition.
[14] Blood total calcium at 12 hours after parturition.
[15] Blood total calcium at 24 hours after parturition.

Table 4.7. Incidence of health events following parturition for primiparous cows.

	Primiparous (n=30)			
	Treatments[1]			
	Low Starch		High Starch	
	Control	P169	Control	P169
Health Event				
Twins	0	0	0	0
Metritis[2]	2	0	2	5
Mastitis[3]	0	1	2	1
Ketosis[4]	0	0	0	0
Retained placenta[5]	0	0	0	0
Milk fever[6]	0	0	0	0
Displaced abomasum	0	0	0	0
Downer[7]	0	0	0	0

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + P169 pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + P169 pre- and postpartum (HSM).
[2] Excessive dark brown or white discharge, possible foul odor and temperature greater than 103°F.
[3] Clinical mastitis, antibiotic therapy required.
[4] Clinical ketosis, diagnosed with keto stick, treated with dextrose.
[5] Placenta retained more than 24 hours.
[6] Clinical milk fever, diagnosed by lethargic activity and difficulty getting up, treated with CMPK IV solution.
[7] Cow was unable to stand for more than 24 hours.

Table 4.8. Calf weight and colostrum quantity and quality for primiparous cows.

	Treatments[1]							
	Low Starch		High Starch			*P*-value		
Variable	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]
Calf weight, kg	40.1	42.4	40.9	42.8	2.3	0.80	0.36	0.92
Colostrum								
Yield[6], kg	3.5	3.5	5.0	4.5	0.7	0.09	0.74	0.66
Specific gravity[7], g/dL	28.3	26.0	24.2	24.0	1.2	0.02	0.31	0.37
IgG[8], mg/dL	5665.7	5308.4	3705.6	4201.3	496.3	<0.01	0.89	0.38

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + P169 pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + P169 pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.
[6] Colostrum measured within 12 hours after parturition
[7] Colostrum specific gravity measured using Brix refractometer
[8] Colostrum IgG concentration measured at Colorado State Veterinary Diagnostic Lab, Fort Collins, CO

Table 4.9. Primiparous cow (n=30) feeding behavior using ten minute scans prepartum on d -21, -20 and -19 and postpartum on d 21, 22, 23.

	Treatments[1]							
	Low Starch		High Starch			*P*-value		
Variable	Control	P169	Control	P169	SEM[2]	S[3]	P[4]	S × P[5]
				Prepartum				
Eating, h/d	3.7	3.9	3.2	3.3	0.3	0.05	0.64	0.86
Ruminating, h/d	8.0	8.4	8.5	8.3	0.6	0.68	0.91	0.52
Standing, h/d	10.7	10.3	9.8	10.8	0.8	0.80	0.69	0.39
				Postpartum				
Eating, h/d	3.5	3.0	3.0	3.2	0.3	0.48	0.58	0.21
Ruminating, h/d	8.1	7.8	8.7	8.0	0.6	0.48	0.31	0.78
Standing, h/d	11.4	12.1	10.7	11.3	0.9	0.40	0.45	0.92

[1]Diets fed from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + P169 pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + P169 pre- and postpartum (HSM).
[2] Largest standard error.
[3] Low starch vs. high starch.
[4] DFM carrier vs. DFM.
[5] Interaction of starch concentration and DFM supplementation.

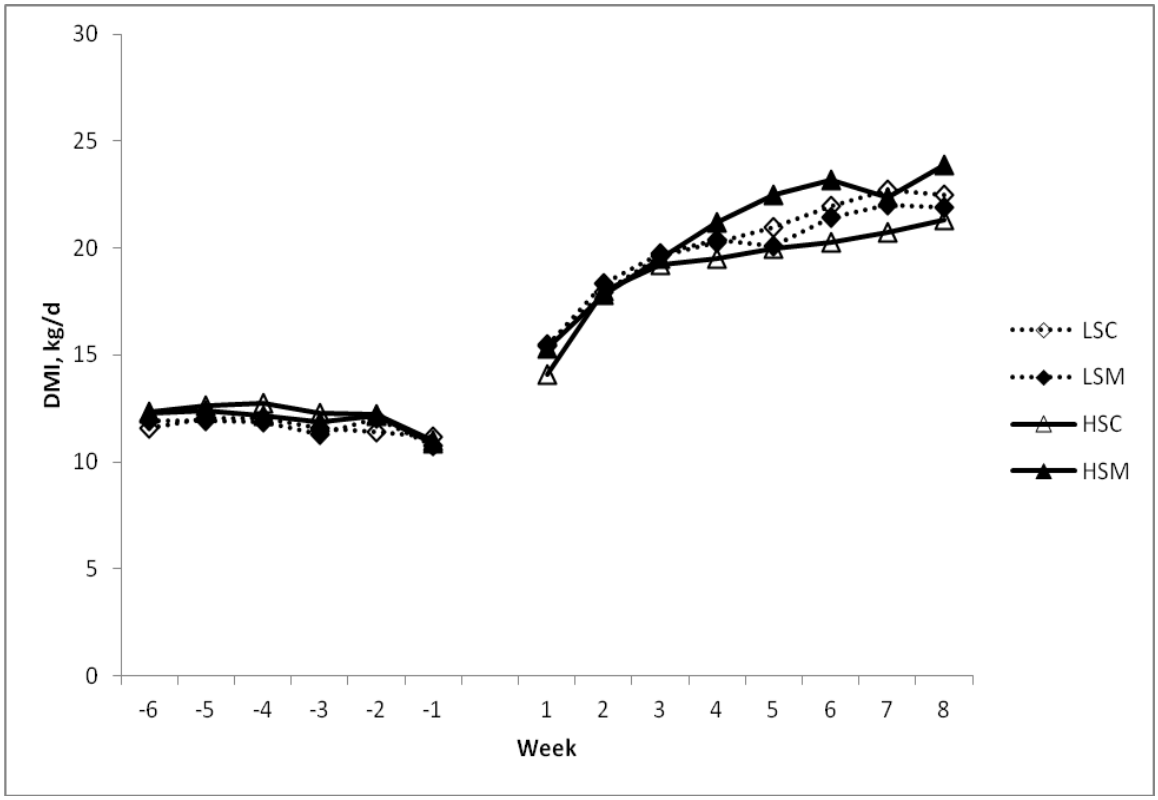

Figure 4.1. Effect of treatment on pre- and postpartum DMI, kg/d by week from -42 d to 56 d. Treatment from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.43$; DFM, $P = 0.83$; Starch × DFM, $P = 0.84$; Week, $P < 0.01$. Largest SEM = 0.6 kg/d. Postpartum: Starch, $P = 0.91$; DFM, $P = 0.57$; Starch × DFM, $P = 0.44$; Week, $P < 0.01$. Largest SEM = 1.2 kg/d. For clarity, SEM bars have been omitted.

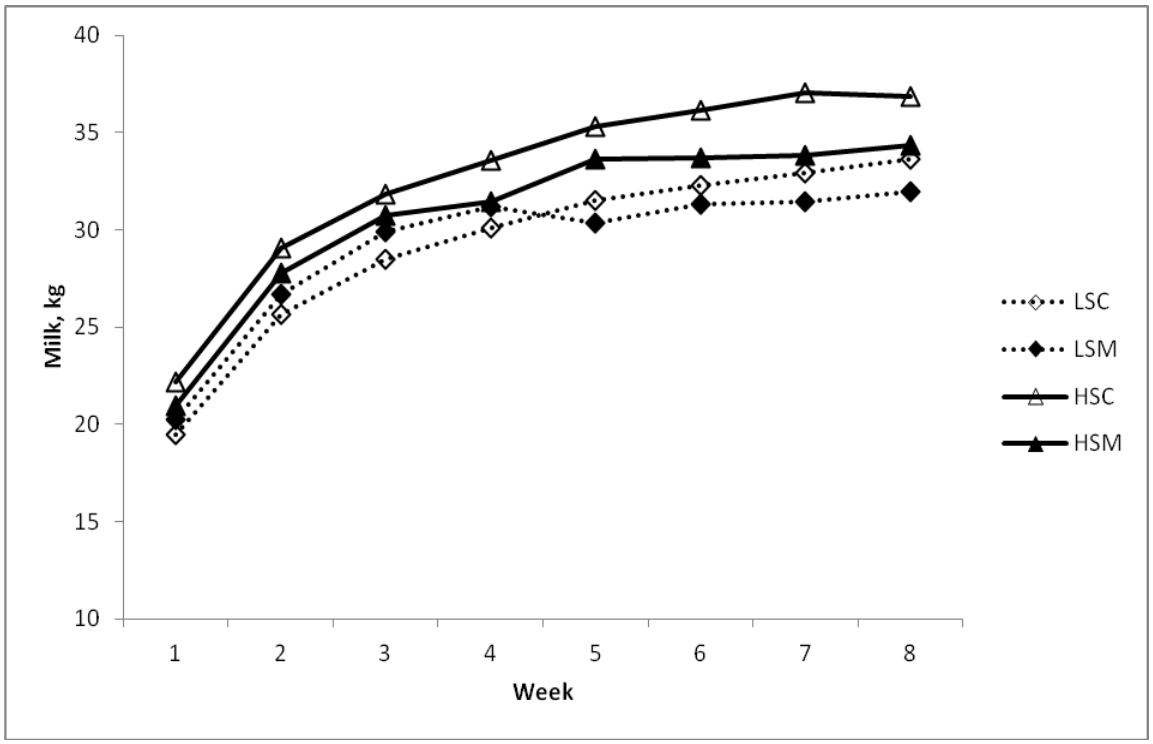

Figure 4.2. Effect of treatment on milk yield, kg by week. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Starch, $P = 0.17$; DFM, $P = 0.58$; Starch × DFM, $P = 0.62$; Week, $P < 0.01$. Largest SEM = 1.9 kg. For clarity, SEM bars have been omitted.

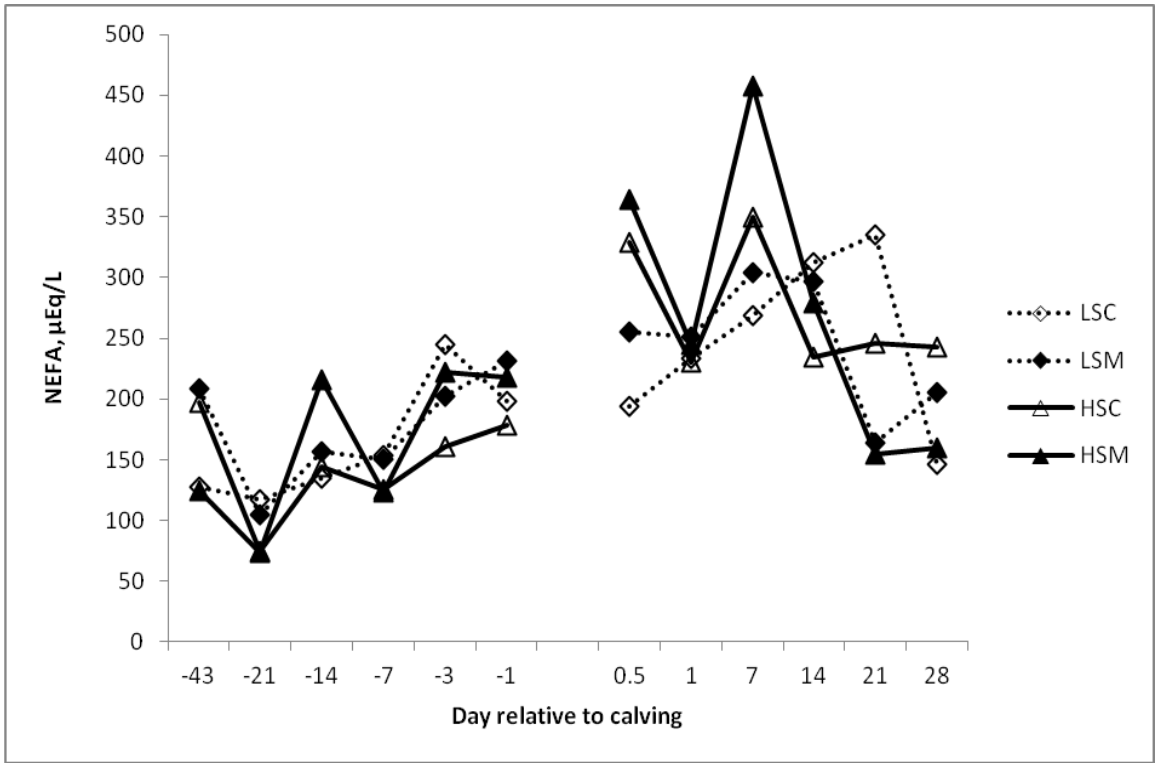

Figure 4.3. Effect of treatment on blood serum NEFA, µEq/L pre- and postpartum. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.48$; DFM, $P = 0.47$; Starch × DFM, $P = 0.90$; Day, $P < 0.01$. Largest SEM = 22.6 µEq/L. Postpartum: Starch, $P = 0.47$; DFM, $P = 0.98$; Starch × DFM, $P = 0.92$; Day, $P < 0.01$. Largest SEM = 38.8 µEq/L. For clarity, SEM bars have been omitted.

