Risk Factors for and Treatment of Ketosis in Lactating Dairy Cattle

by

Jessica Lynn Gordon

A Thesis
Presented to
the University of Guelph

In partial fulfillment of requirements
for the degree of
Doctorate of Veterinary Science
in
Population Medicine

Guelph, Ontario, Canada

ABSTRACT

RISK FACTORS FOR AND TREATMENT OF KETOSIS IN LACTATING DAIRY CATTLE

Jessica Gordon
University of Guelph, 2013

Co-advisors:
Dr. T.F. Duffield
Dr. S.J. LeBlanc

This thesis was conducted to investigate risk factors for ketosis development in lactating dairy cattle and evaluate treatments for affected animals. Four main studies were carried out. A systematic review of the ketosis treatment literature was performed to analyze the current body of literature available and provide guidance for treatments to be used in future studies. Secondly, a randomized clinical trial was performed on seventeen commercial dairy farms to determine the effectiveness of a combination butaphosphan cyanocobalamin product, insulin, and propylene glycol for ketosis treatment. A second randomized clinical trial was performed on nine commercial dairy herds to further evaluate the usefulness of a combination butaphosphan cyanocobalamin product and two durations of propylene glycol treatment on ketosis resolution and early lactation milk production. Finally, records from five commercial dairy farms were analyzed to evaluate individual cow risk factors associated with ketosis development.

Evaluation of the ketosis treatment literature revealed the lack of well-designed ketosis treatment studies and the need for further investigation to determine an effective treatment regimen. Both treatment trials showed an effect of blood glucose concentrations at the time of enrollment on the efficacy of study treatments that had not

been previously described in the literature. Animals that had blood glucose $\leq$ 2.2 mmol/L at the time of ketosis diagnosis were more likely to cure and produced more milk when treated with insulin, butaphosphan cyanocobalamin, or extended duration of propylene glycol than untreated controls with blood glucose $\leq$ 2.2 mmol/L. Treatment benefits did not extend to animals with blood glucose > 2.2 mmol/L at the time of enrollment. Older age at first calving, extended days open in the previous lactation, longer dry period, and increased parity increased ketosis risk. Also, animals that were ketotic during a lactation were more likely to become ketotic in the subsequent lactation. The information contained in this thesis helps increase understanding of ketosis risk and proper treatment. The novel interaction of the level of blood glucose and ketosis sheds light on previous inconsistent results on ketosis impacts and will change approaches to understanding and treatment of the condition in the field.

ACKNOWLEDGEMENTS

This research, thesis, and degree could not have been completed without the help of many individuals, to all of whom I am eternally grateful. I must start by thanking my co-advisors, Drs. Todd Duffield and Stephen LeBlanc, who allowed me the freedom to pursue my interests, reigning me in and guiding me when necessary, but allowing my own motivation to be the only limiting factor to my success. This style of advising has allowed me to develop the interests and skills that will be essential for my future career. Thank you Stephen for encouraging me to remember to balance work and fun, while encouraging productivity. And thank you for finding me in the AABP exhibit hall and taking me on when I'm pretty sure you knew what you were getting into. Thank you Todd for taking a chance on an unknown and providing endless opportunities to further my development. Thank you also for the book discussions; I look forward to many more in the future. Thank you to the other members of my advisory committee, Drs. Dave Kelton and Tom Herdt. Thank you Dave for providing and unbiased ear and a keen epidemiological eye. Thank you Tom for taking me on many years ago and awakening my interest in cows. It has been surreal to work with you through all my degrees. I appreciate all the opportunities and guidance you have given me.

They say it takes a village to raise a child. I felt this sentiment during my time in Ruminant Field Service, where the entire group was nurturing and supportive (when I wasn't being teased!). I cannot express the level of thanks I have for Dr. Ken Bateman who has worked to mentor me throughout my time, taught me about the beef industry, and become a friend and confidant. Thank you also to Dr. Paula Menzies who has taught me about the small ruminant industry and guided my progress, Dr. Cynthia Miltenburg

who has shared in the challenges of being a DVSc, and Shelley James who listened to my endless chatter. A special thanks to Dr. Jeff Rau and Rachel Skippen, my partners in crime and great friends.

My research could not have been completed without the assistance of a hoard of summer students who put a tremendous amount of time and effort into the trials. Thank you Dave Renaud, Jennifer Reynen, Colleen Sczentimrey, Brittany Scace, Robyn Thompson, Amy Thomson, Lindsay Oxby, Brett Gamble, and Laura Macrae at the University of Guelph and Stephanie Ross, Jennifer Smith, Vanessa Kane, and Jeff Arthur at Michigan State University for all your hard work and enthusiasm. I must also thank all the dairies and veterinary clinics that helped with the trial and allowed us access to their farms on a weekly basis to disturb their routines. Of course, thanks must also be given to Bayer Animal Health, Vetoquinol, the American Association of Bovine Practitioners Research Assistantship, and Ontario Ministry of Agriculture and Food for funding this research.

I am extremely lucky to have had the opportunity to pursue my graduate degree in Population Medicine. The faculty, staff, and students in the department provide a welcoming and friendly environment that feels like home. The numbers of people that have touched my life during my three years in the department are innumerable, but I have to mention a few. To my cards group, a diverse group of people who I spent many hours with, Laura Pieper, Les Gonzalez, Khaled Gohary, Brittany Foster, Rafael Neves, and Felipe Cardoso, I appreciate the chance you all gave me to forget about grad school and laugh until my stomach hurt. Thank you also to Julie Wittrock, my fellow HP addict and

an amazingly witty person. You are all great friends that I hold dear to my heart and I look forward to many years as friends and colleagues.

I think your first class always holds a special place in your heart. Thank you to the 2011 OVC foodies and mixed animals students. Your enthusiasm and curiosity helped me uncover my love for teaching. I set out to guide you, little knowing that you all would actually guide me and teach me that respect of authority and friendship are far from mutually exclusive.

Finally, I must thank my family for the support that I have received throughout my life. To all the strong women in my family that made me believe that I could do anything that I put my mind to, thank you for the strength to do so. Thank you to my parents and in-laws for putting up with our continuous moving and my perpetual lack of phone calls. Last but not least I must thank my husband Troy. Without his continuous support and encouragement, not to mention dinner deliveries and car fueling trips, I don't know how I would have completed my three (yes I swear this is the last) degrees. My appreciation for your love and understanding cannot be put in words.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

LITERATURE REVIEW

Ketosis in dairy cattle occurs due to a period of negative energy balance (NEB) that occurs almost universally at the beginning of lactation (Herdt, 2000). High demands for energy, fat, and protein to support lactation are coupled with a decrease in dry matter intake around the time of calving (Baird, 1982). As lactation continues, dry matter intake increases at a slower rate than milk production and body stores are used to support milk production (Herdt, 2000). Breakdown of body stores to support lactation occurs in many species, but when excessive catabolism is present, pathologic levels of blood ketone bodies can accumulate. Build up of ketone bodies in the blood can cause inappetance and further exacerbate the problem.

Adaptation to milk production occurs through many pathways in the body (Bauman and Currie, 1980). Successful adaptation occurs due to coordinated changes in the body to support the dominant physiologic state of lactation. This orchestrated flow to a new equilibrium is defined as homeorhesis (Bauman and Currie, 1980). If animals are unable to adapt, due to management factors, concurrent disease, or a multitude of other known and unknown reasons, negative effects can carry into lactation. These negative effects may include displaced abomasum, increased culling risk, lower milk production, and impaired reproductive performance (Butler and Smith, 1989; Collard et al., 2000; LeBlanc et al., 2005; Duffield et al., 2009; McArt et al., 2012b).

Ketosis is a common disease on many farms (Duffield, 2000; Geishauser et al., 2000). The cumulative lactational incidence varies greatly between farms, averages about 40%, and can be as high as 80% in some herds (Duffield, 2000). Though ketosis

has been extensively studied since the early 1900's, objective data regarding appropriate ketosis treatment is scarce. Even in trials where treatments were placed under rigorous scrutiny, the focus of many studies was on treating ketone levels, not necessarily on improving health and production. Thus, the most appropriate treatment for ketosis remains unclear and treatment does not prevent negative sequelae in all animals. Both of these factors help stress the importance of prevention. Prevention should begin with a thorough investigation of management practices around the time of calving. General recommendations can be given to ensure adequate bunk space, limit overcrowding, and ensure proper feed delivery and adequate water access (Overton et al., 2011). However, the exact practices that need to be examined and the ideal management situation vary widely between operations. This makes universal recommendations nearly impossible and increases the frustration on the part of the producer.

Even on the best-managed operation, there will be animals at high risk of development of ketosis. Cow-level risk factors for ketosis may include previous disease, breed, lactation, body condition score, and season of calving (Dohoo and Martin, 1984). However, many potential risk factors have not been examined and the risk of an individual cow developing ketosis is still largely unknown. By better understanding which animals are at risk and recommending appropriate treatment regimens, dairy advisors can help improve health, production, and welfare of dairy cattle and the economic viability of farm operations.

Etiology of Ketosis

Negative energy balance is nearly ubiquitous in early lactation in dairy cattle. This is due to the homeorhetic drive to sustain high milk production levels coupled with a

decrease in dry matter intake around the time of calving (Bauman and Currie, 1980; Baird, 1982; Herdt, 2000). This leads to a period of increased reliance on alternative fuels and body stores to meet the needs of production and maintenance (Ingvartsen, 2006). A significant proportion of body energy demands are met using ketone bodies during this period. The three major ketone bodies seen in cattle are acetone, acetoacetate, and beta-hydroxybutyrate (BHBA). Ketone bodies can be used for as fuel for many tissues, sparing glucose for dependent functions such as milk production (Ingvartsen, 2006).

Presence of ketone bodies in the blood is not a pathologic finding in the ruminant. Ketone bodies are produced daily in the rumen epithelium from the volatile fatty acid butyrate that is a normal product of ruminant digestion (Hird and Symons, 1961). Butyrate may be high in silages that are not properly preserved, which may lead to increased blood ketone concentrations due to increased uptake from the rumen or decrease in feed intake due to palatability (Ingvartsen, 2006).

Ketone bodies are also generated from incomplete oxidation of nonesterified fatty acids (NEFA) liberated during fat catabolism. Glucose demand is high during early lactation due to the high requirement of lactose for milk synthesis (Herdt and Emery, 1992). This is exacerbated by the limited ability of the ruminant to absorb glucose directly from the diet. Thus, ruminants rely heavily on gluconeogenesis, especially during early lactation (Herdt and Emery, 1992). Glucose cannot be produced directly from NEFA or ketone bodies (Herdt and Emery, 1992). However, both of these substances play an important role in glucose balance in early lactation (Ingvartsen, 2006). NEFA liberated from fat catabolism travel to the liver where they are re-esterified to

triglycerides (TG) or oxidized to acetyl CoA (Herdt and Emery, 1992). When completely oxidized, acetyl CoA stimulates the Krebs cycle and provides energy for gluconeogenesis from pyruvate (Ingvartsen, 2006). Acetyl CoA may also be converted to acetoacetate, the parent ketone body, which can be further converted to BHBA or acetone. Finally, TG may be transferred to other tissues with the help of very low-density lipoproteins (VLDL) or stored in the liver (Herdt, 2000). Beyond the stimulation of gluconeogenesis that occurs due to NEFA metabolism, NEFA, TG, and ketone bodies can all act as alternative fuel sources for many body tissues to spare glucose.

The exact causes of pathologic hyperketonemia are not fully understood (Herdt, 2000). One theory is that the demand for glucose exceeds the glucoeneogenic ability of the liver, which is generally true in early lactation (Holtenius and Holtenius, 1996). According to this theory, gluconeogenic pathways in the liver are maximally stimulated but blood glucose remains low, likely due to a lack of sufficient glucose precursors. Low blood glucose and insulin maintain high levels of fat catabolism and promote ketone body formation. A second theory involves signal breakdown at the level of fat catabolism (Herdt, 2000). In this case, fat breakdown, fueled by insulin resistance commonly observed in early lactation dairy cattle, occurs at a higher rate than can be processed in the liver (Hayirli, 2006). A sequela to this theory would be that fat accumulation occurs in the liver without maximal stimulation of gluconeogenesis (Holtenius and Holtenius, 1996). Fatty acids that are not completely oxidized or converted to ketone bodies are re-esterified to TG, leading to fatty liver due to the low capacity for the ruminant liver to synthesize VLDL for TG transport (Emery et al., 1992). Fatty liver may lead to further impairment of gluconeogenesis due to destruction of liver

parenchyma. Fat accumulation in the liver prepartum or even during the previous lactation may play a significant role in ketosis development, though more research is needed to determine the exact mechanisms (Holtenius and Holtenius, 1996; Drackley et al., 2001; Hayirli, 2006).

Ketosis Categorization

The terminology regarding ketosis classification has changed multiple times as more information is uncovered regarding the etiology and timing of hyperketonemia. Historically, ketosis was classified as primary or secondary based on when the signs commenced and what concurrent diseases the animal was experiencing (Baird, 1982). Primary ketosis was defined as ketosis that occurred due to a lack of sufficient glucose to support the demands of milk production (Baird, 1982). This may be due to lack of sufficient carbohydrates in the diet (nutritional) or due to the high requirements of dairy cattle for glucose in early lactation (spontaneous). Secondary ketosis was defined as ketosis due to a period of anorexia from concurrent disease (Baird, 1982).

Holtenius and Holtenius (1996) proposed a classification scheme, which aligned bovine ketosis with human diabetes. According to their classification, cattle with low blood glucose and insulin at the time of hyperketonemia diagnosis should be classified as type I ketosis. This form of ketosis was the equivalent of primary spontaneous ketosis in earlier classification schemes. Type I ketosis occurs due to the high demand for glucose to support milk production (Holtenius and Holtenius, 1996). The ability of the animal to absorb glucose from the diet is maximized in this situation and body stores, including protein, are utilized for gluconeogenesis. The animal's ability to generate glucose from amino acids is limited to protect the body from excessive protein degradation, so fatty

acids and ketones are used as fuels to spare glucose use (Holtenius and Holtenius, 1996). Type I ketosis was defined as ketosis seen around peak lactation, without concurrent disease or fat accumulation in the liver.

Type II ketosis was described as hyperketonemia with concurrent hyperinsulinemia and hyperglycemia (Holtenius and Holtenius, 1996). It was described as ketosis that occurred during early lactation and was generally observed with other disease. In this type of ketosis, fat accumulates in the liver due to insufficient stimulation of gluconeogenesis and ketogenesis. It is hypothesized that the accumulation of fat decreases the functionality of liver tissue, further exacerbating the problem. This form of ketosis occurred earlier in lactation than type I and overfeeding in the dry period was a significant risk factor for development (Holtenius and Holtenius, 1996).

Both of these classification systems have since fallen out of favor due to the difficulty of placing many animals with hyperketonemia in these categories. A majority of ketosis in North America occurs in the first 10 days after calving and may or may not be accompanied by concurrent disease (McArt et al., 2012b). Additionally, few cows diagnosed with ketosis are hyperinsulinemic or hyperglycemic at the time of diagnosis (Herdt, 2000) and levels of fat accumulation in the liver vary widely.

Currently, the terms subclinical and clinical are favored for ketosis categorization (Duffield, 2000). Clinical ketosis is characterized by an increase in blood, urine, or milk ketone bodies in conjunction with other visible signs, such as inappetence, obvious rapid weight loss, and dry manure. Subclinical ketosis (SCK) is defined as an increase in blood, urine, or milk ketone bodies above a threshold associated with undesirable outcomes in the absence of obvious clinical signs.

In large groups of loose-housed cattle, it has become difficult or impossible to determine if a specific animal is showing clinical signs of ketosis. Attempts have been made to classify ketosis as clinical or subclinical based on blood BHBA concentrations (Kauppinen, 1983b; McArt et al., 2011). However clinical and research experience has shown that when examined, animals with high levels of ketonemia may show no clinical signs and animals with relatively low levels may be obviously ill. The severity of clinical signs appears to depend on the individual animal's ability to process and tolerate ketone bodies (Herdt, 2000). The disease may therefore be best described as hyperketonemia rather than trying to distinguish clinical from subclinical.

Classification of ketosis is most relevant for clarity and consistency in comparing incidence risk rates. Depending on the methods and frequency of screening, incidence rates of clinical ketosis are expected to be 2 – 15% in the first month of lactation, while 40% cumulative incidence of SCK is typical if cows are screened weekly during the same time period (Duffield, 2000). There is some evidence that greater concentrations of blood ketone bodies are associated with higher risk of negative outcomes such as subsequent disease and culling (McArt et al., 2012b). McArt et al. (2012b) found that each 0.1 mmol/L increase in blood BHBA in animals with blood BHBA between 1.2 and 2.9 mmol/L increased the risk of DA by 1.1 times (95% CI 1.0 to 1.2) and increased the risk of culling by 1.4 times (95% CI 1.1 to 1.8). However, the importance of the distinction between clinical and SCK with regard to treatment is unclear.

Ketosis Diagnosis

In affected animals, ketone bodies are present in blood, milk, and urine and tests have been developed that detect ketone bodies in all three body fluids. Urine ketone

body concentrations are about four times higher than blood concentrations (Schultz, 1971), while milk BHBA concentrations are one-eighth and milk acetoacetate concentrations are about half that of blood concentrations (Andersson, 1984). The gold standard for ketosis diagnosis is measurement of ketone bodies in serum or plasma photometrically in a diagnostic laboratory. BHBA is used most frequently due to its stability in samples (Duffield, 2000; Herdt, 2000). The concentration of blood BHBA that defines hyperketonemia varies from 1.0 mmol/L to 1.4 mmol/L in various studies (Kelly, 1977; Whitaker et al., 1983; Whitaker et al., 1993; Nielen et al., 1994; Geishauser et al., 1997; LeBlanc et al., 2005; Duffield et al., 2009; McArt et al., 2012b). During the first week of lactation, Duffield et al. (2009) found that BHBA concentrations $\geq$ 1.2 mmol/L were associated with decreased milk production and increased risk of DA (OR = 2.6, 95% CI = 1.3 to 5.1) and metritis (OR = 3.4, 95% CI = 1.6 to 7.2). However, concentrations $\geq$ 1.4 mmol/L led to larger milk production losses at first test, -1.9 versus -1.2 kg for a cut point at $\geq$ 1.2 mmol/L, and increased risk of clinical ketosis (OR = 4.3 versus 3.5, 95% CI = 1.4 to 12.8 and 1.2 to 10.6, respectively) and LDA (OR = 2.8 versus 2.6, 95% CI = 1.3 to 6.0 and 1.2 to 5.2, respectively). Blood BHBA concentrations greater than 1.8 mmol/L were required to significantly decrease 305-d milk yield by 333.7 kg.

Though considered the gold standard, laboratory diagnosis of hyperketonemia is expensive, inconvenient, and the delay in reporting makes it impractical for individual animal diagnosis and treatment. Numerous cow-side tests have been developed since the 1950's to allow for more rapid diagnosis of ketotic animals. However, many of these tests have been plagued with low sensitivity or specificity (Geishauser et al., 1998;

Carrier et al., 2004). There are four cow-side tests that are currently accepted as sufficiently accurate for routine use: Ketostix (Bayer, Pittsburgh, PA) for measurement of urine acetoacetate, KetoTest (Sanwa Kagaku Kenkyusho Co., Nagoya, Japan) or PortaBHB (PortaCheck Inc., Moorestown, NJ) for measurement of milk BHBA, and Precision Xtra (Abbott Laboratories, Abbott Park, IL) for measurement of blood BHBA (Geishauser et al., 1998; Geishauser et al., 2000; Carrier et al., 2004; Iwersen et al., 2009; Denis-Robichaud et al., 2011). Though the accuracy of the Ketostix test strip is high (90% sensitivity and 86% specificity when read at 5 seconds with a cut-point of "trace"; Carrier et al., 2004), the development of highly accurate cow-side milk and blood tests has decreased the use of this test due to the relative challenge of obtaining urine from all animals.

A relatively recent addition to cow-side ketone testing is an electronic hand-held ketone and glucose meter that is used in human patients with diabetes (Precision Xtra). Oxidation of BHBA to acetoacetate causes reduction of NAD^+ to NADH in the test strip. When NADH is reoxidized, the current generated is measured and is directly proportional to the amount of BHBA in the sample (Iwersen et al., 2009). In a study comparing serum BHBA levels determined photometrically (gold standard) and Precision Xtra results, the Precision Xtra was found to be 100% sensitive and specific (95% CI = 69 to 100% and 94 to 100%, respectively) when all samples were obtained by a single research group and ketosis was defined as $\geq$ 1.4 mmol/L (Iwersen et al., 2009). However, when the same study was completed at multiple locations, the sensitivity and specificity dropped to 96% (95% CI = 95 to 98%) and 97% (95% CI = 96 to 98%) respectively. In the multicenter study, some of the tests were performed on dry cows and cows with unknown lactational

status whereas all animals were 4 to 40 DIM in the initial study. This suggests that stage of lactation or variation between users may affect the accuracy of this test. Konkol et al. (2009) also used serum BHBA concentrations $\geq$ 1.4 mmol/L as the gold standard to determine the accuracy of the Precision Xtra. However, they found a much lower sensitivity (85.2%) and similar specificity (99.4%) when using 1.4 mmol/L as the threshold for ketosis using the Precision Xtra. The highest accuracy was obtained by using a threshold of 1.3 mmol/L on the Precision Xtra (sensitivity, specificity, positive predictive value, and negative predictive value of 96.3%, 98.8%, 99.4%, and 92.9% respectively). It is unclear what causes variation in the accuracy of the Precision Xtra and more research is needed to determine the optimal threshold and protocol for use of this tool in dairy cattle.

Milk ketone tests are often preferred for routine use on farm due to ease of milk collection and the minimal training that is required to perform the test. Both commercially available milk test strips (KetoTest and PortaBHB) use the same principle, a test strip is dipped into the milk sample and the semi-quantitative result is read one minute later using a chart provided on the bottle (0, 50, 100, 200, or 500 μmol/L; Carrier et al., 2004; Denis-Robichaud et al., 2011). Both test strips measure BHBA and use a colormetric reaction to estimate the concentration of BHBA in the sample. Carrier et al. (2004) reported that the KetoTest strip had a sensitivity of 73% and a specificity of 96% when using a cut-point of 100 μmol/L on the test strip and ketosis was defined as blood BHBA $\geq$ 1.4 mmol/L. Similar accuracy was reported for the PortaBHB (Denis-Robichaud et al., 2011) with the same cut-point and definition of ketosis. However, the PortaBHB appears to be more sensitive (92%) and less specific (78%) than the KetoTest.

It should be noted that the results reported for the PortaBHB were obtained using Precision Xtra results as the referent (Denis-Robichaud et al., 2011). Though the Precision Xtra is considered highly accurate, sensitivity and specificity are not always 100% (Konkol et al., 2009) and it is not considered the gold standard. This could affect the results obtained for the PortaBHB.

The preferred test for farm use depends on the management of the operation and the ease of collecting the respective samples. For example, milk may be the most convenient sample to collect in a tie-stall facility where the animals are restrained and samples can be taken without interfering with the milking schedule. However, in a large freestall operation, milk would have to be collected in the parlor for testing, which may slow parlor throughput down to an unacceptable level during milking. In this situation, it may be easier to collect blood or urine when the cows are restrained in headlocks or sort cows out for testing as they leave the parlor. In order to achieve the highest farm compliance, the best choice of test is the test that is most likely to be utilized consistently.

Disease Risk Due to Hyperketonemia

Numerous studies have demonstrated an association between ketosis and various periparturient diseases (Dohoo and Martin, 1984; Curtis et al., 1985; Gröhn et al., 1989; LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010a; McArt et al., 2012b; a). However, in many studies it is difficult to determine if ketosis is caused by the disease, the disease is caused by ketosis, or if the relationship is causal at all. In addition, the diagnostic method for ketosis is not always consistent between or even within studies. More recent studies have emphasized the need for clear disease definitions (Kelton et al., 1998) and determination of the time sequence of disease diagnosis.

The disease most commonly associated with ketosis is DA (LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010a; McArt et al., 2012b). The risk of DA in ketotic animals varies widely from 2.6 (Duffield et al., 2009) to 19.3 (McArt et al., 2012b) times greater than in non-ketotic animals in the same study. It is unclear exactly why the risk is vastly different between studies, but it is likely due in part to the fact that the calculation by Duffield et al. included all animals in the study (treated or not treated with a controlled release monensin capsule prepartum) and the calculation by McArt et al. included only control animals in the study (i.e. measuring the risk of DA in animals with untreated SCK). Considering that treatment with propylene glycol in the McArt et al. study (2012a) significantly decreased the risk of DA, it is likely that the relative risk would be decreased if all animals had been included. However, McArt et al. only included animals that were subclinically ketotic (no clinical signs and Precision Xtra BHBA 1.2 to 2.9 mmol/L). If a producer was not monitoring for SCK and thus the animals were not being treated, it would lead to a greatly increased DA rate on the farm based on the large increase in risk in untreated ketotic animals compared to non-ketotic animals.

The time of onset (or at least first detection) of ketosis is important. Animals that developed SCK during the first week of lactation had increased risk of DA and removal from the herd relative to animals that developed SCK in the second week of lactation (McArt et al., 2012b). This information suggests that animals that are afflicted earlier in lactation are more poorly adapted to the period of negative energy balance seen at the beginning of lactation.

Production Losses Due to Hyperketonemia

The relationship between hyperketonemia and milk production can be challenging to define. Many studies have examined this relationship with mixed results. Most early studies showed a negative correlation between test day milk acetone levels and test day milk production (Andersson and Emanuelson, 1985; Gustafsson et al., 1993; Miettinen and Setälä, 1993; Steen et al., 1996). Dohoo and Martin (1984) showed that SCK decreased test day milk yield by 1.0 to 1.4 kg depending on the level of ketone bodies in the milk. However, Kauppinen elucidated the complexity of this relationship by showing a positive correlation between test day blood BHBA and acetoacetate concentrations and milk production (Kauppinen, 1983a) and SCK and higher annual milk yields (Kauppinen, 1984). The difference between the design of these studies and the others was that the blood and milk samples were not necessarily collected on the same day as production was measured. Taking all of these studies as a group, early understanding was that SCK decreased production at the time of diagnosis, but that cows could make up for the production loss over the lactation.

Several studies have since described the detrimental effects of ketosis on production (Duffield et al., 2009; Ospina et al., 2010b; McArt et al., 2012b). A recent study conducted in New York and Wisconsin (McArt et al., 2012b) reported that each 0.1 mmol/L increase in blood BHBA (based on Precision Xtra results) above 1.2 mmol/L at 3 to 16 DIM decreased milk production by 0.5 kg/d for the first 30 days of lactation. A herd level study in which herds were classified as high risk if > 15% of mature cows had blood BHBA $\geq$ 1 mmol/L or > 20% of heifers had blood BHBA $\geq$ 1.2 mmol/L) showed

high risk herds had 358 and 534 kg decreases in projected 305 ME milk yield for cows and heifers respectively (Ospina et al., 2010c).

Recent studies have reinforced the challenges in understanding the relationship between milk production and SCK (Duffield et al., 2009; Chapinal et al., 2012). Duffield et al. (2009) showed that blood BHBA $\geq$ 1.2 mmol/L in either of the first two weeks postpartum decreased milk yield at first DHI test by 1.2 to 3.3 kg. However, cows with SCK during the second week postpartum had similar yields at the second DHI test and surpassed cows that had not had SCK at the third DHI test. By the third DHI test animals classified with SCK during the second week after calving had significantly higher projected 305 ME compared to cows that had not experienced SCK. Chapinal et al. (2012) similarly found that animals diagnosed with SCK in the first two weeks after calving had 1.5 to 2.4 kg less milk at first DHI test. However when the first four DHI tests were examined together, animals classified as SCK had higher milk production than those without SCK. Though a bit surprising, this finding has a plausible physiologic explanation. Cows in early lactation require use of body stores, NEFA, and BHBA to support milk production (Herdt, 2000). It follows that cows that are naturally low producers do not require as much break down of body stores to support lactation or conversely, cows with low BHBA are not metabolizing sufficient body fat to support high levels of production. This highlights the need to better define the relationship between ketosis and milk production.

