THE EPIDEMIOLOGY, ECONOMICS, AND TREATMENT OF SUBCLINICAL KETOSIS IN EARLY LACTATION DAIRY CATTLE

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

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January 2013

THE EPIDEMIOLOGY, ECONOMICS, AND TREATMENT OF SUBCLINICAL KETOSIS IN EARLY LACTATION DAIRY CATTLE

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Cornell University 2013

The ability of dairy cattle to adapt to the natural change of energy balance in early lactation is an important aspect of the transition period, as the demands for milk production cannot be met by feed intake alone. Cattle unable to adequately transition from late gestation to early lactation are at a higher risk for subclinical ketosis (SCK), an excess of circulating ketone bodies without clinical signs of hyperketonemia. Cows with SCK are at an increased risk of postpartum diseases such as displaced abomasum and metritis, and SCK has been found to decrease milk yield in early lactation and may adversely affect reproduction. The objectives herein were to: 1) describe the epidemiology of SCK in cows diagnosed with SCK in early lactation; 2) determine important dry and parturient period risk factors of hyperketonemia development; 3) determine the effect of oral administration of propylene glycol in cows diagnosed with SCK on disease development, removal from the herd, reproduction, and milk production; and 4) estimate the cost per case of SCK and evaluate different on-farm testing and treatment strategies based on herd SCK incidence. Peak incidence and prevalence of SCK was found to occur at 5 days in milk (DIM) with a median time to resolution of 5 days. Cows developing SCK from 3 to 7 DIM were more likely to suffer from negative disease and production outcomes than cows that developed SCK from 8 to 16 DIM. Treatment of SCK positive cows with propylene glycol decreased disease incidence, improved reproduction, and enhanced milk production over non-treated control cows. Risk factors associated with development of hyperketonemia included advanced parity, high prepartum non-esterified fatty

acid concentrations, and calving difficulty. The cost per case of SCK in the first 30 DIM was estimated at $67. Testing of fresh cows 2 days per week from 3 through 9 DIM and treatment of SCK positive cows with propylene glycol was found to be the most cost-effective treatment in herds with SCK incidences between 15 and 50%; above 50% blanket treatment of all fresh cows with propylene glycol was more economically beneficial.

BIOGRAPHICAL SKETCH

Jessica Anne Allerton Smith was born and raised in Anchorage, Alaska and spent much of her early life in swimming pools and on cross-country skis. She attended Dimond High School from 1991 to 1995 where she excelled in sports and became interested in science and math. In the fall of 1995, Jessica matriculated to Dartmouth College, located in Hanover, New Hampshire, where she joined the cross-country ski team and studied a little on the side.

Early in her college career, Jessica thought she might be interested in biomedical research and volunteered in a biomedical engineering lab at the Thayer School of Engineering. One year later she realized that was not her calling and decided to take her medical school entrance exams. Trying to get a little experience under her belt, Jessica volunteered at Dartmouth-Hitchcock Medical Center for a term which led her to realize medical school was not for her either. Jessica was then convinced by friends of the family to ride with their large-animal veterinarian, Dr. Barbara LeClair, and forty-five minutes into her first day with Dr. LeClair Jessica knew this was her calling. In May of 1999, Jessica graduated from Dartmouth, having majored in biochemistry and molecular biology with a minor in engineering.

Jessica spent the next four years riding with Dr. LeClair every spring and working on her brothers' dairy farm, the McNamara Dairy, where her love for cows was cultivated. The rest of the year Jessica spent training and cross-country ski racing around the world in hopes of qualifying for the 2002 Olympic Games. An ill-timed bout of pneumonia kept Jessica from racing at the 2002 Olympic Trials, and Jessica decided it was time to send her application in for veterinary school.

In the fall of 2003, Jessica returned to the classroom at the Cornell University College of Veterinary Medicine where she pursued her interest in bovine health and production medicine. At the end of her junior year she married Scott McArt, a fellow Dartmouth cross-country skier

whom she had been dating for 8 years. Jessica graduated from Cornell in May, 2007 and began an internship and residency in the Cornell Ambulatory and Production Medicine Clinic. Two years later, at the suggestion of Dr. Daryl Nydam, Jessica began work on her PhD in Comparative Biomedical Sciences. Fortunately for her, Jessica's course and research schedule allowed her to continue providing clinical service to farms one day a week until the arrival of her first child, Sigrid, in November 2010. After almost two more years of coursework, research, and paper writing, Jessica and Scott welcomed their second daughter, Tazlina, in September 2012.

Jessica plans to defend her thesis in early December 2012 and then move with her family to Fort Collins, Colorado, where she will begin a job as an assistant professor in the Department of Clinical Sciences at Colorado State University. As a faculty member in dairy population health management, Jessica will continue her work on farms while teaching veterinary students and conducting applied research in dairy production medicine.

Dedicated to my mother, Linda Lee Dyer Smith,

who has inspired me more than she will ever know.

ACKNOWLEDGMENTS

The writing of this thesis would not have been possible without the encouragement of my husband, Scott, who listened to me complain, cry, and cheer during various stages of my PhD – thank you for always being there. I appreciate the support and excitement given by my parents, Michael and Linda Smith, who have helped me immensely through this journey and have always been willing to lend me money to support my continuing education. A big thank you to my daughters, Sigrid and Tazlina, whose love has helped me put life into perspective and shown me what is really important at the end of the day.

This PhD would not have been possible without the amazing encouragement and support of my friend and advisor, Dr. Daryl Nydam. Over the years Daryl has given me many "learning opportunities" to make me better prepared for a faculty job, taught me how to cut a cow with a left displaced abomasum from the left side, picked me up countless times at the end of my driveway for a ride in to school, high-fived me both times I told him I was pregnant, and reminded me that my husband is not the most tardy person on Earth. I would also like to thank the other members of my committee: Dr. Chuck Guard, who always tells me the truth; Dr. Tom Overton, who has supported me at all my conferences; Dr. Julia Felippe, who welcomed me into her immunology lab and also gave me tips on life with a newborn; and Dr. Yrjö Gröhn, whom I was fortunate to have placed on my committee. This experience would not nearly have been as much fun without the help of my friends and colleagues who went through it with me: Dr. Paula Ospina, Dr. Jennifer Zambriski, Dr. Derek Cavatorta, and Dr. Takashi Yasui.

None of my data collection could have happened without the help of Judy Alishauskas, Emily Bold, Stephanie Brittin, Mark Fagan, and Caitlin Jeffrey, who met me every morning at 6am to go bleed cows – your enthusiasm was contagious. A big thank you as well to the dairy producers who agreed to participate in my trials – the dairy industry will benefit from your

generosity. I would also like to thank Dr. Gary Oetzel for being my step-advisor, for showing me around a few Wisconsin dairies, and for being my dinner date at ADSA. I feel fortunate to have learned the basics of epidemiology by an amazing group of people in the Department of Population Medicine and Diagnostic Sciences, namely Dr. Hollis Erb, Dr. Hussni Hohammed, and Dr. Yrjö Gröhn, and hope that I can excite students about epidemiology as much as you did me. I would also like to acknowledge the USDA-NRI for financial support, without which this research project would not have been possible.

And finally, a thank you to my dog, Paxson, who has previewed all of my lectures and spent countless hours next to me on the couch while I write. I am glad you are here to see this finished.