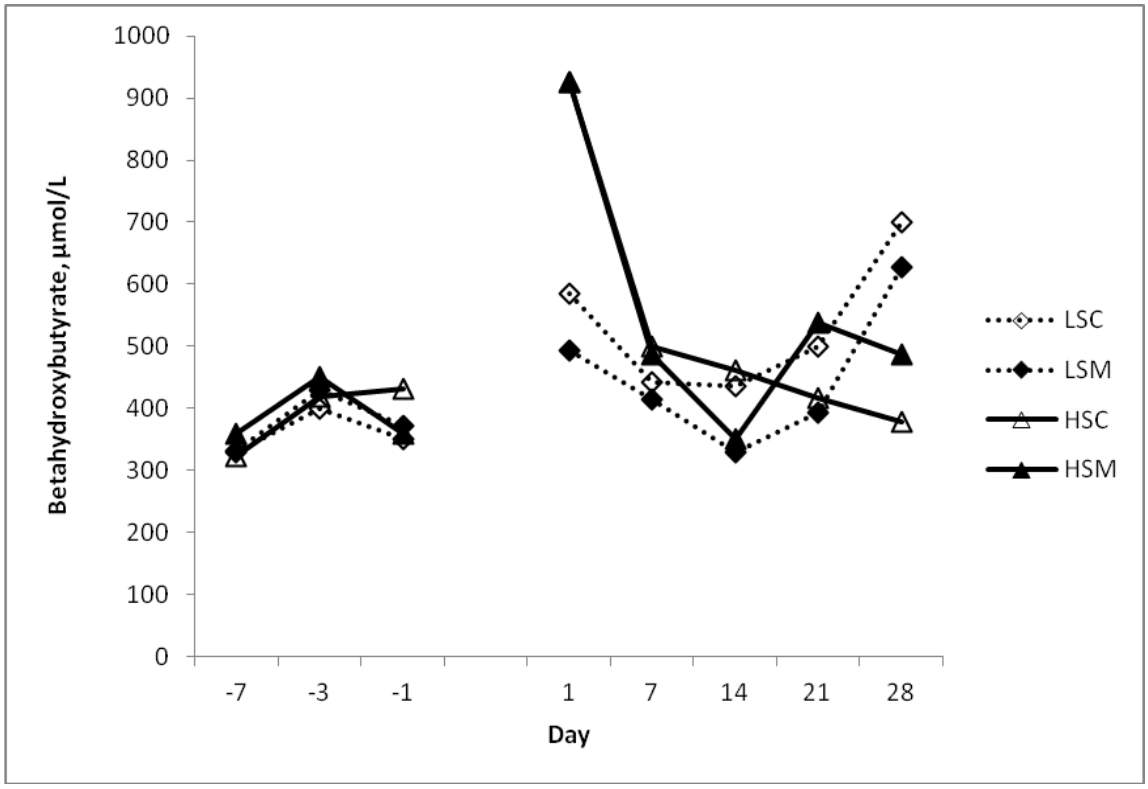

Figure 4.4. Effect of treatment on blood serum BHBA, µmol/L pre- and postpartum. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.53$; DFM, $P = 0.81$; Starch × DFM, $P = 0.79$; Day, $P = 0.13$. Largest SEM = 40.8 µmol/L. Postpartum: Starch, $P = 0.37$; DFM, $P = 0.62$; Starch × DFM, $P = 0.40$; Day, $P < 0.01$. Largest SEM = 61.6 µmol/L. For clarity, SEM bars have been omitted.

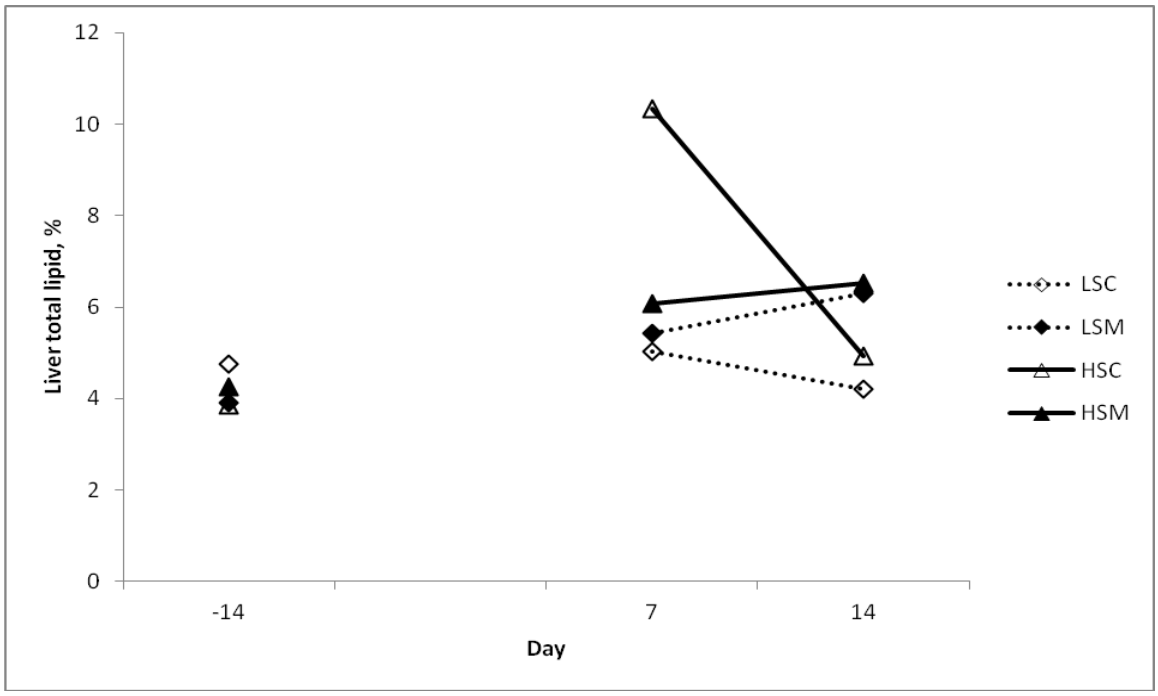

Figure 4.5. Effect of treatment on liver total lipid percent d -14, 7 and 14. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.44$; DFM, $P = 0.31$; Starch × DFM, $P = 0.18$. Largest SEM = 0.2 % of wet wt. Postpartum: Starch, $P = 0.02$, DFM, $P = 0.45$; Starch × DFM, $P = 0.22$; Day, $P = 0.07$. Largest SEM = 0.9 % of wet wt. For clarity, SEM bars have been omitted.

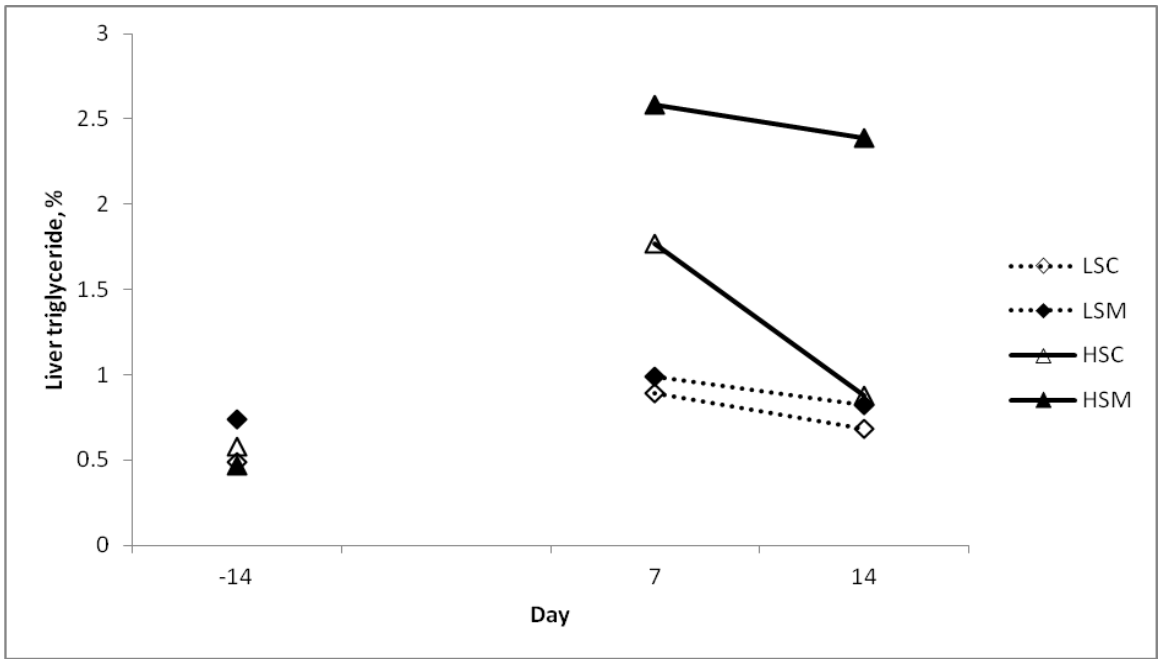

Figure 4.6. Effects of treatment on liver triaclyglycerol percent d -14, 7 and 14. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, P = 0.52; DFM, P = 0.61; Starch × DFM, P = 0.21. Largest SEM = 0.2 % wet wt. Postpartum: Starch, P = 0.03; DFM, P = 0.10; Starch × DFM, P = 0.17; Day, P = 0.09. Largest SEM = 0.4 % wet wt. For clarity, SEM bars have been omitted.