Ketosis and Reproductive Efficiency

Early investigations into the relationship between SCK and reproduction reported mixed results. Two Scandinavian studies examining the effects of SCK on reproduction

reported no negative effect on individual cow fertility (Kauppinen, 1984; Andersson and Emanuelson, 1985). However, one of these studies showed a significant increase in the herd mean calving to first service interval in herds with a higher prevalence of SCK (Andersson and Emanuelson, 1985). This study also showed an increased risk of cystic ovaries with increasing milk acetone level on an individual cow basis (Andersson and Emanuelson, 1985). These results are challenging to interpret due to advancements in diagnosis of ketosis and statistical methods. Tests used during this period had a low sensitivity, so animals that would be classified as ketotic today may have been misclassified in these studies. This may lead to increased similarities between the affected and unaffected group, making it more challenging to find statistical differences between groups. Furthermore, statistical methods were not available to account for confounding variables such as lactation and herd.

More recent studies have examined the effects of SCK on reproduction at both the individual cow (Walsh et al., 2007a; Walsh et al., 2007b; Chapinal et al., 2012; McArt et al., 2012b) and herd (Ospina et al., 2010b) levels. Walsh et al. (2007b) found that cows that were subclinically ketotic during the first two weeks of lactation were 50% less likely to conceive at first service and remained open for 16 to 22 days more than animals that were not diagnosed with SCK. They also showed that the higher the BHBA concentrations, the less likely an animal was to become pregnant, suggesting it is not just the presence of ketone bodies but the degree of ketosis that is important to future reproductive success. In another study, the same investigators found that cows diagnosed with SCK based on milk BHBA during the first two weeks after calving had 1.5 greater odds to be anovulatory at 46 and 60 DIM (Walsh et al., 2007a). McArt et al. (2012b)

showed that cows that were diagnosed with SCK based on blood BHBA concentrations between 3 and 16 DIM were 30% less likely to conceive to first service than unaffected herd mates. Interestingly, a study examining the relationship between reproduction and SCK diagnosis in 4 regions of the US found no association between blood BHBA levels and pregnancy at first service (Chapinal et al., 2012). It is unclear why Chapinal et al. reported differing results from previous studies. The studies that showed a difference in first service conception rate were performed in the Northeastern and Midwest US and Eastern Canada. The multiregion study included herds from the Western and Southeastern US, as well as the regions included in previous studies, and samples were collected from the regions at different times of the year. It is possible that there are differences between regions or seasons that affect the relationship between SCK and reproduction. However, it is more likely that differences in breeding management on enrolled farms (i.e. AI versus bull breeding and synchronization versus heat detection for first AI) accounted for the differences. More research is required to understand this complex relationship.

Ospina et al. (2010b) classified herds as high risk for metabolic disease if > 15% of animals sampled once between 3 and 14 DIM had blood BHBA concentrations of $\geq$ 1.2 mmol/L. They found that high-risk herds had a 0.8% lower pregnancy rate than low-risk herds. Though this is not a large difference, it shows that higher prevalence of SCK can affect herd performance. Considering that most herds have a cumulative lactational incidence higher than 15% (Duffield, 2000), it would be interesting to determine the mechanisms by which an increased incidence of SCK affects overall herd reproductive performance.

Ketosis Treatment

Ketosis treatment has been an active area of research since the early 1900's. During this period, numerous treatments have been examined with varying results from the perspective of efficacy. A systematic review of the ketosis treatment literature is presented in Chapter 2 and will not be discussed further in this chapter.

Risk Factors for Development of Ketosis

With a high incidence of ketosis on many farms (Duffield, 2000), the negative effects on health and production (Duffield et al., 2009; McArt et al., 2012b), and the difficulty with effective treatment regimens, there is an increased interest in ketosis prevention. Information regarding nutrition and management of transition cows has greatly increased over the last few years. However, there is still a lack of information on many ketosis risk factors regarding individual cows and herds.

Many cow-level factors include things about the cow that cannot be changed, such as breed (Erb and Martin, 1978; Andersson and Emanuelson, 1985; Bendixen et al., 1987) and parity (Dohoo and Martin, 1984; Gröhn et al., 1989; McArt et al., 2013). Though these factors are interesting to examine, they have little bearing on ketosis management.

Other factors that affect ketosis risk reside at the cow-level but reflect herd management decisions and can be altered. These include body condition score (Gillund et al., 2001; Busato et al., 2002; McArt et al., 2013) and dry period length (Rastani et al., 2005; Watters et al., 2008; Santschi et al., 2011). Generally, body condition score (BCS) at calving higher than 3.25 or 3.5 on a 5-point scale is associated with a higher risk of development of ketosis (Gillund et al., 2001; Busato et al., 2002). Recently McArt et al.

(2013) found that cows with a body condition score during the close up period at or above the herd median had a significantly increased risk of development SCK between 3 and 16 DIM, though the difference was small (1.1 and 1.2 times respectively). However, interpretation of this information is challenging as the median BCS and the exact timing of scoring were not reported. Furthermore, the amount of body condition lost after calving was not examined and this appears to be more important than the absolute BCS in some studies (Gillund et al., 2001).

There has been interest in shortened dry periods to decrease the amount of unproductive time in the life of a dairy cow (Rastani et al., 2005). Studies have found a decreased risk of ketosis in animals that have a shortened dry period (35 days or less) with little to no effect on production or reproduction in the subsequent lactation (Rastani et al., 2005; Watters et al., 2008; Santschi et al., 2011). It should be noted that there were differences between herds in days open in response to shortened dry period (Santschi et al., 2011), suggesting that other management factors may affect the success of a short dry period in a herd.

Nutritional management of the transition cow has been examined as an important area for ketosis prevention. Gustafsson et al. (1995) found that the amount of concentrate and how it was fed were important for determining the risk of ketosis in a herd. They found that herds that fed fewer meals throughout the day had an increased risk of ketosis, especially herds with diets high in concentrate levels (Gustafsson et al., 1995). They hypothesized that higher levels of concentrate led to periods of acidosis, which decreased overall feed consumption. Richert et al. (2013) also found that increasing levels of concentrates in the diet was associated with an increase in farmer reported ketosis

incidence. The negative effect of increased concentrate was more pronounced in herds that used grazing to meet a portion of their energy requirements potentially due to these herds being more likely to feed concentrate separately and at fewer times during the day, increasing the risk of acidosis (Richert et al., 2013). However, as the incidence of disease in this study was producer reported, it may be that there are differences in management style and producer ability that affect the accuracy of disease reporting in different types of herds. Silages high in butyric acid can increase ketosis risk, as butyric acid can be converted to acetoacetate and BHBA in the rumen (Tveit et al., 1992). High levels of butyric acid can also decrease palatability of the feed, increasing the risk of NEB and ketosis (Tveit et al., 1992; Grant and Albright, 1995). Digestibility of the diet and decreased palatability due dietary components (i.e. excessive supplementary fat) may also affect ketosis risk (Grant and Albright, 1995).

Many other herd-level management factors may affect ketosis risk such as housing type (Dohoo and Martin, 1984; Bendixen et al., 1987), grouping strategies, and pen moves. Many of these factors have not been studied to determine their effects and interactions. Furthermore, several risk factors often combine to determine ketosis risk and these factors may vary between herds and studies (McArt et al., 2013). More research is required to further define management risk factors for ketosis development.

RESEARCH OBJECTIVES

The overall goal of the research described in this thesis was to increase the knowledge of ketosis risk factors and treatment in lactating dairy cattle.

The first objective was to summarize the current body of literature on ketosis treatment, elucidate gaps and areas for future research, and develop a generalized recommendation for an appropriate treatment regimen.

A second objective was to assess the efficacy of a butaphosphan-cyanocobalamin combination product, long acting insulin, and propylene glycol in ketosis treatment.

The final objective was to investigate selected cow-level risk factors for ketosis development to better understand ketosis risk and make recommendations for prevention through management or early treatment of high-risk cows.

REFERENCES

Andersson, L. 1984. Concentrations of blood and milk ketone bodies, blood isopropanol and plasma glucose in dairy cows in relation to the degree of hyperketonaemia and clinical signs. Zentralbl. Veterinarmed. A. 31:683-693.

Andersson, L. and U. Emanuelson. 1985. An epidemiological study of hyperketonaemia in Swedish dairy cows; determinants and the relation to fertility. Prev. Vet. Med. 3:449-462.

Baird, G. D. 1982. Primary ketosis in the high-producing dairy cow: Clinical and subclinical disorders, treatment, prevention, and outlook. J. Dairy Sci. 65:1-10.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bendixen, P. H., B. Vilson, I. Ekesbo, and D. B. Astrand. 1987. Disease frequencies in dairy cows in Sweden. IV. Ketosis. Prev. Vet. Med. 5:99-109.

Busato, A., D. Faissler, U. Küpfer, and J. W. Blum. 2002. Body condition scores in dairy cows: associations with metabolic and endocrine changes in healthy dairy cows. J. Vet. Med. Ser. A. 49:455-460.

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

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.

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301-1309.

Collard, B. L., P. J. Boettcher, J. C. M. Dekkers, D. Petitclerc, and L. R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. J. Dairy Sci. 83:2683-2690.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347-2360.

Denis-Robichaud, J., L. DesCôteaux, and J. Dubuc. 2011. Accuracy of a new milk strip cow-side test for diagnosis of hyperketonemia. Bovine Practitioner. 45:97-100.

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

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:E100-E112.

Duffield, T. F. 2000. Subclinical ketosis in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 16:231-253.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

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.

Erb, H. N. and S. W. Martin. 1978. Age, breed and seasonal patterns in the occurrence of ten dairy cow diseases: a case control study. Can. J. Comp. Med. 42:1-9.

Geishauser, T., K. Leslie, T. Duffield, and V. Edge. 1997. Evaluation of aspartate transaminase activity and β-hydroxybutyrate concentration in blood as tests for prediction of left displaced abomasum in dairy cows. Am. J. Vet. Res. 58:1216-1220.

Geishauser, T., K. E. Leslie, D. Kelton, and T. F. Duffield. 1998. Evaluation of five cowside tests for use with milk to detect subclinical ketosis in dairy cows. J. Dairy Sci. 81:438-443.

Geishauser, T., K. Leslie, J. Tenhag, 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.

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

Grant, R. J. and J. L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. J. Anim. Sci. 73:2791-2803.

Gröhn, Y. T., H. N. Erb, C. E. McCulloch, and H. S. Saloniemi. 1989. Epidemiology of metabolic disorders in dairy cattle: association among host characteristics, disease and production. J. Dairy Sci. 72:1876-1885.

Gustafsson, A. H., L. Andersson, and U. Emanuelson. 1993. Effect of hyperketonaemia, feeding frequency and intake of concentrate and energy on milk yield in dairy cows. Anim. Prod. 56:51-60.

Gustafsson, A. H., L. Andersson, and U. Emanuelson. 1995. Influence of feeding management, concentrate intake and energy intake on the risk of hyperketonaemia in Swedish dairy herds. Prev. Vet. Med. 22:237-248.

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.

Herdt, T. H. and R. S. Emery. 1992. Therapy of diseases of ruminant intermediary metabolism. Vet. Clin. North Am. Food Anim. Pract. 8:91-106.

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Vet. Clin. North Am. Food Anim. Pract. 16:215-230.

Hird, F. J. R. and R. H. Symons. 1961. The mode of formation of ketone bodies from butyrate by tissue from the rumen and omasum of the sheep. Biochim. Biophys. Acta. 46:457-467.

Holtenius, P. and K. Holtenius. 1996. New aspects of ketone bodies in energy metabolism of dairy cows: A review. J. Vet. Med. Ser. A. 43:579.

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

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.

Kauppinen, K. 1983a. Correlation of whole blood concentrations of acetoacetate, β-hydroxybutyrate, glucose and milk yield in dairy cows as studied under field conditions. Acta Vet. Scand. 24:337-348.

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

Kauppinen, K. 1984. Annual milk yield and reproductive performance of ketotic and non-ketotic dairy cows. Zentralbl. Veterinarmed. A. 31:694-704.

Kelly, J. M. 1977. Changes in serum beta-hydroxybutyrate concentrations in dairy cows kept under commercial farm conditions. Vet. Rec. 101:499-502.

Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. J. Dairy Sci. 81:2502-2509.

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.

LeBlanc, S. J., K. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci. 88:159-170.

McArt, J. 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.

McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2012a. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. J. Dairy Sci. 95:2505-2512.

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

McArt, J. 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.

Miettinen, P. V. A. and J. J. Setälä. 1993. Relationships between subclinical ketosis, milk production and fertility in Finnish dairy cattle. Prev. Vet. Med. 17:1-8.

Nielen, M., M. G. A. Aarts, A. G. M. Jonkers, T. Wensing, and S. Y.H. 1994. Evaluation of two cowside tests for the detection of subclinical ketosis indairy cows. Can. Vet. J. 35:229-232.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546-554.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93:1596-1603.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010c. 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.

Overton, M. W., W. G. Boomer, and P. J. Gorden. 2011. Transition management checklist. Accessed Mar. 1, 2013. http://www.extension.org/pages/25100/transition-management-checklist/.

Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. J. Dairy Sci. 88:1004-1014.

Richert, R. M., K. M. Cicconi, M. J. Gamroth, Y. H. Schukken, K. E. Stiglbauer, and P. L. Ruegg. 2013. Risk factors for clinical mastitis, ketosis, and pneumonia in dairy cattle on organic and small conventional farms in the United States. J Dairy Sci 96:4269-4285.

Santschi, D. E., D. M. Lefebvre, R. I. Cue, C. L. Girard, and D. Pellerin. 2011. Incidence of metabolic disorders and reproductive performance following a short (35-d) or conventional (60-d) dry period management in commercial Holstein herds. J. Dairy Sci. 94:3322-3330.

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

Steen, A., O. Østerås, and H. Grønstøl. 1996. Evaluation of additional acetone and urea analyses, and of the fat-lactose-quotient in cow milk samples in the herd recording system in Norway. J. Vet. Med. Ser. A. 43:181-191.

Tveit, B., F. Lingaas, M. Svendsen, and Ø. V. Sjaastad. 1992. Etiology of acetonemia in Norwegian cattle 1. Effect of ketogenic silage, season, energy level, and genetic factors. J. Dairy Sci. 75:2421-2432.

Walsh, R. B., D. F. Kelton, T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2007a. Prevalence and risk factors for postpartum anovulatory condition in dairy cows. J Dairy Sci 90:315-324.

Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007b. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90:2788-2796.

Watters, R. D., J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Crump, P. W. Clark, and R. R. Grummer. 2008. Effects of dry period length on milk production and health of dairy cattle. J. Dairy Sci. 91:2595-2603.

Whitaker, D. A., J. M. Kelly, and E. J. Smith. 1983. Subclinical ketosis and serum beta-hydroxybutyrate levels in dairy cattle. Br. Vet. J. 139:462-463.

Whitaker, D. A., E. J. Smith, G. O. d. Rosa, and J. M. Kelly. 1993. Some effects of nutrition and management on the fertility of dairy cattle. Vet. Rec. 133:61-64.

CHAPTER TWO

As previously published

Gordon, J.L., LeBlanc S.J., Duffield, T.F. 2013. Ketosis treatment in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 29: 433-445.

KETOSIS TREATMENT IN LACTATING DAIRY CATTLE

Keywords

- Ketosis
- Treatment
- Systematic review
- Propylene glycol

Synopsis

This article provides an update on ketosis treatment regimens. The ketosis treatment literature is reviewed and the findings summarized. Current treatment recommendations and areas for future research are provided.

Key Points

- Ketosis is a common disease in dairy cattle in early lactation
- Multiple treatments have been used in dairy cattle, with varying levels of support of efficacy and varying results
- There is a lack of well-designed ketosis treatment clinical trials
- Currently, the best recommendation for treatment is 300 ml of 100% propylene glycol orally once daily for 5 days
- Further research is required to determine the most effective ketosis treatment regimen for economically important outcomes

INTRODUCTION

Subclinical ketosis is a common disease of the transition period in dairy cattle, affecting approximately 40% of lactations in North America (Duffield, 2000; McArt et al., 2012b). The incidence on individual farms varies widely and may be as high as 80% (Duffield, 2000). The costs associated with ketosis include treatment of the disease, increased risk and treatment of other diseases, decreased milk production, worse reproductive performance, and higher risk of culling in the first 30 days of lactation (McArt et al., 2011; McArt et al., 2012a).

Classification

Historically, ketosis was classified as primary or secondary based on when signs commenced and what concurrent diseases were facing the animal (Herdt, 2000). Recently, this nomenclature has fallen out of favor, as most ketosis is seen in the first 10 days after calving in North America and may or may not be accompanied by other disease (McArt et al., 2012b). Currently, the terms subclinical and clinical are favored for ketosis definition. Clinical ketosis is characterized by an increase in blood, urine, or milk ketone bodies in conjunction with other visible signs, such as inappetence, obvious rapid weight loss, and dry manure. Subclinical ketosis is defined as an increase in blood, urine, or milk ketone bodies, above a threshold demonstrated to be associated with undesirable outcomes, in the absence of obvious clinical signs.

Due to the housing system utilized in many North American dairies (large groups of loose-housed cattle), it has become difficult or impossible to determine if a specific animal is showing clinical signs of ketosis. Attempts have been made to classify ketosis as clinical or subclinical based on blood BHBA concentrations (McArt et al., 2011).

However, it has been our experience that when examined, animals with high levels of ketonemia may show no clinical signs and animals with relatively low levels may be obviously ill. The severity of clinical effect appears to depend on the individual animal's ability to process and tolerance to ketone bodies (Herdt, 2000). The disease may therefore be best described as hyperketonemia rather than trying to distinguish clinical from subclinical.

Classification of ketosis is most relevant for clarity and consistency in comparing incidence risk rates. Depending on the methods and frequency of screening, incidence rates of clinical ketosis are expected to be 2 – 15% in the first month of lactation, while 40% cumulative incidence of subclinical ketosis is typical if cows are screened weekly during the same time period (Duffield, 2000). There is some evidence that greater ketonemia is associated with higher risk of negative outcomes such as subsequent disease and culling (McArt et al., 2012b). However, the importance of the distinction between clinical and subclinical ketosis with regard to treatment is unclear. To the authors' knowledge, there are no well-designed studies that have shown a difference in efficacy of treatments based on the initial level of ketonemia.

Physiology of Early Lactation and Ketosis

When considering effective treatment for ketosis, it is critical to consider the physiology of the animal during this period. At the beginning of lactation, animals are faced with a sudden and drastic increase in energy demand (Herdt, 2000). This is coupled with a decrease in feed intake that generally starts in the dry period. The rate of increase of feed intake postpartum lags behind the demands of lactation, leading to a period of negative energy balance. Fat is mobilized from body stores in the form of

non-esterified fatty acids (NEFA) to meet energy requirements. NEFA travel to the liver destined for one of three pathways, complete oxidation for energy, incomplete oxidation to ketone bodies, or re-esterification to fatty acids. All of these pathways are stimulated in the transition animal, but the magnitude of fat breakdown and tolerance of the individual determine the relative distribution of the paths (Herdt, 2000).

In early lactation, homeorhesis is the driving physiologic force (Bauman, 2000). Homeorhesis was defined by Bauman and Currie as "the orchestrated or coordinated changes in metabolism of body tissues necessary to support a physiological state" (Bauman and Currie, 1980). These processes facilitate break down of body stores of fat and protein in excess of what would be allowed based on homeostatic regulation. This leads to a period of insulin resistance that is nearly universal in early lactation animals (Bauman, 2000). Milk production requires large amounts of glucose. Because ruminants absorb only minimal amounts of glucose from their diet, gluconeogenesis is required to meet this need. This process is generally diminished in animals affected by ketosis, leading to hypoglycemia. Providing glucose, stimulating gluconeogenesis, and decreasing fat breakdown form the foundation for rational ketosis treatment (Herdt and Emery, 1992).

SYSTEMATIC REVIEW OF KETOSIS TREATMENT

Background

Reviews have long been used to summarize the body of literature on a given topic. This can be especially helpful for practitioners who have limited time to read primary scientific articles or require the information in a short period of time while

working on a clinical case (Vandeweerd et al., 2012a). Historically, these were narrative reviews conducted by an expert on the subject (Cook et al., 1997; Sargeant et al., 2006). Even high quality reviews are inherently biased, as selection of papers and interpretation of the information are consciously or unconsciously influenced by the author's opinions at the outset.

Systematic reviews help remove the bias of the reviewers by following a rigorous method in selection of materials to be included (Sargeant et al., 2006; Vandeweerd et al., 2012a). Authors provide a detailed framework for conducting the review that can be repeated and examined for accuracy. A specific question is formulated and an exhaustive search of the literature is performed. Methods for inclusion of materials are clearly defined and laid out prior to initiation of the review. The quality of all materials included is determined through specified criteria. Inclusion of all high quality relevant material is the framework for a systematic review, so small studies that are well designed are not excluded due to lack of power.

Materials and Methods

A systematic review of ketosis treatment was performed in February 2011 to determine the most effective treatment(s) for ketosis in lactating dairy cattle. The search phrases "ketosis treatment cattle" and "acetonemia treatment cattle" were entered into four databases: CAB, Pub Med, Agricola, and Google Scholar. These databases included references from 1900 to present in all languages. A complete list of references from the search was obtained from each database and abstracts were obtained for all references. Titles and abstracts were used to determine the relevance of each reference to the question. If abstracts were unavailable and the title was suggestive that the reference was

relevant, the full reference was obtained and analyzed. Materials that appeared relevant based on the title and/or abstract were obtained in full and analyzed. In addition, a manual search of relevant conference proceedings (American Dairy Science Association, American Association of Bovine Practitioners) was conducted and studies known to the authors that were not yet published in the peer-reviewed domain were solicited for evaluation (Carrier et al., 2011; McArt et al., 2011; McArt et al., 2012a).

The following criteria were used to determine the appropriateness of materials for the review:

1. Study animals were lactating dairy cattle
2. Animals experienced naturally occurring ketosis
3. Animals were diagnosed prior to initiation of treatment and method of diagnosis was clearly defined
4. Inclusion of a control group that was positive for ketosis
5. Control group was untreated or treated with a baseline treatment common to both groups (e.g. dextrose versus dextrose and insulin)
6. Any intervention was considered – oral, injectable, or feed additive
7. Any outcome was considered, but must be clearly defined – ketosis cure, health data, milk production, reproductive performance

Results of the Review

A total of 1,395 references were obtained from the search (Figure 2.1). These included journal articles, theses, conference proceedings, abstracts, and book chapters. Of these, 660 were excluded because they covered another topic, such as ketosis prevention, and 360 were excluded as duplicate citations. A further 186 were excluded as

they reviewed the literature without presenting novel data. Of the 189 that remained, 179 did not include a control group, leaving just 10 articles considered appropriate for the review (Table 2.1).

One of the most striking aspects of this venture was the lack of well-designed ketosis treatment literature. During the past 15 years, the extent of ketosis observed in North America has been clearly defined and the prevalence of ketosis initially surprised many veterinarians and producers. Due to the relative frequency of clinical and subclinical ketosis, it is surprising that there has been so little advancement in the body of evidence for treatment of a ketotic cow. Much treatment of ketosis is based on disease principles or past experience. Though both of these are critical components for development of treatment strategies, stronger evidence is required to ensure rational and effective treatment (Vandeweerd et al., 2012b). Due to the small number of studies that met the inclusion criteria and the large number of treatments represented by these studies, it is difficult to provide concrete information on many common treatments. However, some of the common treatments will be discussed below in relation to the findings of the review.

DEXTROSE

Background

The presence of hypoglycemia in ketosis was well established by the 1930's (McSherry et al., 1960). Since this time, dextrose has been considered a staple in ketosis treatment. This treatment seems physiologically sound, as the requirement for glucose for milk production drives fat metabolism and hypoglycemia (Herdt and Emery, 1992).

There are concerns that the amount of glucose in a standard 500 ml bottle of 50% dextrose is excessive. A bolus of 500 ml 50% dextrose increases the blood glucose concentrations to about 8 times normal immediately after administration and returns to pre-treatment concentrations by about 2 hours after administration (Sakai et al., 1996). This is paired with an immediate 5-fold increase in circulating insulin concentration and a 12-fold increase after 15 minutes (Sakai et al., 1996). Any glucose not utilized by the animal during this period is excreted via the kidneys, increasing the excretion of electrolytes and potentially increasing the risk of electrolyte imbalances (Wagner and Schimek, 2010). The decrease in blood BHBA levels due to dextrose treatment are short lived (< 24 hours) and dextrose administration must be repeated or followed with another treatment for lasting effect (Wagner and Schimek, 2010).

Some have expressed concern with the high level of glucose leading to abomasal dysfunction. There is evidence that high levels of glucose can lead to decreased abomasal motility and displaced abomasum has been correlated with hyperglycemia (Holtenius et al., 2000; Zadnik, 2003; Sahinduran and Albay, 2006; Samanac et al., 2009). However, such effects in relation to one treatment with dextrose have not been established.

Systematic Review

Dextrose was studied extensively early on (1940's and 50's) in case series or small studies without controls where all affected animals were treated and the number that improved with treatment was determined. Since then, dextrose has only been studied in combination with other treatments or as the baseline for a positive control group. It has never been studied in a randomized clinical trial to determine efficacy as a standard

treatment for all cases. None of the articles successfully passing the review process examined the efficacy of dextrose without the addition of other treatments.

Recommendations

Use of dextrose should be considered a second-line treatment for cases of ketosis. Animals with severe ketonemia with concurrent hypoglycemia may benefit from treatment with dextrose. Animals with ketosis suffering from nervous signs (such as abnormal licking, chewing on pipes or concrete, gait abnormalities, and aggression) should also be treated promptly with dextrose to alleviate hypoglycemia and nervous signs. These animals should then be followed up with other treatments for longer-term effectiveness (Herdt and Emery, 1992; Wagner and Schimek, 2010).

GLUCOCORTICOIDS

Background

Glucocorticoids have been used in ketosis treatment due to their ability to produce hyperglycemia due to changes in glucose utilization (Herdt and Emery, 1992). Steroids also block the effects of insulin, allowing for increased catabolism of fat and protein stores. Plasma concentrations of both glucose and insulin increase significantly about 48 hours after injection with dexamethasone (Jorritsma et al., 2004).

Systematic Review

Two studies in the review utilized a glucocorticoid alone. One of these was the oldest study of the group and well designed (Cote et al., 1966; Kronfeld, 1966; Robertson, 1966). In this study, enrolled animals were randomly assigned to receive dexamethasone or flumethasone, dexamethasone or flumethasone plus protamine zinc

insulin or no treatment. Animals were then followed for 5 days after treatment to determine milk production, presence of clinical signs of ketosis, and appetite. There was no difference between dexamethasone and flumethasone, so these treatments were grouped together as glucocorticoids. Treated animals were more than twice as likely to improve clinically as compared to untreated controls based on appetite, behavior, and digestive exam coded subjectively (68% for glucocorticoid + insulin and 55% for glucocorticoid alone versus 23% for untreated animals). Treated animals also had increased milk yields in the first week after treatment (6.07 $\pm$ 0.79 kg/d for glucocorticoid + insulin and 3.73 $\pm$ 1.04 kg/d for glucocorticoid alone versus 1.11 $\pm$ 0.91 kg/d for untreated animals). This study was revolutionary in its time due to the use of an untreated control group, something not previously utilized in ketosis treatment studies. The major downfalls of this study are the extremely short follow-up time and the lack of proper statistical methods available at the time to examine the influence of potential confounders such as parity.

The second study points to concerns for use of glucocorticoids (Seifi et al., 2007). Animals enrolled in this study were randomly assigned to treatment with 20 mg of isoflupredone (Predef 2x, Zoetis, Madison, NJ), 20 mg of isoflupredone plus 100 IU insulin, or a placebo, each treatment given once between calving and 8 DIM. This study was not designed as a ketosis treatment study, as all fresh animals were enrolled. However, blood was collected before enrollment and animals were later classified as subclinically ketotic or not based on serum BHBA $\geq$ 1.4 mmol/L. Animals that were ketotic at enrollment and were treated with isoflupredone and insulin were more likely than controls to remain ketotic in the 2 weeks after treatment. Animals that were not

ketotic at the start and were treated with isoflupredone alone or isoflupredone and insulin were respectively 1.6 and 1.7 times more likely to become ketotic 1 week after treatment. There was no effect of treatment on reproduction or test-day milk production. This study suggests that there is no benefit of routine use of corticosteroids at the time of calving and metabolic state may be impaired with their use. Furthermore, care should be taken when utilizing these products for ketosis treatment, as they may impair the animal's ability to overcome the disease.

Recommendations

At this time, evidence for corticosteroids as therapy for ketosis is at best equivocal and indicates that steroids with insulin decreases cure. The lack of efficacy, combined with the risk of adverse side effects, does not support inclusion of corticosteroids in treatment of ketosis.