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LIST OF ABBREVIATIONS

BCS	Body condition score
BCSG	Body condition score group
BHBA	Beta-hydroxybutyrate
CEASE	Calving ease
CSEX	Calf sex
DA	Displaced abomasum
DIM	Days in milk
DMI	Dry matter intake
HR	Hazard ratio
LACT	Lactation
LS	Locomotion score
ME305	Mature equivalent 305 day milk yield
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
OR	Odds ratio
PDCC	Previous days carried calf
PG	Propylene glycol
RR	Risk ratio
SCK	Subclinical ketosis
TAG	Triacylglycerols
TMR	Total mixed ration
USDA	United States Department of Agriculture
VWP	Voluntary waiting period

CHAPTER ONE

ELEVATED NON-ESTERIFIED FATTY ACIDS AND β-HYDROXYBUTYRATE AND THEIR ASSOCIATION WITH TRANSITION COW PERFORMANCE: A REVIEW

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The Veterinary Journal: in review

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ABSTRACT

Dairy cows pass through a period of negative energy balance as they transition from late gestation to early lactation. Poor adaptation through this period, expressed as elevated concentrations of non-esterified fatty acids pre or postpartum and elevated concentrations of β-hydroxybutyrate postpartum, increases an individual animal's risk of: postpartum disease, removal from the herd, reproductive difficulty, and reduced milk production. Field studies have shown that subclinical ketosis often affects 40% of cows in a herd although the incidence can be as high as 80%. Peak incidence occurs at 5 days in milk, and cows that develop subclinical in the first week of lactation have a higher risk of negative effects and reduced milk production than cows that develop subclinical ketosis in the second week of lactation. Herds with more than 15 to 20% of their cows experiencing elevated concentrations of non-esterified fatty acids and β-hydroxybutyrate in early lactation have higher rates of negative downstream health events, poor reproduction, and reduced milk yield than herds with a lower prevalence of negative energy balance. This paper reviews testing of energy related metabolites and current research on the impact, individual animal and herd consequences, treatment, and economics associated with poor adaptation to negative energy balance in dairy cows. Dairy cows pass through a period of negative energy balance as they transition from late gestation to early lactation. Poor adaptation through this period, expressed as elevated concentrations of non-esterified fatty acids pre or postpartum and elevated concentrations of β-hydroxybutyrate postpartum, increases an individual animal's risk of: postpartum disease, removal from the herd, reproductive difficulty, and reduced milk production. Field studies have shown that subclinical ketosis often affects 40% of cows in a herd although the incidence can be as high as 80%. Peak incidence occurs at 5 days in milk, and cows that develop subclinical ketosis in the first week of lactation have a higher risk of negative effects and reduced milk production than cows that develop subclinical

ketosis in the second week of lactation. Herds with more than 15 to 20% of their cows experiencing elevated concentrations of non-esterified fatty acids and β-hydroxybutyrate in early lactation have higher rates of negative downstream events, poor reproduction, and reduced milk yield than herds with a lower prevalence of negative energy balance. This paper reviews: 1) strategies for testing of energy-related metabolites, 2) consequences of poor adaptation to negative energy balance (for individual animals and for herds), 3) treatment approaches for affected cows, and 4) economic considerations for testing and treating cows with poor adaptation to negative energy balance.

Keywords: negative energy balance; subclinical ketosis; non-esterified fatty acids; β-hydroxybutyrate; dairy cow

INTRODUCTION

Negative energy balance (NEB) is a normal occurrence in dairy cattle as they transition from late gestation to early lactation. This transition period is often considered to occur from 3 weeks pre-partum to 3 weeks post-partum (Grummer, 1995; Drackley, 1999), as during this timeframe homeorhetic regulation of metabolic functions are necessary in order to accommodate parturition and lactogenesis (Bauman and Currie, 1980). In addition, it has recently been shown that nutritional management in the early dry period, i.e. after cessation of milking, is also very important to maintain the health and productivity of transition cows (Dann et al., 2006). Dry matter intake (DMI) decreases by over 30% in the last 3 weeks of gestation (Hayirli et al., 2002) which limits the availability of energy sources during a time of increased demand; within 4 days post-partum, the demands for glucose, amino acids, and fatty acids are several times higher than pre-partum requirements due to milk production (Bell, 1995). For these reasons, the transition

from late gestation to early lactation is a dynamic period for dairy cattle, during which most infectious and metabolic diseases are likely to occur (Goff and Horst, 1997; Ingvartsen et al., 2003; Mallard et al., 1998). Cows unable to adapt to this challenging time are more prone to negative downstream events, and the associations between excessive NEB in dairy cows and these detrimental health effects have been reviewed by several authors (Andersson, 1988; Kehrli et al., 1989; Duffield, 2000; Hammon et al., 2006). The economic impacts of mal-adaptation are not trivial and include increased risk of metabolic disease, reduced milk production, early removal from the herd, and poor reproductive performance. This paper reviews testing of NEB related metabolites and current research on the ecology of subclinical ketosis (SCK), individual animal and herd consequences, treatment, and economics associated with poor adaptation to NEB in dairy cows.

ADAPTATION TO NEGATIVE ENERGY BALANCE

The period of NEB occurs as the energy demands for milk production cannot be met by feed intake alone (Bauman and Currie, 1980; Baird, 1982; Herdt, 2000). Glucose, a fundamental nutrient required for normal brain function in addition to use by other tissues, is under tight homeostatic control in order to allow for basic functioning of the animal. In ruminants, ingested carbohydrates are fermented to short-chain fatty acids by rumen microbes, and thus most glucose must be synthesized by the liver (Reynolds et al., 1988). As glucose is a major component in milk, gluconeogenesis is closely linked to lactogenesis as the amount of available glucose will determine the quantity of milk produced (Mepham, 1993). After parturition, there is a decrease in insulin production by the pancreas (Drackley et al., 2001) which results in decreased glucose utilization by insulin sensitive organs (e.g. adipose tissue and muscle) and allows the mammary gland to have additional glucose for milk production (Komatsu et al., 2005). Thus alternative

fuel sources are needed for certain tissues in the body to maintain normal function during this period of increased milk production.

In response to a decrease in available glucose, an increase in lipolysis releases nonesterified fatty acids (NEFA) which circulate throughout the body in the blood (McNamara, 1991; Bertics et al., 1992; Herdt, 2000). Nonesterified fatty acids can be used directly as a fuel source by various tissues such as muscle, used for milk fat synthesis by the mammary gland, or taken up by the liver (Palmquist et al., 1969; Herdt, 2000). The liver removes approximately 15 to 20% of NEFA from the blood (Drackley and Andersen, 2006) where it can be completely oxidized to provide energy for the liver, partially oxidized to produce ketone bodies (acetone, acetoacetic acid, and beta-hydroxybutyric acid (BHBA)), converted into triacylglycerols (TAG) and packaged into very low density lipoproteins for transport back to the adipose tissue, or stored in the liver as TAG. Ketone bodies released by the liver act as an alternate fuel source for tissues such as the brain and heart (Herdt, 2000; Drackley and Andersen, 2006). Thus, a certain concentration of NEFA and BHBA in the blood is part of a normal adaptation to NEB in early lactation. However, excessive NEFA or BHBA concentrations indicate an excess of NEB at which point detrimental health and production outcomes are more likely to occur. In addition, elevated levels of NEFA and BHBA can be detrimental to immune function (Hammon, 2006; Contreras et al., 2010; Ster et al., 2012) and decrease appetite (Dale et al., 1979).

When excessive NEFA enter the liver, its capacity to fully oxidize NEFA to energy is overwhelmed and some of these NEFA are re-esterified into liver TAG. As ruminants have a poor capability of exporting liver TAG as very low density lipoproteins, fat accumulates in the liver (hepatic lipidosis). Excessive fat accumulation in the liver impairs normal liver function (Rukkwamsuk et al., 1999; Jorritsma et al., 2001; Murondoti et al., 2004), which may lead to hyperketonemia (Herdt, 2000). Hyperketonemia can be associated with clinical signs such as a

decrease in appetite, weight loss, and a decrease in milk production; unfortunately the practical evaluation of these clinical signs is largely subjective. Cows can also suffer from SCK which is defined as an excess of circulating ketone bodies without obvious clinical signs (Andersson, 1988). The term hyperketonemia will be used throughout this review to encompass animals with either SCK or clinical ketosis (i.e. blood BHBA ≥1.2 mmol/L); it is important to note that SCK and clinical ketosis are not different diseases, just variations in severity of a single disorder. The distinction between SCK and clinical ketosis can either be based subjectively on clinical assessment or more objectively through measurement of BHBA. Most hyperketonemic cows (greater than 95%) are subclinically ketotic (Duffield et al., 1998; J. McArt, unpublished data; P. Ospina, unpublished data). This review will focus on the associations of elevated blood NEFA and BHBA on downstream outcomes; the impacts of clinical ketosis and its treatment will not be discussed.