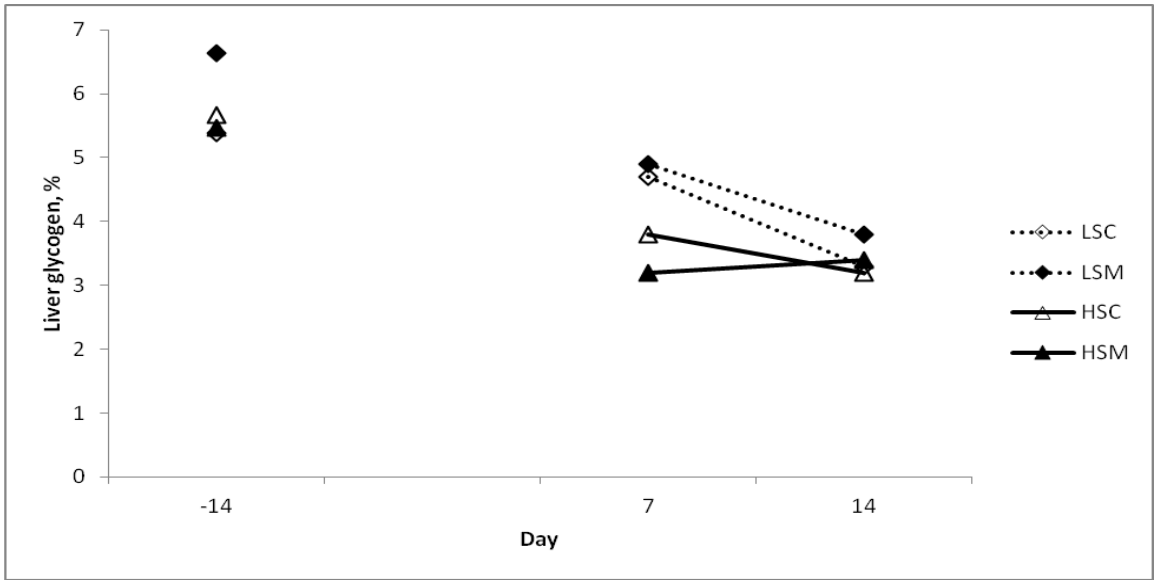

Figure 4.7. Effects of treatment on liver glycogen percent d -14, 7 and 14. Treatments from 42 d prepartum through 56 d postpartum; low starch + control (DFM carrier) pre- and postpartum (LSC); low starch + DFM (P169 + carrier) pre- and postpartum (LSM); high starch + control (DFM carrier) pre- and postpartum (HSC); high starch + DFM (P169 + carrier) pre- and postpartum (HSM). Prepartum: Starch, $P = 0.28$; DFM, $P = 0.19$; Starch × DFM, $P = 0.08$. Largest SEM = 0.5 % wet wt. Postpartum: Starch, $P = 0.08$; DFM, $P = 0.88$; Starch × DFM, $P = 0.52$; Day: $P = 0.11$. Largest SEM = 0.5 % wet wt. For clarity, SEM bars have been omitted.

Evaluation of low cost on farm predictors of individual cow risk for subclinical ketosis.

SUMMARY

Early lactation data from 3 studies (n=161 cows) were composited to determine on farm predictors for cows at risk for subclinical ketosis based on serum beta-hydroxybutyrate (**BHBA**) above 1.2 mmol/L, non-esterified fatty acids (**NEFA**), and liver triglycerides (**TG**). In experiment 1 the objectives of this retrospective analysis were to determine if low-cost, on-farm measures of colostrum yield (**CY**), colostrum specific gravity (**CSG**) and body condition score (**BCS**) at calving are predictors of subclinical ketosis. Pearson correlation coefficients were calculated for CY, CSG, BCS, BHBA, NEFA, and ME 305d milk production. In this data set, CY was significantly correlated with serum BHBA concentration on d 1 and d 7 postpartum. Serum NEFA was positively correlated on d 1, 7 and 14 with CSG using Brix refractometer. Both CY and CSG were positively correlated with liver TG on d 7 and CY and CSG were negatively correlated with DMI during the first week postpartum. BCS was negatively correlated with serum calcium 24 h postpartum and can identify cows at risk for hypocalcemia. BCS is positively correlated with serum BHBA at d 1 and d 14, serum NEFA at d 7 and d 14 and ME 305 day milk production. In experiment 2 we applied significant correlations found in experiment 1 to identify variables that predispose cows to exceed threshold for subclinical ketosis on commercial dairy farms. A field trial (n=328 cows) was conducted using two commercial dairy farms to develop the subclinical ketosis prediction model (Pre-K). Analysis of field data was conducted using multiple logistic regression with an ROC curve to determine sensitivity and specificity of individual and multiple predictors of subclinical ketosis identified in experiment 1. Based on data from experiment 2, day of

calving data of CY, BCS, and parity were included in the model. The Pre-K model resulted in a sensitivity of 60% and a specificity of 64% for subclinical ketosis prediction. Low cost, on farm measures of CY, BCS, and parity provides insight into rates of body reserve mobilization without blood or liver sample collection. The implementation of these variables together can allow dairy producers to quickly identify cows at risk for subclinical ketosis shortly after parturition. Rapid, low cost identification of cows at high risk for subclinical ketosis might allow for early treatment or implementation of tailored feeding and management strategies to minimize the incidence and severity of subclinical fresh cow disorders.

INTRODUCTION

The transition period is a critical time three weeks before and three weeks after parturition (Grummer et al., 1995; Drackley, 1999). In early lactation cows enter negative energy balance due to high energy demands for milk production and an inability to meet those demands through dry matter intake. Around the time of calving, there is an increase in somatotropin which reflects a reduction in DMI (Bell, 1995; Grum et al., 1996). Lower DMI in early lactation leads to a reduction in circulating insulin and an increase in glucagon (Ingvartsen and Anderson, 2000; Ingvartsen, 2006). Also, adipose tissue becomes less sensitive to insulin (Hayirli, 2006; Locher et al., 2011). In early lactation, an increase in glucagon, catecholamines, and adrenocorticotropic hormone stimulate hormone-sensitive lipase (Vaughan et al., 1964; Slavin et al., 1994; Holtenius et al., 1996) which shifts metabolism in adipose tissue from lipogenesis to lipolysis to provide non-esterified fatty acid (**NEFA**) as a energy source (McNamara, 1991). The greater amount of NEFA being mobilized results in greater uptake of NEFA by the liver (Emery et al., 1992). The liver can oxidize NEFA to produce ATP and ketones, or

reconvert NEFA to produce triglycerides and store them in the liver (Drackley, 1999; Ingvartsen, 2006). If cows are not able to adapt to negative energy balance successfully, excess body reserves are mobilized resulting in an increase in NEFA which leads to an increase in circulating ketones primarily beta-hydroxybutyrate (**BHBA**) due to oxidation in the liver. An elevated BHBA level above 1.2 mmol/L is a common cut point for cows experiencing symptoms of subclinical ketosis (Iwersen et al., 2009; Konkol et al., 2009, McArt et al., 2011).

Recent research has focused on measuring and detecting elevated BHBA concentrations in early lactation. The "gold standard" is measuring blood BHBA concentration (Oetzel, 2007). Urine (Carrier et al., 2004; Oetzel, 2007) and milk (Carrier et al., 2004; Geishauser et al., 2000) can also be used to measure ketones, but samples are typically difficult to obtain and have a lower sensitivity than blood (Carrier et al. 2004; Oetzel et al., 2012). Subclinical ketosis affects 26.4-55.7% (McArt et al., 2011) of cows and is estimated to cost $78 per case (Geishauser et al., 2001). More recently, McArt et al. (2014) estimated $104 per case of subclinical ketosis. Several studies have looked at the effects of elevated BHBA concentration on health and production in early lactation. Elevated BHBA concentration above 1.0 mmol/L increased the likelihood of a displaced abomasum (Geishauser et al., 1997). In addition, BHBA concentration above 1.4 mmol/L increased risk for metritis (Dohoo and Martin, 1984), severity of mastitis (Kremer et al., 1993), a 1.4 kg/d decrease in early lactation milk yield (Dohoo and Martin, 1984), decreased annual milk yield by 393 kg for multiparous cows (Ospina et al., 2010), and increased days from calving to conception (Cook et al., 2001).

Since subclinical ketosis increases the risk for metabolic and health disorders in early lactation, it is hard to estimate a cost for each case. Based on previous research conducted by McArt et al. (2011), the number of cows affected in a herd ranges from 26.4-55.7%. Therefore, the cost of subclinical ketosis can quickly add up on modern commercial dairy farms.

There are various tests available to detect cows with subclinical ketosis: blood BHBA (Precision Xtra Meter; Abbott Laboratories, Inc., North Chicago, IL), urine acetone and acetoacetate test (Uristick, Bayer Corp., Pittsburgh, PA), milk acetone and acetoacetate test (Afimilk, Afimilk USA, Inc., Massillon, OH), and DHIA first test fat:protein ratio. All of these tests can accurately identify cows at risk for subclinical ketosis. However, these tests all detect cows that are already experiencing negative effects of subclinical ketosis because they are measuring circulating ketone bodies. Therefore, a tool is needed to predict cows at risk for developing subclinical ketosis at calving in order to administer a preventative treatment and reduce the negative effects of subclinical ketosis.

In order for a subclinical ketosis prediction model to be beneficial, it must be; 1) easy to use, 2) economically feasible, and 3) able to accurately predict cows to allow dairy producers to proactively implement ketosis preventive treatments. Previously Nordlund (2006) proposed a Transition Cow Index™ (**TCI**) that predicts an individual cow's milk production in the current lactation based off of her individual history. If a cow produced above her predicted value then she transitioned into lactation extremely successfully, if a cow produced to her predicted value then she transitioned successfully, and if a cow produced below her predicted value then she transitioned poorly. This tool

can assist dairy producers in evaluating and managing their transition cow success. Objectives of this experiment were to; 1) Identify low cost on-farm at the time of calving predictors that positively correlate with postpartum ketosis, 2) develop the Pre-K model, and determine the ability of the model to accurately identify cows that will develop subclinical ketosis at the time of calving. We hypothesize that the Pre-K model will accurately identify cows at risk for developing subclinical ketosis.

Experiment I. Association of colostrum yield, colostrum specific gravity, and body condition score with calcium homeostasis, NEFA, BHBA, liver TG, DMI, and milk yield

MATERIALS AND METHODS

Animal housing and management

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. One hundred sixty-one multiparous Holstein and Holstein-cross (Holstein × Montbeliard × Swedish Red) periparturient dairy cows calving from three experiments from 2009 – 2011 were utilized from Farm A. Cows were housed in individual tie stalls with rubber mattresses and bedded with sawdust in a mechanically cross-ventilated barn. Cows were managed to have a 45 d dry period. Cows were moved to a calving area once calving appeared imminent and were returned to their tie-stalls after calving. Cows were milked twice daily (0200 and 1400 h) in a milking parlor and cows were fed once daily during the dry period (1300 h) and once daily after calving (1100 h).

Body Condition Score

Body condition score (**BCS**) was assigned using 0.25-unit increments on a scale from 1- 5 (1 = thin, 5 = obese) (Ferguson et al., 1994) for each cow weekly from dry-off to fifty-six DIM. Two trained individual scorers assigned BCS weekly before morning feeding.

Colostrum Yield and Composition

Colostrum was harvested after calving and individual cows were milked last at the next milking session after calving. Colostrum yield was recorded and a sample from the first milking was collected and frozen at -20°C, and analyzed at room temperature for specific gravity using a digital brix refractometer (Misco PA302, Cleveland, OH).

Milk Yield and Composition

Milk weights were recorded from 1 to 56 DIM and composite samples were obtained from consecutive a.m. and p.m. milking's weekly from 4 to 56 DIM. Milk samples were preserved (800 Broad Spectrum Microtabs II; D and F Control Systems, Inc. San Ramon, CA) and were analyzed for fat, protein, lactose, milk urea N, and SCC using mid-infrared procedures (AOAC, 1995) at a commercial laboratory (DHIA, Zumbrota, MN). Projected annual 305 d ME milk yield was calculated when cow reached 100 DIM.