INSULIN

Background

Dairy cattle in early lactation are inherently insulin resistant (Bauman, 2000). This is part of the complex mechanism of homeorhesis that allows dairy cattle to produce large amounts of milk during a period of negative energy balance. Animals with ketosis show increased insulin resistance compared to their healthy herd mates (Sakai et al., 1996). Insulin is used in the treatment of ketosis due to the anabolic effects of the hormone (Hayirli, 2006). Insulin decreases fat breakdown, increases fat synthesis, and increases use of ketone bodies as energy sources, which should decrease the level and consequences of ketonemia.

Systematic Review

Insulin is never given as the sole treatment for ketosis due to the risk of hypoglycemia. There were 3 studies from the review of the literature that used insulin as an adjunct therapy where the added benefits of insulin could be examined. Two of these studies have been discussed in the glucocorticoid section (Robertson, 1966; Seifi et al., 2007). Results from the Robertson study indicated that the addition of insulin increased cure rate and milk production over treatment with glucocorticoids alone (Robertson, 1966). Beyond the concerns that were mentioned previously regarding this study, the insulin used was an animal-source protamine zinc formulation that is no longer available. It is difficult to say if recombinant human forms of insulin would yield the same results. In Seifi et al. (2007), treatment with a recombinant insulin (Humulin Ultralente, Eli Lily, Indianapolis, IN) increased the risk of animals developing and remaining ketotic over animals treated with isoflupredone alone (Seifi et al., 2007).

The third study was performed by Sakai and colleagues to examine the effects of insulin when added to dextrose therapy (Sakai et al., 1993). Animals were diagnosed with ketosis using a combination of urine ketone body concentrations and clinical signs. All animals received 500 ml 50% dextrose IV once a day for 5 days after diagnosis. Half of the cows were assigned to receive 200 IU of lente insulin subcutaneously for 3 days from day 2 to 4 after enrollment. On day 6 after enrollment, urine ketone body concentrations were measured to determine the effectiveness of treatment. Blood was collected at enrollment and at day 6 and later analyzed for BHBA concentrations. In this study, animals treated with insulin had significantly lower blood BHBA concentrations and significantly higher glucose and insulin concentrations at day 6 after enrollment than

cows treated with dextrose alone. However, this study had an extremely short follow-up period and did not look at any economically important outcomes such as milk production and culling.

We conducted a ketosis treatment study in the summer of 2011 in which we used insulin glargine (Lantus, Sanofi Aventis, Laval, Quebec, Canada) or a placebo in addition to propylene glycol in animals diagnosed with ketosis using a cut-point of $\geq$ 1.2 mmol/L blood BHBA with a validated handheld meter (Precision Xtra, Abbott, Abbott Park, IL). Based on preliminary results, insulin had no effect on blood ketone body concentrations one or two weeks after treatment or the likelihood of cure of ketosis based on blood BHBA concentrations (Gordon et al., 2012).

Recommendations

There is limited evidence in support of insulin therapy as part of a ketosis treatment regimen. This, coupled with the high cost of most insulin preparations precludes wide-scale use of insulin in ketosis treatment. It is possible that there may be some benefit in refractory cases, especially those involving hepatic lipidosis (Hayirli, 2006), but more research is needed in this area.

VITAMIN B12 PHOSPHORUS COMBINATION PRODUCT

Background

Cyanocobalamin (a form of vitamin B12) has been used as an adjunct therapy in ketosis treatment due to its role in gluconeogenesis. It has been hypothesized that administration of vitamin B12 may increase gluconeogenesis by increasing the activity of methylmalonyl-CoA mutase, a vitamin B12-dependent enzyme and important component of the Krebs or tricarboxylic acid (TCA) cycle (Kennedy et al., 1990). With an increase

in the activity of this enzyme, energy may be produced more efficiently and TCA cycle activity and gluconeogenesis may be increased.

Butaphosphan, an organic phosphorus source, has been also been used due to its presumed role in gluconeogenesis (Rollin et al., 2010). Phosphorus is required at many stages in the gluconeogenic pathway, as all intermediate compounds must be phosphorylated to continue the cycle. However, it is unclear if this form of phosphorus is available to the animal.

Systematic Review

One study from the review used a vitamin B12 product (Lohr et al., 2006). One hundred and twenty lactating cows were enrolled in the study when they were presented to veterinary clinics in Germany for left displaced abomasum (LDA) and were determined to have ketosis based on a urine test. After correction of the LDA, animals were randomly assigned to receive 3 days of a commercial combination butaphosphan and cyanocobalamin (Catosal, Bayer Animal Health, Shawnee Mission, KS) product or a placebo. Blood samples were collected during treatment and animals were monitored for feed intake, milk production, and rumination. Animals were considered to have healthy rumination if there were at least 3 ruminations/min. Individuals evaluating the animals were blind to treatment. Treatment with this product resulted in a significant increase in proportion of animals with healthy rumination at days 2 (65% versus 48%) and 3 (82% versus 63%) after treatment. Treated animals also tended to have a larger decrease in plasma BHBA concentrations as compared to values on the day of enrollment.

It is challenging to determine the usefulness of a butaphosphan cyanocobalamin combination product for ketosis treatment based on this study. The outcomes found to be

different between treatments are subjective (ruminations) and have questionable economic significance. It is also difficult to determine if animals with ketosis, but without an LDA would respond in the same manner. A large study using this product at calving showed a decreased risk of ketosis in treated cows that were in their 3rd or higher lactation (Rollin et al., 2010), but this does not prove efficacy in ketosis treatment.

In a recent study completed by the authors (Gordon et al., 2012), we treated animals with 3 days of butaphosphan and cyanocobalamin or a placebo; all cows received propylene glycol. Based on preliminary results, this product tends to increase the likelihood of cure of ketosis (blood BHBA < 1.2 mmol/L in the week after treatment), decrease blood BHBA concentrations one week after treatment, and increase milk production in the first 30 days (Gordon et al., 2012).

Recommendations

This butaphosphan cyanocobalamin combination product may prove useful in ketosis treatment in the future, if effects on milk production, culling and disease risk can be confirmed. At this time, there is insufficient evidence to suggest routine use of this product for ketosis treatment.

PROPYLENE GLYCOL

Background

Propylene glycol was first described as a treatment for ketosis in 1954 (Johnson, 1954; Maplesden, 1954). It is generally given as an oral drench once a day. When propylene glycol enters the rumen, it is either absorbed directly or converted to propionate (Nielsen and Ingvartsen, 2004). Propylene glycol that is absorbed directly enters the TCA cycle to increase oxidation of acetyl co-A and stimulate gluconeogenesis.

Propionate from propylene glycol digestion can also be used for gluconeogenesis and helps stimulate insulin release (Studer et al., 1993). There is a significant increase in insulin by 15 minutes after administration and insulin remains increased for 2 hours or more after drenching (Studer et al., 1993). This spike in insulin helps decrease fat breakdown and hepatic ketone body production.

Due to the physical labor required to administer propylene glycol, many producers and veterinarians have expressed interest in propylene glycol feed additives (Nielsen and Ingvartsen, 2004). The concern with this method of delivery is that there is no resultant insulin spike due to the small, relatively steady amount of propylene glycol that is supplied to the rumen throughout the day (Nielsen and Ingvartsen, 2004). This chronic delivery of propylene glycol also alters the environment in the rumen to favor more propionate production (Nielsen and Ingvartsen, 2004). According to the hepatic oxidation theory, this would likely decrease feed intake, increasing fat mobilization and perpetuating the problem of ketosis, although the clinical relevance of this has not been determined (Allen et al., 2009).

Systematic Review

Previously, much of the work done with propylene glycol and ketosis involved prevention of ketosis (Nielsen and Ingvartsen, 2004). Two of the studies that remained at the end of the systematic review selection process used propylene glycol without other treatments (Ruegsegger and Shultz, 1986; McArt et al., 2011; McArt et al., 2012a). In the study conducted by Ruegsegger et al (1986), cows from study herds were tested once a week for milk ketone bodies and enrolled if they had ketosis without other complicating diseases (Ruegsegger and Shultz, 1986). Enrolled animals were randomly assigned to

receive no treatment, 125 ml propylene glycol, or 125 ml propylene glycol with 12 g of niacin daily for 7 days. There were no differences in blood BHBA, milk production, or milk composition between any of the groups. The small sample size may have resulted in a lack of power to detect these differences and the low amount of propylene glycol used in this trial is insufficient for lactating cattle (Nielsen and Ingvartsen, 2004).

One of the best designed and highest impact trials on ketosis treatment was conducted in 2010 by researchers at Cornell (McArt et al., 2011; McArt et al., 2012a). For this trial, all animals 3 to 16 DIM were tested for ketosis on Mondays, Wednesdays, and Fridays using a handheld meter (Precision Xtra). All animals with blood BHBA 1.2 to 2.9 mmol/L that had not been previously treated for ketosis by farm personnel were enrolled. Cows were randomly assigned to receive 300 ml (310 g) propylene glycol or 300 ml water daily until their blood BHBA levels were < 1.2 mmol/L or ≥ 3.0 mmol/L or they reached 16 DIM. Blood BHBA was measured 3 times a week until 16 DIM and daily milk weights, culling and reproductive records were collected.

Animals that were treated with propylene glycol were 1.5 times more likely to be cured of subclinical ketosis (blood BHBA < 1.2 mmol/L) and half as likely to progress to blood BHBA ≥ 3.0 than control cows. Treated animals were also 40% less likely to develop a DA and half as likely to die or be sold in the first 30 days of lactation. Milk production was increased by 1.3 and 1.6 kg/d in treated cows in the first 30 days of lactation in two of the herds, though the third herd showed no difference in milk production between groups.

This study clearly demonstrated the potential benefits of oral propylene glycol in treatment of subclinical ketosis. The identification of significant differences in

economically important outcomes was a first for the ketosis treatment literature and is a trait that future studies should strive to emulate. A limitation of this study was the failure to treat animals with blood BHBA levels $\geq$ 3.0 mmol/L. It can be expected that propylene glycol would be efficacious in animals with higher blood BHBA levels, but higher initial BHBA levels might decrease the cure rate and milk response. The variable amount of time that animals were treated in this study can also prove challenging for interpretation. Although the median time for treatment was 5 days, treatment time varied from 2 to 13 days. Many producers would like a specific protocol for treatment and few would likely be willing to drench animals for 13 days.

Recommendations

Treatment of ketotic animals with 300 g of propylene glycol daily should be considered the base of ketosis treatment. The exact length of time that animals should be treated still needs to be determined, but based on results of the McArt study (McArt et al., 2011; McArt et al., 2012a) and other studies in propylene glycol use (Nielsen and Ingvartsen, 2004), 5 days of treatment would appear to be sufficient without being overly taxing on farm labor. When choosing a product, it is critical examine the concentration of propylene glycol in the product and ensure that animals are being treated with sufficient volume to provide 300 g of propylene glycol. Propylene glycol should be considered for treatment of all ketotic animals, though more research is needed to determine the efficacy for animals with BHBA above 3.0 mmol/L.

COMBINATION THERAPIES

Background

Many studies of ketosis treatment have utilized combinations of therapies. Many of these studies have shown that animals treated with more than one product have better outcomes that animals treated with only one treatment. However, many of these studies have used short follow-up periods and outcomes that were not economically important.

Systematic Review

An excellent example of a trial involving multiple treatment modalities was conducted at the University of Minnesota (Carrier et al., 2011). Urine was collected daily from all animals in the first 15 days of lactation for ketone body testing using Ketostix (Bayer, Pittsburgh, PA). Animals that were classified with a "small" level of ketosis or higher were enrolled in the study and randomly assigned to the treatment or control groups. Treated cows (n=279) were given 20 mg dexamethasone, 500 ml 50% dextrose, 5 mg vitamin B12 all intravenously once on the day of enrollment and 500 ml propylene glycol orally on the day of enrollment and for 2 days following enrollment. Control animals (n = 282) were left untreated. In this study, treatment tended (P = 0.1) to lower milk production (1 kg/d over the lactation) and significantly increased the risk of culling (by 40%) in the first 60 days. It is important to note that although outcomes were not different or poorer for treated animals, treatment did decrease BHBA and NEFA values in treated animals in the first week after treatment compared to controls. This study is a critical addition to the ketosis treatment literature for two reasons: it illustrates the importance of long-term follow-up and use of economically important outcomes, and it requires that we reconsider common ketosis treatment regimens.

Recommendations

There is no combination therapy that can be recommended at this time. It is essential that future work test individual treatments alone or in factorial study designs, so the efficacy of each product can be determined. Any combination that is studied should be based on treatments previously proven efficacious (i.e. propylene glycol) and with the addition of one other treatment. By taking this stepwise approach, the efficacy of treatment combinations can be established.

SUMMARY OF CURRENT TREATMENT RECOMMENDATIONS

The only treatment for ketosis that has currently been shown to improve resolution of ketosis, cow health, and productivity is oral propylene glycol (McArt et al., 2011; McArt et al., 2012a). It is important to note the concentration of propylene glycol in the product used to ensure that animals are receiving 300 g once a day for 5 days. Once a day dosing is sufficient and will decrease the labor requirement for treatment. Use of a drenching gun increases the ease with which animals can be treated, increasing producer compliance. Subclinically ketotic animals should not receive other treatments as the risk of detriment likely outweighs the benefit. Animals experiencing nervous signs of clinical ketosis may also benefit from a single treatment with 500 ml 50% dextrose IV.

CONCLUSIONS

There is a scarcity of well-designed ketosis treatment trials and information on effective ketosis treatment. In the past, the focus was on treating ketonemia, rather than improving productivity. Research has shown that blood BHBA concentrations

$\geq$ 1.2 mmol/L in the first two weeks post-partum increases the risk of disease and culling and decreases milk production (Ospina et al., 2010a; b; Chapinal et al., 2011; Chapinal et al., 2012). Increased emphasis on economically important outcomes (disease risk, milk production, culling and reproductive performance) is required in subsequent research to increase understanding of effective ketosis treatment.

REFERENCES

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.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: Homeostasis and homeorhesis revisisted. Pages 311-328 in Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction P. B. Cronje, ed. Oxford University Press, Oxford, England.

Carrier, J., S. Godden, J. P. Fetrow, and P. Rapnicki. 2011. Clinical trial of early ketosis detection and therapy in fresh cows. Page 157 in Proc. 44th Annual Conf., Am. Assoc. Bovine Pract., St. Louis, Missouri. VM Publishing Co., Stillwater, OK.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897-4903.

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301-1309.

Cook, D. J., C. D. Mulrow, and R. B. Haynes. 1997. Systematic reviews: synthesis of best evidence for clinical decisions. Annal. Intern. Med. 126:376-380.

Cote, J. F., R. A. Curtis, and B. J. McSherry. 1966. The clinical operation of a therapeutic trial on bovine ketosis. J. Am. Vet. Med. Assoc. 149:1617-1619.

Duffield, T. F. 2000. Subclinical ketosis in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 16:231-253.

Fetrow, J. P., J. W. Pankowski, J. L. Vicini, and T. R. Ames. 1999. Use of bovine somatotropin at the time of surgery for left displacement of the abomasum in dairy cows. J. Am. Vet. Med. Assoc. 214:529-531.

Gordon, J. L., S. J. LeBlanc, L. Neuder, T. H. Herdt, D. F. Kelton, and T. F. Duffield. 2012. Efficacy of a combination butaphosphan and cyanocobalamin product and insulin for ketosis treatment. J. Dairy Sci. 95 (Suppl. 2):177. (Abstract).

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.

Herdt, T. H. and R. S. Emery. 1992. Therapy of diseases of ruminant intermediary metabolism. Vet. Clin. North Am. Food Anim. Pract. 8:91-106.

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Vet. Clin. North Am. Food Anim. Pract. 16:215-230.

Holtenius, K., K. Sternbauer, and P. Holtenius. 2000. The effect of the plasma glucose level on the abomasal function in dairy cows. J. Anim. Sci. 78:1930-1935.

Johnson, R. B. 1954. The treatment of ketosis with glycerol and propylene glycol. Cornell Vet. 44:6-21.

Jorritsma, R., J. Thanasak, M. Houweling, J. P. T. M. Noordhuizen, and K. E. Mü ller. 2004. Effects of a single dose of dexamethasone-21-isonicotinate on the metabolism of heifers in early lactation. Vet. Rec. 155:521-523.

Kennedy, D. G., A. Cannavan, A. Molloy, F. O'Harte, S. M. Taylor, S. Kennedy, and W. J. Blanchflower. 1990. Methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthetase (EC 2.1.1.13) in the tissues of cobalt-vitamin 12 deficient sheep. Br. J. Nutr. 64:721-732.

Kronfeld, D. S. 1966. The design of a therapeutic trial on bovine ketosis. J. Am. Vet. Med. Assoc. 149:1610-1616.

Lohr, B., B. Brunner, H. Janowitz, M. Hummel, T. Seeger, I. Weber, T. Wittek, B. Schmidt, and K. Hellmann. 2006. Clinical efficacy of Catosal® in the treatment of ketosis in cows with left abomasal displacement. Tierärztliche Umschau 61:187-190.

Maplesden, D. C. 1954. Propylene glycol in the treatment of ketosis. Can. J. Comp. Med. Vet. Sci. 18:287-293.

McArt, J. 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.

McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2012a. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. J. Dairy Sci. 95:2505-2512.

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

McSherry, B. J., D. C. Maplesden, and H. D. Branion. 1960. Ketosis in cattle - A review. Can. Vet. J. 1:208-213.

Nielsen, N. I. and K. L. Ingvartsen. 2004. Propylene glycol for dairy cows. Anim. Feed Sci. Technol. 115:191-213.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546-554.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. 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.

Robertson, J. M. 1966. The evaluation of a therapeutic trial on bovine ketosis. J. Am. Vet. Med. Assoc. 149:1620-1623.

Rollin, E., R. D. Berghaus, P. Rapnicki, S. M. Godden, and M. W. Overton. 2010. The effect of injectable butaphosphan and cyanocobalamin on postpartum serum beta-hydroxybutyrate, calcium, and phosphorus concentrations in dairy cattle. J. Dairy Sci. 93:978-987.

Ruegsegger, G. J. and L. H. Shultz. 1986. Use of a combination of propylene glycol and niacin for subclinical ketosis. J. Dairy Sci. 69:1411-1415.

Sahinduran, S. and M. K. Albay. 2006. Haematological and biochemical profiles in right displacement of abomasum in cattle. Rev. Med. Vet. 157:352-356.

Sahoo, S. S., R. C. Patra, P. C. Behera, and D. Swarup. 2009. Oxidative stress indices in the erythrocytes from lactating cows after treatment for subclinical ketosis with antioxidant incorporated in the therapeutic regime. Vet. Res. Commun. 33:281-290.

Sakai, T., T. Hayakawa, M. Hamakawa, K. Ogura, and S. Kubo. 1993. Therapeutic effects of simultaneous use of dextrose and insulin in ketotic dairy cows. J. Dairy Sci. 76:109-114.

Sakai, T., M. Hamakawa, and S. Kubo. 1996. Glucose and xylitol tolerance tests for ketotic and healthy dairy cows. J. Dairy Sci. 79:372-377.

Samanac, H., V. Stojic, D. Kirovski, P. Pudlo, and I. Vujanac. 2009. Glucose tolerance test in the assessment of endocrine pancreatic function in cows before and after surgical correction of left diplaced abomasum. Acta Vet. (Beograd) 59:513-523.

Sargeant, J. M., A. Rajic, S. Read, and A. Ohlsson. 2006. The process of systematic review and its application in agri-food public-health. Prev. Vet. Med. 75:141-151.

Seifi, H. A., S. J. LeBlanc, E. Vernooy, K. E. Leslie, and T. F. Duffield. 2007. Effect of isoflupredone acetate with or without insulin on energy metabolism, reproduction, milk production, and health in dairy cows in early lactation. J. Dairy Sci. 90:4181-4191.

Shpigel, N. Y., R. Chen, Y. Avidar, and E. Bogin. 1996. Use of corticosteroids alone or combined with glucose to treat ketosis in dairy cows. J. Am. Vet. Med. Assoc. 208:1702-1704.

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

Vandeweerd, J. M., P. Clegg, V. Hougardy, and S. Buczinski. 2012a. Using systematic reviews to critically appraise the scientific information for the bovine veterinarian. Vet. Clin. North Am. Food Anim. Pract. 28:13-21.

Vandeweerd, J. M., P. Gustin, and S. Buczinski. 2012b. Evidence-based practice? An evolution is necessary for bovine practitioners, teachers, and researchers. Vet. Clin. North Am. Food Anim. Pract. 28:133-139.

Wagner, S. A. and D. E. Schimek. 2010. Evaluation of the effect of bolus administration of 50% dextrose solution on measures of electrolyte and energy balance in postpartum dairy cows. Am. J. Vet. Res. 71:1074-1080.

Zadnik, T. 2003. A comparative study of the hemato-biochemical parameters between clinically healthy cows and cows with displacement of the abomasum. Acta Vet. (Beograd) 53:297-309.

Figure 2.1 Flowchart showing the total number of articles found during the review and the number of articles excluded by reason for exclusion.

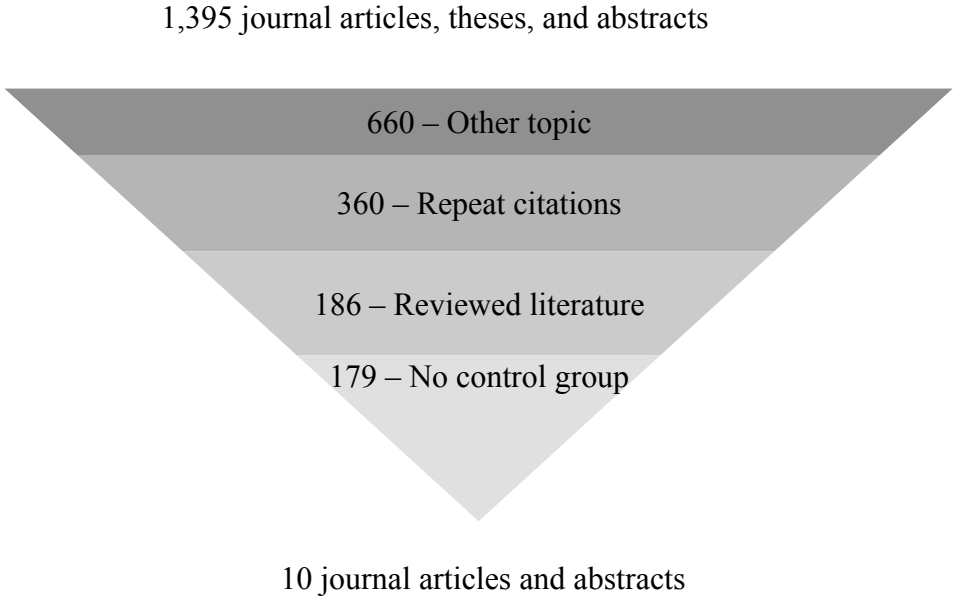

Table 2.1 Studies remaining after exclusion criteria were applied.

Study	Treatment	Control Group	Random
McArt et al., 2011, 2012	Propylene glycol	Untreated	Yes
Carrier et al, 2011	Dextrose + dexamethasone + B12 + propylene glycol	Untreated	Yes
Sahoo et al., 2009	Dextrose + dexamethasone, Dextrose + dexamethasone + E/Se	Untreated	No
Seifi et al., 2007	Isoflupredone, Isoflupredone + insulin (ultralente)	Untreated	Yes
Lohr et al., 2006	Catosal	Untreated	Yes
Fetrow et al., 1999	rBST	Untreated	Yes
Shpigel et al., 1996	Dextrose + dexamethasone, Dextrose + flumethasone	Dexamethasone or Flumethasone	No
Sakai et al., 1993	Dextrose + insulin (lente)	Dextrose	No
Ruegsegger & Shultz, 1986	Propylene glycol, Propylene glycol + niacin	Untreated	Yes
Robertson (Kronfeld, Cote et al.), 1966	Dexamethasone/flumethasone + insulin (protamine zinc), Dexamethasone/flumethasone alone	Untreated	Yes

CHAPTER THREE

EFFECTS OF A COMBINATION BUTAPHOSPHAN AND CYANOCOBALAMIN PRODUCT AND INSULIN ON KETOSIS RESOLUTION AND MILK PRODUCTION

ABSTRACT

The objective of this study was to determine the effects of butaphosphan-cyanocobalamin (B+C), glargine insulin, and propylene glycol (PG) on resolution of ketosis and average daily milk yield after treatment. Cows from 16 herds in Ontario and 1 herd in Michigan were tested at weekly intervals between 3 and 16 DIM. Ketosis was defined as blood β-hydroxybutyrate (BHBA) $\geq$ 1.2 mmol/L. All ketotic cows were given a baseline treatment of 3 days of 300 g PG orally. Animals were then randomly assigned to treatment with 3 doses of either 25 ml B+C or 25 ml saline placebo and 1 dose of either 2 ml (200 IU) glargine insulin or 2 ml saline placebo in a 2 x 2 factorial arrangement. Outcomes of interest on all farms were ketosis cure (blood BHBA < 1.2 mmol/L 1 week post enrollment), maintenance of ketosis cure (blood BHBA < 1.2 mmol/L 1 and 2 weeks post enrollment), and blood BHBA concentrations at 1 and 2 weeks post enrollment. Milk weights were collected daily in 1 large freestall herd. Repeated measures ANOVA was utilized to evaluate blood BHBA concentrations 2 weeks after treatment and milk production for 30 days after treatment. Poisson regression was used to examine the effect of treatment on cure and maintenance of cure. Due to a regulatory delay causing temporary unavailability of B+C in Canada, data was analyzed in 2 sets of models: 1 for insulin and the corresponding placebo (n = 620) and 1 for the full trial (n = 380). Animals with blood glucose concentrations $\leq$ 2.2 mmol/L at

the time of ketosis diagnosis were 2.1 times more likely (95% CI = 1.2 to 3.7) to cure if treated with B+C. Animals in lactation 3 or higher that had blood glucose concentrations < 2.2 mmol/L at enrollment produced 4.2 kg/d (95% CI = 1.4 to 7.1) more milk if treated with insulin versus placebo and 2.8 kg/d (95% CI = 0.9 to 4.7) more milk if treated with B+C versus placebo. Animals in lactation 3 or higher with blood glucose $\geq$ 2.2 mmol/L that were treated with insulin produced 2.3 kg/d (95% CI = 0.3 to 4.4) less milk than untreated controls. There was no interaction between treatments. This evidence suggests that B+C and insulin may be beneficial for ketosis treatment in animals with blood glucose < 2.2 mmol/L at ketosis diagnosis. It also suggests that blood glucose concentration may be an important predictor of success of ketosis treatment.

INTRODUCTION

The transition from the late dry period to early lactation requires a highly orchestrated series of physiological processes to occur in order to facilitate a smooth start to lactation (Bauman and Currie, 1980). In some animals, proper adaptation does not occur. These animals are prone to metabolic disease, especially hyperketonemia (Herdt, 2000).

Effective treatment of cows with ketosis is challenging. Recently, there has been renewed interest in identifying efficacious ketosis treatments. One recent study examined the effects of propylene glycol (PG) treatment and found that treated cows were more likely to resolve ketosis, had increased milk production, and were less likely to have a displaced abomasum (DA) or be culled during the first 30 days of lactation (McArt et al., 2011; McArt et al., 2012a). This study provided a good base from which to investigate whether additional treatments would further alleviate the negative effects of ketosis.

A combination butaphosphan-cyanocobalamin (B+C, Catosal, Bayer, Shawnee, KS) product has been investigated for ketosis prevention and treatment (Lohr et al., 2006; Furll et al., 2010; Rollin et al., 2010). Cyanocobalamin is a form of vitamin B12 that has been shown to be decreased in cows around the time of parturition (Kincaid and Socha, 2007). It has been hypothesized that administration of vitamin B12 may increase gluconeogenesis by increasing the activity of methylmalonyl-CoA mutase, a vitamin B12-dependent enzyme in the tricarboxylic acid (TCA) cycle (Kennedy et al., 1990). Treatment of first lactation animals with injectable B12 was reported to increase milk production in one study (Girard and Matte, 2005), but not in another (Akins et al., 2013). Butaphosphan, an organic phosphorus source, may contribute to gluconeogenesis (Rollin et al., 2010) because intermediate compounds in gluconeogenesis must be phosphorylated to continue the cycle. However, it is unclear if this form of phosphorus is biologically available to the animal.

Rollin et al. (2010), gave B+C on the day of calving and the following day. Treatment with B+C significantly reduced the odds of development of subclinical ketosis development in cows in third lactation or greater, but it had no effect in younger animals. A German study examined the effects of B+C when it was administered to cows with a DA and reported that treated cows had an increase in rumination compared to untreated cows (Lohr et al., 2006). However, these data were collected subjectively and the clinical and economic importance of these outcomes is unclear. B+C has never been utilized in a large-scale clinical trial for the treatment of subclinical ketosis.