TESTING METHODS

Blood NEFA concentrations are a more accurate measure of NEB than ketone bodies. Blood NEFA concentrations are also a stronger indicator of disease, reproductive performance, and milk production than blood BHBA concentration (Ospina et al., 2010c; Huzzey et al., 2011). However, with current technology, collection and processing of blood samples to get accurate NEFA concentrations can be comparatively difficult in the field (Stokol and Nydam, 2006) and expensive; in addition, the stress of sample collection on cows can increase their NEFA concentration (Holmes and Lambourne, 1970; Leroy et al., 2011). Samples for NEFA analysis should be collected in EDTA or non-anticoagulant tubes, placed on ice immediately after collection, kept at 4˚C until processing, and the serum separated within 24 hours (Stokol and Nydam, 2005); samples with a hemolytic index ≥ 300 should be interpreted with caution.

Additionally, pre-partum NEFA values are commonly measured between 14 and 3 days prior to calving because NEFA naturally rise a few days before parturition (LeBlanc et al., 2005). This is a very narrow time window and difficult to predict because of the normal variability of gestation length. The current cost at the New York State Animal Health Diagnostic Center is US$11[1] per sample[2]. Time of collection is also important as NEFA concentrations are generally highest before feeding (Quiroz-Rocha et al., 2010); however, NEFA seems to be less sensitive to time of sample collection than BHBA concentrations (Eicher et al., 1999) which fluctuate throughout the day and are usually highest 4 to 5 hours after feeding (Oetzel, 2004).

Testing for ketone bodies is less expensive and more practical when compared to NEFA; urine, milk, or blood can be used as test substrates. The gold standard is analysis of blood BHBA in a laboratory by kinetic enzymatic assay, as BHBA are the most stable ketone bodies in the blood (Tyopponen and Kauppinen, 1980). However, there are many relatively accurate cow-side tests available which make on-farm testing very practical. It is important to note that the sensitivity and specificity of the different tests can have a large impact on individual test results and, as a consequence, herd diagnosis and treatment. A urine test for acetoacetic acid, Ketostix (Bayer), had 78% sensitivity and 96% specificity when the strip reading was ≥15 mg/dL ("small") and 49% sensitivity and 99% specificity when the strip reading was ≥40 mg/dL ("moderate") when compared to a concentration of 1.4 mmol/L BHBA by laboratory analysis. Unfortunately, only 50% of cows could typically be induced to urinate while sampling (Carrier et al., 2004). The cost is approximately US$0.20[3] per strip. KetoCheck (Great States Animal Health), an acetoacetic acid milk test, costs approximately US$0.60 per test and had a sensitivity of 41% and a specificity of 99% (Carrier et al., 2004). The accuracy for KetoTest (Elanco), a milk test for BHBA, costs approximately US$1.70 per test. Using a cut-point of 100 μmol/L, sensitivities and specificities have ranged from 73 to 80% and 76 to 96%, respectively; use of a

[1] US$1 = approx. £0.62, €0.77 at 26 October 2012.
[2] See: http://ahdc.vet.cornell.edu
[3] See: www.mwivet.com

200 μmol/L cut-point returned sensitivities from 27 to 59% and specificities from 90 to 99% (Geishauser et al., 2000; Carrier et al., 2004). PortaBHB (PortaCheck), a newer cow-side milk test for BHBA, had a sensitivity and specificity of 89 and 80%, respectively, when using a threshold of 100 μmol/L and 40 and 100% when using a threshold of 200 μmol/L when compared to a blood BHBA concentration of ≥1.4 mmol/L (Denis-Robichaud, 2011). It costs approximately US$2.05 per strip.

Precision Xtra Meter (Abbott Laboratories), a whole blood test for BHBA, has reported sensitivities ranging from 85 to 96% and 90 to 100% and specificities ranging from 94 to 98% and 98% to 100% when compared to cut-points of 1.2 mmol/L and 1.4 mmol/L blood BHBA, respectively (Iwersen et al., 2009; Konkol et al., 2009; Voyvoda and Erdogan, 2010). Cost per test strip is approximately US$1.30; the meter can be purchased for less than US$30.

Several recent studies have measured blood or serum BHBA concentrations to diagnose hyperketonemia due to its superior accuracy over milk or urine ketones. Although numerous studies using milk or urine ketones have reported incidence and prevalence of hyperketonemia as well as the consequences of poor adaptation to NEB (Emery et al., 1964; Dohoo and Martin, 1984; Simensen et al., 1990), only studies measuring blood or serum BHBA concentrations will be discussed further.

DEFINING THE CUT-POINT CONCENTRATIONS FOR HIGH NEFA AND BHBA

Recent studies measuring the association of different cut-points of elevated NEFA and BHBA with disease outcomes, reproductive measures, and milk production, have assisted in more accurately identifying animals with poor adaptation to NEB. Multiple studies have reported that the prepartum NEFA cut-points with the highest sensitivity and specificity for the prediction of postpartum health problems ranged from 0.3 mEq/L to 0.5 mEq/L (Cameron et al.,

1998; LeBlanc et al., 2005; Ospina et al., 2010b; Ospina et al., 2010c; Chapinal et al., 2011; Roberts et al., 2012). Reported postpartum NEFA cut-points for the prediction of postpartum health problems ranged from 0.70 to 1.0 mEq/L (LeBlanc et al., 2005; Ospina et al., 2010b; Ospina et al., 2010c; Chapinal et al., 2011; Roberts et al., 2012). The only prepartum BHBA cut-point reported was by Chapinal et al. (2011), who indicated that prepartum BHBA concentrations $\geq$0.8 mmol/L were best associated with postpartum problems. Postpartum BHBA concentration cut-points that maximize accuracy of disease and production measures range from 0.9 mmol/L to 1.6 mmol/L with the majority between 1.2 to 1.4 mmol/L (LeBlanc et al., 2005; Walsh et al., 2007; Duffield et al., 2009; Ospina et al., 2010b; Ospina et al., 2010c; Chapinal et al., 2011; Seifi et al., 2011; Roberts et al., 2012). Classification of an animal as having elevated NEFA or BHBA using lower cut-points typically produces a higher sensitivity and lower specificity, while use of higher cut-points produce a lower sensitivity and higher specificity. Thus, the choice of a cut-point within these ranges depends on the users preference for a higher sensitivity or specificity.

INCIDENCE AND PREVALENCE OF HIGH NEFA AND BHBA

Determining the incidence and prevalence of high NEFA or BHBA

There are two important measurements which help evaluate the frequency of a disease in a farm population: incidence and prevalence. Incidence is the number of cows that develop a disease at any time during a given period, i.e. the number of new cases divided by the total number of cows that have gone through this time period. Incidence testing must be completed often enough to ensure that all cows developing the disease during the time period will be correctly identified (e.g. if a disease typically lasts 5 days, incidence testing should occur at least

two times per week). As an example, if 15 cows within a group of 50 cows that were monitored between 3 and 16 days in milk (DIM) developed hyperketonemia for the first time sometime between 3 and 16 DIM, the incidence of hyperketonemia during that time period would be 30% (15/50).