Concentrations of Serum NEFA, BHBA, and total blood calcium

In Experiment 1, subclinical ketosis was defined with a BHBA $\geq$ 1.2 mmol/L. Blood was sampled from the coccygeal vein or artery on d +1, +7, and +14. Blood was sampled from the coccygeal vein or artery 12 h and 24 h after parturition for total blood calcium analysis. Blood samples were collected before feeding (0800) into evacuated serum tubes (SST; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing

clot activator. Serum was obtained by centrifugation for 20 minutes at 1,300 × *g*. Aliquots of serum were frozen at -20°C until later analysis for contents of non-esterified fatty acid (**NEFA**) (NEFA-HR(2) kit; Wako chemicals USA, Inc., Richmond VA), BHBA (Precision Xtra Meter; Abbott Laboratories, Inc., North Chicago, IL) (Iwersen et al., 2009; Konkol et al., 2009), and total blood calcium (Calcium Arsenazo III kit, Pointe Scientific, Inc., Canton, MI)

Liver composition

Liver was sampled via puncture biopsy (Hughes, 1962;Veenhuizen et al., 1991) from cows under local anesthesia at approximately 0700 h on d -14 (to determine effects of dry period treatments), +7 and +14 (to determine effect of dry period treatments on the immediate lipid accumulation following parturition). Before biopsy, local anesthesia was infiltrated in the intercostal space posterior to the tenth rib followed by aseptic preparation of the surgical site with Vedadine® (Vedco Inc., St. Joseph, MO). Liver cores were frozen immediately in liquid N, transferred to a -80°C freezer and later analyzed for contents of total lipids (Hara and Radin, 1978), triaclyglycerol (Fletcher, 1968; Foster and Dunn, 1973).

Statistical analysis

In Experiment 1, retrospective correlation analysis was completed using SAS® version 9.3 (SAS Institute Inc., Cary, NC). Before analyses, data were tested for normality using Proc Univariate. Cows were then assigned to one of two groups, cows with and without subclinical ketosis on d 7 and/or d 14 based on blood BHBA ≥ 1.2 mmol/L or < 1.2 mmol/L. Pearson correlation coefficients were calculated using Proc Corr in SAS to determine correlations among health and production parameters and

postpartum subclinical ketosis. Significant correlations were declared at $P < 0.05$ and trends are discussed when $P < 0.10$. Data were then analyzed using multiple logistic regression with an receiver operating characteristic (**ROC**) curve with 95% bootstrap confidence interval in statistical software R version 3.1.1 (The R Foundation for Statistical Computing, Vienna, Austria) to determine sensitivity and specificity of BCS, CY, and CSG to predict cows that are going to develop subclinical ketosis. Data were also subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996) to compare cows with and without subclinical ketosis. Significant data were declared at $P < 0.05$ and trends are discussed when $P < 0.10$.

RESULTS

Correlations of CY, CSG, and BCS with various health and production parameters

Colostrum yield (**CY**) was positively correlated ($P < 0.05$) with serum NEFA concentration on d 1 postpartum, and positively correlated ($P < 0.05$) with blood serum BHBA concentration on d 1 and d 7 (Table 5.1). Liver TG concentration on d 7 was positively correlated ($P < 0.05$) and tended ($P < 0.10$) to be correlated on d 14 with CY (Table 5.1). Week one after parturition, CY was negatively correlated ($P < 0.05$) with DMI (Table 5.1).

Colostrum specific gravity (**CSG**) was positively correlated ($P < 0.05$) with NEFA d 1, 7, and 14 (Table 5.1). Liver TG concentration on d 7 was positively correlated ($P < 0.01$) with CSG. Cows with greater DMI week one of lactation positively correlated ($P < 0.01$) with CSG (Table 5.1).

Prepartum body condition score (**BCS**) was negatively correlated ($P < 0.05$) with total blood serum calcium 24h after parturition (Table 5.1). Prepartum BCS was

positively correlated ($P < 0.01$) with serum NEFA concentration on d 7 and 14, and ($P < 0.01$) BHBA concentration d 1. BCS at calving tended to be positively correlated ($P < 0.10$) with 305 d ME milk yield (Table 5.1).

Accuracy of ketosis prediction measures of CY, CSG, and BCS

Sensitivity is the determination of an actual positive test being identified positive, and specificity is the determination of an actual negative test being identified negative as defined by Parikh et al. (2008). Based on Experiment 1, the threshold for ketosis detection for CY is 8.0 kg, CSG is 28.0 g/dL, and BCS is 3.75 for determining the highest accuracy based on sensitivity and specificity. When the individual parameters were tested, BCS had the highest sensitivity and specificity with 48% and 82%, then CY with 43% and 73%, and CSG with 33% and 67% for sensitivity and specificity respectively. The highest sensitivity was achieved with the combination of CY and CSG with 80% and 60% for specificity. The highest sensitivity and specificity was achieved with the combination of CY and BCS with 75% and 83%. The combination of all three parameters had a reduced accuracy with 60% and 74% for sensitivity and specificity (Table 5.2).

Effects of ketosis on CY, CSG, and milk yield

In Experiment 1, the incidence of subclinical ketosis was 36 %, and BHBA for cows without subclinical ketosis averaged 0.7 ± 0.25 mmol/L and cows with subclinical ketosis averaged 1.7 ± 0.25 mmol/L on d 7. Cows that developed subclinical ketosis tended ($P = 0.09$) to have greater colostrum yield than cows that did not develop subclinical ketosis (Table 5.3). However, colostrum specific gravity was similar ($P = 0.84$) among cows (Table 5.3). Cows that developed subclinical ketosis had greater ($P <$

0.01) body condition score than cows that did not develop subclinical ketosis (Table 5.3). Milk yield based on ME 305d lactation was similar (P = 0.90) among cows (Table 5.3).

Experiment II Development and validation of the Pre-K model on-farm

MATERIALS AND METHODS

Animal housing and management

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Three hundred twenty-eight multiparous Holstein cows from two commercial dairy farms in South Eastern Minnesota were utilized to validate if CY, CSG, and BCS are adequate predictors of subclinical ketosis on commercial dairy farms. Multiparous cows that calved from November 2013 through June 2014 were utilized. Farm B milks 450 cows with a rolling herd average of 13,030 kg of milk and are housed in a freestall barn with mattresses bedded with sawdust. Cows were managed for a 45 d dry period and were moved to a calving pen when calving appeared eminent. Fresh cows were moved to a fresh cow group and monitored daily through 14 DIM. There were one hundred and fifty-six cows tested with a subclinical ketosis incidence of 27.0% (Table 5.4). Farm C milks 550 cows with a rolling herd average of 15,198 kg of milk and are housed in a freestall barn with sand bedded stalls. Cows were managed for a 45 d dry period and were moved to a close-up bedded pack group calving pen two weeks before expected calving. Fresh cows were placed in a fresh cow group and monitored daily through 14 DIM. There were one hundred and seventy-two cows tested with a subclinical ketosis incidence of 36.6% (Table 5.4). All cows were milked three times daily in a parlor and fed TMR once daily. Both farms had similar management with a far-off and a close-up dry cow group with a mixed (primiparous and

multiparous) fresh cow group after parturition. Cows were managed to have a forty-five day dry period and were fed wheat straw based dry cow diets to meet NRC nutrient requirements. All multiparous cows were included in this study if they were treated for ketosis or not. After testing positive for subclinical ketosis (BHBA ≥ 1.2 mmol/L), cows received 300 g of oral propylene glycol on farm B, and 300 g of oral propylene glycol and 85.0 g of choline (Reashure®, Balchem Corporation, New Hampton, NY.) on farm C.

Body Condition Score

Body condition score (**BCS**) was assigned using 0.25-unit increments on a scale from 1- 5 (1 = thin, 5 = obese) (Ferguson et al., 1994) for each cow weekly once they entered the close-up group (21 d before expected calving). A trained individual scorer assigned BCS weekly before morning feeding.

Colostrum Yield and Composition

Colostrum was harvested after calving and individual cows were milked with the fresh cow group at the next milking session after calving. Colostrum yield was weighed using a digital scale (CPWplus 35, Adam Equipment Co. Ltd, Danbury, CT) and a sample from the first milking was collected and stored at 2°C. Samples were analyzed at room temperature for specific gravity using a digital brix refractometer (Misco PA302, Cleveland, OH) then stored at -20°C.

Milk Yield

Milk weights were recorded via monthly DHIA milk sample testing. Projected annual 305 d ME milk yield was calculated when cows reached 100 DIM.

Concentrations of Serum BHBA

Blood samples from individual cows were collected via venipuncture of the coccygeal vein or artery once between d 5 and d 9 and once between d 12 and d 16 for analysis of serum concentrations of BHBA to determine cows that have subclinical ketosis. Samples were collected before feeding (0800) into evacuated serum tubes (SST; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing clot activator. Serum was obtained by centrifugation for 20 minutes at 1,300 × *g*. Aliquots of serum were analyzed for contents of BHBA (Precision Xtra Meter; Abbott Laboratories, Inc., North Chicago, IL).

Statistical analysis

Statistical analysis was completed using statistical software R version 3.1.1 (The R Foundation for Statistical Computing, Vienna, Austria). Before analyses, data were tested for normality and distribution using a Q-Q plot. Data were subjected to Anova using general linear model. Model included colostrum yield, colostrum specific gravity, body condition score, parity, days dry, calving ease, and twining. Variables that had a *P*-value < 0.15 were considered significantly related to subclinical ketosis. Significant variables were then analyzed using multiple logistic regression model with a smoothed ROC curve (Robin et al., 2011) with 95% bootstrap confidence interval to determine sensitivity and specificity of colostrum yield, body condition score, and parity to predict cows that are going to develop subclinical ketosis. In addition, cows were divided into two groups, cows with and without subclinical ketosis on d 7 and/or d 14 defined by having a BHBA concentration lower than or greater than or equal to 1.2 mmol/L. Data were subjected to ANOVA by using the MIXED procedure of SAS® version 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 1996) to compare cows with and without

subclinical ketosis on each farm. Significant data were declared at $P < 0.05$ and trends are discussed when $P < 0.15$.

RESULTS

Effects of subclinical ketosis on farm B and farm C

Cows that developed subclinical ketosis on farm B had greater ($P < 0.01$) colostrum yield than non-ketotic cows (Table 5.4). However, cows that developed subclinical ketosis had lower ($P = 0.03$) colostrum specific gravity (Table 5.4). Cows that developed subclinical ketosis tended to have greater ($P = 0.11$) ME 305d milk yield compared to non-ketotic cows (Table 5.4).

On farm C cows that developed subclinical ketosis had greater ($P = 0.01$) body condition score, and greater ($P = 0.05$) days dry than non-ketotic cows (Table 5.4). In addition, colostrum yield tended ($P = 0.07$) to be greater. Cows that developed subclinical ketosis tended ($P = 0.09$) to produce lower ME 305d milk yield compared to cows below the subclinical ketosis threshold (Table 5.4).

Pre-K model development

Using the general linear model analysis, colostrum yield ($P < 0.001$), parity ($P = 0.01$), and body condition score ($P = 0.13$) at the day of calving were associated with the development of subclinical ketosis (Table 5.5). Other measurements of colostrum specific gravity, days dry, calving ease, and twins were not correlated with cows developing subclinical ketosis (Table 5.5).

Using multiple logistic regression model analysis containing CY, BCS, and parity; farm B colostrum yield ($P < 0.001$) and parity ($P = 0.15$) were related to subclinical ketosis development (Table 5.6). Farm C colostrum yield ($P = 0.02$), body

condition score ($P = 0.02$), and parity ($P < 0.01$) were correlated to subclinical ketosis (Table 5.6). In the overall model, colostrum yield ($P < 0.001$), body condition score ($P = 0.02$), and parity ($P = 0.001$) were correlated to ketosis (Table 5.6).

Pre-K model sensitivity and specificity

Using multiple logistic regression models (CY, BCS, and parity) with a smoothed ROC curve (Figure 5.1), the model achieved a sensitivity of 60% and a specificity of 64% (Table 5.7). Applying the model to each farm resulted in a sensitivity of 60% and a specificity of 66% on farm B, and a sensitivity of 60% and a specificity of 62% on farm C (Table 5.7).