Insulin might be useful in ketosis treatment by suppressing fat mobilization and slowing the production of ketone bodies (Hayirli, 2006). Tissue insulin responsiveness

decreases around the time of parturition to spare glucose for milk production (Hayirli, 2006). This decrease in responsiveness to insulin is exacerbated by the presence of ketone bodies in the blood (Sakai et al., 1993). Robertson (1966) found that the addition of insulin to steroid administration for treatment of ketosis increased milk production and improved appetite in treated animals over 5 days. Sakai et al. (1993) studied the effects of intravenous glucose in conjunction with subcutaneous insulin compared to glucose treatment alone and found the addition of the insulin decreased blood ketones and increased blood glucose. Seifi et al. (2007) reported that animals treated with insulin were more likely to develop ketosis and treated animals that were subclinically ketotic at time of enrollment were less likely to resolve their ketosis than untreated animals. However, the animals treated in this study were not given a glucose source in conjunction with the administration of the insulin. Use of insulin in ketosis treatment is uncommon, likely due to the risk of severe hypoglycemia if administered without a glucose source (Hayirli, 2006).

The purpose of this study was to examine the effects of B+C and insulin administration, individually and in combination, on subclinical ketosis cure and early lactation milk production.

MATERIALS AND METHODS

Study Population

Data were collected from 16 dairy herds in Ontario (Farms A through P) and 1 dairy herd in Michigan (Farm Z) from May 13 to September 14, 2011. Herds were purposively selected based on their proximity to study sites and willingness to comply with the proposed ketosis testing and treatment protocol. To be eligible for enrollment,

herds were required to be enrolled in monthly milk testing through a Dairy Herd Improvement (DHI) organization or collect daily milk weights on farm. Enrolled herds included tie-stall (n = 8) and freestall housed herds (n = 8) ranging in size from 50 to 3,200 lactating animals. All herds fed a total mixed ration to all lactating cattle.

Data Collection and Study Design

Herds were visited weekly on the same day of the week and at approximately the same time of day throughout the study period. All cows 3 to 16 DIM were tested for ketosis using the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). Ketosis was defined *a priori* as $\geq$ 1.2 mmol/L of BHBA. The Precision Xtra meter is a hand-held device that measures BHBA in whole blood. This meter has been previously validated for use in cattle and has a reported sensitivity of 88% and a specificity of 96% at this cut-point (Iwersen et al., 2009). Cows were excluded from testing if they had been previously diagnosed with ketosis or a DA during the current lactation or had been enrolled in the study the previous week. This testing scheme provided 2 opportunities for enrollment for each animal, once at 3 to 9 and once at 10 to 16 DIM.

Blood was drawn from the coccygeal vessels using a 20 gauge x 2.54 cm needle and 3 cc syringe. Ketone testing was performed immediately after blood collection according to manufacturer's instructions. A ketone test strip was inserted into the meter and the lot number displayed on the meter was confirmed to be the lot number on the strip. Once the "add blood" symbol appeared, blood was added to the strip until the meter showed the test chamber was full. After 10 seconds, the blood BHBA concentration was displayed on the screen and the number was recorded.

In animals that were classified as ketotic, blood glucose concentrations were also measured using a second Precision Xtra test. Blood was tested for glucose immediately after the ketone results were displayed. The procedure for glucose testing is the same as for ketone testing, except that the results are displayed in 5 seconds. The use of Precision Xtra for glucose determination has also been validated in dairy cattle (Wittrock et al., 2013).

All animals classified as ketotic ($\geq$ 1.2 mmol/L blood BHBA) were treated with 300 g PG per day for 3 days via oral drench. The concentration of PG in the drench varied by study site, so animals were treated with 450 ml of liquid (67% PG, Glycol-P, Vetoquinol, Lavaltrie, QC, Canada) in Ontario herds and 300 ml of liquid in the Michigan herd. Additional treatment with 25 ml B+C or 25 ml saline placebo subcutaneously (SQ) for 3 consecutive days and 2 ml (200 IU) long-acting insulin (Lantus, Sanofi-Aventis, Laval, QC, Canada) or 2 ml saline placebo SQ once at the time of enrollment were applied in a 2 x 2 factorial arrangement. These doses were selected for convenience and to provide consistency between study sites. Subcutaneous injections were split so a maximum of 10 ml was administered per site. Randomization was performed using a random number generator so that sequential blocks of 4 cows included 1 cow of each treatment combination. In the Michigan herd, treatments were randomized separately for primiparous and multiparous animals. In Ontario herds, all animals were included on the same randomization list. Individuals performing the testing, assigning the treatment group, and administering treatments, farm personnel, and veterinarians were blinded to B+C and insulin treatment.

Enrolled cows were tested for blood BHBA and glucose concentrations at weekly intervals for 2 weeks after treatment. Further data collected included lactation number, DIM at enrollment, and (at Farm Z only) daily milk weights. Milk weights were exported from DairyComp 305 daily throughout the study period. Milk weights recorded as "0" were re-entered as missing data points. Animals were excluded from analysis if they did not receive the assigned treatments, were not tested at 1 and 2 weeks after treatment, or died or were sold before 2 weeks after treatment. Animals were excluded from daily milk yield analysis if they did not have at least 10 daily milk weights during the study period.

The personnel responsible for maintaining the health of the animals on the farm were allowed to treat enrolled animals for other disease conditions as needed. They were asked to record any treatments given and this was included in the analysis. Study personnel asked herd owners and workers about the treatment status of all enrolled animals on a weekly basis.

This study aimed to screen 1,600 cows. With an expected ketosis incidence rate of 20%, this would yield 320 cows with ketosis and 80 cows per treatment group. Given 95% confidence, 80% power, and 0.45 mmol/L standard deviation, this sample size would allow for detection of a 0.2 mmol/L difference in blood BHBA between treatment groups. This study was reviewed and approved by the University of Guelph Animal Care Committee (11R036) and the Michigan State University Institutional Animal Care and Use Committee (#04/11-078-00).

There was a period of unavailability of B+C in Ontario during most of the study due to a regulatory approval delay. During this period, the randomization specified

above was maintained except that the animals were treated with only insulin or the corresponding placebo. Thus, each sequential block of four animals would include 2 treated with insulin and 2 treated with placebo. No placebo for B+C was given to these animals to prevent unnecessary discomfort. Due to the length of the approval delay, there were insufficient animals treated with B+C in Ontario herds. All analysis of B+C was completed using data from 1 large herd (Farm Z). Analysis of insulin included data from all herds where at least eight animals were enrolled in the study (4 blocks of treatment with insulin and placebo). Herds with fewer than eight animals enrolled were excluded from analysis.

Statistical Analysis

All statistical analysis was performed in SAS (Version 9.3, SAS Institute, Cary, NC). The outcomes of interest were ketosis cure at 1 week post-treatment, maintenance of ketosis cure at the second week post-treatment, blood BHBA concentrations at 1 and 2 weeks post-treatment, and average daily milk production in the first 30 days after treatment. Ketosis cure and numerical decrease of BHBA were both of interest. Ketosis cure is a measure of whether or not the blood BHBA of an animal dropped below a predefined cut-point during the specified time period, but it does not capture the amount the blood BHBA decreased after treatment. Animals with high blood BHBA at the time of enrollment may have a large decrease in blood BHBA after treatment, but may not drop below the cut-point. This decrease may still be important to health and production. Separate models were constructed for the full trial (B+C and insulin) and the insulin trial. Ketosis cure was defined as blood BHBA concentrations < 1.2 mmol/L at 1 week post-treatment. Maintenance of cure was defined as blood BHBA concentrations

< 1.2 mmol/L at 1 and 2 weeks post-treatment. Continuous variables (DIM at enrollment, BHBA and glucose at all 3 time points) were examined for normality. DIM was right skewed and was categorized in two ways based on previous literature (Duffield et al., 2009; McArt et al., 2012b), enrollment at week 1 (3 to 9 DIM) versus week 2 (10 to 16 DIM) and early (3 to 5 DIM) versus late (6 to 16 DIM). Glucose was categorized into $\geq$ 2.2 mmol/L and < 2.2 mmol/L (low) based on sensitivity analysis (Appendix 3.1) and was offered to models in two ways, as either a linear or categorical predictor. A natural log transformation was used to normalize BHBA values 1 and 2 weeks post-treatment. An appropriate transformation could not be found for enrollment BHBA concentrations, so it was categorized into moderate (1.2 to 2.4 mmol/L) and high ($\geq$ 2.4 mmol/L) based on sensitivity analysis (Appendix 3.2). Parity groups were set as 1, 2 and 3+ lactations. Descriptive statistics were generated with PROC FREQ and PROC MEANS in SAS. All variables were examined for association with the selected outcomes using contingency tables and the Chi-square statistic using PROC GLM. Any variable with a univariable association to the outcome ($P \leq 0.2$) was offered for inclusion in multivariable models.

For the outcomes cure and maintenance of cure, Poisson regression was used (PROC GENMOD in SAS), with a log link and Poisson distribution with no offset. Clustering by herd and overdispersion were accounted for in the model with an exchangeable correlation structure. This type of model was utilized to allow for the associations to be expressed as risk ratios (RR) rather than odds ratios (OR, Ospina et al., 2012). Risk ratios report probabilities directly, are easier to interpret, and are less likely to overestimate the true effect when outcomes of interest are common (Ospina et al., 2012). A mixed model (PROC MIXED in SAS) was used to examine the effects of

treatment on BHBA concentrations 1 and 2 weeks post-treatment and average daily milk production in the first 30 days after treatment with cow as a repeated measure and a first order autoregressive correlation structure. Numerous correlation structures were examined and the model with the lowest Akaike Information Criterion (AIC) was selected.

All variables with a univariable association with the outcome ($P \leq 0.2$) were offered to the respective models and the models were built manually using a backward stepwise elimination process. The variable with the highest *P*-value was removed until only variables significantly associated with the outcome ($P \leq 0.05$) remained in the model. Treatments (B+C and insulin) were retained in all of the applicable models regardless of *P*-value. Each model was examined for evidence of confounding (changes in coefficients of the final model by > 20%) at the removal of each variable. Biologically relevant interaction terms were formed between variables in the final model and retained if significant ($P \leq 0.05$). If both the continuous and dichotomized form of a predictor variable were significantly associated with an outcome, they were tested in separate backward stepwise multivariable models and the form of the predictor in the model with the lowest AIC was selected. If treatment was part of a significant interaction with another predictor, the outcome was stratified by the predictor and stratum specific results were presented. Effects of B+C, insulin, and possible interactions between the two were examined using data from the 1 large herd (Farm Z). There was no interaction between B+C and insulin in these models, so models for the effects of insulin alone were constructed using data from all herds with at least eight animals enrolled.

RESULTS

Descriptive Statistics

Of the 1,653 animals tested, 712 (43%) were classified as ketotic (blood BHBA concentrations $\geq$ 1.2 mmol/L). Due to labor constraints, there was a maximum number of animals that could be enrolled per farm per week, so 14 of these animals were not enrolled. Of the 698 animals enrolled, 42 were excluded because they did not receive the full course of treatment (n = 6), were enrolled twice (n = 1), were not tested at 1 and 2 weeks post-treatment (n = 19), or they died or were euthanized (n = 12) or were sold (n = 4) before the end of the study period. Six farms had less than eight animals enrolled (Farms C, E, G, H, I, and O), so animals from these herds were removed from analysis (n = 36).

The cumulative lactational incidence for ketosis in the study was 43%, but the herd incidence ranged from 14% (Farm O) to 79% (Farm P, Appendix 3.3). Twelve of the 16 herds had a cumulative lactation incidence of more than 40%. The variation in ketosis risk between herds had no effect on ketosis cure or blood BHBA concentrations after treatment.

Descriptive statistics for the 620 animals enrolled in the insulin study are provided in Appendix 3.4. The control group was composed of 57, 99, and 160 animals in lactation 1, 2, and $\geq$ 3 respectively. The insulin group was composed of 50, 95, and 159 animals in lactation 1, 2 and $\geq$ 3 respectively. There were no differences (P = 0.7 to 0.9) in lactation number, DIM at enrollment, or enrollment BHBA concentration between treatment groups.

Descriptive statistics for the 380 animals enrolled in the full trial are provided in Table 3.1. The control group was composed of 15, 29, and 55 animals in lactation 1, 2, and ≥ 3 respectively. The insulin group was composed of 15, 30, and 51 animals in lactation 1, 2, and ≥ 3 respectively. The B+C group was composed of 13, 35, and 48 animals in lactation 1, 2, and ≥ 3 respectively. The insulin + B+C group was composed of 14, 28, and 47 animals in lactation 1, 2, and ≥ 3 respectively. There were no differences (P = 0.8 to 0.9) in lactation number, DIM at enrollment, or enrollment BHBA concentration between treatment groups.

Insulin Study

Effect of Insulin Treatment on Ketosis Cure. Cure risk was 42% (n = 127) for cows treated with insulin and 38% (n = 120) for controls. Based on significant associations in the univariable analysis, variables offered to the insulin model for ketosis cure risk included parity (P = 0.004), enrollment BHBA group (P < 0.0001), glucose group (P = 0.004), and insulin treatment (P = 0.8). High enrollment BHBA concentrations, low enrollment glucose, and increased parity were all significantly associated with decreased ketosis cure (Table 3.2). Insulin treatment was not associated with ketosis cure. There were no significant interactions between model variables.

Effect of Insulin Treatment on Maintenance of Ketosis Cure. A total of 247 animals were classified as cured and could be used in the maintenance of cure model. The risk of maintenance of cure was 74% (n = 94) for animals treated with insulin and 67% (n = 80) for control animals. Variables offered to the insulin model for maintenance of ketosis cure included parity (P = 0.004) and insulin treatment (P = 0.2). After

accounting for parity, there was no effect of insulin on maintenance of ketosis cure (RR = 1.0, 95% CI = 0.9 to 1.2, $P = 0.1$).

Effect of Insulin Treatment on Blood BHBA Concentrations. Variables offered to the models to examine the effect of treatment on blood BHBA concentrations 1 and 2 weeks after treatment included parity ($P < 0.0001$), enrollment BHBA group ($P < 0.0001$), glucose group ($P = 0.004$), and insulin treatment ($P = 0.5$). Multiparous cows had significantly higher blood BHBA concentrations at 1 and 2 weeks post treatment ($P = 0.0003$ and $P < 0.0001$ respectively, Appendix 3.5 and 3.6). Higher blood BHBA concentrations and lower blood glucose concentrations at enrollment were also associated with significantly higher blood BHBA concentrations at 1 and 2 weeks post-treatment ($P = 0.0009$ to 0.01). Insulin had no effect on blood BHBA concentrations at either time point ($P = 0.2$ and 0.8). There were no significant interactions among model variables.

Full Trial

Effect of Treatment on Ketosis Cure. Variables offered to the model for ketosis cure included parity ($P = 0.0001$), enrollment BHBA group ($P = 0.0007$), early enrollment (3 to 5 DIM, $P = 0.02$), glucose group ($P < 0.0001$), B+C treatment ($P = 0.2$), and insulin treatment ($P = 0.9$). Animals with a higher blood BHBA at enrollment ($P = 0.004$), those enrolled in the first 3 to 5 days of lactation ($P = 0.02$), and multiparous animals ($P = 0.002$) were less likely to cure (Table 3.3). There was a significant interaction between B+C and glucose group, so a stratified analysis was performed (Table 3.4). Cows that had blood glucose < 2.2 mmol/L at enrollment were significantly more likely to cure if they were treated with B+C. However, B+C had no effect on cure

risk in animals that had blood glucose $\geq$ 2.2 mmol/L at enrollment (RR = 1.0, 95% CI = 0.7 to 1.3, $P = 0.3$). There were no other significant interactions in the model, including between treatments ($P = 0.8$)

Effect of Treatment on Maintenance of Ketosis Cure. One hundred and fifty-two animals were categorized as cured and were used for analysis of maintenance of cure. Variables offered to the model for the maintenance of ketosis cure were parity ($P < 0.0001$), enrollment BHBA group ($P = 0.1$), glucose group ($P = 0.0008$), B+C treatment ($P = 0.9$), and insulin treatment ($P = 0.5$). Parity was the only significant predictor of maintenance of ketosis cure with multiparous animals being less likely to remain cured than primiparous animals (RR = 0.7, 95% CI = 0.5 to 0.9, $P = 0.01$). Accounting for parity, neither treatment had an effect on maintenance of cure (B+C: RR = 1.1, 95% CI = 0.9 to 1.3, $P = 0.9$ and insulin: RR = 0.9, 95% CI 0.7 to 1.1, $P = 0.7$). There were no significant interactions among model variables, including between treatments ($P = 0.7$).

Effect of Treatment on Blood BHBA Concentrations. Variables offered to the model to examine the effect of treatment on blood BHBA concentration 1 week after treatment included parity ($P < 0.0001$), enrollment BHBA group ($P < 0.0001$), B+C treatment ($P = 0.2$), and insulin treatment ($P = 0.9$). Variables offered to the model to examine the effect of treatment on blood BHBA concentrations 2 weeks after treatment included parity ($P < 0.0001$), enrollment BHBA group ($P = 0.005$), glucose group ($P < 0.0001$), B+C treatment ($P = 0.5$), and insulin treatment ($P = 0.5$). Neither treatment had any effect on blood BHBA concentrations at either time point (B+C: week + 1 $P = 0.2$, week + 2 $P = 0.5$, insulin: week + 1 $P = 0.9$, week + 2 $P = 0.1$, Appendix 3.7 and

3.8). There were no significant interactions among model variables, including between treatments ($P = 0.8$). Multiparous cows and cows with enrollment BHBA concentrations $\geq$ 2.4 mmol/L had significantly increased blood BHBA concentrations at 1 week post-treatment ($P < 0.0001$). Multiparous cows also had significantly increased blood BHBA concentrations at 2 weeks post-treatment ($P < 0.0001$), though enrollment BHBA group was not associated with increased BHBA concentrations at this time point ($P = 0.1$). Low glucose at enrollment was associated with increased blood BHBA concentrations at 2 weeks post-treatment ($P = 0.01$), but not with blood BHBA concentrations at 1 week post-treatment ($P = 0.1$).

Effect of Treatment on Early Lactation Milk Production. A treatment by parity interaction was identified for the effect of treatment on milk production, so a model for milk production was constructed for each parity group. Milk models were examined in two ways: 1. All animals included, 2. Animals treated with the opposite treatment excluded (i.e. B+C versus control, insulin and B+C and insulin cows excluded). This was to ensure that the effects of 1 treatment were not masking the effects of the opposite treatment. The analysis was conducted in this manner despite the fact that the interaction between the 2 treatments was not significant in any model, including the milk models ($P = 0.6$ to 0.9). There was no difference among the models, except for parity 3 animals. In parity 3 animals, the effects of all variables in the model remained the same except for treatment variables and interactions including treatment. Treatment effects are thus given from 2 models comparing treated (B+C or insulin) to control (no treatment) animals (Table 3.6).

Variables offered to the model for effect of treatment on milk production in first lactation animals included month of calving ($P = 0.0004$), early enrollment ($P = 0.0007$), enrollment glucose group ($P = 0.1$), B+C treatment ($P = 0.06$), and insulin treatment ($P = 0.001$). Early enrollment was associated with significantly decreased average daily milk production ($P = 0.0005$, Table 3.5). Milk production also decreased as month of calving was later in the calendar year (May to August, $P = 0.0006$). B+C had no effect on milk production ($P = 0.5$), but treatment with insulin increased milk production by 3.8 kg/d (95% CI = 2.1 to 5.5). There were no interactions between variables.

Variables offered to the model for milk production in second lactation animals included month of calving ($P < 0.0001$), early enrollment ($P < 0.0001$), enrollment BHBA group ($P < 0.0001$), B+C treatment ($P = 0.1$), and insulin treatment ($P = 0.4$). Neither treatment had any significant effect on production in second lactation animals ($P = 0.5$, Appendix 3.9).

For third and greater lactation animals, variables offered to the model for milk production included month of calving ($P < 0.0001$), early enrollment ($P < 0.0001$), enrollment BHBA group ($P < 0.0001$), enrollment glucose group ($P = 0.05$), B+C treatment ($P = 0.01$), and insulin treatment ($P = 0.2$). There was a significant interaction between glucose group and both insulin and B+C. When evaluated based on glucose group at enrollment, animals with low blood glucose at enrollment had a significant increase in production when treated with either B+C (2.8 kg/d, $P = 0.05$) or insulin (4.2 kg/d, $P = 0.0003$, Table 3.6). However, B+C had no effect on animals with blood glucose $\geq$ 2.2 mmol/L at enrollment (1.0 kg/d, $P = 0.3$) and insulin treatment led to a significant decrease in production (-2.3 kg/d, $P = 0.03$) in those animals. Again, early

enrollment, enrollment BHBA > 2.4 mmol/L, and month of calving later in the year were all associated with decreased average daily milk production ($P < 0.0001$, Appendix 3.10).

DISCUSSION

The objective of this study was to evaluate the effects of insulin and B+C on ketosis cure, blood BHBA concentrations following treatment, and average daily milk production in the first 30 days after treatment. This is the first large-scale multi-herd randomized blind clinical trial that has utilized either of these products for treatment of ketosis. Although no difference in ketosis cure or blood BHBA concentrations was observed with insulin treatment, the effect of insulin on milk production in the first 30 days after treatment suggests it may be useful in some animals. Analysis of B+C suggests that it may be useful for ketosis cure and milk production in animals with low blood glucose at enrollment, especially animals in their third or greater lactation. It should be stated that no animals in our trial were left completely untreated. All animals were given a 3-day treatment regimen of PG due to the documented benefits of PG treatment and the risks associated with failure to treat ketosis (McArt et al., 2011; McArt et al., 2012a). Though this can make analysis more challenging, it was done to improve animal welfare of enrolled animals and increase compliance with study protocols on enrolled farms. Thus, we are assuming that PG treatment afforded the same benefits to all enrolled animals and we are examining the additional benefits of B+C or insulin. Based on this study it is not possible to comment on the efficacy of either product in the absence of PG treatment.

The ketosis incidence in this study was consistent with several published studies (Duffield et al., 1998; McArt et al., 2011). The variation in ketosis incidence among

herds was expected (Duffield, 2000; McArt et al., 2011) and illustrates the differences in disease risk among herds.

Insulin Study

Insulin had no effects on ketosis cure, maintenance of ketosis cure, or blood BHBA concentrations at 1 or 2 weeks post-treatment. This is in agreement with some studies (Seifi et al., 2007), but not others (Robertson, 1966; Sakai et al., 1993). Studies that have shown a benefit of insulin administration have generally had short follow-up periods (5 days or less). Our first follow-up test was 7 days after treatment, so our protocol may have failed to identify short-term benefits of insulin treatment. Insulin works by suppressing fat mobilization. This may be useful in animals where gluconeogenesis and ketogenesis are maximized and excess fat is accumulated in the liver (Herdt, 2000). However, in animals where gluconeogenesis is not maximized, administration of insulin may exacerbate the problem. These animals will still have a strong intrinsic drive for milk production and correspondingly high glucose requirements. Administration of insulin to these animals may temporarily decrease fat mobilization, further decrease body glucose levels, and lead to a subsequent increase in fat mobilization due to low blood glucose levels. We did not measure fat content of the liver in our study. However, the fact that about 40% of animals were hypoglycemic (< 2.2 mmol/L, based on reference ranges) and none of the animals were hyperglycemic (> 5.5 mmol/L) suggests that gluconeogenesis may not be maximally stimulated in most animals in the study. Furthermore, peripartum animals are insulin resistant and the level of resistance is exacerbated by increased blood ketone levels (Sakai et al., 1993), so it may be that administration of 1 dose of insulin is insufficient to decrease fat catabolism.

Multiparous cows were less likely to cure, to maintain cure and had increased blood BHBA concentrations at 1 and 2 weeks post-treatment. This is not surprising as older cows are more likely to become ketotic, (Gröhn et al., 1989; Rasmussen et al., 1999) likely due to higher milk yield (Andersson and Emanuelson, 1985; Gröhn et al., 1989; Hardeng and Edge, 2001).

The relationship between higher blood BHBA concentration at enrollment and decreased cure risk and increased blood BHBA concentrations 1 and 2 weeks after treatment is also expected. Higher concentrations of blood ketone bodies likely reflect greater difficulty adapting to lactation and these animals will take more time to adjust. Also if treatment decreased blood BHBA concentrations by the same numeric amount in all animals, animals that were more severely affected would be less likely to have BHBA reduced to < 1.2 mmol/L.

The relationship between low blood glucose at diagnosis and ketosis cure and blood BHBA concentrations at 1 and 2 weeks post-treatment is interesting. It has been suggested that animals with high blood ketone and low blood glucose concentrations have maximally stimulated gluconeogenic pathways, but glucose precursors are insufficient to meet the glucose demands of high production (Holtenius and Holtenius, 1996). Limitations on gluconeogenesis provide a defense mechanism to prevent excessive breakdown of body protein stores (Holtenius and Holtenius, 1996). It would follow that cows afflicted with this type of ketosis during a period when milk production is still increasing would remain ketotic for longer periods, as glucose demands would continue to increase with increasing milk production. However, it has been suggested that these animals are also hypoinsulinemic (Holtenius and Holtenius, 1996). Treatment

with insulin should help alleviate some of the symptoms in these animals, but no effects of treatment on ketosis cure or blood BHBA concentrations were realized in this study. Again, it may be that the effects of the insulin treatment are short-term and the weekly follow-up did not allow for observation of any effects. Unfortunately, more frequent follow-up was not possible in this study due to study design and labor constraints.

Full Trial

In the full trial, not only were blood glucose concentrations at enrollment significantly associated with ketosis cure risk, there was also a significant interaction between blood glucose concentrations and B+C treatment. Though B+C treatment had no effect in animals with blood glucose $\geq$ 2.2 mmol/L at enrollment, animals with blood glucose < 2.2 mmol/L at enrollment were 2 times more likely to cure than non-treated animals. Though a mechanism for this is unclear, it has been hypothesized that B+C stimulates gluconeogenesis (Rollin et al., 2010). Animals with low blood glucose would be more likely to respond to stimulation of these pathways, where animals with normal blood glucose would not have a strong homeorhetic drive to increase glucose production. In animals where glucose precursors were limited, stimulation of gluconeogenesis would likely not occur to preserve body protein stores (Holtenius and Holtenius, 1996). However, in animals with impaired gluconeogenesis from fat accumulation in the liver, stimulation of gluconeogenesis may result in decreased fat catabolism and decreased ketogenesis (Herdt, 2000). Low blood glucose at enrollment was also associated with higher blood BHBA concentrations at 2 weeks post-treatment. The explanatory hypothesis for this has been discussed previously.

Probability of cure was decreased and post-treatment blood BHBA concentrations were increased for older animals and animals with high blood BHBA concentrations at enrollment. The reasons for these findings have been discussed previously. Animals that were enrolled at 3 to 5 DIM were 0.6 times as likely to cure as animals that were enrolled over the following 11 days. This suggests that animals that are diagnosed closer to calving are more severely affected than animals that are diagnosed later in lactation, even accounting for BHBA concentration at diagnosis. Animals that are diagnosed in the first few days after calving are likely experiencing effects that started during the dry period and may be more likely to develop fatty liver (Herdt, 2000). It is interesting to note that there were no significant differences observed when enrollment was categorized as week 1 (3 to 9 DIM) versus week 2 (10 to 16 DIM) of the study. This suggests that this difference in severity of the effects of ketosis lasts for a short time after calving. Increased risk of worse outcomes in animals diagnosed with ketosis in the first few days of lactation has been reported previously (McArt et al., 2012b).

Average daily milk yield for 30 days after treatment was decreased in animals that were enrolled in the first 3 to 5 days of lactation in all lactation groups. This translated to decreases of 2.7 kg/d (95% CI = -4.1 to -1.2), 7.2 kg/d (95% CI = -7.8 to -3.9) and 5.8 kg/d (95% CI = -7.0 to -4.7) in first, second and third and higher lactation, respectively. Thus not only does early diagnosis affect ketosis cure risk, but this carries over into effects on production. If these animals are afflicted with fatty liver, this may explain some of this difference. Animals with fatty liver would be less able to appropriately adjust to lactation and would take longer to adapt (Herdt, 2000). The difference in the impact of ketosis on production seen in first lactation animals is in

agreement with findings of another recent study (McArt et al., 2012b). However, the decrease observed in second and greater lactation animals in the present study is much greater than that found in the previous study. McArt et al. (2012b) did not separate study animals by lactation group, so this may explain some of the difference.