Prevalence is a snapshot of the amount of a disease existing in a herd at a point in time; it is determined by 1 test and is thus easier to determine than incidence. Prevalence is calculated by dividing the number of disease positive cows by the number of cows sampled at that point or period in time. For example, if 3 cows were SCK positive out of 20 cows tested that day, the prevalence would be 15%. At the herd level, the recommendation is to test at least 15 to 20 cows in the appropriate DIM range each time you conduct a prevalence test. For example, if there are 50 fresh cows at risk for SCK and you expect the prevalence to be 15%, sampling 20 cows will allow you to be 90% confident that the prevalence you obtain is +/- 10% of the true herd prevalence. This is a relatively small number of animals to test, but it is only useful for determining whether a herd has a very low or very high prevalence of the disease. More cows should be tested in order to more confidently estimate the true herd prevalence.

Knowing a herd's estimated incidence of hyperketonemia is more useful than knowing the prevalence because the best strategy for testing cows for hyperketonemia depends on the herd incidence. However, a direct determination of the herd incidence is practically challenging because it requires repeated testing of individual cows. A reasonable alternative is to conduct a herd prevalence test and then estimate the herd incidence from the herd prevalence. Fortunately, the incidence of hyperketonemia has consistently reported to be about twice its prevalence (Duffield et al., 1998; McArt et al., 2012a). For example, if 100 early lactation cows from a herd were tested and 15 of these cows had blood BHBA ≥1.2 mmol/L, the herd prevalence would be 15% and the estimated herd incidence would be 30%.

Non-esterified fatty acids

Due to the cost of testing and difficulty of sampling, few large field trials have reported the incidence of increased prepartum or postpartum NEFA concentrations. Cameron et al. (1998) found herd incidences of increased prepartum NEFA in 67 herds in Michigan, USA, classified as a serum concentration of > 0.30 mEq/L between 3 and 35 days before expected calving, to range from 0 to 90%.

Cow-level prevalence of increased prepartum NEFA, classified as a serum concentration of ≥ 0.30 mEq/L from 3 to 14 days before expected calving, using a total of 1439 cows from 104 herds in New York, Pennsylvania, and Vermont, USA was 34%. Median herd prevalence of increased prepartum NEFA in 61 of these herds with at least 15 cows sampled was 29% with a range of 0 to 88% (Ospina et al., 2010a; P. Ospina, unpublished data). The prevalence of herds in which more than 15% of the sampled cows had an increased prepartum NEFA concentration of ≥0.30 mEq/L was 75% (Ospina et al., 2010a). LeBlanc et al. (2005) reported a cow-level prevalence of 40% using a similar cut point in 977 cows from 20 herds in Ontario, Canada. Mean herd prevalence of increased prepartum NEFA using a cut-off of 0.30 mEq/L was found to be 19 and 15% in 2 herds in New York milking at least 1,500 cows, with 256 and 288 cows sampled per herd, respectively, from 3 to 14 days before expected calving (McArt et al., 2012c; J. McArt, unpublished data).

Cow-level prevalence of increased postpartum NEFA in 1318 cows from the 104 herds in New York, Pennsylvania, and Vermont, USA was 32% when using a cutoff of 0.70 mEq/L from 3 to 14 DIM; herd prevalence of increased postpartum NEFA in 61 of these herds had a median of 24% with a range of 0 to 90% (unpublished data from Ospina et al., 2010a). The prevalence of herds in which more than 15% of the sampled cows had an increased postpartum NEFA ≥0.70 mEq/L was 65% (Ospina et al., 2010a). Seifi et al. (2011) reported a cow-level prevalence of

37% in the first week postpartum and 34% in the second week postpartum when using a cutoff of 1.0 mmol/L in 849 cows from 16 herds in Ontario, Canada. In 55 sampled herds in the USA and Canada, Chapinal et al. (2012) reported that 17% of the herds had at least 30% of cows with increased postpartum NEFA ≥1.0 mEq/L.

β-hydroxybutyrate

On an individual cow basis, peak incidence and prevalence of SCK (diagnosed as a BHBA concentration of 1.2 to 2.9 mmol/L) were found to occur at 5 DIM (Figure 1) after intensive monitoring of 1,717 cows on 4 large TMR-fed freestall dairies in New York and Wisconsin, USA (McArt et al., 2012a). A similar peak in prevalence of hyperketonemia (diagnosed as a BHBA concentration of ≥1.2 mmol/L) at 1 week postpartum was reported by Duffield et al. (1998) using data from 1,010 cows on mostly component-fed tiestall dairies in southwest Ontario, Canada. As the median time to resolution of SCK was reported to be 5 days (McArt et al., 2012a), farms that test less frequently than twice per week will underestimate the true incidence.

Herd-level incidence of hyperketonemia appears to range between 25 and 60% with an average of approximately 45%. The incidence of SCK in the 4 herds from New York and Wisconsin, which tested for SCK 3 times per week, ranged from 26 to 56% with a mean of 43% using a blood BHBA concentration of 1.2 to 2.9 mmol/L to diagnose SCK (McArt et al., 2011). The incidence of hyperketonemia in the same study ranged from 27 to 57% with a mean of 44% (J. McArt, unpublished data). A study by Duffield et al. (1998) in 25 herds reported a similar incidence of 49% using a BHBA cut-point of 1.2 mmol/L, although this study tested cows only once weekly and thus underestimated the true incidence.

LeBlanc et al. (2005) reported a cow-level hyperketonemia prevalence of 20% in 1063

cows from 20 herds in Ontario, Canada sampled between 1 and 7 DIM when using a cutoff of ≥1.2 mmol/L. In a study of 849 cows in 16 herds in Ontario, Canada, Seifi et al. (2011) reported a hyperketonemia prevalence of 31% using a BHBA concentration cutoff of ≥1.0 mmol/L. In 1318 cows from 104 herds in New York, Pennsylvania, and Vermont, USA, the cow-level prevalence of hyperketonemia from 3 to 14 DIM was 18% when using a cut-off of ≥1.2 mmol/L (Ospina et al., 2010a; P. Ospina, unpublished data); herd-level prevalence from 61 of these herds ranged from 0 to 71% with a median of 14%. Chapinal et al. (2012a) reported that 12% of 2,069 cows from 45 herds had blood BHBA ≥1.4 mmol/L the first week after calving and that 20% of cows had blood BHBA ≥1.2 mmol/L the second week after calving.

The prevalence of herds in which more than 15% of sampled cows had increased postpartum BHBA concentrations of ≥1.2 mmol/L was 40% (Ospina et al., 2010a). Chapinal et al. (2012b) reported that the prevalence of herds in which more than 25% of sampled cows had increased postpartum BHBA concentrations of ≥1.4 mmol/L was 15% in 55 herds in the US and Canada tested within a week postpartum.

CONSEQUENCES OF ELEVATED NEFA AND BHBA

Numerous studies have reported on the consequences of cows having elevated NEFA or BHBA. These results will be divided into four categories: high prepartum NEFA, high postpartum NEFA, high prepartum BHBA, and high postpartum BHBA. The downstream outcomes of these elevations can be expressed as odds or risk of displaced abomasum (DA), odds or risk of culling or removal from herd, the degree of reproductive impairment, and the degree of lost milk production in early lactation.

High prepartum NEFA

Multiple studies have reported that high prepartum NEFA concentrations are associated with an approximately two-fold increase in the odds or risk of developing a DA (Cameron et al., 1998; LeBlanc et al., 2005; Ospina et al., 2010c; Chapinal et al., 2011). In addition, LeBlanc et al. (2005) showed that the odds of DA development increased at higher NEFA concentrations. Similarly, it has been found that cows with high prepartum NEFA are almost twice as likely to be culled within 30 days (P. Ospina, unpublished data) or 60 days of calving (Roberts et al., 2012). In addition, Ospina et al. (2010b) found that cows with increased prepartum NEFA were less likely to conceive within 70 days post-voluntary waiting period and had lower ME305 milk production than cows with lower prepartum NEFA concentrations. Increased prepartum NEFA have also been found to increase the risk of clinical ketosis and metritis development (Ospina et al., 2010c) as well as retained placenta (Chapinal et al., 2011). Huzzey et al. (2011) found that the odds of development of at least one disorder (DA, SCK, or culling) in multiparous cows increased with increasing prepartum NEFA concentrations. A summary of these associations is in Table 1.