The individual variables in the model were also analyzed. In the overall model, CY had a sensitivity of 57% and a specificity of 65% (Table 5.8). When the model was applied to each farm, the sensitivity was 62% and specificity of 66% for farm B, and farm C had a sensitivity of 53% and a specificity of 65% (Table 5.8). Using BCS resulted in a sensitivity of 77% and a specificity of 27% for the model, sensitivity of 74% and a specificity of 29% for farm B, and a sensitivity of 79% and specificity of 24% for farm C (Table 5.8). Using parity resulted in a sensitivity of 66% and a specificity of 49% for the model, sensitivity of 60% and a specificity of 50% for farm B, and a sensitivity of 71% and a specificity of 49% for farm C.

Prediction model developed from and applied to each farm individually

Using multiple logistic regression models (CY, BCS, and parity) with a smoothed ROC curve, the model when developed from farm B achieved a sensitivity of 55% and a specificity of 68% (Table 5.9). When the model was applied to farm C it achieved a sensitivity of 59% and a specificity of 71%. Using farm C to develop the model resulted

in a sensitivity of 59% and a specificity of 69%. When this model was applied to farm B it resulted in a sensitivity of 55% and a specificity of 71%.

DISCUSSION

In order for a subclinical ketosis prediction model to be beneficial, it must be; 1) easy to use, 2) economically feasible, and 3) able to accurately predict cows to allow dairy producers to proactively implement ketosis preventive treatments. Therefore, measures of colostrum yield (**CY**), colostrum specific gravity (**CSG**), and body condition score (**BSC**) were chosen as variables to determine if they are able to predict cows at risk for subclinical ketosis.

The proposed Pre-K model is a simple on-farm model that uses day of calving data as a predictor of cows at risk for subclinical ketosis. The Pre-K model with be further validated on two commercial dairy farms utilizing day of calving information (CY, CSG, BCS, parity, days dry, calving ease, and twins). Simply state, the proposed model is based on the representation of using measures of CY and CSG to predict the slope (rate) and BCS predicts the y-intercept (potential) of lipid mobilization expressed as BHBA. Greater BCS indicates greater amounts of potentially mobile body fat stores where CSG and CY predicts the rate (dependent upon energy intake) at which adipose tissue is mobilized to support colostrogenesis and lactation. Various other day of calving measures of parity, days dry, calving ease, and twins may contribute to an individual cow's risk factor for developing subclinical ketosis.

The current tests available to determine subclinical ketosis measure ketone bodies circulating in blood, urine, or milk. However, in order to help limit the effects of subclinical ketosis we need to identify cows at risk for ketosis before their blood BHBA

concentration is above 1.2 mmol/L. Evaluating a cow at the time of calving might be a way to identify high risk cows for developing subclinical ketosis. Colostrogenesis takes a lot of energy, and cows that produce high levels of colostrum and have high quality colostrum begin to mobilize body condition to meet these energy demands. Therefore, higher colostrum yield and higher colostrum specific gravity correlated with blood NEFA and BHBA after calving in experiment 1.

Cows with greater body condition have been shown to be at greater risk for developing subclinical ketosis (Kauppinen, 1983; Gillund et al., 2001). Compared with moderate BCS, cows with greater BCS have a linear decrease in DMI as calving approaches (Hayirli et al., 2002). Over conditioned cows are at risk for developing subclinical ketosis (Kauppinen, 1983; Gillund et al., 2001) as well as having lower blood calcium levels after calving (Roche and Berry, 2006). This is due to an increase in milk production supported by catabolism of adipose tissue (Roche et al., 2005). Due to an increase in milk production, there is also an increase in milk protein production and calcium is a major component contained in the casein micelle (McMahon and Brown, 1984). Therefore, cows with a body condition score greater than 3.5 had a 30 percent greater chance for hypocalcemia compared to a cow with a BCS of 3.0 (Roche and Berry, 2006). Our data supports these previous studies as increased BCS was positively correlated with NEFA and BHBA concentrations and lower total blood calcium 24 h after parturition. Cows that developed subclinical ketosis had greater BCS compared to the healthy cows on farm A (3.6 vs. 3.3 respectively). In support of previous research, cows that experienced subclinical ketosis had lower blood calcium 24h after parturition and had a higher BCS at parturition predisposing them to hypocalcemia on farm A.

Evaluating each individual variable based on sensitivity and specificity is a way to measure accuracy. The best individual variable was BCS to predict which cows were going to develop subclinical ketosis on farm A. Using BCS provides us with an estimate on how much potential body condition an individual cow has available to mobilize. Previous research has shown that cows with a BCS greater than or equal to 3 at dry-off produce greater milk yield compared to cows with a BCS less than 3 at dry-off (Machado et al., 2010). This suggests that cows that have potential body reserves to mobilize, will mobilize body condition to meet the energy demands of high milk yield in early lactation. Due to potential body condition mobilization, it is important to monitor the rate at which an individual cow is mobilizing body reserves. Evaluating the CY and CSG may provide us an insight on the rate a cow may potentially be mobilizing body condition at the time of calving. Therefore when CY and BCS are combined, the highest combination of sensitivity and specificity is achieved on farm A.

The Pre-K model was developed using data from two commercial dairy farms. Based on preliminary data from farm A (experiment 1), we expected that CY and BCS would be viable predictors for cows that are at risk for developing subclinical ketosis. We found (based on experiment 2) that CY, BCS, and parity at the time of calving were significant risk factors associated with cows developing subclinical ketosis. Cows that have greater BCS have larger amounts of adipose tissue to mobilize, and the greater CY provides insight into the rate at which individual cows are mobilizing adipose tissue to support colostrogenesis and milk production. Cows that are older are also at greater risk for developing subclinical ketosis and hypocalcemia as reported by McArt et al. (2013). Utilizing the Pre-K model resulted in a sensitivity of 60% and specificity of 64%. A

previous study conducted by McArt et al. (2013) found a predictive concordance value of 64% when a model was used to predict cows that developed subclinical ketosis between 3 and 16 DIM. McArt et al. (2012) reported 75% of the cows that developed subclinical ketosis, developed it between 3 and 7 DIM with peak prevalence at 5 DIM. Therefore, models that focus on using day of calving data to predict cows that are at risk for developing subclinical ketosis maybe more accurate at predicting cows 3 to 7 DIM rather than 3 to 16 DIM as other factors post calving may affect cows developing subclinical ketosis.

Various other tools have been tested in the field to determine sensitivity and specificity for ketosis identification using tools that measure circulating ketones. Looking at blood BHBA test, data from three combined studies (Burke et al. 2008, Oetzel and McGuirk, 2008, and Iwersen et al. 2009) reported a sensitivity of 91% and specificity is 94% when cut point is 1400 μmol/L using Precision Xtra Meter (Abbott Laboratories, Inc., North Chicago, IL) according to Oetzel (2012). Carrier et al. (2004) measured urine using Ketocheck powder (Great Lakes Animal Health, St. Joseph, MO) and found a sensitivity of 41% and a specificity of 99%; Ketostix (Bayer Corporation, Elkhart, IN) at small cut point has a sensitivity of 78% and a specificity of 96% and at moderate cut point had a sensitivity of 49% and a specificity of 99%. Carrier et al. (2004) also used milk Keto test strip (Sanwa Kagaku Kenkyusho Co. Ltd, Nagoya, Japan) with a cut point of 100 μmol/L and found a sensitivity of 73% and specificity of 96%, and cut point of 200 μmol/L had a sensitivity of 27% and specificity of 99%.

Although the current Pre-K model consisting of CY, BCS, and parity does not have the specificity of these current tools, 64% compared to > 94%, the Pre-K model may

be useful in predicting cows that are high risk for developing subclinical ketosis at the time of calving. The current ways for determining subclinical ketosis using blood BHBA concentration, urine acetone and acetoacetate, and milk acetone and acetoacetate can be used in conjunction with the proposed Pre-K model to implement a ketosis management strategy in early lactation on-farm. Further validation and modification of the Pre-K model needs to be conducted on-farm with multiple farms over various seasons and various management practices. In addition, various other day of calving data (locomotion score, days carried calf, calf gender, stillborn, season, previous days in lactation, previous ME 305 d milk yield) needs to be tested and added to the Pre-K model to make it more robust and improve the sensitivity and specificity of predicting cows at risk for subclinical ketosis. Also, once high risk cows are identified; further research needs to be conducted to determine appropriate subclinical ketosis prevention strategy.

CONCLUSION

Utilizing day of calving data of CY, BCS, and parity has potential to be able to predict cows that are at risk for developing subclinical ketosis. Further development and validation of the Pre-K model is needed over various seasons and multiple farms. Rapid, low cost identification of cows at high risk for subclinical ketosis allows for early treatment or implementation of tailored feeding and management strategies to minimize the incidence and severity of subclinical ketosis in fresh cows.

Table 5.1. Pearson correlation values (r) for colostrum yield (CY), colostrum specific gravity (CSG), and body condition score (BCS) with serum metabolites, liver triaclyglycerol concentration, dry matter intake, and 305d ME milk yield.

Item	CY	CSG	BCS
Serum calcium, total, 12h	0.01	-0.14	-0.03
Serum calcium[1], total, 24 h	-0.06	-0.20	-0.31*
NEFA[2] d 1	0.20*	0.17*	-0.04
d 7	0.11	0.29**	0.39**
d 14	0.03	0.20**	0.29**
BHBA[3] d 1	0.19*	0.01	0.38**
d 7	0.16*	-0.02	0.13
d 14	0.02	-0.01	0.39**
Liver triaclyglycerol[4], % d 7	0.20*	0.13**	0.23
d 14	0.15t	0.07	0.16
DMI week 1[5]	-0.18*	0.32**	-0.16
ME 305d milk yield[6]	-0.08	0.08	0.29t

t *P*-value < 0.1

* *P*-value < 0.05

** *P*- value < 0.01

[1] Serum total calcium at 24 hours after parturition.

[2] NEFA analysis of serum from days 1, 7, and 14 relative to parturition.

[3] BHBA analysis of serum from days 1, 7, and 14 relative to parturition.

[4] Percent of liver total lipid from day 7 and 14 relative to parturition.

[5] Dry matter intake week 1 after parturition.

[6] 305 day mature equivalent milk yield.

Table 5.2. Sensitivity and specificity with 95% confidence interval for colostrum yield, colostrum specific gravity, and body condition score to predict cows that developed subclinical ketosis in Holstein and Holstein-crossbred cows (n = 161) Farm A.

Variable	Sensitivity[1], %			Specificity[2], %		
	Low[6]	Median[7]	High[8]	Low[9]	Median[10]	High[11]
CY[3]	34.0	43.0	51.0	65.0	73.0	83.0
CSG[4]	23.0	33.0	43.0	59.0	67.0	77.0
BCS[5]	36.0	48.0	58.0	73.0	82.0	92.0
CY and CSG	71.0	80.0	87.0	50.0	60.0	69.0
CY and BCS	66.0	75.0	82.0	73.0	83.0	92.0
CSG and BCS	35.0	44.0	51.0	69.0	79.0	88.0
CY, CSG, and BCS	51.0	60.0	67.0	64.0	74.0	83.0

[1] Sensitivity is the determination of an actual positive test being identified positive.
[2] Specificity is the determination of an actual negative test being identified negative.
[3] Colostrum yield is the measure of total milk yield from first milking after parturition.
[4] Colostrum specific gravity is measured from first milking after parturition.
[5] Body condition score assigned one week before parturition.
[6] Lowest value for sensitivity based on 95% CI.
[7] Median value for sensitivity.
[8] Highest value for sensitivity based on 95% CI.
[9] Lowest value for specificity based on 95% CI.
[10] Median value for specificity.
[11] Highest value for specificity based on 95% CI.

Table 5.3. Retrospective analysis of the effects of subclinical ketosis on colostrum yield, colostrum specific gravity, body condition score, and milk yield at Farm A.