Milk production was also decreased in mature (lactation 2 and higher) animals that had enrollment BHBA concentrations above 2.4 mmol/L. The association between high blood BHBA concentrations and short-term milk yield has been well established (Duffield et al., 2009; McArt et al., 2012b). McArt et al. (2012b) found that for each 0.1 mmol/L increase in BHBA above 1.2 mmol/L at ketosis diagnosis, milk production decreased by 0.5 kg/d in the first 30 days of lactation. Thus, at 2.4 mmol/L BHBA, milk production would be decreased by 6 kg/d, which is consistent with the results of this study. It is unclear why this same effect was not seen in first lactation animals in the present study, though it may be due to lack of significant power to detect a difference.

Treatment had no significant effect on early lactation milk production in second lactation animals ($P = 0.5$ for both treatments). Treatment with insulin significantly increased milk production in primiparous animals (3.8 kg/d, 95% CI = 2.1 to 5.5). Though the reason for this difference is unclear, it may be that there was insufficient power to detect a treatment effect in second lactation animals or it may be due to a difference in the type of ketosis that is experienced in first lactation animals compared to older animals. Ketosis incidence in first lactation animals is generally low. Most animals in their first lactation do not have sufficient levels of production to develop ketosis due to production alone. However, animals with increased body condition at calving or decreased DMI around calving are at risk of ketosis development (Rasmussen et al.,

1999; Gillund et al., 2001; Hayirli and Grummer, 2004). In animals where ketosis is due to high levels of fat metabolism (high BCS), insulin may be useful as it would decrease breakdown of body fat. Measurement of body condition score and differences between animals in various lactations may help explain some of this difference.

Both treatments significantly increased milk production in animals in their third and greater lactation, but only in cows that had low blood glucose at enrollment. Treatment with B+C increased milk production by 2.8 kg/d in animals with blood glucose levels < 2.2 mmol/L at enrollment, whereas treatment in animals with blood glucose $\geq$ 2.2 mmol/L did not have a significant effect on production. Again, if B+C stimulates gluconeogenesis, it may be more likely to do so in animals that have low blood glucose concentrations. Increased blood glucose would likely increase milk production, as glucose is often limiting in ruminant animals (Herdt, 2000). Increases in blood glucose should lead to both decreased lipolysis and ketogenesis and to decreased accumulation of fat in the liver, which could decrease the negative effects of ketonemia on production as well. Recent studies have found that supplementation of vitamin B12 increases blood glucose and milk production (Preynat et al., 2009a; b). However, it should be noted that vitamin B12 was given in conjunction with folic acid in those studies, so the effect of vitamin B12 alone is unknown. Also, the dose of vitamin B12 given in the current study was 1.25 mg per injection, for a total dose of 3.75 mg over 3 days. This is much lower than the 10 mg weekly dose administered by Preynat et al. (2009a, b). More research is required to understand the mechanism of action of B+C and the relationship of this mechanism with blood glucose levels. Based on the results of this study, administration of B+C to animals with low (< 2.2 mmol/L) blood glucose levels at

enrollment (40% of ketotic cows in this study) may help with ketosis cure and increase milk production in third or greater lactation animals. Though there were no adverse effects of treatment in animals with blood glucose $\geq$ 2.2 mmol/L at enrollment, B+C treatment did not show any benefit.

Insulin also significantly increased milk production by 4.3 kg/d in animals with low blood glucose concentrations at enrollment. However, in animals with blood glucose > 2.2 mmol/L at enrollment, insulin led to a decrease in production of 2.3 kg/d. Animals with low blood glucose may have decreased circulating insulin (Holtenius and Holtenius, 1996). These animals may benefit from insulin administration to decrease ketogenesis and fat catabolism. However, it is unclear how this would increase milk production. A decrease in fat catabolism in a cow in negative energy balance in early lactation should decrease milk production, as less energy is available for production and maintenance requirements. It may be that decreases in blood BHBA concentrations lead to increased DMI. Though cows with increased weight loss after calving have decreased DMI and increased ketosis risk (Busato et al., 2002; Janovick and Drackley, 2010), increased blood BHBA alone has not been shown to decrease DMI. If DMI is increased, this would lead to more energy available for milk production and gluconeogenesis.

There are two possible mechanisms that might explain why cows with blood glucose > 2.2 mmol/L may respond with a decrease in production. Cut-points associated with defining ketosis are based on average responses and may not be valid for all animals. Some animals that are classified as ketotic, especially at the lower end of the range of blood BHBA concentrations, are likely able to cope well with the increase in blood ketones as a normal component of their own adaptive response to negative energy

balance. In these animals there are no problems with homeorhetic mechanisms; it is just that their individual cut-points for abnormal ketone concentrations are higher. These cows are utilizing body fat stores to help support high levels of milk production and are often the higher producing cows in the herd (Andersson, 1984). Decreasing catabolism in these animals would actually limit production without any positive effects on health, which may explain some of the difference observed in this study. Animals with high insulin levels and insulin resistance may show no benefit from additional insulin administration. Alternatively, administration of insulin in animals with high blood insulin may override some of the inherent decrease in insulin binding ability of the liver around parturition and increase fat accumulation in the liver (Holtenius and Holtenius, 1996). This may lead to a further decrease in gluconeogenic ability of the liver and exacerbate ketonemia (Holtenius and Holtenius, 1996; Herdt, 2000), which would likely further limit milk production. Measurement of blood insulin levels, insulin resistance, and liver fat content may help in understanding of the mechanisms involved in the loss of production seen in animals with blood glucose levels > 2.2 mmol/L at the time of enrollment. Whatever the mechanism is, based on the results of this study treatment with glargine insulin in mature (third lactation and greater) animals should be considered if their blood glucose at ketosis diagnosis is < 2.2 mmol/L and care should be taken when treating animals with blood glucose $\geq$ 2.2 mmol/L.

CONCLUSIONS

Blood glucose concentrations at ketosis diagnosis may be an important predictor of cure of subclinical ketosis and early lactation milk production. Variations in blood glucose concentrations may also be useful in determining an appropriate treatment

regimen for ketotic animals. Use of 200 IU of glargine insulin SQ once had no effect on ketosis cure, but may increase early lactation milk production in first lactation animals when given with PG. Glargine insulin may also increase milk production in mature animals with low blood glucose concentrations at ketosis diagnosis when given with PG, but should be avoided in animals with blood glucose concentrations $\geq$ 2.2 mmol/L due to decreases in milk production. B+C may be beneficial when given with PG for ketosis cure in all animals and for early lactation milk production in mature animals with low blood glucose concentrations at the time of ketosis diagnosis. More research is required to examine the relationship between blood glucose concentrations and the effects of elevated blood ketone body concentrations.

REFERENCES

Akins, M. S., S. J. Bertics, M. T. Socha, and R. D. Shaver. 2013. Effects of cobalt supplementation and vitamin B(12) injections on lactation performance and metabolism of Holstein dairy cows. J. Dairy Sci. 96:1755-1768.

Andersson, L. 1984. Concentrations of blood and milk ketone bodies, blood isopropanol and plasma glucose in dairy cows in relation to the degree of hyperketonaemia and clinical signs. Zentralbl. Veterinarmed. A. 31:683-693.

Andersson, L. and U. Emanuelson. 1985. An epidemiological study of hyperketonaemia in Swedish dairy cows; determinants and the relation to fertility. Prev. Vet. Med. 3:449-462.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Busato, A., D. Faissler, U. Küpfer, and J. W. Blum. 2002. Body condition scores in dairy cows: associations with metabolic and endocrine changes in healthy dairy cows. J. Vet. Med. Ser. A. 49:455-460.

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

Duffield, T. F. 2000. Subclinical ketosis in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 16:231-253.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

Furll, M., A. Deniz, B. Westphal, C. Illing, and P. D. Constable. 2010. Effect of multiple intravenous injections of butaphosphan and cyanocobalamin on the metabolism of periparturient dairy cows. J. Dairy Sci. 93:4155-4164.

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

Girard, C. L. and J. J. Matte. 2005. Effects of intramuscular injections of vitamin B12 on lactation performance of dairy cows fed dietary supplements of folic acid and rumen-protected methionine. J. Dairy Sci. 88:671-676.

Gröhn, Y. T., H. N. Erb, C. E. McCulloch, and H. S. Saloniemi. 1989. Epidemiology of metabolic disorders in dairy cattle: association among host characteristics, disease and production. J. Dairy Sci. 72:1876-1885.

Hardeng, F. and V. L. Edge. 2001. Mastitis, ketosis, and milk fever in 31 organic and 93 conventional Norwegian dairy herds. J. Dairy Sci. 84:2673-2679.

Hayirli, A. and R. R. Grummer. 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. Can. J. Anim. Sci. 84:337-347.

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.

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Vet. Clin. North Am. Food Anim. Pract. 16:215-230.

Holtenius, P. and K. Holtenius. 1996. New aspects of ketone bodies in energy metabolism of dairy cows: A review. J. Vet. Med. Ser. A. 43:579.

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, N. A. and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086-3102.

Kennedy, D. G., A. Cannavan, A. Molloy, F. O'Harte, S. M. Taylor, S. Kennedy, and W. J. Blanchflower. 1990. Methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthetase (EC 2.1.1.13) in the tissues of cobalt-vitamin 12 deficient sheep. Br. J. Nutr. 64:721-732.

Kincaid, R. L. and M. T. Socha. 2007. Effect of cobalt supplementation during late gestation and early lactation on milk and serum measures. J. Dairy Sci. 90:1880-1886.

Lohr, B., B. Brunner, H. Janowitz, M. Hummel, T. Seeger, I. Weber, T. Wittek, B. Schmidt, and K. Hellmann. 2006. Clinical efficacy of Catosal® in the treatment of ketosis in cows with left abomasal displacement. Tierärztliche Umschau 61:187-190.

McArt, J. 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.

McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2012a. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. J. Dairy Sci. 95:2505-2512.

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

Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. North Am. Food Anim. Pract. 20:651-674.

Ospina, P. A., D. V. Nydam, and T. J. DiCiccio. 2012. Technical note: The risk ratio, an alternative to the odds ratio for estimating the association between multiple risk factors and a dichotomous outcome. J. Dairy Sci. 95:2576-2584.

Preynat, A., H. Lapierre, M. C. Thivierge, M. F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009a. Effects of supplements of folic acid, vitamin B12, and rumen-protected methionine on whole body metabolism of methionine and glucose in lactating dairy cows. J. Dairy Sci. 92:677-689.

Preynat, A., H. Lapierre, M. C. Thivierge, M. F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009b. Influence of methionine supply on the response of lactational performance of dairy cows to supplementary folic acid and vitamin B12. J. Dairy Sci. 92:1685-1695.

Rasmussen, L. K., B. L. Nielsen, J. E. Pryce, T. T. Mottram, and R. F. Veerkamp. 1999. Risk factors associated with the incidence of ketosis in dairy cows. Anim. Sci. 68:379-386.

Robertson, J. M. 1966. The evaluation of a therapeutic trial on bovine ketosis. J. Am. Vet. Med. Assoc. 149:1620-1623.

Rollin, E., R. D. Berghaus, P. Rapnicki, S. M. Godden, and M. W. Overton. 2010. The effect of injectable butaphosphan and cyanocobalamin on postpartum serum beta-hydroxybutyrate, calcium, and phosphorus concentrations in dairy cattle. J. Dairy Sci. 93:978-987.

Sakai, T., T. Hayakawa, M. Hamakawa, K. Ogura, and S. Kubo. 1993. Therapeutic effects of simultaneous use of dextrose and insulin in ketotic dairy cows. J. Dairy Sci. 76:109-114.

Seifi, H. A., S. J. LeBlanc, E. Vernooy, K. E. Leslie, and T. F. Duffield. 2007. Effect of isoflupredone acetate with or without insulin on energy metabolism, reproduction, milk production, and health in dairy cows in early lactation. J. Dairy Sci. 90:4181-4191.

Wittrock, J. A. M., T. F. Duffield, S. Riuzzi, and S. J. LeBlanc. 2013. Evaluation of an electronic cow-side glucose meter for diagnosing insulin resistance in Holstein dairy cows. J. Dairy Sci. 96:4514-4518.

Table 3.1 Descriptive statistics by treatment group for 380 animals enrolled in a ketosis treatment trial utilizing B+C and insulin.

	Control	B+C[1]	Insulin	Both
n	99	95	96	90
BHBA (mmol/L, $\bar{x} \pm$ SD)				
Enrollment	2.2 ± 1.1	2.4 ± 1.3	2.3 ± 1.1	2.1 ± 1.0
Week +1	1.9 ± 1.4	1.8 ± 1.5	1.9 ± 1.3	1.7 ± 1.4
% BHBA ≥ 1.2	40%	40%	33%	48%
Week + 2	1.6 ± 1.2	1.6 ± 1.1	1.7 ± 1.3	1.9 ± 1.5
% BHBA ≥ 1.2	53%	53%	56%	51%
Maintenance of cure	88%	70%	91%	70%
Glucose (mmol/L, $\bar{x} \pm$ SD)				
Enrollment	2.3 ± 0.5	2.3 ± 0.6	2.4 ± 0.6	2.3 ± 0.6
Week +1	2.7 ± 0.7	2.7 ± 0.7	2.8 ± 0.7	2.7 ± 0.7
Week + 2	2.9 ± 0.7	3.0 ± 0.7	2.8 ± 0.7	2.8 ± 0.7

[1]10% butaphosphan cyanocobalamin

Table 3.2 Final Poisson regression model of ketosis cure in 620 Holsteins from 11 herds. Cows were randomly assigned to treatment with 200 IU insulin glargine SQ once (n = 304) or control (n = 316) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L and cure was defined as blood BHBA < 1.2 mmol/L 1 week after treatment.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-0.39	0.07	--	--	--
Enrollment BHBA					
1.2 to 2.4 mmol/L	--[1]	--	--	--	--
> 2.4 mmol/L	-0.43	0.10	<0.0001	0.7	0.5 to 0.8
Glucose group					
< 2.2 mmol/L	-0.32	0.09	0.0006	0.7	0.6 to 0.9
≥ 2.2 mmol/L	--[1]	--	--	--	--
Parity					
1	--[1]	--	--	--	--
2	-0.34	0.12	<0.0001	0.7	0.6 to 0.9
3+	-0.53	0.12	<0.0001	0.6	0.5 to 0.7
Insulin					
Yes	0.09	0.08	0.2	1.1	0.9 to 1.3
No	--[1]	--	--	--	--

[1]Reference group

Table 3.3 Final Poisson regression model variables used to predict ketosis cure in 380 Holsteins from 1 herd. Cows were randomly assigned to treatment with B+C (n = 95), insulin (n = 96), B+C and insulin (n = 90), or control (n = 99) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L and cure was defined as blood BHBA < 1.2 mmol/L 1 week after treatment.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-0.001	0.20	0.8	--	--
BHBA at enrollment					
1.2 to 2.4 mmol/L	--[1]	--	--	--	--
> 2.4 mmol/L	-0.55	0.19	0.004	0.6	0.4 to 0.8
DIM at enrollment					
3 to 5 days	-0.35	0.15	0.02	0.7	0.5 to 0.9
6 to 16 days	--[1]	--	--	--	--
Glucose at enrollment					
< 2.2 mmol/L	-0.34	0.16	0.04	0.7	0.5 to 1.0
$\geq$ 2.2 mmol/L	--[1]	--	--	--	--
Parity					
1	--[1]	--	--	--	--
2	-0.46	0.17	0.002	0.6	0.4 to 0.9
3+	-0.49	0.16	0.002	0.6	0.4 to 0.8
Insulin					
Yes	-0.002	0.13	0.9	1.0	0.8 to 1.3
No	--[1]	--	--	--	--
B+C[2]					
Yes	0.33	0.15	0.03	1.3	1.1 to 1.9
No	--[1]	--	--	--	--
B+C[2] x glucose group			0.02		

[1]Reference group
[2]10% butaphosphan cyanocobalamin

Table 3.4 Stratum specific Poisson models of ketosis cure in Holsteins dairy cows with blood glucose ≥ 2.2 mmol/L (n = 237) and < 2.2 mmol/L (low, n = 143) at enrollment. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L and cure was defined as blood BHBA < 1.2 mmol/L 1 week after treatment.

Variable	β	SE	*P*-value	RR	95% CI
Glucose at enrollment < 2.2 mmol/L					
Intercept	-0.85	0.46	0.07	--	--
Enrollment BHBA					
1.2 to 2.4 mmol/L	--[1]	--	--	--	--
> 2.4 mmol/L	-0.44	0.29	0.1	0.6	0.4 to 1.1
DIM at enrollment					
3 to 5 days	--[1]	--	--	--	--
6 to 16 days	-0.12	0.38	0.8	0.9	0.5 to 1.7
Parity					
1	--[1]	--	--	--	--
2	-0.57	0.46	0.2	0.6	0.2 to 1.4
3+	-0.83	0.45	0.2	0.4	0.2 to 1.0
Insulin					
Yes	0.05	0.28	0.9	1.0	0.6 to 1.8
No	--[1]	--	--	--	--
B+C[2]					
Yes	0.72	0.31	0.02	2.1	1.2 to 3.7
No	--[1]	--	--	--	--
Glucose at enrollment ≥ 2.2 mmol/L					
Intercept	-0.02	0.20	0.2	--	--
Enrollment BHBA					
1.2 to 2.4 mmol/L	--[1]	--	--	--	--
> 2.4 mmol/L	-0.67	0.29	0.02	0.5	0.3 to 0.9
DIM at enrollment					
3-5 days	-0.42	0.17	0.01	0.7	0.5 to 0.9
6-16 days	--[1]	--	--	--	--
Parity					
1	--[1]	--	--	--	--
2	-0.46	0.17	0.01	0.6	0.4 to 0.9
3+	-0.49	0.16	0.01	0.6	0.4 to 0.8
Insulin					
Yes	-0.005	0.14	0.9	1.0	0.8 to 1.3
No	--[1]	--	--	--	--
B+C[2]					
Yes	-0.03	0.14	0.3	1.0	0.7 to 1.3
No	--[1]	--	--	--	--

[1]Reference group
[2]10% butaphosphan cyanocobalamin

Table 3.5 Final model for milk production (kg/d) in the first 30 days after treatment for 57 first lactation Holstein dairy cows from 1 herd. Animals were randomly assigned to treatment with B+C (n = 13), insulin (n = 15), B+C and insulin (n = 14), or control (n = 15) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L.

Variable	Milk difference per day (kg)	SE	*P*-value	95% CI
DIM at enrollment				
3 to 5 days	-2.7	0.72	0.0005	-4.1 to -1.2
6 to 16 days	--[1]	--	--	--
Month of calving				
May	--[1]	--	--	--
June	-1.2	1.0	0.2	-3.2 to 0.8
July	-3.1	0.88	0.0006	-4.9 to -1.4
August	-3.9	1.0	0.0006	-5.9 to -1.8
Insulin				
Yes	3.8	0.84	0.002	2.1 to 5.5
No	--[1]	--	--	--
B+C[2]				
Yes	0.8	0.63	0.5	-1.4 to 2.9
No	--[1]	--	--	--

[1]Reference group
[2]10% butaphosphan cyanocobalamin

Table 3.6 Stratum specific model for milk production (kg/d) in the first 30 days after treatment for 154 third or greater lactation Holstein dairy cows from 1 herd. Animals were randomly assigned to treatment with B+C (n = 48), insulin (n = 51), or control (n = 55). Animals treated with both treatments were excluded and each treatment was compared to the control group. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L. Blood glucose was divided into $\geq$ 2.2 mmol/L and < 2.2 mmol/L (low) groups.

Variable	Milk difference per day (kg)	SE	*P*-value	95% CI
Enrollment glucose < 2.2 mmol/L				
Insulin				
Yes	4.2	1.4	0.0003	1.4 to 7.1
No	--[1]	--	--	--
B+C[2]				
Yes	2.8	0.97	0.05	0.9 to 4.7
No	--[1]	--	--	--
Enrollment glucose $\geq$ 2.2 mmol/L				
Insulin				
Yes	-2.3	1.0	0.03	-4.4 to -0.3
No	--[1]	--	--	--
B+C[2]				
Yes	1.0	0.98	0.3	-0.9 to 2.9
No	--[1]	--	--	--

[1]Reference group
[2]10% butaphosphan cyanocobalamin

Appendix 3.1 Sensitivity analysis to determine a cut-point for blood glucose at enrollment based on clinical disease diagnosis in the 2 weeks after ketosis diagnosis using records from 334 cows with complete disease information.

Glucose (mmol/L)	% at/below cut-point	Sensitivity (Sn, %)	Specificity (Sp, %)	Sum of Sn and Sp
1.6	14.6	29.3	93.8	123.1
1.8	20.6	41.9	90.8	132.7
2.0	29.6	51.6	84.9	136.5
2.1	34.6	61.3	79.8	141.1
2.2*	42.7	74.2	74.3	148.5
2.3	50.8	77.4	68.4	145.8
2.4	57.4	83.9	58.1	142
2.6	67.4	85.4	40.9	126.3
2.8	77.6	87.1	36.8	123.9

*Optimum cut-point based on sum of sensitivity and specificity

Appendix 3.2 Sensitivity analysis to determine a cut-point for blood BHBA at enrollment based on clinical disease diagnosis in the 2 weeks after ketosis diagnosis using records from 334 cows with complete disease information.

BHBA (mmol/L)	% at/above cut-point	Sensitivity (Sn, %)	Specificity (Sp, %)	Sum of Sn and Sp
2.0	44.4	60.0	56.5	116.5
2.2	36.6	56.9	62.5	119.4
2.4*	30.3	44.6	76.2	120.8
2.6	27.3	38.6	80.7	119.3
2.8	23.0	29.2	87.7	116.9
3.0	20.6	24.3	91.1	145.8

*Optimum cut-point based on sum of sensitivity and specificity

Appendix 3.3 Weekly ketosis incidence for 1,653 Holstein dairy cows from 17 herds on a weekly testing schedule for ketosis from 3 to 16 DIM. Ketosis was defined as blood BHBA concentrations $\geq$ 1.2 mmol/L.

Herd	Number tested N	Total ketotic N (%)	Week 1 (3-9 DIM) N (% of ketotic)	Week 2 (10-16 DIM) N (% of ketotic)
A	76	41 (54%)	29 (71%)	12 (19%)
B	48	17 (35%)	10 (59%)	7 (41%)
C	13	6 (46%)	6 (100%)	0 (0%)
D	53	32 (60%)	16 (50%)	16 (50%)
E	6	4 (67%)	4 (100%)	0 (0%)
F	19	9 (47%)	6 (67%)	3 (33%)
G	13	7 (54%)	3 (43%)	4 (57%)
H	12	6 (50%)	5 (83%)	1 (17%)
I	9	6 (67%)	5 (87%)	1 (17%)
J	18	12 (67%)	11 (92%)	1 (8%)
K	134	80 (60%)	61 (76%)	19 (24%)
L	79	16 (20%)	6 (38%)	10 (62%)
M	70	22 (31%)	19 (86%)	3 (14%)
N	67	22 (33%)	19 (86%)	3 (14%)
O	49	7 (14%)	3 (43%)	4 (57%)
P	14	11 (79%)	8 (73%)	3 (27%)
Z	973	414 (43%)	314 (76%)	100 (24%)
Total	1,653	712 (43%)	525 (74%)	187 (26%)

Appendix 3.4 Descriptive statistics for 620 animals enrolled in a ketosis treatment trial utilizing insulin.

Variable	Unit of measurement	Mean	Median	Standard Deviation	Min	Max
Lactation		2.8	3	1.4	1	8
DIM at enrollment	days	7.8	7	3.3	3	16
BHBA at enrollment	mmol/L	2.3	1.8	1.3	1.2	7.9
BHBA at 1 wk post-treatment	mmol/L	1.9	1.4	1.4	0.2	7.8
BHBA at 2 wk post-treatment	mmol/L	1.8	1.2	1.4	0.2	7.4
Glucose at enrollment	mmol/L	2.3	2.3	0.6	0.7	4.4
Glucose at 1 wk post-treatment	mmol/L	2.7	2.6	0.7	0.6	4.8
Glucose at 2 wk post-treatment	mmol/L	2.9	2.8	0.7	1.0	6.7

Appendix 3.5 Final linear regression model for blood BHBA concentrations 1 week after treatment, accounting for repeated measures, in 620 Holsteins from 11 herds. Cows were randomly assigned to treatment with 200 IU insulin glargine SQ once (n = 304) or placebo (n = 316) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L.

Variable	β	SE	*P*-value
Intercept	0.42	0.18	0.02
Enrollment BHBA			
1.2 to 2.4 mmol/L	--[1]	--	--
> 2.4 mmol/L	0.36	0.07	0.0009
Glucose group			
< 2.2 mmol/L	0.20	0.07	0.01
≥ 2.2 mmol/L	--[1]	--	--
Parity			
1	--[1]	--	--
2	0.43	0.09	0.0003
3+	0.55	0.08	0.0003
Insulin			
Yes	-0.09	0.06	0.2
No	--[1]	--	--

[1]Reference group

Appendix 3.6 Final linear regression model for blood BHBA concentrations 2 weeks after treatment, accounting for repeated measures, in 620 Holsteins from 11 herds. Cows were randomly assigned to treatment with 200 IU insulin glargine SQ once (n = 304) or placebo (n = 316) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L.

Variable	β	SE	*P*-value
Intercept	0.22	0.24	0.35
Enrollment BHBA			
1.2 to 2.4 mmol/L	--[1]	--	--
> 2.4 mmol/L	0.26	0.07	0.005
Glucose group			
< 2.2 mmol/L	0.21	0.07	0.01
≥ 2.2 mmol/L	--[1]	--	--
Parity			
1	--[1]	--	--
2	0.42	0.09	<0.0001
3+	0.61	0.08	<0.0001
Insulin			
Yes	0.015	0.06	0.8
No	--[1]	--	--

[1]Reference group

Appendix 3.7 Final linear regression model for blood BHBA concentrations 1 week after treatment, accounting for repeated measures, in 380 Holsteins from 1 herd. Cows were randomly assigned to treatment with B+C (n = 95), insulin (n = 96), B+C and insulin (n = 90), or control (n = 99) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L.

Variable	β	SE	*P*-value
Intercept	0.49	0.04	<0.0001
Enrollment BHBA			
1.2 to 2.4 mmol/L	--[1]	--	--
> 2.4 mmol/L	0.46	0.08	<0.0001
Parity			
1	--[1]	--	--
2	0.54	0.10	<0.0001
3+	0.58	0.11	<0.0001
Insulin			
Yes	-0.01	0.07	0.9
No	--[1]	--	--
B+C[2]			
Yes	-0.10	0.07	0.2
No	--[1]	--	--

[1]Reference group
[2]10% butaphosphan cyanocobalamin

Appendix 3.8 Final linear regression model for blood BHBA concentrations 2 weeks after treatment, accounting for repeated measures, in 380 Holsteins from 1 herd. Cows were randomly assigned to treatment with B+C (n = 95), insulin (n = 96), B+C and insulin (n = 90), or control (n = 99) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L.

Variable	β	SE	*P*-value
Intercept	0.46	0.03	<0.0001
Glucose group			
< 2.2 mmol/L	0.21	0.08	0.01
$\geq$ 2.2 mmo/L	--[1]	--	--
Parity			
1	--[1]	--	--
2	0.53	0.11	<0.0001
3+	0.64	0.10	<0.0001
Insulin			
Yes	0.17	0.11	0.1
No	--[1]	--	--
B+C[2]			
Yes	-0.05	0.07	0.5
No	--[1]	--	--

[1]Reference group
[2]10% butaphosphan cyanocobalamin

Appendix 3.9 Final model for milk production (kg/d) in the first 30 days after treatment for 122 second lactation Holstein dairy cows in 1 herd. Animals were randomly assigned to treatment with B+C (n = 35), insulin (n = 30), B+C and insulin (n = 28), or control (n = 29) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L.

Variable	Milk difference per day (kg)	SE	*P*-value	95% CI
Enrollment BHBA				
1.2 to 2.4 mmol/L	--[1]	--	--	--
> 2.4 mmol/L	-6.2	0.86	<0.0001	-7.9 to -4.6
DIM at enrollment				
3 to 5 days	-7.2	0.73	<0.0001	-7.8 to -3.9
6 to 16 days	--[1]	--	--	--
Month of calving				
May	--[1]	--	--	--
June	-4.2	0.97	<0.0001	-6.2 to -2.3
July	-5.9	0.98	<0.0001	-7.8 to -3.9
August	-5.2	0.93	<0.0001	-7.0 to -3.4
Insulin				
Yes	-0.54	0.68	0.5	-1.8 to 1.0
No	--[1]	--	--	--
B+C[2]				
Yes	-0.79	0.64	0.5	-2.0 to 1.1
No	--[1]	--	--	--

[1]Reference group
[2]10% butaphosphan cyanocobalamin

Appendix 3.10 Final model for milk production (kg/d) in the first 30 days after treatment for 201 third lactation Holstein dairy cows in 1 herd. Animals were randomly assigned to treatment with B+C (n = 48), insulin (n = 51), B+C and insulin (n = 47), or control (n = 55) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L.