High postpartum NEFA

While postpartum NEFA have not been measured in as many studies as prepartum NEFA, Ospina et al. (2010c) found an almost ten-fold increase in the risk of DA development in cows with elevated postpartum NEFA; an almost five-fold increase in the odds of DA development with elevated postpartum NEFA was reported by Chapinal et al. (2011). In relation to culling, studies have shown an increased risk or odds of culling in the first 30 to 60 DIM ranging from 2.6 to 3.8 with elevated postpartum NEFA (Ospina et al., 2010c; Seifi et al., 2011; P. Ospina, unpublished data). Increased postpartum NEFA have also been found to increase the

risk of clinical ketosis and metritis (Ospina et al., 2010c) and increase time to conception compared to cows with lower NEFA concentrations (Ospina et al., 2010b). The association of elevated postpartum NEFA with milk production has been reported to differ by parity group. Mature equivalent milk production was increased in first lactation cows but was decreased in multiparous animals with elevated postpartum NEFA (Ospina et al., 2010b).

High prepartum BHBA

Few studies have reported on the association of elevated prepartum BHBA concentrations with postpartum health and production outcomes. Chapinal et al. (2011) reported that cows with elevated prepartum BHBA were at an almost 4 times higher odds of developing a DA than cows with lower prepartum BHBA concentrations. Additionally, cows sampled one week prepartum with elevated BHBA concentrations have been shown to have an approximately 2 times higher odds of being culled within the first 60 DIM than their herdmates with lower BHBA values (Roberts et al., 2012).

High postpartum BHBA

All studies reporting the association of postpartum BHBA concentrations with DA development have shown an increased risk or odds of DA with elevated concentrations; the reported associations range from 3 to 25 (Geishauser et al., 1997; LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010c; Chapinal et al., 2011; Seifi et al., 2011; McArt et al., 2012a). In addition, LeBlanc et al. (2005) found that the odds of DA increased with increasing concentrations of BHBA. Similarly, cows with elevated BHBA concentrations had between 2 and 6 times higher odds or risk of being culled or removed from the herd in the first 30 DIM

(McArt et al., 2012a; P. Ospina, unpublished data) or 60 DIM (Roberts et al., 2012) than their herdmates with lower BHBA concentrations.

Studies reporting associations between elevated postpartum BHBA and reproductive measures have not shown consistent results. Time to conception (within 70 days of the voluntary waiting period) was decreased by 13% for cows with elevated (Ospina et al., 2010b). Walsh et al. (2007) reported a decrease in conception to first service for cows with elevated BHBA in week 1 or 2 postpartum, with the pregnancy per AI ratio decreasing as BHBA concentrations increased. Other studies have shown no association between elevated postpartum BHBA and conception to first service or days open (Kessel et al., 2008; McArt et al., 2012a), however the statistical power of these studies may have been too low to detect a true difference in reproductive measures.

Most studies have reported a decrease in milk production in cows with elevated postpartum BHBA concentrations compared to cows with lower BHBA values. Duffield et al. (2009) showed that cows with hyperketonemia produced 1.9 and 3.3 kg less milk per day at their first Dairy Herd Improvement Association (DHIA) test when diagnosed at 1 and 2 week postpartum, respectively, than their non-ketotic herdmates. Similarly, using average daily milk weights, McArt et al. (2012a) found that cows diagnosed with SCK produced 1.2 kg less milk per day for the first 30 DIM than non-ketotic cows. Based on mature equivalent milk yield assessed at 120 DIM using DHIA test data, Ospina et al. (2010b) found that in animals diagnosed with hyperketonemia, heifers were projected to produce 403 kg more milk and cows were projected to produce 393 kg less milk per lactation when compared to non-ketotic heifers and cows, respectively. This finding agrees with the association reported earlier for elevated postpartum NEFA and milk yield by parity group. In contrast, Kessel et al. (2008) found no difference in milk production through 100 DIM for hyperketonemic versus non-hyperketonemic

cows, although the small sample size of this study most likely precluded finding a true difference if one existed. A summary of these associations is in Table 2.

The time of onset of SCK and the BHBA concentration at first positive test are also important indicators of individual cow performance. McArt et al. (2012a) found that cows first diagnosed with SCK from 3 through 7 DIM were more likely to develop a DA or be removed from the herd, less likely to conceive to first service, and produced less milk than cows first diagnosed with SCK at 8 DIM or later. These adverse outcomes were also greater as the BHBA concentration at first positive test increased.

HERD-LEVEL ALARM RATES

Two studies have reported on the association of the proportion of positive animals sampled within a herd and downstream outcomes at the herd level. Ospina et al. (2010a) sampled 60 herds and reported that if ≥15% of sampled animals in a herd had ≥0.27 mEq/L prepartum NEFA, the herd had a higher risk of cows with DA and clinical ketosis, increased time to conception, and produced less milk compared to herds with less than 15% of sampled animals above the threshold. Similar results were found for herd with greater than 15% of samples animals with postpartum NEFA concentrations ≥0.70 mEq/L or postpartum BHBA concentrations ≥1.2 mmol/L. Chapinal et al. (2012b) sampled 45 herds and reported a reduced milk production based on first DHIA test of 4.4 kg per cow per day when ≥15% of sampled animals had prepartum BHBA concentrations ≥0.8 mmol/L. In addition, herds where ≥25% of sampled animals had postpartum BHBA concentrations ≥1.4 mmol/L had an increase odds of DA, and herds with ≥30% postpartum NEFA ≥1.0 mEq/L had a decreased odds of conception to first service. Results from these studies suggest that herds with ≥15 to 20% of sampled animals having increased pre or postpartum NEFA or BHBA concentrations may have more difficulty

with postpartum disease, poor reproduction, and lower milk production than herds with a lower proportion of positive animals.

TREATMENT OF INDIVIDUAL COWS WITH HYPERKETONEMIA

Much research has been completed on the use of treatment and management modalities to prevent cows from developing hyperketonemia, however, few studies have analyzed the benefits of treatment of cows once diagnosed. A systematic review of hyperketonemia treatment in lactating dairy cattle was completed by Gordon et al. (2011), and produced only 10 articles that reported a clearly defined method of diagnosis of naturally occurring hyperketonemia prior to initiation of treatment, included a control group, had clearly defined outcomes of interest concerning negative energy balance, disease or production measures, and used adequate statistical methodology. Results from this search and subsequently published articles will be described below.

A study using 741 cows to compare oral administration of propylene glycol (PG) to SCK positive cows (diagnosed by blood BHBA) with a no-treatment SCK positive control group reported many benefits to treating individual cows with PG (McArt et al., 2011; McArt et al., 2012b). Cows treated with PG were at a decreased risk of developing clinical ketosis, were more likely to resolve their SCK, less likely to develop a DA, less likely to be removed from the herd in the first 30 DIM, more likely to conceive to first service, and on some farms made more milk than control cows. Carrier et al. (2011) and Ruegsegger and Schultz (1986) found no beneficial impact of treatment of SCK positive cows with PG. The study by Ruegsegger and Schultz (1986) used only 26 cows diagnosed for SCK by a less accurate test (milk ketones), thus the power to detect a difference between treatment groups was quite low. Although the study by Carrier et al. (2011) enrolled 561 cows for analysis, the diagnosis of SCK was by a less accurate

test (urine acetoacetate) and additional treatment with dextrose, dexamethasone, and cyanocobalamin (vitamin B12) was used in conjunction with PG.