	Treatments			
Item	No ketosis[1]	Ketosis[2]	SEM	*P*-value
n	103	58		
Days dry	44.3	43.6	1.2	0.62
Calving ease score[3]	1.5	1.8	0.2	0.14
Colostrum yield[4], kg	6.9	8.0	0.5	0.09
Colostrum specific gravity[5], g/dL	23.0	23.2	0.9	0.84
Body condition score[6]	3.3	3.6	0.1	<0.01
ME 305 d milk yield[7], kg	11,108.0	11,075.0	215.3	0.90

[1] Cows with a BHBA level below 1.2 mmol/L.

[2] Cows with a BHBA level greater than or equal to 1.2 mmol/L.

[3]Calving ease score on a 1-5 scale, 1 = no assistance and 5 = severe dystocia.

[4] Colostrum yield is the measure of total milk yield from first milking after parturition.

[5] Colostrum specific gravity measured using Brix refractometer.

[6] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.

[7] Projected 305 d mature equivalent milk yield.

Table 5.4. Effects of ketosis on colostrum yield, colostrum specific gravity, body condition score, and milk yield.

	Treatments			
Item	No ketosis[1]	Ketosis[2]	SEM	*P*-value
Farm B				
n	114	42		
Days dry	48.1	48.1	1.1	0.98
Calving ease score[3]	1.3	1.3	0.2	0.66
Colostrum yield[4], kg	3.5	6.3	0.5	<0.01
Colostrum specific gravity[5], g/dL	27.3	25.4	0.7	0.03
Body condition score[6]	3.2	3.2	0.03	0.35
ME 305 d milk yield[7], kg	10,831.0	11,443.0	328.9	0.11
Farm C				
n	109	63		
Days dry	59.8	67.2	2.9	0.05
Calving ease score[3]	2.3	2.5	0.1	0.20
Colostrum yield[4], kg	4.0	5.0	0.5	0.07
Colostrum specific gravity[5], g/dL	29.0	29.1	0.6	0.93
Body condition score[6]	3.2	3.3	0.03	0.01
ME 305 d milk yield[7], kg	13,273.0	12,859.0	194.4	0.09

[1] Cows with a BHBA level below 1.2 mmol/L.
[2] Cows with a BHBA level greater than or equal to 1.2 mmol/L.
[3] Calving ease score on a 1-5 scale, 1 = no assistance and 5 = severe dystocia.
[4] Colostrum yield is the measure of total milk yield from first milking after parturition.
[5] Colostrum specific gravity measured using Brix refractometer.
[6] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.
[7] Projected 305 d mature equivalent milk yield projected at 100 DIM.

Table 5.5. General linear model using combined data from farm B and C evaluating factors related to ketosis at the time of parturition.

Variable	Coefficient[1]	SE[2]	*P*-value
Colostrum yield[3]	0.18	0.05	<0.001
Colostrum specific gravity[4]	-0.03	0.03	0.37
Body condition score[5]	1.12	0.73	0.13
Parity	0.33	0.13	0.01
Days dry	0.01	0.01	0.50
Calving ease[6]	0.12	0.13	0.39
Twins	0.25	0.51	0.63

[1] Coefficient determined using general linear model.
[2] Standard error of coefficient.
[3] Colostrum yield is the measure of total milk yield from first milking after parturition.
[4] Colostrum specific gravity measured using Brix refractometer.
[5] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.
[6] Calving ease score on a 1-5 scale, 1 = no assistance and 5 = severe dystocia.

Table 5.6. General linear model analysis utilizing colostrum yield, body condition score, and parity for farm B, farm C, and farm B and C combined.

Variable	Coefficient[1]	SE[2]	*P*-value
Farm B			
Colostrum yield[3]	0.24	0.06	<0.001
Body condition score[4]	1.01	0.96	0.30
Parity	0.28	0.20	0.15
Farm C			
Colostrum yield[3]	0.14	0.06	0.02
Body condition score[4]	2.21	0.96	0.02
Parity	0.44	0.15	<0.01
Farm B and C			
Colostrum yield[3]	0.19	0.04	<0.001
Body condition score[4]	1.55	0.67	0.02
Parity	0.39	0.12	0.001

[1] Coefficient determined using general linear model.

[2] Standard error of coefficient.

[3] Colostrum yield is the measure of total milk yield from first milking after parturition.

[4] Body condition score on a 1-5 scale, 1 = thin and 5 = obese.

Table 5.7. Multiple logistic model developed from farm B and C combined and applied to each farm individually with a 95% confidence interval represented by the lowest and highest values.

		Sensitivity,%			Specificity,%		
Farm	Threshold[1]	Low[3]	Median[4]	High[5]	Low[6]	Median[7]	High[8]
Model[2]	6.99	50	60	70	58	64	71
Farm B	6.99	45	60	74	57	66	75
Farm C	6.99	48	60	72	53	62	72

[1] Cut point for determining subclinical ketosis risk based on model.
[2] Model for determining subclinical ketosis risk based on [(0.19*colostrum yield) + (1.55*body condition score) + (0.39*parity)].
[3] Lowest value for sensitivity based on 95% CI.
[4] Median value for sensitivity.
[5] Highest value for sensitivity based on 95% CI.
[6] Lowest value for specificity based on 95% CI.
[7] Median value for specificity.
[8] Highest value for specificity based on 95% CI.

Table 5.8. Individual variables (colostrum yield (CY), body condition score (BCS), and parity)in the multiple logistic model developed from farm B and C combined and applied to each farm individually with a 95% confidence interval represented by the lowest and highest values.

		Sensitivity,%			Specificity,%		
Farm	Threshold[1]	Low[5]	Median[6]	High[7]	Low[8]	Median[9]	High[10]
CY							
Model[2]	4.26	47	57	67	59	65	71
Farm B	4.26	48	62	76	56	66	75
Farm C	4.26	41	53	66	56	65	74
BCS							
Model[3]	3.25	69	77	85	21	27	32
Farm B	3.25	60	74	86	21	29	37
Farm C	3.25	70	79	89	17	24	33
Parity							
Model[4]	2.76	56	66	74	43	49	56
Farm B	2.76	45	60	74	40	50	60
Farm C	2.76	59	70	81	40	49	58

[1] Cut point for determining subclinical ketosis risk.
[2] Model for determining subclinical ketosis risk based on farm B and C combined (0.19*colostrum yield). [3] Model for determining subclinical ketosis risk based on farm B and C combined (1.55*body condition score).
[4] Model for determining subclinical ketosis risk based on farm B and C combined (0.39*parity).
[5] Lowest value for sensitivity based on 95% CI.
[6] Median value for sensitivity.
[7] Highest value for sensitivity based on 95% CI.
[8] Lowest value for specificity based on 95% CI.
[9] Median value for specificity.
[10] Highest value for specificity based on 95% CI.

Table 5.9. Multiple logistic model developed from each individual farm (B or C) and applied to the opposite farm with a 95% confidence interval represented by the lowest and highest values.

		Sensitivity,%			Specificity,%		
Farm	Threshold[1]	Low[4]	Median[5]	High[6]	Low[7]	Median[8]	High[9]
Model[2]	5.25	41	55	69	59	68	77
Farm C	5.25	47	59	71	62	71	79
Model[3]	9.15	47	59	72	59	69	77
Farm B	9.15	38	55	71	63	71	79

[1] Cut point for determining subclinical ketosis risk.
[2] Model for determining subclinical ketosis risk based on farm B [(0.24*colostrum yield) + (1.01*body condition score) + (0.28*parity)].
[3] Model for determining subclinical ketosis risk based on farm C [(0.14*colostrum yield) + (2.21*body condition score) + (0.44*parity)].
[4] Lowest value for sensitivity based on 95% CI.
[5] Median value for sensitivity.
[6] Highest value for sensitivity based on 95% CI.
[7] Lowest value for specificity based on 95% CI.
[8] Median value for specificity.
[9] Highest value for specificity based on 95% CI.

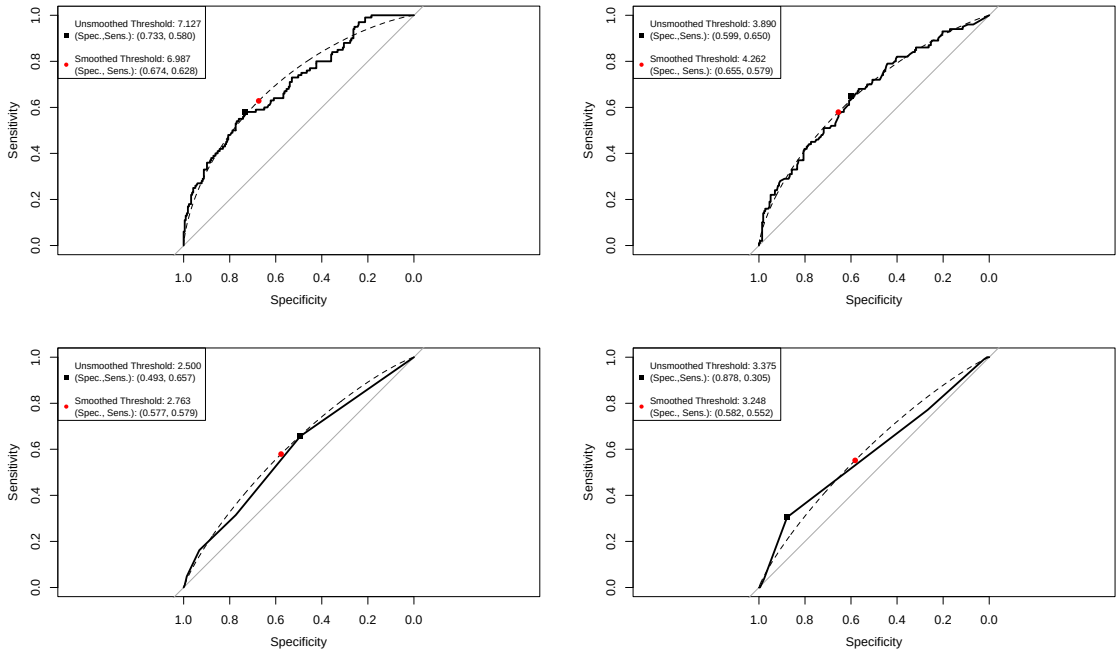

Figure 5.1. Comparison of sensitivity and specificity for the Pre-K model (colostrum yield, body condition score, and parity) from farm B and C using unsmoothed (solid line) and smoothed (dashed line) ROC curves. Top left graph is the ROC curve for the Pre-K model. Top right graph is ROC curve for colostrum yield only. Bottom left graph is ROC curve for parity only. Bottom right graph is ROC curve for body condition score only.

Overall Conclusions

Utilizing bacterial inoculants:

1) Treating corn silage with an inoculant that contains a mix of homo- and heterolactic bacteria is beneficial to fermentation and aerobic stability even at low packing densities. This may especially be beneficial when storing forage in a bunker or pile where packing densities typically vary throughout the forage.

2) Make sure that forage is packed as dense as possible to promote proper fermentation and reduce the risk for aerobic deterioration and loss of dry matter at feed-out.

3) Increasing packing density and utilizing an inoculant does not have an additive effect.

Feeding varying starch diets and supplementation with P169

Multiparous cows:

1) Feeding high starch diets achieved by feeding coarsely ground dry corn with P169 had the greatest milk production starting 5 weeks after parturition. As the demand for glucose increases in early lactation and towards peak milk production, supplementation with P169 can help increase propionate production in the rumen and increase glucogenic precursors and thus increase milk production.

2) Low starch diets can successfully be fed throughout the transition period and still meet the demands for high milk production.

3) Cows fed high starch diet spent an additional two hours per day standing compared to cows fed low starch. This suggests that overcrowding in the fresh cow pen maybe a bigger concern when feeding high starch diets to your fresh cows.

4) Supplementation with P169 increased feed efficiency.

Primiparous cows:

1) Feeding high starch diets tended to improve 3.5 % fat corrected milk. However, feeding high starch diets in early lactation came at a risk as primiparous cows had increased lipid mobilization and increased risk for metritis, mastitis, and subclinical ketosis.