Variable	Milk difference per day (kg)	SE	*P*-value	95% CI
Enrollment BHBA				
1.2 to 2.4 mmol/L	--[1]	--	--	--
> 2.4 mmol/L	-5.8	0.80	<0.0001	-7.4 to -3.5
DIM at enrollment				
3 to 5 days	-5.8	0.63	<0.0001	-7.0 to -4.7
6 to 16 days	--[1]	--	--	--
Month of calving				
May	--[1]	--	--	--
June	-5.0	0.88	<0.0001	-6.7 to -3.2
July	-4.6	0.96	<0.0001	-6.5 to -2.7
August	-6.9	0.93	<0.0001	-8.7 to -5.0
Glucose group				
< 2.2 mmol/L	--[1]	--	--	--
$\geq$ 2.2 mmol/L	-5.0	0.88	0.0002	-6.7 to -3.2
Insulin				
Yes	0.96	0.61	0.1	-0.3 to 2.1
No	--[1]	--	--	--
B+C[2]				
Yes	-0.45	0.79	0.5	-2.0 to 1.1
No	--[1]	--	--	--
Insulin x glucose group	--	--	0.002	--
B+C[2] x glucose group	--	--	0.002	--

[1]Reference group
[2]10% butaphosphan cyanocobalamin

CHAPTER FOUR

RANDOMIZED CLINICAL FIELD TRIAL ON THE EFFECTS OF BUTAPHOSPHAN-CYANOCOBALAMIN AND PROPYLENE GLYCOL ON KETOSIS RESOLUTION AND MILK PRODUCTION

ABSTRACT

The purpose of this study was to determine the effects of a butaphosphan cyanocobalamin combination product (B+C) and 2 durations of propylene glycol treatment (PG; 3 versus 5 days) on ketosis resolution and early lactation milk yield. Cows from 9 freestall herds (8 in Ontario and 1 in Michigan) were tested at weekly intervals between 3 and 16 DIM. Ketosis was defined as blood β-hydroxybutyrate (BHBA) $\geq$ 1.2 mmol/L. Ketotic cows were randomly assigned to treatment with 25 ml B+C or 25 ml saline placebo and 3 or 5 days of 300 g PG orally in a 2 x 2 factorial arrangement. Outcomes evaluated for all farms included ketosis cure (blood BHBA < 1.2 mmol/L 1 week post enrollment), maintenance of ketosis cure (blood BHBA < 1.2 mmol/L 1 and 2 weeks post enrollment), and blood BHBA concentrations at 1 and 2 weeks post enrollment. Daily milk weights were collected in 3 herds. Poisson regression was used to evaluate cure and maintenance of cure, while repeated measures ANOVA was used to evaluate blood BHBA concentrations in the 2 weeks after enrollment and average daily milk production in the 30 days after treatment. A total of 594 animals were enrolled in the study with 124 treated with B+C and 5 days PG, 176 treated with B+C and 3 days PG, 128 treated with saline and 5 days PG, and 166 treated with saline and 3 days PG. Animals with blood BHBA > 2.4 mmol/L at the time of enrollment were 1.7 times more likely (95% CI = 1.4 to 2.2) to cure and had a decrease of

0.25 $\pm$ 0.11 mmol/L blood BHBA at 1 week after enrollment if treated with 5 days of PG compared to 3, though this response was not seen in animals with BHBA 1.2 to 2.4 mmol/L at enrollment. Cows with blood glucose concentrations < 2.2 mmol/L at enrollment produced 3.1 kg/d (95% CI = 1.3 to 5.0) more milk if treated with B+C and 3.4 kg/d (95% CI 1.7 to 5.1) more milk if treated with 5 days of PG compared to their respective controls. This response was not seen in animals with blood glucose $\geq$ 2.2 mmol/L at enrollment and there was no interaction between treatments. These results indicate that extended PG treatment is beneficial in decreasing blood BHBA concentrations in more severely affected animals. Additionally, each of B+C and extended PG treatment improved milk yield in animals with low blood glucose at the time of ketosis diagnosis.

INTRODUCTION

Metabolic disease is common in early lactation dairy cattle due to a period of negative energy balance that occurs at the beginning of lactation for nearly every animal (Bauman and Currie, 1980; Baird, 1982; Herdt, 2000). Lactation is given a high priority in metabolic demands, to the point that body stores are heavily utilized to support lactation (Bauman and Currie, 1980). Adaptations occur in many animals that allow changes in physiology and metabolism to support lactation without subsequent disease through a process of homeorhesis (Bauman and Currie, 1980). However, in some animals these adaptations are inadequate and metabolic disease results, often in the form of hyperketonemia (Bauman and Currie, 1980; Herdt, 2000).

Production of ketone bodies is part of the homeorhetic changes that allow lactation to proceed and support higher levels of production (Herdt, 2000). However,

pathologic levels of ketone bodies are associated with increased risk of displaced abomasum (DA), poorer reproductive performance, and decreased milk production (Duffield et al., 2009; Ospina et al., 2010a; b; McArt et al., 2012b). All of these outcomes can lead to decreased animal welfare and economic losses for the producer.

Propylene glycol (PG) was first described for ketosis treatment in 1954 (Johnson, 1954; Maplesden, 1954). When PG enters the rumen, it is either absorbed directly or converted to propionate (Nielsen and Ingvartsen, 2004). PG that is absorbed directly stimulates gluconeogenesis by entering the tricarboxylic acid (TCA) cycle (Nielsen and Ingvartsen, 2004). Propionate produced from metabolism of PG can be used as a precursor for gluconeogenesis and helps stimulate insulin release to decrease fat catabolism (Studer et al., 1993). Though PG has often been studied (Nielsen and Ingvartsen, 2004) many of these studies were small or poorly designed. Recently, a large-scale clinical field trial illustrated the benefits of PG use for treatment of subclinical ketosis (McArt et al., 2011; McArt et al., 2012a). Cows in this study were tested for ketosis 3 times a week and were treated with PG until ketosis was resolved, from 2 to 16 days. Though this study showed several benefits of treatment with PG, there were two challenges with the study design. Giving an oral drench of PG is labor-intensive and the results do not identify a minimum effective duration of treatment. Additionally, animals with BHBA $\geq$ 3.0 mmol/L were removed from the trial, so it is unclear if more severely affected animals benefit from PG treatment.

Recently, a combination butaphosphan and cyanocobalamin product (B+C, Catosal, Bayer, Shawnee, KS) has been investigated for ketosis treatment (Lohr et al., 2006; Gordon et al., 2012). Cyanocobalamin is a form of vitamin B12, which has been

hypothesized to increase gluconeogenesis by increasing the activity of methylmalonyl-CoA mutase, a vitamin B12-dependent enzyme and important component of the TCA cycle (Kennedy et al., 1990). Butaphosphan, an organic phosphorus source, might also stimulate gluconeogenesis by phosphorylating intermediate compounds in the process (Rollin et al., 2010). However, it is unclear if this form of phosphorus is biologically available to the animal. The use of this combination product has been shown to increase rumination in cows with after surgical correction of an LDA (Lohr et al., 2006) and to increase ketosis cure and milk production in multiparous cows (Gordon et al., 2012). Data from these studies was collected subjectively (Lohr et al., 2006) or from a single herd (Gordon et al., 2012), so it is unclear how efficacious B+C would be in various commercial herds.

The objective of this study was to examine the effects of B+C and varying durations of PG treatment for ketosis resolution and the effects on early lactation milk production.

MATERIALS AND METHODS

Study Population

Data were collected from 8 dairy herds in Ontario (Farms A through H) and 1 in Michigan (Farm Z) from May 14 to August 27, 2012. Herds were purposively selected due to their proximity to study sites and willingness to participate. To be eligible for inclusion, herds were required to be enrolled in monthly milk testing through their local Dairy Herd Improvement (DHI) organization or collect daily milk weights on farm. Enrolled herds were housed in freestall facilities ranging from 100 to 3,200 lactating cows. All herds used a total mixed ration to all lactating cows.

Data Collection and Study Design

Herds were visited weekly during the study period. Individual herds were visited on the same day of the week and at the same time of day. At each visit, cows 3 to 16 DIM were tested for ketosis using the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). Cows were excluded from enrollment if they had been previously diagnosed with ketosis or a DA or had been enrolled in the study the previous week. Ketosis was defined *a priori* as blood BHBA $\geq$ 1.2 mmol/L. The Precision Xtra meter is a hand-held device that measures BHBA in whole blood. This meter has been previously validated for use in cattle and has 88% sensitivity and 96% specificity at this cut-point (Iwersen et al., 2009). This testing scheme provided two opportunities for enrollment for each animal, once at 3 to 9 DIM and again at 10 to 16 DIM.

Blood was drawn from the coccygeal vessels using a 20 gauge x 2.54 cm needle and 3 cc syringe. Ketone testing was performed immediately according to manufacturer's instructions. A ketone test strip was inserted into the meter and the lot number displayed on the meter was checked against the lot number on the strip. Once the "add blood" symbol appeared, blood was added to the strip until the meter showed the test chamber was full. After 10 seconds, the blood BHBA concentration was displayed on the screen and the number was recorded. In animals that were classified as ketotic, blood glucose concentration was measured using a second Precision Xtra meter. Blood was tested for glucose immediately after the ketone results were displayed. The procedure for glucose testing is the same as for ketone testing, except that the results are displayed in 5 seconds. The use of Precision Xtra for glucose determination has also been validated in cattle (Wittrock et al., 2013).

Animals classified as ketotic (≥ 1.2 mmol/L blood BHBA) were randomly assigned to 1 of 4 treatment groups in a 2 x 2 factorial arrangement. Treatment groups were: 1) 25 ml B+C subcutaneously once a day for 3 days and 300 g PG via oral drench once a day for 5 days, 2) 3 days of B+C and 3 days of PG, 3) 25 ml placebo (sterile 0.9% saline) subcutaneously once a day for 3 days and PG for 5 days, or 4) 3 days of placebo and 3 days of PG. In 3 of the study herds, 5 days of administration of PG was not possible due to labor constraints and animals were randomly assigned to treatment with 3 days B+C and 3 days PG or 3 days placebo and 3 days PG. The concentration of PG in the drench varied by study site, so animals were treated with 450 ml of liquid (67% PG, Glycol-P, Vetoquinol, Lavaltrie, QC, Canada) in herds in Ontario and 300 ml of liquid (100% PG) in the herd in Michigan. The label dose for B+C is 2 ml/45 kg body weight. A fixed volume dose of 25 ml was selected for convenience, to maintain consistency across study sites, and to facilitate blinding. Subcutaneous injections were split so a maximum of 10 ml was administered per site. Randomization was performed using a random number generator so that sequential blocks of four cows included 1 cow of each treatment group. In the Michigan herd, treatments were randomized separately for primiparous and multiparous animals. In Ontario herds, all animals within a herd were included on the same randomization list. Individuals performing the testing and assigning and administering treatments, farm personnel, and veterinarians were blinded to B+C treatment. Farm personnel and veterinarians were blinded to the duration of PG treatment.

Enrolled cows were tested for blood BHBA and glucose concentrations at weekly intervals for 2 weeks after treatment. Further data collected included lactation number,

DIM at enrollment, and daily milk weights (Farms C, H, and Z). Milk weights were exported from DairyComp 305 (Valley Agricultural Software, Tulare, CA) or AfiFarm (Afimilk, Kibbutz Afikim, Israel) throughout the study period. Milk weights recorded as "0" were re-entered as missing data points. Animals were excluded from analysis if they did not receive treatments, were not tested at 1 and 2 weeks after treatment, or died or were sold before 2 weeks after treatment. Animals were excluded from milk analysis if they had fewer than 10 daily milk weights during the study period.

The people responsible for health of the animals on the farm were allowed to treat enrolled animals for other significant diseases as needed after the first 5 days of study treatment. They were asked to record any additional treatments given and this was included in the analyses as covariates. Study personnel verbally confirmed whether or not any additional treatments had been administered to enrolled animals on a weekly basis.

Based on sample size calculations, this study was designed to screen 1,600 cows. With an expected ketosis incidence rate of 20%, this would yield 320 cows with ketosis and 80 cows per treatment group. Given 95% confidence, 80% power, and 0.45 mmol/L standard deviation, this sample size would allow for detection of a 0.2 mmol/L difference in blood BHBA between treatment groups. The proposal was approved by the University of Guelph Animal Care Committee (11R036) and the Michigan State University Institutional Animal Care and Use Committee (#04/11-078-00).

Statistical Analysis

Statistical analyses were completed in SAS (Version 9.3, SAS Institute, Cary, NC). The outcomes of interest were cure at 1 week post-treatment, maintenance of cure

at the second week post-treatment, blood BHBA concentrations at 1 and 2 weeks post-treatment, and milk production for 30 days after treatment. Cure was defined as blood BHBA < 1.2 mmol/L at 1 week post-treatment. Maintenance of cure was defined as blood BHBA < 1.2 mmol/L at 1 and 2 weeks post-treatment. Continuous variables (DIM at enrollment, BHBA, and glucose at all 3 time points) were examined for normality and linearity. DIM was categorized in two ways, enrollment at week 1 (3 to 9 DIM) versus week 2 (10 to 16 DIM) and early (3 to 5 DIM) versus late (6 to 16 DIM). Glucose was categorized into $\geq$ 2.2 mmol/L and < 2.2 mmol/L (low) based on previous work (Chapter 3) and was offered to models in one of two forms, either as a linear or a categorical predictor. A natural log transformation was utilized with BHBA values 1 and 2 weeks post-treatment. An effective transformation could not be identified to normalize enrollment BHBA concentrations, so it was categorized into moderate (1.2 to 2.4 mmol/L) and high (> 2.4 mmol/L) based on previous work (Chapter 3). Parity was divided into 1, 2 and 3+ lactations. Descriptive statistics were generated using PROC FREQ and PROC MEANS in SAS. Each variable was examined for association with the selected outcomes using contingency tables and the Chi-square statistic for categorical variables and univariable linear regression for continuous variables using PROC GLM. Any variable $P \leq 0.2$ was offered to multivariable models.

For the outcomes cure and maintenance of cure, Poisson regression was used (PROC GENMOD in SAS) with a log link, Poisson distribution, and an exchangeable correlation structure. Clustering by herd and overdispersion were accounted for in the model by including a random herd effect in the model. A mixed model (PROC MIXED in SAS) was used to examine the effects of treatment on BHBA concentrations 1 and

2 weeks post-treatment and milk production in the first 30 days after treatment with cow as a repeated measure and a first order autoregressive correlation structure. Numerous correlation structures were examined and the model with the lowest Akaike Information Criterion (AIC) was selected.

All variables with a univariate association with the outcome ($P \leq 0.2$) were offered to the respective multivariable models. Models were built manually via backward stepwise elimination, with the variables with the highest *P*-values removed first until only variables associated with the outcome ($P \leq 0.05$) remained. At removal of each variable, the model was examined for evidence of confounding (changes in coefficients by > 20%). Treatments (B+C and PG duration) were retained in the models regardless of *P*-value. In the case that two forms of a variable (linear and categorical or two categorization schemes) where both significantly associated with the outcome, they were tested in separate backwards stepwise multivariable models and the model with the lowest AIC or quasi-likelihood under the independence model criterion (QIC) was selected. Interactions were formed between treatments and retained if significant. Next, interactions were formed between other variables in the final model and retained if biologically relevant and significant ($P \leq 0.05$). If treatment was part of a significant interaction with another predictor, the outcome was stratified by the predictor and stratum specific results were presented.

RESULTS

Descriptive Statistics

Of the 1,742 cows tested, 763 were diagnosed with ketosis (blood BHBA $\geq$ 1.2 mmol/L, 44%). Herd incidence is listed in Appendix 4.1 and ranged from 31% to

69%. There were 601 cows enrolled (78%) between 3 and 9 DIM and 162 enrolled (22%) between 10 and 16 DIM. Due to labor constraints, there was a maximum number of cows that could be enrolled per herd per week, so 120 of these animals were not enrolled. A further 49 animals were lost to follow up because they did not receive study treatments (n = 12), were missed on follow up testing (n = 15), were enrolled in the study twice (n = 2), died (n = 12), or were sold (n = 8). This left 594 animals for analysis.

Descriptive statistics for the 594 animals retained in the study are provided in Appendix 4.2. There was no difference in lactation number (2.7 $\pm$ 1.5), DIM at enrollment (7.3 $\pm$ 3.3 days), or BHBA concentrations (2.2 $\pm$ 1.1 mmol/L) at enrollment between study groups ($P > 0.5$). One hundred sixty-seven cows (28%) had enrollment BHBA > 2.4 mmol/L and 220 (37%) had enrollment glucose < 2.2 mmol/L. There was no difference in these two measures between treatment groups ($P > 0.5$).

Effect of Treatment on Ketosis Resolution

Variables offered to the model for ketosis cure included enrollment BHBA group (1.2 to 2.4 versus > 2.4 mmol/L, $P = 0.001$), parity ($P = 0.0002$), glucose group (< 2.2 versus $\geq$ 2.2 mmol/L, $P = 0.002$), week of enrollment ($P = 0.2$), early (3 to 5 DIM) versus late (6 to 16 DIM) enrollment ($P = 0.1$), B+C ($P = 0.3$), and length of PG treatment (3 versus 5 d, $P = 0.5$). Overall, 42% of cows had resolved ketosis 1 week after diagnosis. Enrollment in the first week (3 to 9 DIM), blood glucose concentrations < 2.2 mmol/L at enrollment, and parity greater than 1 were associated with significantly decreased cure risk (Table 4.1). B+C treatment had no effect on cure risk ($P = 0.5$) and there was no interaction between treatments ($P = 0.2$). There was a significant interaction between blood BHBA at enrollment and PG treatment ($P < 0.0001$). PG treatment did

not affect cure risk in animals with blood BHBA 1.2 to 2.4 mmol/L at enrollment ($P = 0.7$, Table 4.2), but animals with blood BHBA > 2.4 mmol/L at enrollment were 1.7 times more likely to cure if treated with 5 days of PG versus 3 days (95% CI 1.4 to 2.2, $P < 0.0001$, 30% versus 18%, respectively).

Variables offered to the model for maintenance of ketosis cure (n = 252 cows) included enrollment BHBA group ($P = 0.0002$), parity ($P < 0.0001$), glucose group ($P < 0.0001$), week of enrollment ($P = 0.1$), early versus late enrollment ($P = 0.1$), B+C ($P = 0.9$), and length of PG treatment ($P = 0.8$). None of the variables measured had a significant association with maintenance of ketosis cure ketosis cure ($P = 0.2$ to 0.9)

Effect of Treatment on Blood BHBA Concentrations

Variables offered to the models for effect of treatment on blood BHBA concentrations at 1 and 2 weeks post-enrollment included enrollment BHBA group ($P < 0.0001$), parity ($P < 0.0001$), glucose group ($P < 0.0001$), B+C ($P = 0.2$), and PG treatment length ($P = 0.6$). In addition, week of enrollment ($P = 0.06$) or early versus late enrollment ($P = 0.1$) were offered to the model for the effects on blood BHBA concentrations at 1 week post-enrollment.

The final models for blood BHBA concentration 1 week and 2 weeks after enrollment are presented in Table 4.3 and Table 4.4, respectively. Low blood glucose at diagnosis and parity greater than 1 were associated with significantly higher blood BHBA concentrations at 1 and 2 weeks after enrollment. B+C treatment had no significant effect on blood BHBA concentrations at either time point and there was no interaction between treatments ($P = 0.4$ and 0.9).

In the model for blood BHBA concentrations 1 week after enrollment there was a significant interaction between BHBA concentrations at enrollment and length of PG treatment ($P = 0.03$). Extended PG treatment had no significant effect on blood BHBA concentrations at 1 week after enrollment (1.5 $\pm$ 1.2 mmol/L) for animals with moderate blood BHBA concentrations at enrollment ($P = 0.7$). However, animals with blood BHBA > 2.4 mmol/L at enrollment had a significantly greater reduction (-0.25 $\pm$ 0.11 mmol/L) in blood BHBA concentrations 1 week after enrollment when treated with 5 days of PG ($P = 0.02$).

There was no effect of PG duration on BHBA concentrations at the second follow-up test. Though blood BHBA concentrations > 2.4 mmol/L at enrollment were associated with significantly higher BHBA concentrations at the second follow-up test ($P = 0.02$), there were no interactions between enrollment BHBA concentrations and treatment at this time point ($P = 0.6$).

Effect of Treatment on Daily Milk Production for 30 Days After Enrollment

Variables offered to the model for milk production in the 30 days after enrollment included parity ($P < 0.0001$), glucose group ($P < 0.0001$), month of calving ($P < 0.0001$), week of enrollment ($P < 0.0001$), early versus late enrollment ($P = 0.1$), B+C ($P = 0.2$), and PG duration (3 versus 5 d, $P = 0.03$).

The final model for milk production is presented in Table 4.5. Milk production per day decreased as animals calved later in the summer. Animals that were diagnosed with ketosis at the first test (3 to 9 DIM) produced 2.4 kg/d less milk than animals that were diagnosed at the second test (10 to 16 DIM). There was no interaction between treatments ($P = 0.5$). However, there were significant interactions between blood glucose

at enrollment and both B+C treatment and PG treatment length. Treatment effects stratified by blood glucose at enrollment are provided in Table 4.6. Neither treatment had a significant effect on milk production in animals with blood glucose ≥ 2.2 mmol/L at enrollment. However among animals with low blood glucose at enrollment, extended PG treatment increased milk production by 3.4 kg/d (95% CI = 1.7 to 5.1, $P = 0.0001$) and B+C treatment increased milk production by 3.1 kg/d (95% CI = 1.3 to 5.0, $P = 0.01$).

DISCUSSION

The purpose of this study was to determine the effects of a butaphosphan cyanocobalamin combination product and varying lengths of propylene glycol treatment on ketosis cure risk, blood BHBA concentrations after treatment, and daily milk production in the first 30 days after enrollment. This is the first large-scale field trial to utilize B+C for ketosis treatment and test the difference between 2 discrete durations of PG treatment in multiple herds. Extended PG treatment improved ketosis cure risk and decreased blood BHBA concentrations after treatment in animals that had high blood BHBA concentrations at enrollment and increased milk production in animals with low blood glucose at enrollment. Though B+C did not affect ketosis cure risk or blood BHBA concentrations, the effect of B+C on milk production suggests it may be beneficial in animals with low blood glucose at enrollment. It is important to note that no animals were left completely untreated in this study due to the well-established impacts of untreated ketosis (McArt et al., 2011; McArt et al., 2012a). Thus, we are assuming that 3 days of PG treatment afforded some benefit to all enrolled animals and we are examining the additional effects of B+C or extended PG treatment.

Extended PG treatment improved ketosis cure risk and decreased blood BHBA concentrations 1 week after enrollment in animals with higher blood BHBA concentrations at the time of enrollment (> 2.4 mmol/L). Animals treated with PG for 5 d were 1.7 times more likely to cure than animals treated with 3 days of PG. However, in animals with blood BHBA concentrations from 1.2 to 2.4 mmol/L at enrollment there was no difference between groups. It is not possible to infer whether 3 d of PG is sufficient for cows with BHBA from 1.2 to 2.4 mmol/L and additional days of treatment provide no further benefit, or whether neither duration of PG therapy is beneficial in these cows. PG helps in the treatment of ketosis by providing precursors for and stimulating gluconeogenesis (Nielsen and Ingvartsen, 2004). Increased glucose leads to decreased fat catabolism and decreased ketogenesis. The difference in effect seen based on enrollment BHBA concentrations is likely due to animals with higher BHBA concentrations being more severely affected. These animals may benefit from the effects of additional treatments with PG. However in animals with lower blood BHBA concentrations, the stress of being handled and drenched 2 additional times might outweigh any benefits of the PG treatment.

B+C had no effects on ketosis cure, maintenance of cure, or blood BHBA concentrations at 1 and 2 weeks after treatment. This is counter to previous studies that showed that B+C decreased blood BHBA concentrations (Lohr et al., 2006) and increased ketosis cure risk (Gordon et al., 2012). In the study by Lohr et al. (2006), the significant decrease in blood BHBA concentrations after B+C treatment was as a percent of the BHBA concentration at enrollment, not as an absolute decrease in BHBA concentrations. In fact, there was no difference between treatment groups at any time

point when examined as actual BHBA concentrations. In the preceding study (Gordon et al., 2012), B+C significantly increased ketosis cure in animals with low blood glucose at enrollment, though it had no effect in animals with blood glucose $\geq$ 2.2 mmol/L at enrollment. It is unclear why this interaction was not present in the current study, as blood glucose concentrations were similar in both studies.

As in the previous study conducted by our group (Chapter 3), B+C significantly increased milk production in the first 30 days after treatment in animals with low blood glucose concentrations at enrollment. Treatment with B+C increased milk production by 3.1 kg/d (95% CI = 1.3 to 5.0) in animals with blood glucose < 2.2 mmol/L at the time of enrollment, but had no effect on production in animals with higher blood glucose. The mechanism for this increase in milk production is not completely clear. It has been hypothesized that animals with early lactation ketosis have some level of fat accumulation in the liver and a subsequent decrease in gluconeogenic capabilities of liver tissue (Holtenius and Holtenius, 1996; Herdt, 2000) and that B+C stimulates gluconeogenesis (Rollin et al., 2010). Additionally, glucose is often limiting in cows in early lactation (Herdt, 2000), so an increase in blood glucose would likely increase milk production. Stimulation of gluconeogenesis in animals with normal blood glucose may not have a significant effect, as there is less homeorhetic drive to increase glucose levels. However, in animals where gluconeogenesis is impaired, stimulation may increase blood glucose levels and decrease ketogenesis and fat accumulation in the liver (Herdt, 2000).

A study examining the effects of administration of vitamin B12 around the time of calving (Preynat et al., 2009a; b) showed administration of vitamin B12 increased blood glucose, which corresponded to an increase in milk production. However, animals

in this study were treated with folic acid in conjunction with vitamin B12, so the effects of vitamin B12 alone are unknown. Furthermore, animals in the study by Preynat et al. (2009a; b) were treated weekly with 10 mg cyanocobalamin, a much higher dose than that administered in the current study (1.25 mg per injection for a total of 3.75 mg). More research is required to understand the mechanism of B+C and the relationship of this mechanism with blood glucose levels. However, the increased milk production seen in treated animals in 2 separate studies and in more than one herd suggests that B+C may be beneficial for ketotic animals with low blood glucose at enrollment (< 2.2 mmol/L).

There was also a significant interaction between blood glucose concentrations at enrollment and treatment with PG. Animals with blood glucose < 2.2 mmol/L at the time of ketosis diagnosis produced 3.4 kg/d (95% CI 1.7 to 5.1) more milk in the 30 days after treatment if treated with 5 days of PG versus 3 days. In contrast, animals that had blood glucose at enrollment $\geq$ 2.2 mmol/L tended ($P = 0.06$) to produce less milk (-1.6 kg/d, 95% CI -3.2 to 0) per day if treated with 5 days of PG. Since PG acts as a glucose precursor, this would likely increase the amount of glucose available and subsequently increase milk production (Nielsen and Ingvartsen, 2004). Again, in animals with low blood glucose the effect would likely be larger, as there is a homeostatic and homeorhetic drive to increase blood glucose concentrations. Increased blood glucose should decrease lipolysis and ketogenesis to decrease the effects of hyperketonemia on production as well. A recent study found that treatment with PG increased milk production in animals treated for ketosis, but this increase was dependent on herd of origin (McArt et al., 2011). We did not see similar effects of herd in this study. The increase in milk we observed in response to PG treatment in the current study was higher than observed by McArt et al.

(2011). However, they did not measure blood glucose and thus could not look at the conditional association between glucose concentrations and milk production. Additionally, animals in the study by McArt et al. (2011) were tested three times a week and treated until blood BHBA concentrations were < 1.2 mmol/L, ≥ 3.0 mmol/L or the animals reached 16 DIM (2 to 13 days duration of treatment). Thus, though the time of eligibility for enrollment in both studies was similar, there are too many differences in study design to make a direct comparison of the magnitude of treatment effects.