Glucocorticoids have long been used to treat hyperketonemia; it is believed that glucocorticoids stimulate gluconeogenesis by decreasing glucose use in the muscle and increasing proteolysis, thus providing the cow with gluconeogenic precursors (Foster, 1988; Bruss, 1997). However, few studies have scientifically analyzed the use of glucocorticoids in hyperketonemia treatment on disease risks and production parameters. Seifi et al. (2007) found that administration of isoflupredone had no effect on serum BHBA concentrations or risk of clinical disease on cows previously diagnosed with SCK, although treated cows tended to remain hyperketonemic longer than non-treated control cows. Robertson (1966) found an improvement in serum acetone and acetoacetate and plasma NEFA concentrations as well as an increase in milk yield after administration of dexamethasone, although hyperketonemia diagnosis was based on clinical signs and not biochemical parameters.

Few studies have analyzed the use of B vitamins in treating hyperketonemia. Two studies have reported that Catosal (butaphosphan and cyanocobalamin, Bayer) tended to decrease blood BHBA levels in cows diagnosed with SCK compared to control cows not given the butaphosphan-cyanocobalamin combination (Lohr et al., 2006; Gordon et al., 2012). Ruegsegger and Shultz (1986) reported no difference in plasma BHBA concentrations after treatment of SCK positive cows (identified by milk ketones) with niacin, although the study provided limited statistical power with only 26 animals included in the analysis.

The use of insulin has been studied in conjunction with other treatments for hyperketonemia. The use of insulin when combined with Catosal or steroids was reported to be ineffective at lowering BHBA concentrations in cows with SCK (Seifi et al., 2007; Gordon et al., 2012). However, Sakai et al. (1993) reported that insulin reduced blood ketone bodies when

used in conjunction with intravenous dextrose. Robertson (1966) found that insulin, in combination with dexamethasone, improved clinical signs and milk yield; however, as mentioned above, this study did not make a standard diagnosis of hyperketonemia before treatment was initiated.

One study reported on the effect of recombinant bovine somatotropin on hyperketonemia in cows diagnosed with DA. There was no difference in serum BHBA concentrations between cows that received recombinant bovine somatotropin and control cows 3 to 5 days post-treatment (Fetrow et al., 1999).

ECONOMIC IMPACT AND HERD ECONOMIC DECISIONS

The economic impact of hyperketonemia in a herd is not trivial and includes treatment costs, increased culling, and decreased milk production. A case of clinical ketosis has been estimated to cost US$211 (Charles Guard, personal communication, 2012). McLaren et al. (2006) found that an economic index of return over feed decreased by US$0.015 per day for every 1% increase in herd incidence of SCK, which was more costly than any of the other diseases studied. Lactational estimates of the per case cost of SCK range from US$50 to US$100 (Duffield et al., 1998; Duffield, 2000; Geishauser et al., 2001), although recent research shows a similar cost per case for outcomes only through the first 30 DIM; lactational estimates are likely much higher (J. McArt, unpublished data).

As most cows that develop SCK will do so in the first 2 weeks postpartum (Duffield et al., 2009; McArt et al., 2012a), early and accurate identification of cows with SCK is a large determinant of the economic impact of a program to test and treat cows with SCK.

Treatment of cows diagnosed with SCK with oral PG has been shown to be economically beneficial, although herd benefits vary based on SCK incidence and the intensity and accuracy of

diagnostic testing. Geishauser et al. (2001) reported the cost-benefit ratio of testing all fresh cows twice in the first 2 weeks postpartum followed by treatment with PG twice daily for 3 days to be 1 to 3.2 in herds with a 40% incidence of SCK. An economic return of approximately US$1,000 per 100 fresh cows over their first 30 DIM was found when cows were tested 2 times between 3 and 9 DIM and positive cows treated with PG in a herd with a 40% incidence of SCK (J. McArt, unpublished data).

There is trade-off between the increased cost of labor and supplies to test cows for SCK and a blanket treatment of all fresh cows with PG; this balance depends on the incidence of SCK in a herd. A recent analysis (J. McArt, unpublished data) indicates that testing cows for SCK once per week is less beneficial than twice per week due to the average time of resolution of a case of SCK, and that testing cows three times per week is too expensive due to labor and other testing costs. Testing of fresh cows 2 days per week from 3 through 9 DIM and treatment of SCK positive cows with PG was found to be the most cost-effective treatment in herds with SCK incidences between 15 and 50%; above a 50% incidence it was more economically beneficial to forego SCK diagnosis and to blanket treat all fresh cows with PG.

PREDICTING HYPERKETONEMIA

Prediction of cows more likely to develop hyperketonemia in early lactation would allow for more targeted testing strategies as well as focused preventative measures. Studies completed on large data sets in Finland and Sweden reported that increased parity, calving season, and 15 disorders were associated with an increased risk of clinical ketosis development. However, these studies defined clinical ketosis only as a veterinary diagnosis before 50 DIM, and the authors noted that most of these cases were likely secondary to one of the 15 disorders (Grohn et al., 1989; Emanuelson et al., 1993).

More recent data from a large field study was used to identify dry and parturient period risk factors to help explain which cows went on to develop hyperketonemia, which was diagnosed by blood BHBA concentration ≥1.2 mmol/L between 3 and 16 DIM with 3 times weekly testing (McArt et al., 2012c). Of the models presented, the model predicting hyperketonemia development between 3 and 5 DIM had a higher predictive value than the model predicting hyperketonemia development in cows between 3 and 16 DIM. The 3 to 5 DIM model is more clinically practical as most cows first develop SCK during this time and these cows are at a higher risk of health events than cows that first develop SCK later (McArt et al., 2012a). Elevated pre-calving NEFA (≥ 0.30 mEq/L), birth of a male calf, a calving ease ≥ 3 on a scale of 1 to 5, birth of a stillborn calf, and parity ≥3 were all risk factors for hyperketonemia development from 3 to 5 DIM. Thus for herds that chose to focus their hyperketonemia testing rather than test all fresh cows, special attention should be paid to cows that have any of these risk factors.

Table 1.1. Summary of recent research on elevated prepartum non-esterified fatty acid concentrations in dairy cows and their association with displaced abomasum (DA), culling, reproduction, and milk production. Results compare cows with prepartum non-esterified fatty acid concentrations above the study cut-point to cows below the cut-point. Sensitivity (Se) and specificity (Sp) of cut-point concentrations for outcome risks are listed where reported.

Study	Herds sampled	Cows sampled	Outcome/cut-point (mEq/L)	Se	Sp	Result	*P* - value
Cameron et al., 1998	67	1,170	DA				
			>0.3	-	-	RR[1] = 2.0	0.007
LeBlanc et al., 2005	20	1,131	DA				
			≥0.3	63	56	OR[2] = 2.3	0.002
			≥0.4	50	72	OR = 2.6	0.003
			≥0.5	46	82	OR = 4.1	<0.001
			≥0.6	30	89	OR = 3.0	<0.001
			≥0.8	17	93	OR = 2.6	0.007
			≥1.0	15	96	OR = 4.1	<0.001
Ospina et al., 2010b	91	1,164	Time to conception[3]				
			≥0.27	-	-	HR[4] = 0.8	0.01
	91	1,183	ME305[5]				
			≥0.27	-	-	-683 kg	<0.001
Ospina et al., 2010c	100	1,440	DA				
			≥0.27	57	62	RR = 2.0	0.03
	100	1,440	Culling[6]				
			≥0.27	-	-	RR = 1.8	0.001
Chapinal et al., 2011	22	2,365	DA				
			≥0.50	49	75	OR = 2.9	<0.001
Roberts et al., 2012	69	5,979	Culling				
			≥0.40	53	63	OR = 1.8	<0.001