2) Dietary starch concentration had no effect on DMI pre- or postpartum.

3) Supplementation with P169 had no effect on primiparous cow health or production.

Ketosis prediction utilizing the Pre-K model

1) The Pre-K model utilizing colostrum yield, body condition score, and parity is an accurate way to identify high risk cows for subclinical ketosis at the time of calving. This will allow dairy producers to implement a subclinical ketosis preventative treatment strategy and be proactive instead of reactive to help reduce the effects of subclinical ketosis and thus increase cow health and productivity.

2) The Pre-K model utilizes measurements that are inexpensive and not labor intensive to measure. This will allow the model to be consistently incorporated into fresh cow protocols on commercial dairy farms.

3) The Pre-K model can be used in conjunction with current ketosis testing protocols to help dairy producers identify cows with subclinical ketosis early.

COMPREHENSIVE BIBLIOGRAPHY

Aksu, T., E. Baytok, and D. Bolat. 2004. Effects of a bacterial silage inoculant on corn silage fermentation and nutrient digestibility. Small Rumin. Res. 55:249-252.

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

Allen, D. 2001. Dry matter and neutral detergent fiber intake of dairy cattle. Ph. D. Dissertation. University of Minnesota.

Allen, M. S. 2012. Adjusting concentration and ruminal digestibility of starch through lactation. Pages 24-30 Proc. Four-State Dairy Nutr. and Mgmt Conf. Dubuque, IA.

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87: 3317-3334.

Analytical Methods Guide, Orion Research, Cambridge, MA, May, 1977.

AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, Virginia.

AOAC. 1996. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, Virginia.

AOAC. 1998. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, Virginia.

AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Arlington, Virginia.

AOAC. 2005. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Arlington, Virginia.

Arriola, K. G., S. C. Kim, and A. T. Adesogan. 2011. Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. J. Dairy Sci. 94:1511-1516.

ASAE, 1993. Method of determining and expressing fineness of feed materials by sieving. ASAE Standard ASAE S319.2.

Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon, and G. B. Penner. 2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. IUBMB life. 62:869-877.

Axelsson, L. 2004. Lactic acid bacteria: classification and physiology. *Lactic Acid Bacteria: Microbiology and Functional Aspects*.(S. Salminen, A. von Wright, and A. Ouwehand)(Eds). Third edition, Marcel Dekker, Inc., New York. 1-66.

Barkema, H. W., Y.H. Schukken, T.J. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. J. Dairy Sci. 81:411-419.

Batajoo, K. and R. D. Shaver. 1994. Impact of nonfiber carbohydrate on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 77:1580-1588.

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

Blowey, R. 2005. Factors associated with lameness in dairy cattle. In Practice 27:154-162.

Broderick, G., D. Mertens, and R. Simons. 2002. Efficacy of carbohydrate sources for milk production by cows fed diets based on alfalfa silage. J. Dairy Sci. 85:1767-1776.

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

Burke, C. M., K. E. Leslie, and L. M. Neuder. 2008. Test comparison of Precision Xtra and Ketostix for ketosis in dairy cows. Page 287 in Proc. 41st Annu. Conf., Am. Assoc. Bov. Pract. AABP, Auburn, Alabama.

Canale, A., M. E. Valente, and A. Ciotti. 1984. Determination of volatile carboxylic acids (C_1–C_{5i}) and lactic acid in aqueous acid extracts of silage by high performance liquid chromatography. J. Sci. Food Agric. 35:1178–1182.

Carrier, J., S. Stewart, S. Godden, J. Fetrow, and P. Rapnicki. 2004. Evaluation and use of three cowside tests for detection of subclinical ketosis in early postpartum cows. J. Dairy Sci. 87:3725-3735.

Chase, L. E. 2007. Can we feed less starch to our cows? Pages 213-220 in Proc. Cornell Nutr. Conf. Feed Manuf., East Syracuse, NY. Cornell Univ., Ithaca, NY.

Cleale, R. M., J. L. Firkins, F. Van Der Beek, J. H. Clark, E. H. Jaster, G. C. McCoy, and T. H. Klusmeyer. 1990. Effect of inoculation of whole plant corn forage with

Pediococcus acidilactici and *Lactobacillus xylosus* on preservation of silage and heifer growth. J. Dairy Sci. 73:711-718.

Contreras-Govea, F. E., and R. E. Muck. 2006. Microbial inoculants for silage. Focus on Forage, 8:1-4.

Cook, N. B., W. R. Ward, and H. Dobson. 2001. Concentrations of ketones in milk in early lactation, and reproductive performance of dairy cows. Vet. Rec. 148:769-772.

Dann, H. M., K. W. Cotanch, P. D. Krawczel, C. S. Mooney, R. J. Grant, and T. Eguchi. 2008. Evaluation of low starch diets for lactating Holstein dairy cattle. J. Dairy Sci. 91(E-Suppl. 1):530.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563-3577.

Derias, R. E. 1961. Method for determination water soluble carbohydrates. J. Sci. Fd. Agric. 12:152.

Dohoo, I. R., and S. W. Martin. 1984. Subclinical ketosis: prevalence and associations with production and disease. Can. J. Comp. Med. 48:1-5.

Douglas, G. N., J. Rehage, A. D. Beaulieu, A. O. Bahaa, and J. K. Drackley. 2007. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. J. Dairy Sci. 90:2941-2959.

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

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

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

Endres, M. I., T. J. DeVries, M. A. G. Von Keyserlingk, and D. M. Weary. 2005. Short communication: Effect of feed barrier design on the behavior of loose-housed lactating dairy cows. J. Dairy Sci. 88:2377-2380.

Erdman, R. 1988. Forage pH effects on intake in early lactation dairy cows. J. Dairy Sci. 71:1198-1203.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695-2703.

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.

Ferraretto, L. F., R. D. Shaver, S. Massie, R. Singo, D. M. Taysom, and J. P. Brouillette. 2014. Effect of ensiling time on fermentation profile and starch digestibility in whole plant corn silage from two different hybrid types. J. Dairy Sci. 97(E-Suppl):(Abstr).

Fletcher, M. J. 1968. A colorimetric method for estimating serum triglycerides. Clin. Chim. Acta. 22:393-397.

Foster, L. B. and R. T. Dunn. 1973. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clin. Chem. 19:338-340.

Francisco, C. C., C. S. Chamberlain, D. N. Waldner, R. P. Wettemann, and L. J. Spicer. 2002. Propionibacteria fed to dairy cows: Effects on energy balance, plasma metabolites and hormones, and reproduction. J. Dairy Sci. 85:1738-1751.

Geishauser, T., K. Leslie, T. Duffield, and V. Edge. 1997. An evaluation of milk ketone tests for the prediction of left displaced abomasums in dairy cows. J. Dairy Sci. 80:3188-3192.

Geishauser, T., K. Leslie, J. Ten Hag, and A. Bashiri. 2000. Evaluation of eight cow-side ketone tests in milk for detection of subclinical ketosis in dairy cows. J. Dairy Sci. 83:296-299.

Geishauser, T., K. Leslie, D. Kelton, and T. Duffield. 2001. Monitoring for subclinical ketosis in dairy herds. Compend. Contin. Educ. Pract. Vet. 23:65-71.

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.

Gillund, P., O. Reksen, Y. T. Grohn, K. Karlberg. 2001. Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J. Dairy Sci. 84:1390–1396.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus reagents, procedures, and some applications). Agric. Handbook No. 379ARS USDA, Washington D. C., p. 20.

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

Grummer, R. R., P. C. Hoffman, M. L. Luck, and S. J. Berties. 1995. Effect of prepartum and postpartum dietary energy on growth and lactation in primiparous cows. J. Dairy Sci. 78:172-180.

Grusenmeyer, D. J., C. M. Ryan, D. M. Galton. 2006. Shortening the dry period from 60 to 40 days does not affect colostrum quality but decreases colostrum yield by Holstein cows. J. Dairy Sci. 89(Suppl. 1):336.

Hall, M. B. 2000. Neutral detergent soluble carbohydrates nutritional relevance and analysis: A laboratory manual. University of Florida, Bulletin 339.

Hara, A. and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. J. Anal. Biochem. 90:420-426.

Hamaker, B. R., A. A. Mohamed, J. E. Habben, C. P. Huang, and B. A. Larkins. 1995. Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. Cereal Chem. 72:583-588.

Hayirli, A. 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet. Res. Commun. 30:749-774.

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

Higginbotham, G. E., S. C. Mueller, K. K. Bolsen, and E. J. DePeters. 1998. Effects of inoculants containing propionic acid bacteria on fermentation and aerobic stability of corn silage. J. Dairy Sci. 81:2185-2192.

Hoffman, P., N. Esser, R. Shaver, W. Coblentz, M. Scott, A. Bodnar, and R. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474.

Holm, J., I. Bjork, A. Drews, and N. G. Asp. 1986. A rapid method for the analysis of starch. Starch 7:224-226.

Holtenius, P., G. Olsson, M. Emanuelson, and H. Wiktorsson. 1996. Effects of different energy levels, concentrate/forage ratios and lipid supplementation to the diet on the adaptation of the energy metabolism at calving in dairy cows. J. Vet Med A. 43:427-435.

Hughes, J. P. 1962. A simplified instrument for obtaining liver biopsies in cattle. Amer. J. Vet. Res. 23:1111-1112.

Hunt, C. W., W. Kezar, D. D. Hinman, J. J. Combs, J. A. Loesche, and T. Moen. 1993. Effects of hybrid and ensiling with and without a microbial inoculant on the nutritional characteristics of whole-plant corn. J. Anim. Sci. 71:38-43.

Huntington, G. B. 1997. Starch utilization by ruminants: from basics to the bunk. J. Anim. Sci. 75:852-867.

Ingvartsen, K. L. 2006. Feeding and management related to diseases in the transition cow: physiological adaptations around calving and strategies to reduce feeding-related diseases. J. Dairy Sci. 126:175-213.

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

Ipharraguerre, I. R., R. R. Ipharraguerre, and J. H. Clark. 2002. Performance of lactating dairy cows fed varying amounts of soyhulls as a replacement for corn grain. J. Dairy Sci. 85: 2905-2912.

Iwersen, M., U. Falkenberg, R. Voigtsberger, D. Forderung, and W. Heuwieser. 2009. Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J. Dairy Sci. 92:2618-2624.

Janovick-Guretzky, N. A., N. B. Litherland, K. M. Moyes, and J. K. Drackley. 2006. Prepartum energy intakes affects health and lactational performance in primiparous and multiparous Holstein cows. J. Dairy Sci. (Suppl. 1):267.

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

Jurjanz, S., and V. Monteils. 2005. Ruminal degradability of corn forages depending on the processing method employed. Anim. Res. 3:15-23.

Kang, T. W., A. T. Adesogan, S. C. Kim, and S. S. Lee. 2009. Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage. J. Dairy Sci. 92:732-738.

Kauppinen, K. 1983. Prevalence of bovine ketosis in relation to number and stage of lactation. Acta. Vet. Scand. 24:349–361.

Kleinschmit, D. H. and L. Kung Jr. 2006a. The effects of *Lactobacillus buchneri* 40788 and *Pediococcos pentosaceus* R1094 on the fermentation of corn silage. J. Dairy Sci. 89:3999-4004.

Kleinschmit, D. H. and L. Kung Jr. 2006b. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89:4004-4013.

Knudsen, K. E. B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. J. Anim. Feed Sci. Tech. 67:319-338.

Konkol, K., S. Godden, P. Rapnicki, and M. Overton. 2009. Validation of a rapid cow-side test for the measurement of blood beta-hydroxybutyrate in fresh cows. Page 190 in Proc 42nd Annual Conf. Am Assoc. Bovine Pract., Omaha, NE. VM Publishing Co, Stillwater, OK.