The lack of benefit of 2 additional days of treatment with PG in animals with blood glucose ≥ 2.2 mmol/L at the time of diagnosis may be due to the fact that gluconeogenesis is not stimulated by the administration of PG in these animals or not stimulated to the same amount as animals with low blood glucose. The lack of benefit combined with the fact that restraining animals for administration of an oral drench may be stressful and stress can reduce feed intake (Ingvartsen, 2006) might explain the trend for decreased production in animals with blood glucose ≥ 2.2 mmol/L at ketosis diagnosis.

The relationship of blood glucose concentrations at enrollment with treatment effects on ketosis cure risk, blood BHBA concentrations 1 and 2 weeks after enrollment and milk production in the first 30 days after enrollment is novel. The relationship between blood glucose concentrations and ketosis has been used to classify ketosis (Sakai et al., 1993; Holtenius and Holtenius, 1996). However, the relationship has not been studied in a large-scale clinical trial on ketosis treatment. This is the second study from our group to show that low blood glucose at time of ketosis diagnosis decreases risk of cure, increases blood BHBA concentrations in the 2 weeks after enrollment, and affects

milk production in the 30 days after enrollment. Holtenius and Holtenius (1996) suggested that cows with low blood glucose were animals where gluconeogenesis was maximally stimulated and further production of glucose was limited by lack of gluconeogenic precursors. These animals were generally diagnosed during peak production. They also suggested that animals diagnosed around the time of parturition tended to have normal to high blood glucose and some level of fat accumulation in the liver. We did not measure liver fat content of enrolled animals, so the status of enrolled animals in regards to fatty liver is unknown. However, this suggests that there is more to learn about the relationship between blood glucose and blood BHBA concentrations and the potential differences in mechanisms of ketosis development in early lactation animals.

Although B+C did not affect ketosis resolution or blood BHBA concentrations, it can still be recommended as an adjunct to PG treatment to increase production in animals with low blood glucose (< 2.2 mmol/L) at the time of ketosis diagnosis. Furthermore, 5 d of PG therapy can be recommended in animals with blood BHBA concentrations > 2.4 mmol/L or blood glucose concentrations < 2.2 mmol/L at the time of ketosis diagnosis.

CONCLUSIONS

These results show the benefit to ketosis resolution of extended PG therapy for animals with higher blood BHBA concentrations at enrollment. Each of PG and B+C treatment can increase early lactation milk production in animals with low blood glucose at the time of ketosis diagnosis. Blood glucose may be an important predictor of treatment efficacy for subclinical ketosis and more research is required to understand the relationship between blood BHBA and glucose concentrations in ketotic animals.

REFERENCES

Baird, G. D. 1982. Primary ketosis in the high-producing dairy cow: Clinical and subclinical disorders, treatment, prevention, and outlook. J. Dairy Sci. 65:1-10.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

Gordon, J. L., S. J. LeBlanc, L. Neuder, T. H. Herdt, D. F. Kelton, and T. F. Duffield. 2012. Efficacy of a combination butaphosphan and cyanocobalamin product and insulin for ketosis treatment. J. Dairy Sci. 95 (Suppl. 2):177. (Abstract).

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Vet. Clin. North Am. Food Anim. Pract. 16:215-230.

Holtenius, P. and K. Holtenius. 1996. New aspects of ketone bodies in energy metabolism of dairy cows: A review. J. Vet. Med. Ser. A. 43:579.

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

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.

Johnson, R. B. 1954. The treatment of ketosis with glycerol and propylene glycol. Cornell Vet. 44:6-21.

Kennedy, D. G., A. Cannavan, A. Molloy, F. O'Harte, S. M. Taylor, S. Kennedy, and W. J. Blanchflower. 1990. Methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthetase (EC 2.1.1.13) in the tissues of cobalt-vitamin 12 deficient sheep. Br. J. Nutr. 64:721-732.

Lohr, B., B. Brunner, H. Janowitz, M. Hummel, T. Seeger, I. Weber, T. Wittek, B. Schmidt, and K. Hellmann. 2006. Clinical efficacy of Catosal® in the treatment of ketosis in cows with left abomasal displacement. Tierärztliche Umschau 61:187-190.

Maplesden, D. C. 1954. Propylene glycol in the treatment of ketosis. Can. J. Comp. Med. Vet. Sci. 18:287-293.

McArt, J. 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.

McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2012a. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. J. Dairy Sci. 95:2505-2512.

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

Nielsen, N. I. and K. L. Ingvartsen. 2004. Propylene glycol for dairy cows. Anim. Feed Sci. Technol. 115:191-213.

Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. North Am. Food Anim. Pract. 20:651-674.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93:1596-1603.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. 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.

Preynat, A., H. Lapierre, M. C. Thivierge, M. F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009a. Effects of supplements of folic acid, vitamin B12, and rumen-protected methionine on whole body metabolism of methionine and glucose in lactating dairy cows. J. Dairy Sci. 92:677-689.

Preynat, A., H. Lapierre, M. C. Thivierge, M. F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009b. Influence of methionine supply on the response of lactational performance of dairy cows to supplementary folic acid and vitamin B12. J. Dairy Sci. 92:1685-1695.

Rollin, E., R. D. Berghaus, P. Rapnicki, S. M. Godden, and M. W. Overton. 2010. The effect of injectable butaphosphan and cyanocobalamin on postpartum serum beta-hydroxybutyrate, calcium, and phosphorus concentrations in dairy cattle. J. Dairy Sci. 93:978-987.

Sakai, T., T. Hayakawa, M. Hamakawa, K. Ogura, and S. Kubo. 1993. Therapeutic effects of simultaneous use of dextrose and insulin in ketotic dairy cows. J. Dairy Sci. 76:109-114.

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

Wittrock, J. A. M., T. F. Duffield, S. Riuzzi, and S. J. LeBlanc. 2013. Evaluation of an electronic cow-side glucose meter for diagnosing insulin resistance in Holstein dairy cows. J. Dairy Sci. 96:4514-4518.

Table 4.1 Final Poisson regression model of the probability of ketosis cure in 594 Holsteins from 9 herds. Cows were randomly assigned to treatment with B+C and 5 d PG (n = 124), B+C and 3 d PG (n = 176), placebo and 5 d PG (n = 128), or placebo and 3 d PG (n = 166) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L and cure was defined as blood BHBA < 1.2 mmol/L 1 week after treatment.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-0.29	0.07	<0.0001	--	--
Enrollment BHBA					
1.2 to 2.4 mmol/L	--[1]	--	--	--	--
> 2.4 mmol/L	-0.52	0.14	0.0002	0.6	0.4 to 0.8
Week of enrollment[2]					
1	-0.17	0.06	0.004	0.8	0.7 to 0.9
2	--[1]	--	--	--	--
Glucose group					
< 2.2 mmol/L	-0.42	0.19	0.03	0.7	0.5 to 0.9
$\geq$ 2.2 mmol/L	--[1]	--	--	--	--
Parity					
1	--[1]	--	--	--	--
2	-0.28	0.14	<0.0001	0.8	0.6 to 0.9
3+	-0.39	0.10	<0.0001	0.7	0.6 to 0.8
PG[3]					
3 d	--[1]	--	--	--	--
5 d	0.28	0.07	<0.0001	1.3	1.2 to 1.5
B+C[4]					
No	--[1]	--	--	--	--
Yes	-0.03	0.05	0.5	1.0	0.9 to 1.1
PG[3] X Enrollment BHBA			<0.0001		

[1]Reference group
[2]Week of enrollment was defined as 3 to 9 DIM (week 1) or 10 to 16 DIM (week 2)
[3]Propylene glycol
[4]10% butaphosphan cyanocobalamin

Table 4.2 Stratum specific Poisson regression models of ketosis cure in Holstein dairy cows with moderate (1.2 to 2.4 mmol/L, n = 427) or high (> 2.4 mmol/L, n = 167) blood BHBA at enrollment. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L and cure was defined as blood BHBA < 1.2 mmol/L 1 week after treatment.

Variable	β	SE	*P*-value	RR	95% CI
Enrollment BHBA 1.2 to 2.4					
PG[2]					
3 d	--[1]	--	--	--	--
5 d	0.01	0.03	0.7	1.0	1.0 to 1.1
Enrollment BHBA > 2.4 mmol/L					
PG[2]					
3 d	--[1]	--	--	--	--
5 d	0.55	0.12	<0.0001	1.7	1.4 to 2.2

[1]Reference group
[2]Propylene glycol

Table 4.3 Final model for blood BHBA concentrations 1 week after treatment, accounting for repeated measures, in 594 Holsteins from 9 herds. Cows were randomly assigned to treatment with B+C and 5 d PG (n = 124), B+C and 3 d PG (n = 176), placebo and 5 d PG (n = 128), or placebo and 3 d PG (n = 166) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA $\geq$ 1.2 mmol/L.

Variable	β	SE	*P*-value
Intercept	-0.18	0.08	0.08
Enrollment BHBA			
1.2 to 2.4 mmol/L	--[1]	--	--
> 2.4 mmol/L	0.34	0.07	<0.0001
Glucose group			
< 2.2 mmol/L	--[1]	--	--
$\geq$ 2.2 mmol/L	0.29	0.06	<0.0001
Parity			
1	--[1]	--	--
2	0.23	0.09	0.003
3+	0.30	0.08	0.003
PG[2]			
3 d	--[1]	--	--
5 d	-0.11	0.06	0.08
B+C[3]			
No	--[1]	--	--
Yes	-0.09	0.05	0.1
PG[2] X Enrollment BHBA	--	--	0.03

[1]Reference group
[2]Propylene glycol
[3]10% butaphosphan cyanocobalamin

Table 4.4 Final model for blood BHBA concentrations 2 weeks after treatment, accounting for repeated measures, in 594 Holsteins from 9 herds. Cows were randomly assigned to treatment with B+C and 5 d PG (n = 124), B+C and 3 d PG (n = 176), placebo and 5 d PG (n = 128), or placebo and 3 d PG (n = 166) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L.

Variable	β	SE	*P*-value
Intercept	-0.121	0.10	0.08
Enrollment BHBA			
1.2 to 2.4 mmol/L	--[1]	--	--
> 2.4 mmol/L	0.19	0.08	0.02
Glucose group			
< 2.2 mmol/L	--[1]	--	--
≥ 2.2 mmol/L	0.24	0.07	0.001
Parity			
1	--[1]	--	--
2	0.38	0.10	<0.0001
3+	0.42	0.09	<0.0001
PG[2]			
3 d	-0.04	0.07	0.5
5 d	--[1]	--	--
B+C[3]			
Yes	-0.04	0.07	0.5
No	--[1]	--	--

[1]Reference group
[2]Propylene glycol
[3]10% butaphosphan cyanocobalamin

Table 4.5 Final model, accounting for repeated measures, for milk production (kg/d) in the first 30 days after treatment in 366 Holstein dairy cows from 3 herds. Animals were randomly assigned to treatment with B+C and 5 d PG (n = 89), B+C and 3 d PG (n = 93), placebo and 5 d PG (n = 91), or placebo and 3 d PG (n = 93) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L.

Variable	Milk difference per day (kg)	SE	*P*-value	95% CI
Parity				
1	--[1]	--	--	--
2	12.9	0.75	<0.0001	11.7 to 15.1
3+	15.3	0.79	<0.0001	13.5 to 17.0
Week of enrollment[2]				
1	-2.4	0.57	<0.0001	-1.3 to -3.5
2	--[1]	--	--	--
Month of calving				
May	--[1]	--	--	--
June	-5.3	0.61	<0.0001	-6.5 to -4.1
July	-7.1	0.61	<0.0001	-8.3 to -5.9
Glucose group				
< 2.2 mmol/L	--[1]	--	--	--
≥ 2.2 mmol/L	-1.7	0.47	0.0003	-2.6 to -0.8
PG[3]				
3 d	--[1]	--	--	--
5 d	-0.28	0.45	0.5	-1.2 to 0.6
B+C[4]				
No	--[1]	--	--	--
Yes	0.50	0.45	0.3	-0.4 to 1.4
PG[3] x glucose group	--	--	0.005	--
B+C[4] x glucose group	--	--	0.04	--

[1]Reference group
[2]Week of enrollment was defined as 3 to 9 DIM (week 1) or 10 to 16 DIM (week 2)
[3]Propylene glycol
[4]10% butaphosphan cyanocobalamin

Table 4.6 Stratum specific model, accounting for repeated measures, for milk production (kg/d) in the first 30 days after treatment in Holstein dairy cows with blood glucose < 2.2 mmol/L (low, n = 146) and ≥ 2.2 mmol/L (n = 220) at enrollment.

Variable	Milk difference per day (kg)	SE	*P*-value	95% CI
Glucose at enrollment < 2.2 mmol/L				
PG[2]				
3 d	--[1]	--	--	--
5 d	3.4	0.87	0.0001	1.7 to 5.1
B+C[3]				
No	--[1]	--	--	--
Yes	3.1	0.94	0.01	1.3 to 5.0
Glucose at enrollment ≥ 2.2 mmol/L				
PG[2]				
3 d	--[1]	--	--	--
5 d	-1.6	0.83	0.06	-3.2 to 0
B+C[3]				
No	--[1]	--	--	--
Yes	0.21	0.80	0.8	-1.4 to 1.8

[1]Reference group
[2]Propylene glycol
[3]10% butaphosphan cyanocobalamin

Appendix 4.1 Weekly ketosis incidence for 1,742 Holstein dairy cows from 9 herds on a weekly testing schedule for ketosis from 3 to 16 DIM. Ketosis was defined as blood BHBA concentrations $\geq$ 1.2 mmol/L.

Herd	Number tested N	Total ketotic N (%)	Week 1 (3-9 DIM) N (% of ketotic)	Week 2 (10-16 DIM) N (% of ketotic)
A	135	68 (50%)	59 (87%)	9 (13%)
B	73	24 (33%)	16 (67%)	8 (33%)
C	179	123 (69%)	64 (52%)	59 (48%)
D	35	11 (31%)	11 (100%)	0 (0%)
E	46	26 (57%)	22 (85%)	4 (15%)
F	83	41 (49%)	36 (88%)	5 (12%)
G	64	30 (47%)	28 (93%)	2 (7%)
H	162	55 (50%)	42 (76%)	13 (24%)
Z	965	385 (40%)	323 (83%)	62 (17%)
Total	1,742	763 (44%)	601 (78%)	162 (22%)

Appendix 4.2 Descriptive statistics for 594 animals from 9 herds enrolled in a ketosis treatment trial utilizing B+C and varying lengths of PG treatment.

Variable	Unit of measurement	Mean	Median	Standard Deviation	Min	Max
Lactation		2.7	3.0	1.5	1	12
DIM at enrollment	days	7.3	7.0	3.3	3	16
BHBA at enrollment	mmol/L	2.2	1.8	1.1	1.2	7.5
BHBA at 1 wk post-treatment	mmol/L	1.8	1.3	1.4	0.2	8.7
BHBA at 2 wk post-treatment	mmol/L	1.8	1.2	1.5	0.2	8.7
Glucose at enrollment	mmol/L	2.3	2.3	0.6	0.1	5.9
Glucose at 1 wk post-treatment	mmol/L	2.6	2.6	0.7	0.1	5.1
Glucose at 2 wk post-treatment	mmol/L	2.7	2.7	0.7	0.2	6.2

Appendix 4.3 Descriptive statistics by lactation group for 366 animals from 3 herds enrolled in a ketosis treatment trial utilizing B+C and varying lengths of PG treatment that collected daily milk weights.

Variable	N	Unit of measurement	Mean	Standard Deviation	Min	Max
DIM at enrollment		days				
Lactation 1	60		7	2.8	3	14
Lactation 2	108		8	3.4	3	16
Lactation 3+	198		7	3.3	3	16
Enrollment BHBA		mmol/L				
Lactation 1	60		2.1	1.0	1.2	5.3
Lactation 2	108		2.0	0.9	1.2	6.7
Lactation 3+	198		2.3	1.2	1.2	7.5
Milk production		kg/d				
Lactation 1	60		32.3	11.6	10.4	60.8
Lactation 2	108		44.1	11.3	10.4	88.9
Lactation 3+	198		46.5	7.9	10.4	94.9

Appendix 4.4 Poisson regression model of maintenance of ketosis cure in 252 Holsteins from 9 herds that were classified as cured at 1 week post-treatment. Cows were randomly assigned to treatment with B+C and 5 d PG (n = 58), B+C and 3 d PG (n = 67), placebo and 5 d PG (n = 53), or placebo and 3 d PG (n = 74) at ketosis diagnosis between 3 and 16 DIM. Ketosis was defined as blood BHBA ≥ 1.2 mmol/L and maintenance of cure was defined as blood BHBA < 1.2 mmol/L at 1 and 2 weeks after treatment.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-0.27	0.06	<0.0001	--	--
PG[2]					
3 d	--[1]	--	--	--	--
5 d	-0.09	0.07	0.2	0.9	0.8 to 1.1
B+C[3]					
No	--[1]	--	--	--	--
Yes	0.07	0.09	0.5	1.1	0.9 to 1.3

[1]Reference group
[2]Propylene glycol
[3]10% butaphosphan cyanocobalamin

CHAPTER FIVE

INDIVIDUAL COW PREDICTORS FOR DEVELOPMENT OF SUBCLINICAL KETOSIS IN EARLY LACTATION DAIRY CATTLE

ABSTRACT

The purpose of this study was to identify risk factors for subclinical ketosis in cows in commercial dairy herds. Data including previous lactation days open, milk production, days dry, days pregnant, age at first calving, and parity were collected for cows from 5 freestall herds (4 in New York and 1 in Michigan) and previous lactation ketosis incidence from 1 herd enrolled in a testing schedule for ketosis diagnosis. Blood β-hydroxybutyrate (BHBA) was measured at weekly intervals between 3 and 16 DIM and ketosis was defined *a priori* as ≥ 1.2 mmol/L. Multivariable Poisson regression models were developed to predict ketosis development during the period of 3 to 16 DIM. Separate models were constructed for primiparous and multiparous animals as the data available differed between age groups. A third model was developed for animals from one herd that were tested in two successive years. A total of 4,620 animals were enrolled in the study with 1,386 (30%) being diagnosed with ketosis between 3 and 16 DIM. Age at first calving was an important predictor of ketosis development in primiparous animals. Previous lactation days open, days dry, and parity were important risk factors for ketosis diagnosis in multiparous cows. In animals tested two years in a row, previous lactation ketosis diagnosis and > 60 days dry increased the risk of ketosis in the current lactation. These results may help identify animals at high-risk of ketosis development that could be targeted for prevention and monitoring.

INTRODUCTION

A period of negative energy balance is nearly ubiquitous around the time of calving for dairy cattle due to increased energy demands to support lactation coupled with a slower increase in dry matter intake (Bauman and Currie, 1980; Herdt, 2000). During this period, body stores are utilized to meet energy requirements (Bauman and Currie, 1980) and ketone bodies are formed as a part of this process (Herdt, 2000). Though this is normal, animals that are not able to adequately adapt may experience a period of excessive hyperketonemia (Herdt, 2000).

Recent studies in New York and Wisconsin reported the overall lactational incidence of subclinical ketosis (SCK) to be about 35% with herds ranging from 26 to 54% (McArt et al., 2011) and the onset of significant negative herd effects to be at 15 to 20% of the herd affected (Ospina et al., 2010b). The negative impacts of excessive ketone bodies in the blood have been well documented and include increased risk of displaced abomasum (LeBlanc et al., 2005; Duffield et al., 2009), decreased early lactation milk production (Duffield et al., 2009; McArt et al., 2012), increased culling risk (McArt et al., 2012), increased risk of prolonged anovulation (Walsh et al., 2007a), and decreased pregnancy at first artificial insemination (Walsh et al., 2007b).

Cows in later lactations are generally at an increased risk of developing SCK (Dohoo et al., 1984a; Gröhn et al., 1989; Rasmussen et al., 1999; McArt et al., 2012), though it is not a linear relationship in most studies. Due to the relatively low incidence of ketosis in primiparous animals, little research has examined risk factors specific to development of ketosis in the first lactation. One study found that first lactation animals that were diagnosed with ketosis had a significantly higher age at first calving than those

that were not affected (van Dam et al., 1988). This association is plausible due to the propensity of non-lactating animals to increase body condition as they age, but specific mechanisms that might explain how this would lead to ketosis are lacking.

Dry period length has been associated with ketosis risk in many studies (Markusfeld et al., 1997; Rastani et al., 2005; Watters et al., 2008; Santschi et al., 2011). Studies that have examined a short dry period (35 days or less) have found a decreased risk of ketosis in animals that have a shortened dry period (Rastani et al., 2005; Watters et al., 2008; Santschi et al., 2011) with little to no effect on production or reproduction in the subsequent lactation. It should be noted that herd differences did occur in response to shortened dry period (Santschi et al., 2011), suggesting that other management factors may affect the success of a short dry period in a herd. There has been no research into the association between variables from the previous lactation, such as length of lactation, BCS at the end of the lactating period, and days open, and risk of ketosis in the subsequent lactation.

Ketosis risk is multi-factorial and many risk factors are not well understood. By better understanding what increases the risk of ketosis efforts in prevention and testing can be targeted to high-risk groups. This could potentially decrease the level of hyperketonemia in a herd and the incidence of negative sequelae. The objective of this study was to identify risk factors for development of SCK in early lactation under commercial conditions.

MATERIALS AND METHODS

Study Population

Data were collected from 4 dairy farms in New York (Herds A, B, C, and D) between April 8, 2010 and June 6, 2011 and from 1 dairy farm in Michigan (Herd Z) between May 16, 2011 and August 22, 2011, and again between May 14, 2012 and August 13, 2012. All herds were enrolled in one of 3 randomized clinical trials for ketosis treatment approved by the University of Guelph Animal Care Committee (10R008 and 11R036) and the Michigan State University Institutional Animal Care and Use Committee (#04/11-078-00). Farms were purposively selected due to their proximity to study sites, herd size, use of Dairy Comp 305 farm management software (Valley Agricultural Software, Tulare, CA), and willingness to participate and comply with the ketosis testing and treatment protocols.

Data Collection and Study Design

Herds were visited weekly during the study period. During this time, all animals between 3 and 16 DIM were tested for SCK using the Precision Xtra (Abbott Laboratories, Abbot Park, IL) hand-held meter. Blood was drawn from coccygeal vessels using a 20 gauge x 2.54 cm needle and 3 cc syringe and ketone testing was performed immediately after blood collection according to the Precision Xtra manufacturer's instructions. A ketone test strip was inserted into the device and the lot number on the strip was checked against the lot number displayed on the meter. Once the "add blood" symbol appeared on the meter screen, a drop of blood was added to the strip until the meter showed the test chamber was full. After 10 seconds, the blood BHBA concentration was displayed on the screen and recorded. Ketosis was defined *a priori* as

blood BHBA of $\geq$ 1.2 mmol/L. The Precision Xtra has been previously validated for use in cattle with sensitivity of 88% and specificity of 96% at this cut-point (Iwersen et al., 2009). Cows were excluded from blood testing if they had been diagnosed with a displaced abomasum or ketosis prior to the study visit or if their previous blood test was $\geq$ 1.2 mmol/L.

Data were exported from Dairy Comp 305 throughout the study period for all animals enrolled. These data included calving date (FDAT) and previous gestation length (previous days carried calf, PDCC). For first lactation animals, age at first calving (AFC) was also obtained. Days dry (DDRY), previous lactation total milk yield (PTOTM), and previous lactation 305 mature equivalent milk yield (P305ME) was also obtained for animals in their second or greater lactation. Sample size calculations were performed for the respective clinical trials and records from all eligible animals were used in this study.

Cows were excluded from analysis if their PDCC was less than 260 (n = 78), their DDRY was less than 30 (n = 32), or they had incomplete data (n = 30). Many animals fit into more than one category, so final exclusions included 39 primiparous and 65 multiparous animals.

Statistical Analysis

All statistical analyses were performed using SAS (Version 9.3, SAS Institute, Cary, NC, USA) with cow as the unit of analysis. The binary outcome of interest was diagnosis of SCK during the period from 3 to 16 DIM. Continuous variables (AFC, DCC, DDRY, PDOPN, PTOTM, and P305ME) were assessed for homoscedasticity graphically. PTOTM and P305ME were converted to kilograms and a herd mean was

established for PTOTM and P305ME for each herd. Individual cow PTOTM or P305ME values were subtracted from the mean PTOTM or P305ME for their herd of origin to assign each cow a value relative to the mean of the herd (PTOTMadj and P305MEadj). Correlation between variables was determined using PROC CORR of SAS, with variables considered to be collinear if their Pearson correlation coefficient was ≥ 0.3. PTOTM and PTOTMadj were highly correlated ($r = 0.7$) to both P305ME and PDDRY and were discarded from further analysis. AFC, PDDRY and PDOPN were not normally distributed and were categorized for analysis. DDRY was initially categorized into short (30 to 45 days), planned (45 to 60 days) and long (> 60 days). There was no difference in association to SCK risk between the short and normal dry period groups, so they were combined to form a final categorization of planned (30 to 60 days) and long (> 60 days) dry period. AFC was categorized based on literature and the mean of the data (< 24 and $\geq$ 24 months). PDOPN was categorized using the median of the data (<100 and $\geq$ 100 days). DCC was categorized into short (less than one standard deviation below the mean, < 273 days) and normal to prolonged ($\geq$ 273 days) gestation. Parity was divided into 1, 2 and 3+ lactations. Descriptive statistics were generated with PROC FREQ and PROC MEANS in SAS. Each variable was examined for association with SCK diagnosis with contingency tables and the Chi-square statistic using PROC FREQ or linear regression using PROC GLM. Any variable $P \leq 0.2$ was offered to multivariable models.

Poisson regression was used to predict the development of SCK (PROC GENMOD in SAS) using a log link and Poisson distribution. Clustering by herd and overdispersion were accounted for in the model with an exchangeable correlation structure. This type of model was used to express associations as risk ratios (RR) rather

than odds ratios (OR, Ospina et al., 2012). Risk ratios report probabilities directly, are more accurate when the outcome of interest is common, and are easier to interpret (Ospina et al., 2012). All variables associated with SCK ($P \leq 0.2$) were offered for inclusion in the respective models and the models were built manually via backward stepwise elimination. As data available differed between primiparous and multiparous animals, separate models were constructed to predict SCK in each age group. Models were also examined for changes in association based on DIM (week 1 versus 2 and 3 to 5 versus 6 to 16 DIM) at SCK diagnosis. There were no differences in association among risk factors and SCK incidence between the time periods, so one model was formed for the entire period of 3 to 16 DIM. Herd was also tested as a fixed effect in the model to examine differences in association of predictors by herd. Additionally, a separate model was constructed for animals (n = 334) that had undergone testing two years in a row to examine the effects of previous lactation SCK incidence on current lactation risk. Variables with the highest *P*-value were removed first until only variables with $P < 0.05$ remained in the model. The model was examined for evidence of confounding (changes in coefficients by > 20%) at the removal of each variable. Interactions were formed between all variables in final the model and retained if significant ($P < 0.05$). Models were constructed with DCC as both a linear and dichotomized variable and the form of the variable in the model with the lowest quasi-likelihood under the independence model criterion (QIC) was selected.

RESULTS

A total of 4,620 animals were enrolled in the study, 1,709 primiparous and 2,911 multiparous animals. The overall lactational incidence of SCK was 30% of cows tested

weekly, once or twice, between 3 and 16 DIM. Between 3 and 9 DIM, 1,037 of 4,620 (22%) cows had BHBA $\geq$ 1.2 mmol/L and between 10 and 16 DIM 347 of 3,583 (10%) cows had SCK. The incidence of SCK varied greatly from 15 to 45% among herds (Appendix 5.1). The highest incidence of SCK was observed in animals in their third or later lactation (47%) and incidence decreased with decreasing parity (29% and 16% for lactation 2 and 1 respectively). Of the 334 multiparous cows in one herd tested in two consecutive years, 76 of 126 (60%) of cows that had SCK in the previous lactations had it the following lactation. This is in comparison to 86 of 208 (41%) incidence of SCK among cows that were not diagnosed the previous year (equal to the herd average incidence in the second year). Descriptive statistics are provided in Table 5.1.

Prediction of SCK in First Lactation Animals

The final model for prediction of development of SCK in first lactation animals between 3 and 16 DIM is presented in Table 5.2. When herd was examined as a fixed effect, herd A was chosen as the referent herd because the herd incidence of SCK was the lowest. There was a significant effect of herd on SCK risk ($P < 0.0001$), but there was no interaction between herd and AFC. Ketosis incidence was higher in animals that calved for the first time after 2 years of age.