[1] RR = Risk Ratio
[2] OR = Odds Ratio
[3] Time to conception within 70 days post-voluntary waiting period
[4] HR = Hazard Ratio
[5] ME305 = mature-equivalent 305-day milk yield estimated at 120 days in milk
[6] Culling data unpublished from Ospina et al., 2010c

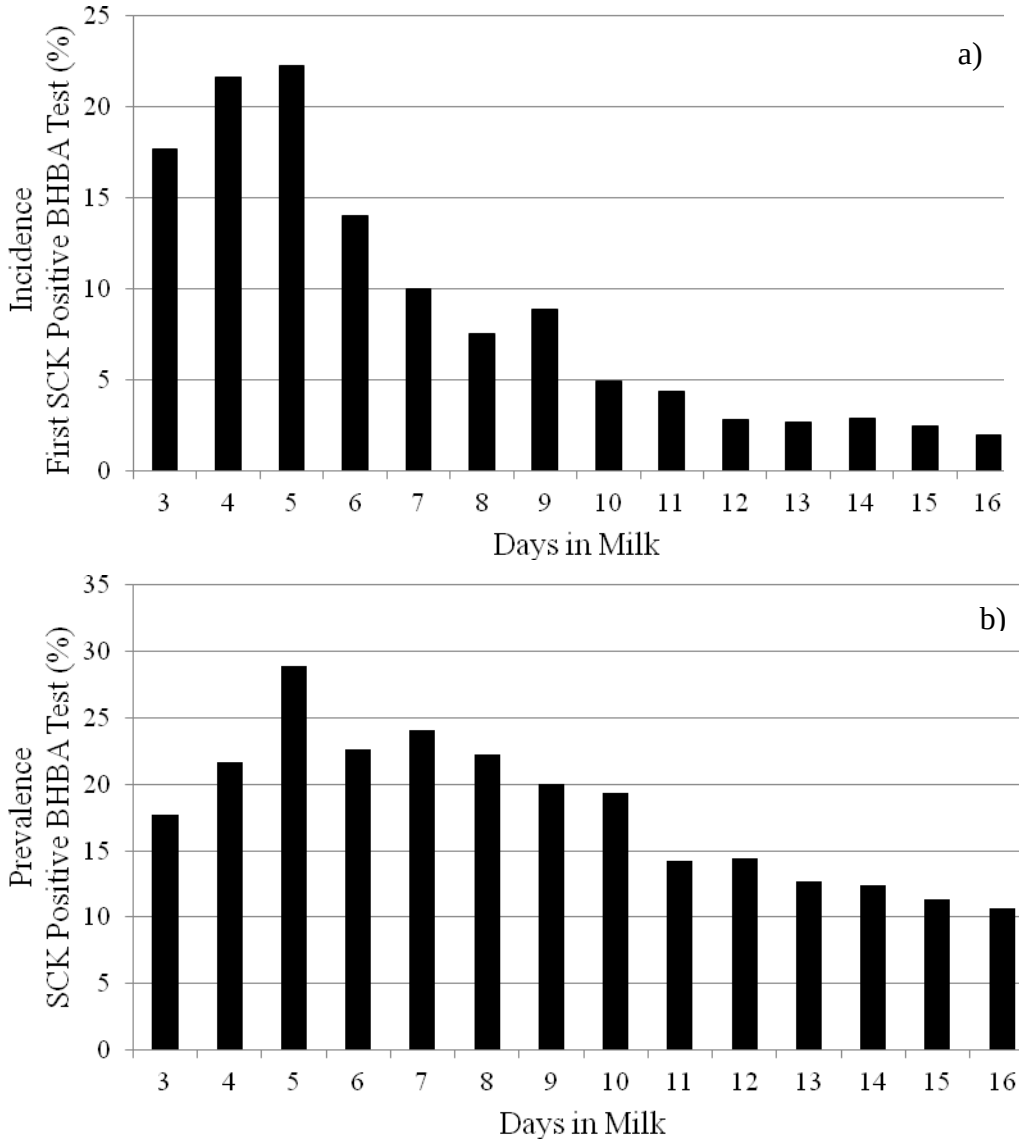
Table 1.2. Summary of recent research on elevated postpartum blood β-hydroxybutyrate concentrations in dairy cows and their association with displaced abomasum (DA), culling, reproduction, and milk production. Results compare cows with postpartum β-hydroxybutyrate concentrations above the study cut-point to cows below the cut-point. Sensitivity (Se) and specificity (Sp) of cut-point concentrations for outcome risks are listed where reported.

Study	Herds sampled	Cows sampled	Outcome/cut-point (mmol/L)	Se	Sp	Result	*P* - value
Geishauser et al., 1997[1]	26	140	DA				
			≥1.0	61	62	OR[2] = 2.4	0.02
			≥1.2	50	75	OR = 2.8	0.01
			≥1.4	39	86	OR = 3.9	<0.001
			≥1.6	31	92	OR = 4.5	<0.001
LeBlanc et al., 2005[1]	20	1,063	DA				
			≥0.6	92	32	OR = 5.4	0.004
			≥0.8	82	57	OR = 6.4	<0.001
			≥1.0	69	74	OR = 6.3	<0.001
			≥1.2	63	82	OR = 8.0	<0.001
			≥1.4	53	88	OR = 8.0	<0.001
			≥1.6	47	91	OR = 9.3	<0.001
			≥1.8	35	94	OR = 8.3	<0.001
			≥2.0	28	95	OR = 7.0	<0.001
Walsh et al., 2007[1]	25	796	Conception to 1st service				
			≥1.0	-	-	OR = 0.7	0.04
Kessel et al., 2008	1	45	Conception to 1st service				
			≥1.0	-	-	-	0.94
Duffield et al., 2009[1]	25	1,010	DA				
			≥0.6	78	24	OR = 1.1	0.08
			≥0.8	66	49	OR = 1.9	0.10
			≥1.0	53	65	OR = 2.1	0.03
			≥1.2	44	77	OR = 2.6	0.008
			≥1.4	34	84	OR = 2.8	0.005
			≥1.6	28	89	OR = 3.1	0.003
			≥1.8	22	91	OR = 2.8	0.02
			≥2.0	13	93	OR = 1.8	0.29
	25	1,010	Milk yield[3] (kg/d)				
			≥0.6	-	-	1.6	0.005
			≥0.8	-	-	0.2	0.56
			≥1.0	-	-	-0.8	0.10
			≥1.2	-	-	-1.2	0.03
			≥1.4	-	-	-1.9	0.003
			≥1.6	-	-	-1.8	0.003
			≥1.8	-	-	-1.7	0.03
			≥2.0	-	-	-1.3	0.13
Ospina et al., 2010b	91	1,095	Time to conception[4]				
			≥1.0	-	-	HR[5] = 0.9	0.1
	91	454	ME305[6] (kg,				

			primiparous)				
			≥0.9	-	-	403	0.04
	91	653	ME305 (kg, multiparous)				
			≥1.0	-	-	-393	0.04
Ospina et al., 2010c	100	1,440	DA				
			≥1.0	71	80	RR = 6.9	<0.001
	100	1,440	Culling[7]				
			≥1.0	-	-	RR = 6.3	0.001
Chapinal et al., 2011	22	2,365	DA				
			≥0.9	57	69	OR = 2.9	<0.001
Seifi et al., 2011[1]	16	899	DA				
			≥0.8	96	57	OR = 27.6	0.001
			≥1.0	91	71	OR = 24.6	<0.001
			≥1.2	68	82	OR = 9.5	<0.001
			≥1.4	59	86	OR = 8.4	<0.001
			≥1.6	50	90	OR = 8.7	<0.001
McArt et al., 2012a	4	1,343	DA				
			≥1.2	-	-	RR[9] = 19.3	<0.001
	4	1,345	Culling				
			≥1.2	-	-	RR = 3.0	< 0.001
	3	751	Conception to 1st service				
			≥1.2	-	-	RR = 0.9	0.55
	3	751	Time to conception[8]				
			≥1.2	-	-	HR = 0.9	0.40
	3	1,115	Milk yield[9] (kg/d)				
			≥1.2	-	-	-1.2	0.006
Roberts et al., 2012	69	5,979	Culling				
			≥1.2	31	82	OR = 1.8	<0.001

[1] Studies reported cut-points for more than one week postpartum; only results from week 1 postpartum are described above.
[2] OR = Odds Ratio
[3] Based on first DHI test
[4] Time to conception within 70 days post-voluntary waiting period
[5] HR = Hazard Ratio
[6] ME305 = mature-equivalent 305-day milk yield estimated at 120 days in milk
[7] Culling data unpublished from Ospina et al., 2010c
[8] Time to conception within 150 days in milk
[9] RR = Risk Ratio
[10] Milk yield per cow per day for the first 30 days in milk

Figure 1.1. Histogram of a) incidence and b) prevalence of subclinical ketosis (SCK) in 1,717 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L.