Kononoff, P. J., A. J. Heinrichs, and D. R. 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.

Kremer, W. D. J., E. N. Noordhuizen-Stassen, F. J. Grommers, Y. H. Schukken, R. Heeringa, A. Brand, and C. Burvenich. 1993. Severity of experimental *Escherichia coli* mastitis in ketonemic and nonketonemic dairy cows. J. Dairy Sci. 76:3428-3436.

Kung Jr, L. 2001. Silage fermentation and additives. Science and Technology in the Feed Industry. Proceedings of Alltech's 17th Annual Symposium: a time for answers. 17:145-159.

Kung Jr., L. and J. Neylon. 2001 Management guidelines during harvest and storage of silages. Tri-State Dairy Nutrition Conference Proceeding, April pp. 103-112.

Kung Jr., L., J. R. Robinson, N. K. Ranjit, J. H. Chen, C. M. Golt, and J. D. Pesek. 2000. Microbial populations, fermentation end-products, and aerobic stability of corn silage treated with ammonia or a propionic acid-based preservative. J. Dairy Sci. 83:1479-1486.

Lawton, J. W. 2002. Zein: A history of processing and use. Cereal Chem. 79:1-18.

Lehloenya, K. V., D. R. Stein, D. T. Allen, G. E. Selk, D. A. Jones, M. M. Aleman, T. G. Rehberger, K. J. Mertz, and L. J. Spicer. 2008. Effects of feeding yeast and

propionibacteria to dairy cows on milk yield and components, and reproduction. J. Anim. Physiol. Anim. Nutr. 92:190-202.

Leonardi, C. and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557-564.

Leonardi, C., F. Giannico, and L. E. Armentano. 2005. Effect of water addition on selective consumption (sorting) of dry diets by dairy cattle. J. Dairy Sci. 88:1043-1049.

Litherland, N. B., D. N. L. da Silva, W. P. Hansen, L. Davis, S. Emanuele, and 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.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger. 1996. SAS System of Mixed Models SAS Institute, Cary, NC.

Lo, S., J. C. Russell, and A. W. Taylor. 1970. Determination of glycogen in small tissue samples. J. Appl. Physiol. 28:234-236.

Locher, L. F., N. Meyer, E. M. Weber, J. Rehage, U. Meyer, S. Dänicke, and K. Huber. 2011. Hormone-sensitive lipase protein expression and extent of phosphorylation in subcutaneous and retroperitoneal adipose tissues in the periparturient dairy cow. J. Dairy Sci. 94:4514-4523.

Machado, V. S., L. S. Caixeta, J. A. A. McArt, and R. C. Bicalho. 2010. The effect of claw horn disruption lesions and body condition score at dry-off on survivability, reproductive performance, and milk production in the subsequent lactation. J. Dairy Sci. 93:4071-4078.

McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95:5056-5066.

McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2013. Dry period and parturient predictors of early lactation hyperketonemia in dairy cattle. J. Dairy Sci. 96:198-209.

McArt, J. A. A., D. V. Nydam, G. R. Oetzel, and C. L. Guard. 2014. An economic analysis of hyperketonemia testing and propylene glycol treatment strategies in early lactation dairy cattle. Prev. Vet. Med. 117:170-179.

McArt, J. A. A., D. V. Nydam, P. A. Ospina, and G. R. Oetzel. 2011. A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis. J. Dairy Sci. 94:6011-6020.

McMahon, D. J. and R. J. Brown. 1984. Composition, structure, and integrity of casein micelles: a review. J. Dairy Sci. 67:499-512.

McNamara, J. P. 1991. Regulation of adipose tissue metabolism in support of lactation. J. Dairy Sci. 74:706-719.

Miltenburg, J. D., D. DeLange, A. P. P. Crauwels, J. H. Bongers, M. J. M. Tielen, Y. H. Schukken, and A. R. W. Elbers. 1996. Incidence of clinical mastitis in a random sample of dairy herds in southern Netherlands. Vet. Rec. 139:204-207.

Muck, R. E. 1996. A lactic acid bacteria strain to improve aerobic stability of silages. Pages 42-43 in Research Summaries. U.S. Dairy Forage Research Center, Madison, WI.

Muck, R. E. 2004. Effects of corn silage inoculants on aerobic stability. Trans ASAE. 47:1011-1016.

Muck, R. E. 2012. Microbiology of ensiling. International Silage Conference. July pp 75 – 86.

Mu-Forster, C, and B. P. Wasserman. 1998. Surface localization of zein storage proteins in starch granules from maize endosperm: Proteolytic removal by thermolysin and in vitro cross-linking of granule-associated polypeptides. Plant Physiol. 116:1563–1571.

Narendranath, N., K. Thomas, and W. Ingledew. 2001. Effects of acetic acid and lactic acid on the growth of saccharomyces cerevisiae in a minimal medium. J. Ind. Microbiol. Biotechnol. 26:171-177.

Nelson, B. H., K. W. Cotanch, M. P. Carter, H. M. Gauthier, R. E. Clark, P. D. Krawczel, R. J. Grant, K. Yagi, K. Fujita, and H. M. Dann. 2011. Effect of dietary starch content in early lactation on the lactational performance of dairy cows. J. Dairy Sci. 94(E-Suppl. 1):637.

Nordlund, K. V. 2006. Transition cow index™. Proc. Am. Assn. Bov. Pract. Conf. 39:36-42.

Norzière, P, W. Steinberg, M. Silberberg, and D. P. Morgavi. 2014. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. J. Dairy Sci. 97:2319-2328.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle (7) Natl. Acad. Sci., Washington DC.

Nyman, A. K., T. Ekman, U. Emanuelson, A. H. Gustafsson, K. Holtenius, K. P. Waller, and C. H. Sandgren. 2007. Risk factors associated with the incidence of

veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and low prevalence of subclinical mastitis. Prev. Vet. Med. 78:142-160.

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

Oba, M. and M. S. Allen. 2003b. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174-183.

Oetzel, G. R. 2007. Herd-level ketosis-diagnosis and risk factors. Pages 67-91 in Pre-conference Seminar 7C: Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting. 40th Annual Conf. Am Assoc. Bovine Pract., Vancouver, BC, Canada.

Oetzel, G. R. 2012. Understanding the impact of subclinical ketosis. Pages 15-25 in Proc. Of the Cornell Nutrition conference for feed manufacturers. East Syracuse, NY. Cornell Univ., Ithaca, NY.

Oetzel, G. R., and S. M. McGuirk. 2008. Evaluation of a handheld meter for cowside evaluation of blood beta-hydroxybutyrate and glucose concentrations in dairy cows. Page 234 in Proc. 41st Annu. Conf., Am. Assoc. Bov. Pract. AABP, Auburn, Alabama.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J. Dairy Sci. 93:3595-3601.

Owens, F., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63:1634-1648.

Parikh, R., A. Mathai, S. Parikh, G. C. Sekhar, and R. Thomas. 2008. Understanding and using sensitivity, specificity and predictive values. Indian J. Ophthalmol, 56:45–50.

Petrie, L., S. D. Acres, and D. H. McCartney. 1984. The yield of colostrum and colostral gammaglobulins in beef cows and the absorption of colostral gammaglobulins by beef calves. Can. Vet. J. 25:273-279.

Rabelo, E. R. L. Rezende, S. J. Bertics, and R. R. Grummer. 2005. Effects of pre- and postfresh transition diets varying in dietary energy density on metabolic status of periparturient dairy cows. J. Dairy Sci. 88:4375-4383.

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

Ranjit, N. K. and L. Kung Jr. 2000. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83:526-535.

Reich, L. J. and L. Kung Jr. 2010. Effects of combining *Lactobacillus buchneri* 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage. J. Anim. Feed Sci. Tech. 159:105-109.

Richards, C. J., J. F. Pedersen, R. A. Britton, R. A. Stock, and C. R. Krehbiel. 1995. In vitro starch disappearance procedure modifications. Anim Feed Sci Tech. 55:35-45.

Robin, X., N. Turck, A. Hainard, N. Tiberti, F. Lisacek, J. C. Sanchez, and M. Müller. 2011. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC bioinformatics, 12:77.

Roche, J. R. and D. P. Berry. 2006. Periparturient climatic, animal, and management factors influencing the incidence of milk fever in grazing systems. J. Dairy Sci. 89:2775-2783.

Roche, J. R., E. S. Kolver, and J. K. Kay. 2005. Influence of precalving feed allowance on periparturient metabolic and hormonal responses and milk production in grazing dairy cows. J. Dairy Sci. 88:677–689.

Ruppel, K. A. 1992. Effect of bunker silo management on hay crop nutrient management. M.S. Thesis, Cornell University, Ithaca, NY.

Seglar, B. 2003. Fermentation analysis and silage quality testing. Pages 119- 136 in Proc. of the Minnesota Dairy Health Conf. Univ. MN. Minneapolis, MN.

Sergeant, E. P. and B. Dempsey. 1979. Ionization constants of organic acids in aqueous, Pergamon Press, Oxford.

Shreve, B., N. Thies, and M. Wolf. 2006. NFTA method 2.2.2.5 Dry Matter by oven drying for 3 hours at 105°C. National Forage Testing Association, Omaha, NE.

Siegfried, V. R., H. Ruckermann, and G. Stumff. 1984. Method for the determination of organic acids in silage by high performance liquid chromatography. Landwirtsch, Forsch, 37:298-304.

Slavin, B. G., J. M. Ong, and P. A. Kern. 1994. Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. J. Lipid Res. 35:1535-1541.

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

Stone, W. C. 1996. Applied topics in dairy cattle nutrition. 1. Soyhulls as either a forage or concentrate replacement in early lactation Holstein cows. PhD. Thesis, Dept. of Animal Sci., Cornell Univ. Ithaca, NY.

Susenbeth, A., T. Dickel, K. H. Südekum, W. Drochner, and H. Steingaß. 2004. Energy requirements of cattle for standing and for ingestion, estimated by a ruminal emptying technique. J. Anim. Sci. 82:129-136.

Vaughan, M. J. E. Berger, and D. Steinberg. 1964. Hormone-sensitive lipase and monoglycerol lipase activities in adipose tissue. J. Biol. Chem. 239:401-409.

Veenhuizen, J. J., J. K. Drackley, M. J. Richards, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J. Dairy Sci. 74:4238-4253.

Voelker, J. and M. S. Allen. 2003. Pelleted beet pulp substituted for high-moisture corn: 1. effects on feed intake, chewing behavior, and milk production of lactating dairy cows. J. Dairy Sci. 86:3542-3552.

Wallace, R. L., G. C. McCoy, T. R. Overton, and J. H. Clark. 1996. Effect of adverse health events on dry matter consumption, milk production, and body weight loss of dairy cows during early lactation. J. Dairy Sci. 79(Suppl 1):205(Abstr.).

Weiss, W. P., D. J. Wyatt, and T. R. McKelvey. 2008. Effect of feeding propionibacteria on milk production by early lactation dairy cows. J. Dairy Sci. 91:646-652.

Wierenga, J. K. 1990. Social dominance in dairy cattle and the influences of housing and management. Appl. Anim. Behav. Sci. 27:201-229.

Windle, M. C., N. Walker, and L. Kung Jr. 2014. Effects of an exogenous protease on the fermentation and nutritive value of corn silage harvested at different dry matter contents and ensiled for various lengths of time. J. Dairy Sci. 97:3053-3060.

Ying, Y. and M. S. Allen. 2005. Effects of corn grain endosperm type and conservation method on site of digestion, ruminal digestion kinetics and microbial nitrogen production of lactating cows. J. Dairy Sci. 88(Suppl. 1):393.

Zaaijer, D. and J. P. T. M. Noordhuizen. 2003. A novel scoring system for monitoring the relationship between nutritional efficiency and fertility in dairy cows. Ir. Vet. J. 56:145-152.