Prediction of SCK in Mature Cows

The final Poisson regression model for multiparous animals is provided in Table 5.3. DCC was not significant when offered to the model as either a linear ($P = 0.3$) or class ($P = 0.5$) variable. Previous lactation days open, DDRY, and parity had a significant effect on SCK risk. When herd was examined as a fixed effect herd A was chosen as the referent herd to maintain consistency; SCK incidence was again lowest in

herd A. Herd had a significant effect ($P < 0.0001$) on SCK risk in the first 3 to 16 DIM in mature animals and was part of significant interactions ($P < 0.0001$) with parity and DDRY (Appendix 5.2). There was no significant interaction between herd and PDOPN ($P = 0.4$).

Prediction of SCK in Animals Tested Two Consecutive Years

The model for prediction of SCK in animals that were tested two years in a row is presented in Table 5.4. After accounting for the effect of dry period length, animals that were diagnosed with SCK the previous year were 2.1 times more likely to become ketotic the subsequent year ($P = 0.002$). There was no interaction between DDRY and previous lactation SCK risk.

DISCUSSION

The important predictors for development of SCK between 3 and 16 DIM were parity, AFC, PDOPN, DDRY, and in a subset of the data SCK in the previous lactation. Herd was also a significant predictor of SCK risk. The difference in herd risk is not surprising as it is well documented that ketosis risk is affected by herd management factors, many of which were not directly measured in this study. Collection of more herd management factors such as diet composition, housing conditions, group sizes and cow factors such as BCS before calving and concurrent disease would help elucidate more of the determinants of SCK.

Age at first calving was also highly associated with SCK risk, with animals that calved for the first time at or after 24 months of age being 1.7 times more likely to become ketotic than those that calved before 24 months of age ($P < 0.0001$). This is in agreement with van Dam et al. (1988), who found that animals that were diagnosed with

SCK in early lactation had a significantly higher age at first calving than their herd mates. This difference may be due to an increase in BCS as the animals age but do not commence lactation. Unfortunately, the study design of the treatment trials did not allow for collection of BCS data. It would be interesting to collect both pieces of information on a group of animals to determine the relationship between BCS and age in nulliparous animals.

The predictive concordance for the model for first lactation animals was 68%. Predictive concordance is calculated to measure the accuracy of the model. In this case, the model was able to correctly identify 68% of first lactation animals that developed SCK using just the herd incidence and age at first calving. This level of predictive concordance is similar to other models recently constructed to predict SCK during the period from 3 to 16 DIM in all lactations (McArt et al., 2013). However, this is the first model constructed for primiparous animals alone. The model constructed by McArt et al. (2013) had information on NEFA and BCS precalving. These measures are known risk factors for development of ketosis (Gillund et al., 2001; Busato et al., 2002; Ospina et al., 2010a) and were not collected in our study. Collection of these measures may help improve the predictive ability of this model.

Longer previous lactation days open and dry period length, and higher parity were all significant predictors of development of SCK in multiparous dairy cattle ($P \leq 0.0004$). It is important to note that when herd was included as a fixed effect in the model, it was part of a significant interaction ($P < 0.0001$) with DDRY and parity. General inferences can still be made about these variables, as the magnitude of the difference in risk based

on dry period or parity varied between herds but the general association did not vary. Ketosis risk was increased in all herds if dry period was > 60 days or parity was $\geq$ 3.

The risk of SCK was 1.3 (95% CI = 1.1 to 1.5) times higher in animals that were open more than 100 days in their previous lactation. There are many factors that could lead to this association. It has been established that the risk of ketosis is increased if an animal was diagnosed with ketosis in the previous lactation (Dohoo and Martin, 1984b; Bendixen et al., 1987; Rasmussen et al., 1999). Additionally, ketosis can lead to an increase in time to pregnancy (Roche, 2006; Walsh et al., 2007b). Thus, it would be expected that an animal that was subclinically ketotic in the previous lactation would have a longer lactation period before becoming pregnant. Previous lactation days open could thus be acting as an indicator or confounder of the relationship between ketosis in successive lactations. However, if this were the case, PDOPN should have remained a significant factor in the model for animals that were tested 2 years in a row. This is especially true since there was no interaction between PDOPN and any other variable in the model.

It is more likely that PDOPN is a marker for lactation length, where animals with a longer open period would have a longer lactation. This could lead to a longer period of consumption of a higher energy lactating cow diet without the expenditure of energy for high levels of milk production and likely lead to increased fat accumulation during the late lactation period. This would be especially true for most herds with 1 lactating group TMR. Body condition score at calving higher than 3.25 or 3.5 on a 5-point scale is associated with a higher risk of development of ketosis (Gillund et al., 2001; Busato et al., 2002). McArt et al. (2013) found that cows with a BCS during the close up period at

or above the herd median had a significantly higher risk of development SCK between 3 and 16 DIM. However, they did not explore the relationship between SCK risk and PDOPN in their model. The relationships between time to pregnancy, lactation length, dry period length, and BCS relative to SCK risk needs to be explored further.

The presence of a herd by parity interaction shows that the effect of parity on the development on SCK varied based on the herd of origin. The risk of SCK in third and later lactations was higher in all 5 herds, though the difference was only significant in 4 of the herds. Previous studies have found that the risk of ketosis increases with increasing parity to a peak incidence during the third to sixth lactation (Dohoo et al., 1984a; Rasmussen et al., 1999). McArt et al. (2013) obtained similar results, including a significant herd by parity interaction, in their model for development of hyperketonemia during the same period and using the same definition of hyperketonemia. The herd where the difference in SCK risk between 2 and 3+ lactation animals was not significant had the highest incidence of SCK in second lactation animals (50%). This may be due to management decisions made during the first lactation in this herd that increase PDOPN or BCS at dry-off. It may also be that resources (i.e. bunk and stall space) are limited in this herd and younger animals would be less able to compete for these resources. Whatever the exact reason for the difference, it shows the importance of herd in SCK risk.

Herd also affects the relationship between dry period length and SCK diagnosis. In 3 of the herds, a long dry period had little effect on SCK risk compared to animals in the same herd with a planned dry period. However, in the other 2 herds, animals with a long dry period were 2 times more likely to become ketotic than their herd mates. The

exact reason for this difference is unclear, but it likely has to do with dry period management. One of the most likely causes for increased ketosis risk would be a higher energy dry cow diet (Janovick and Drackley, 2010; Janovick et al., 2011), especially in the far-off dry group (Dann et al., 2006). All animals would likely be exposed to the close-up dry cow group for the same period of time and thus management differences during this period may explain differences in overall ketosis risk between herds. Animals that were dry for a longer period would spend a longer time in the far-off dry group. If the diet is high in energy, these animals would be at high risk of increasing body condition over the dry period and the increased risk of ketosis that is associated with high BCS at calving (Rasmussen et al., 1999; Gillund et al., 2001). Even if BCS is not increased, the risk of ketosis in animals over-fed in the far-off dry period may be higher (Dann et al., 2006). It is also possible that there are other management factors that are affecting the cows during the far-off dry period such as stocking density or group changes. Overcrowding and more frequent group changes in the prepartum period have been associated with decreased dry matter intake and increased risk of ketosis (Goldhawk et al., 2009; Schirmann et al., 2011; Huzzey et al., 2012), and to the extent that these factors were at play a longer dry period may mean greater exposure to these risk factors. Unfortunately, data on group change timing and frequency were not available in this study.

The model developed to predict risk of SCK in mature cows had a predictive concordance of 75%. This measure is comparable to values found in a recent study (McArt et al., 2013). In this study by McArt et al. (2013), they constructed 4 total models, 2 for development of hyperketonemia at first test (3 to 5 DIM) and 2 for

development any time during the period of 3 to 16 DIM. They found that the models for development of hyperketonemia at the first test had higher concordance (87% and 78%) versus models constructed for the entire time period (64% and 69%). We found no difference in predictive concordance or significant factors in the models based on DIM at diagnosis. However, our testing was less frequent than the testing scheme used by McArt et al. (weekly versus 3 times a week, respectively). It may be that the animals that are at risk during the first few days after calving have different risk factors, but these differences are lost when combined into a slightly longer period.

In the model for prediction of SCK in animals that were tested two years in a row, previous lactation SCK diagnosis and DDRY were significant predictors. Animals that were diagnosed with SCK the first year were 2.1 (95% CI = 1.3 to 3.4) times more likely to become ketotic the following year. This is in agreement with previous studies that found the risk of ketosis to be about 2 times higher in animals previously diagnosed with ketosis (Bendixen et al., 1987; Rasmussen et al., 1999). The predictive concordance of this model was 64% and was the lowest in the study. This may be due to the low number of predictors that were significant in the model and may have improved if more information was collected, such as BCS. This model was built with information from only one herd, so the external validity is likely limited. However it shows that previous SCK occurrence is an important predictor of SCK risk and suggests an area for future research.

CONCLUSIONS

Both herd of origin and age at first calving were important predictors of SCK risk in primiparous animals, stressing the importance of breeding management in primiparous

animals. Previous lactation interval to pregnancy was an important predictor of SCK risk in multiparous animals. Dry period length and parity were also important predictors of SCK risk, after accounting for herd of origin. Examination of these factors for individual cows may help identify high-risk cows so preventive measures can be implemented before SCK diagnosis. Diagnosis with ketosis in a previous lactation may also help identify high-risk animals for SCK in subsequent lactations.

REFERENCES

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bendixen, P. H., B. Vilson, I. Ekesbo, and D. B. Astrand. 1987. Disease frequencies in dairy cows in Sweden. IV. Ketosis. Prev. Vet. Med. 5:99-109.

Busato, A., D. Faissler, U. Küpfer, and J. W. Blum. 2002. Body condition scores in dairy cows: associations with metabolic and endocrine changes in healthy dairy cows. J. Vet. Med. Ser. A. 49:455-460.

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.

Dohoo, I. R., S. W. Martin, I. McMillan, and B. W. Kennedey. 1984a. Disease, production and culling in Holstein-Friesian cows. II. Age, season and sire effects. Prev. Vet. Med. 2:655-670.

Dohoo, I. R. and S. W. Martin. 1984b. Disease production and culling in Holstein-Friesian cows. III. Disease and production as determinants of disease. Prev. Vet. Med. 2:671-690.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

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

Goldhawk, C., N. Chapinal, D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2009. Prepartum feeding behavior is an early indicator of subclinical ketosis. J. Dairy Sci. 92:4971-4977.

Gordon, J. L., S. J. LeBlanc, L. Neuder, T. H. Herdt, D. F. Kelton, and T. F. Duffield. 2012. Efficacy of a combination butaphosphan and cyanocobalamin product and insulin for ketosis treatment. J. Dairy Sci. 95 (Suppl. 2):177. (Abstract).

Gröhn, Y. T., H. N. Erb, C. E. McCulloch, and H. S. Saloniemi. 1989. Epidemiology of metabolic disorders in dairy cattle: association among host characteristics, disease and production. J. Dairy Sci. 72:1876-1885.

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Vet. Clin. North Am. Food Anim. Pract. 16:215-230.

Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2012. The effects of overstocking Holstein dairy cattle during the dry period on cortisol secretion and energy metabolism. J. Dairy Sci. 95:4421-4433.

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, N. A. and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086-3102.

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.

LeBlanc, S. J., K. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci. 88:159-170.

Markusfeld, O., N. Galon, and E. Ezra. 1997. Body condition score, health, yield and fertility in dairy cows. Vet. Rec. 141:67-72.

McArt, J. 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.

McArt, J. 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., 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.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010a. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546-554.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010b. 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.

Ospina, P. A., D. V. Nydam, and T. J. DiCiccio. 2012. Technical note: The risk ratio, an alternative to the odds ratio for estimating the association between multiple risk factors and a dichotomous outcome. J. Dairy Sci. 95:2576-2584.

Rasmussen, L. K., B. L. Nielsen, J. E. Pryce, T. T. Mottram, and R. F. Veerkamp. 1999. Risk factors associated with the incidence of ketosis in dairy cows. Anim. Sci. 68:379-386.

Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. J. Dairy Sci. 88:1004-1014.

Roche, J. F. 2006. The effect of nutritional management of the dairy cow on reproductive efficiency. Anim. Reprod. Sci. 96:282-296.

Santschi, D. E., D. M. Lefebvre, R. I. Cue, C. L. Girard, and D. Pellerin. 2011. Incidence of metabolic disorders and reproductive performance following a short (35-d) or conventional (60-d) dry period management in commercial Holstein herds. J. Dairy Sci. 94:3322-3330.

Schirmann, K., N. Chapinal, D. M. Weary, W. Heuwieser, and M. A. von Keyserlingk. 2011. Short-term effects of regrouping on behavior of prepartum dairy cows. J. Dairy Sci. 94:2312-2319.

van Dam, B. P., P. C. Bartlett, J. H. Kirk, and E. C. Mather. 1988. The effect of age at calving on reproduction, milk production and disease incidence in the first lactation of dairy heifers. Theriogenology 30:583-591.

Walsh, R. B., D. F. Kelton, T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2007a. Prevalence and risk factors for postpartum anovulatory condition in dairy cows. J Dairy Sci 90:315-324.

Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007b. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90:2788-2796.

Watters, R. D., J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Crump, P. W. Clark, and R. R. Grummer. 2008. Effects of dry period length on milk production and health of dairy cattle. J. Dairy Sci. 91:2595-2603.

Table 5.1 Descriptive statistics of continuous variables for 4,620 Holstein dairy cows with complete records for analysis.

Variable	N	Unit of measurement	Mean	Median	Standard Deviation	Minimum	Maximum
Lactation	4,620		2.3	2	1.4	1	12
DCC	4,620	days	278	278	5	260	305
AFC	1,709	months	24	22	2	18	33
DDRY	2,911	days	66	57	29	32	219
PDOPN	2,911	days	119	97	64	39	511
TOTM	2,911	kg	12,204	11,994	3,025	1,184	25,580
305ME	2,911	kg	14,092	14,094	2,342	2,818	31,316

DCC = Days carried calf
AFC = Age at first calving
DDRY = Days dry
PDOPN = Days open in the previous lactation
TOTM = Total milk (kg) produced in the previous lactation
305ME = 305 d mature equivalent adjusted milk production (kg) in the previous lactation

Table 5.2 Final Poisson regression model of the probability of ketosis (blood BHBA ≥ 1.2 mmol/L) diagnosed between 3 and 16 DIM in 1,709 first lactation Holstein dairy cows from 5 herds.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-1.8	0.3	< 0.0001	--	--
AFC[2]					
< 24	--[1]	--	--	--	--
≥ 24	0.62	0.09	< 0.0001	1.7	1.4 to 2.1

[1]Reference group
[2]Age at first calving

Table 5.3 Final Poisson regression model of the probability of ketosis (blood BHBA ≥ 1.2 mmol/L) diagnosed between 3 and 16 DIM in 2,911 mature (lactation 2+) Holstein dairy cows from 5 herds.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-2.4	0.04	< 0.0001	--	--
PDOPN[2]					
< 100	--[1]	--	--	--	--
≥ 100	0.27	0.08	0.0004	1.3	1.1 to1.6
DDRY[3]					
≤ 60	--[1]	--	--	--	--
> 60	0.25	0.09	0.01	1.3	1.1 to 1.6
Parity					
2	--[1]	--	--	--	--
3	0.31	0.16	0.04	1.4	1.0 to 1.9

[1]Reference group
[2]Previous lactation days open
[3]Days dry

Table 5.4 Final Poisson regression model of the probability of ketosis (blood BHBA ≥ 1.2 mmol/L) diagnosed between 3 and 16 DIM in 334 multiparous Holstein dairy cows from 1 herd that were tested 2 years in a row.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-0.5	0.2	0.0008	--	--
DDRY[2]					
≤ 60	--[1]	--	--	--	--
> 60	0.5	0.2	0.02	1.7	1.1 to 2.7
Ketosis Yr 1					
Yes	0.7	0.2	0.002	2.1	1.3 to 3.4
No	--[1]	--	--	--	--

[1]Reference group
[2]Days dry

Appendix 5.1 Weekly ketosis incidence for 4,620 Holstein dairy cows with complete records for analysis from 5 herds on a weekly testing schedule for ketosis from 3 to 16 DIM. Ketosis was defined as blood BHBA concentrations $\geq$ 1.2 mmol/L.

Herd	Total N	Week 1 (3-9 DIM) n (%)		Week 2 (10-16 DIM) n (%)		Total n (%)	
		Ketotic	Non-ketotic	Ketotic	Non-ketotic	Ketotic	Non-ketotic
A	743	59 (8%)	684 (92%)	38 (6%)	646 (94%)	97 (15%)	646 (85%)
B	1,100	98 (9%)	1,002 (91%)	114 (11%)	888 (89%)	212 (19%)	888 (81%)
C	525	201 (38%)	324 (62%)	37 (11%)	287 (89%)	238 (45%)	287 (55%)
D	368	45 (12%)	323 (88%)	15 (5%)	308 (95%)	60 (21%)	308 (79%)
Z	1,883	634 (34%)	1,249 (66%)	143 (11%)	1,106 (89%)	777 (41%)	1106 (59%)
All	4,620	1,037 (22%)	3,583 (78%)	347 (10%)	3,236 (90%)	1,384 (30%)	3,236 (70%)

Appendix 5.2 Final Poisson regression model of the probability of ketosis (blood BHBA ≥ 1.2 mmol/L) diagnosed between 3 and 16 DIM in 2,911 mature (lactation 2+) Holstein dairy cows from 5 herds with herd included as a fixed effect.

Variable	β	SE	*P*-value	RR	95% CI
Intercept	-2.4	0.04	< 0.0001	--	--
PDOPN[2]					
< 120	--[1]	--	--	--	--
≥ 120	0.27	0.08	0.0004	1.3	1.1 to 1.5
DDRY[3] x herd					
≤ 60, A	--[1]	--	--	--	--
≤ 60, B	0.23	0.02	< 0.0001	1.3	1.2 to 1.3
≤ 60, C	1.3	0.01	< 0.0001	2.0	2.7 to 2.9
≤ 60, D	0.52	0.0007	< 0.0001	1.7	1.6 to 1.7
≤ 60, Z	1.1	0.02	< 0.0001	3.7	3.6 to 3.8
> 60, A	0.62	0.03	< 0.0001	1.9	1.8 to 1.9
> 60, B	0.92	0.02	< 0.0001	2.5	2.4 to 2.6
> 60, C	1.3	0.005	< 0.0001	3.6	3.6 to 3.7
> 60, D	0.63	0.0007	< 0.0001	1.9	1.8 to 1.9
> 60, Z	1.5	0.01	< 0.0001	4.6	4.3 to 4.6
Parity x herd					
2, A	--[1]	--	--	--	--
2, B	0.13	0.01	< 0.0001	1.1	1.1 to 1.2
2, C	1.1	0.03	< 0.0001	3.1	2.9 to 3.2
2, D	0.44	0.02	< 0.0001	1.5	1.5 to 1.6
2, Z	1.4	0.02	< 0.0001	3.9	3.8 to 4.1
3, A	0.62	0.004	< 0.0001	1.9	1.8 to 1.9
3, B	0.85	0.02	< 0.0001	2.8	2.7 to 2.9
3, C	1.3	0.01	< 0.0001	4.3	4.2 to 4.5
3, D	0.72	0.01	< 0.0001	2.0	2.0 to 2.1
3, Z	1.4	0.007	< 0.0001	4.2	4.1 to 4.3

[1]Reference group
[2]Previous lactation days open
[3]Days dry

CHAPTER SIX

GENERAL CONCLUSIONS

Conclusions

The goal of the research presented in this thesis was to contribute to the knowledge of effective ketosis treatment and ketosis risk factors in lactating dairy cattle.

The first objective was to synthesize the literature on ketosis treatment and elucidate gaps in the literature. The most significant finding of this part of the research was the lack of well-designed ketosis treatment studies that are available in the literature. A total of 1,395 materials including abstracts, theses, and published papers were examined using a systematic review. Ten journal articles remained after the process of the systematic review was completed, too few for a quantitative analysis. Based on the systematic review, a treatment regimen of 300 g propylene glycol orally for 3 to 5 days is recommended for ketotic cows. However, it remains unclear if other commonly used treatments for ketosis (i.e. 50% dextrose) are efficacious. Recommendations from the review to ensure that future ketosis research includes a control group that is ketotic and left untreated or treated with a baseline product (i.e. dextrose versus dextrose and insulin) and that biologically relevant outcomes such as production, reproduction, and culling risk are examined would help to increase the number of well-designed clinical trials performed for future reviews.

The second objective was to assess the efficacy of a butaphosphan-cyanocobalamin (B+C) product, long acting insulin and propylene glycol for ketosis treatment. Two randomized clinical field trials were utilized to meet this objective. In the first study (Chapter 3), we found that cows treated with B+C with blood glucose

< 2.2 mmol/L at the time of enrollment were significantly more likely to cure (BHBA < 1.2 mmol/L at 1 week after treatment) than controls, though there was no significant difference in cure in animals with blood glucose ≥ 2.2 mmol/L at the time of ketosis diagnosis. Furthermore, multiparous cows in 1 herd with blood glucose < 2.2 mmol/L at the time of ketosis diagnosis produced more milk per day in the first 30 days after treatment if treated with B+C, though this difference was not present in animals with blood glucose ≥ 2.2 mmol/L at the time of ketosis diagnosis. Blood glucose at enrollment was also significantly associated with milk production in animals treated with glargine insulin. Multiparous cows in 1 herd that had blood glucose < 2.2 mmol/L at the time of ketosis diagnosis produced significantly more milk per day when treated with glargine insulin. Conversely, cows with blood glucose ≥ 2.2 mmol/L at the time of ketosis diagnosis produced significantly less milk per day in the 30 days after treatment when treated with glargine insulin. The interaction between blood glucose concentrations at the time of ketosis diagnosis and treatment efficacy was interesting and had not been previously described. The findings from this study suggest that B+C may be helpful in ketosis treatment in animals with blood glucose < 2.2 mmol/L at the time of ketosis diagnosis. Glargine insulin had no effect on ketosis cure or blood BHBA concentrations in our study. Treatment of ketosis with glargine insulin have an effect on average daily milk production in early lactation and the nature of the effect may depend on blood glucose concentrations at the time of ketosis diagnosis. The major limitation of this study was that all B+C analysis and average daily milk production analysis was conducted in 1 herd, so the ability to apply these results to other herds may be limited.

In the 2012 study (Chapter 4), the relationship between blood glucose and treatment expanded to multiple herds and all lactations. Animals with blood glucose < 2.2 mmol/L at enrollment produced more milk in early lactation when treated with B+C or a longer duration (5 days) of propylene glycol compared to controls (saline and 3 days of propylene glycol, respectively). There was no significant difference in milk production in animals with blood glucose $\geq$ 2.2 mmol/L at the time of ketosis diagnosis. The concentration of BHBA also proved important in this study, as animals with blood BHBA > 2.4 mmol/L at the time of treatment were significantly more likely to cure and had a significant reduction in blood BHBA concentrations at 1 week after enrollment when treated with 5 days of propylene glycol versus 3 days. This study helps strengthen the evidence that B+C may be useful for ketosis treatment in animals with low blood glucose at the time of ketosis diagnosis. Though the relationship between blood glucose and ketone bodies has been examined previously for ketosis categorization, this relationship has never been examined in relationship to treatment efficacy. This study also suggests that the concentration of blood BHBA at the time of ketosis diagnosis may help with categorization of ketosis. Blood glucose and blood BHBA may be important predictors of treatment efficacy and more research is required to further understand this relationship.

Though both treatment trials contribute to the body of evidence on ketosis treatment and raise interesting questions for future consideration, there are limitations that are present that may restrict the application of this knowledge. Both trials were completed in one geographic area, on a limited number of purposively selected herds, during one season. The diversity of management systems was increased in the 2011 trial

compared to the 2012 trial, as both tie-stall and freestall facilities were included. However, there were still limitations placed on enrollment, such as use of a total mixed ration, regular milk production monitoring, and distance from study sites that may affect the application of these results to herds in other regions or even all herds in Ontario. The 2012 trial was conducted only in freestall herds, and thus all analysis of B+C was completed in freestall facilities. It is presumed that B+C efficacy would not be affected by housing design, but that can not be proven based on these studies. Also, it is unclear if changes in feed and environmental conditions (such as temperature and humidity) seen in different seasons and different geographical locations would affect the efficacy of the treatments utilized.

The final objective was to investigate selected cow-level risk factors to better understand ketosis risk. Utilizing records collected for other studies, we found that increased age at first calving, previous lactation days open, length of dry period, and parity significantly affected ketosis risk. Herd was also an important predictor of ketosis risk and was involved in interactions with some predictors. Furthermore, animals that were diagnosed with ketosis in the preceding year were twice as likely to be diagnosed with ketosis the subsequent year. Though this study identifies risk factors for ketosis diagnosis, some important factors previously associated with increased risk of ketosis were not measured (such as body condition score). These data can help determine animals at high risk for ketosis so preventive measures can be implemented prior to ketosis development.

Future Research

Despite the knowledge that was obtained from the research described in this thesis, there is a still a lot to learn regarding ketosis treatment and risk.

The role of blood glucose levels in the etiology of ketosis and ketosis treatment requires further investigation. Measurement of blood glucose could assist with determining appropriate treatment regimens and classification of ketotic animals. It could also identify animals that are more severely affected or affected in a different manner that may require more extensive intervention. Though the cut-point used in our studies was based on sensitivity analysis of clinical disease risk, a study that examined blood glucose concentrations in a larger number of ketotic animals and monitored subclinical and clinical disease rates, milk production, and culling risk on more farms may help determine the proper blood glucose cut-point for future use.

Additionally, blood glucose levels may be affected by factors that were not measured in our treatment trials, such as dry matter intake, insulin levels, and liver fat content. This may be especially important in the 2011 trial were insulin was used as a treatment modality. The lack of effect realized with glargine insulin treatment in many of the outcomes monitored may have been due to differences in blood insulin levels in treated animals. A smaller scale intensive trial where dry matter intake is measured daily, along with regular monitoring (at least weekly) of more blood metabolites (such as NEFA and insulin) and liver fat content may help explain the differences in blood glucose levels and elucidate animals at higher risk. If blood glucose can be validated to help predict animals more severely or differently affected by hyperketonemia, this would

give producers and veterinarians a quick and inexpensive tool to use in the field to apply ketosis treatment more effectively.

Though B+C appears to be efficacious in certain animals, there is still much to be learned about appropriate ketosis treatment. A majority of animals diagnosed with ketosis still do not experience ketosis cure after treatment, suggesting that there may be other treatments that are more beneficial. Future treatment trials should include a control group that is affected with ketosis and ideally left untreated. This would allow for investigation of the selected products without the possibility of interactions between treatments that can be difficult to analyze. However, these trials are expensive and require large numbers of cattle to obtain useful results. Furthermore, concerns for animal welfare or compliance may require that all animals in a trial be treated. If this is the case, a baseline treatment with evidence of efficacy (i.e. propylene glycol) should be administered to all groups to allow for investigation of the additional benefits of the tested treatments. Randomization and blinding are two further critical components of the design of future ketosis treatment trials. Finally, economically important outcomes should be investigated, such as culling risk, disease risk and milk production. Biologically plausible treatments should be studied, more of which may become evident as the relationship between blood metabolites is more fully understood. Currently, 50% dextrose and dexamethasone are the logical choices for future study.

Economic analysis is another important component of ketosis treatment evaluation. Though economically important outcomes were measured in the treatment trials presented in this thesis, no economic analysis was completed and future research into the utility of B+C on commercial dairies should include this analysis. Performing an

accurate economic analysis can be challenging as the monetary costs and benefits can vary widely among farms and can fluctuate throughout the year. Inclusion of sensitivity analyses can help determine what factors have the most impact on economic gains and help producers determine the appropriate products to use in their facility.

Finally, though the ketosis risk factor analysis helped define the relationship between ketosis incidence and some cow-level risk factors, there were many risk factors that could not be explored with our study design. The most obvious of these is body condition score at and around calving, but others include dry period diet and management and breeding management in herds. The analysis of dry cow diet composition, grouping strategies, and feed access, in conjunction with ketosis incidence and dry period length on farm may help elucidate some of the differences observed between herds in the magnitude of effect of dry period length of ketosis risk. Observational studies that follow animals throughout lactation and into the subsequent lactation and measure diet composition, body condition score, grouping strategies, and feed access during all management phases may help explain associations between risk factors that are easy to measure and those that are more challenging. Once sufficient numbers of risk factors are understood, herd and individual cow risk assessment tools could be constructed for ketosis to give producers and veterinarians an instrument to determine herds and animals at high risk of ketosis development so screening and prevention programs can become more targeted.