CONCLUSIONS

Negative energy balance is a normal occurrence in dairy cattle during the transition from late gestation to early lactation. It occurs because the energy demands for early lactation milk production cannot be completely met by feed intake. While NEB is a physiologically normal process, excessive NEB reflects poor adaptation and results in adverse health and production effects after calving. Improved identification of cows with poor adaptation should be the goal on any dairy farm. While prevention of NEB is beyond the scope of this review, controlled-energy dry cow diets and feeding of monensin have been found to lessen NEB and help cows transition more smoothly into lactation (Duffield et al., 1998; Dann et al., 2006).

Postpartum NEFA prevalence testing, while expensive and practically challenging, gives the best herd-level evaluation of NEB status. Herd prevalence testing for hyperketonemia allows a quick snapshot of NEB and should be repeated when changes in transition cow nutrition or management occur. If herd prevalence of hyperketonemia is high, accurate and timely identification of cows with hyperketonemia will allow for individual cow treatment interventions in order to decrease the risk of DA, culling, poor reproduction, and low milk yield. In order to most accurately identify cows with hyperketonemia, incidence testing must be performed. Incidence testing of fresh cows with a cow-side blood BHBA meter allows for convenient testing with accurate results and will allow herds to decide which testing and treatment strategy will be most cost effective. Treatment of hyperketonemic cows with PG will decrease the risk of negative downstream health events, improve reproduction, and in some herds will improve milk production. For herds unwilling to test all fresh animals, testing should be focused on animals with high pre-calving NEFA concentrations (if available), cows of advanced parity, and cows with calving difficulty.

CONFLICT OF INTEREST STATEMENT

The authors (Jessica A. A. McArt, Daryl V. Nydam, Garrett R. Oetzel, Thomas R. Overton, and Paula A. Ospina) have no financial or personal relationships with other people or organizations that could inappropriately influence or bias the paper entitled *Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance: A review*.

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CHAPTER TWO

EPIDEMIOLOGY OF SUBCLINICAL KETOSIS IN EARLY LACTATION DAIRY CATTLE

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Journal of Dairy Science 95:5056-5066

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ABSTRACT

The purpose of this study was to describe the epidemiology of subclinical ketosis (SCK) in dairy cows in early lactation and determine the association of (1) days in milk (DIM) at onset of SCK, and (2) blood β-hydroxybutyrate (BHBA) concentration at onset of SCK with development of displaced abomasum (DA) and removal from herd in the first 30 DIM, conception to first service, days to conception within 150 DIM, and early lactation milk yield. Cows from 4 freestall dairy herds (2 in New York and 2 in Wisconsin) were each tested 6 times for SCK from 3 to 16 DIM using the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). Subclinical ketosis was defined as a BHBA concentration of 1.2 to 2.9 mmol/L. Mixed-effects multivariable Poisson regression was used to assess DA, removal from herd, and conception to first service. Semiparametric proportional hazards models were used to evaluate days to conception, and repeated-measures ANOVA was used to evaluate milk yield in the first 30 DIM. A total of 741 of 1,717 (43.2%) eligible cows had a least one BHBA test of 1.2 to 2.9 mmol/L. Peak incidence of SCK occurred at 5 DIM, when 22.3% of cows had their first SCK-positive test. Peak prevalence of SCK occurred at 5 DIM, when 28.9% of cows had a SCK-positive test. Median time from first positive SCK test until BHBA test <1.2 mmol/L was 5 d. Cows first testing SCK positive from 3 to 5 DIM were 6.1 times more likely [95% confidence interval (CI) = 2.3 to 16.0] to develop a DA than cows first testing SCK positive at 6 DIM or later. Cows first testing SCK positive from 3 to 7 DIM were 4.5 times more likely (95% CI = 1.7 to 11.7) to be removed from the herd, were 0.7 times as likely (95% CI = 0.6 to 0.8) to conceive to first service, and produced 2.2 kg less milk per day for the first 30 DIM than cows first testing positive at 8 DIM or later. Each 0.1 mmol/L increase in BHBA at first SCK-positive test increased the risk of developing a DA by a factor of 1.1 (95% CI = 1.0 to 1.2), increased the risk of removal from herd by a factor of 1.4 (95% CI = 1.1 to 1.8), and was associated with a decrease

in milk production by 0.5 kg/d for the first 30 DIM. These results show that time of onset and BHBA concentration of first SCK-positive test are important indicators of individual cow performance.

Key words: dairy cow, ketosis, incidence, prevalence

INTRODUCTION

The ability of dairy cattle to adapt to the natural change of energy balance in early lactation is an important aspect of the transition period, as the demands for milk production cannot be met by feed intake alone (Bauman and Currie, 1980; Baird, 1982; Herdt, 2000). Cattle unable to adequately transition through this period are at a higher risk for metabolic disorders and decreased milk production (Cameron et al., 1998; Drackley, 1999; Herdt, 2000). One of these metabolic disorders, hyperketonemia, develops as a sequela to a poor adaptive response to negative energy balance and occurs when the liver is overwhelmed with NEFA. Hyperketonemia can manifest clinically as a decrease in appetite, weight loss, and a decrease in milk production, but cows are more likely to suffer from subclinical ketosis (SCK), defined as an excess of circulating ketone bodies without clinical signs of ketosis (Andersson, 1988). Early lactational incidence of SCK was found to affect 40 to 60% of cows in herds undergoing repeated testing (Emery et al., 1964; Simensen et al., 1990; Duffield et al., 1998) and is much higher than the 2 to 15% incidence found with clinical ketosis (Duffield, 2000). Cows with SCK are at an increased risk of postpartum diseases such as displaced abomasum (DA) and metritis (Duffield et al., 2009; Ospina et al., 2010a), which may increase their risk of removal from the herd during early lactation. In addition to the effects on disease events, SCK has been found to decrease milk yield in early lactation (Dohoo and Martin, 1984; Ospina et al., 2010b) and may adversely affect

reproduction.

Although studies have reported the prevalence of SCK over time (Dohoo and Martin, 1984; Duffield et al., 1998, 2009), none has adequately described the epidemiology of SCK. Aside from McArt et al. (2011), an interventional trial conducted concurrently with this observational study, no large field trials have reported monitoring BHBA concentrations more frequently than once a week. Practical difficulties of securing adequate labor to repeatedly sample a large number of cows and the lack of a rapid, accurate, and relatively inexpensive cow-side test for SCK may explain why cows were not sampled more frequently in previous studies. In addition, sampling cows only once weekly does not accurately determine SCK incidence, as the median time to resolution of SCK is approximately 5 d (McArt et al., 2011). The recent identification and validation of the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL) by Iwersen et al. (2009) and Konkol et al. (2009) eases many of the previous difficulties associated with intensive ketosis monitoring programs.

The objectives of this study were to describe the epidemiology of SCK in cows diagnosed with SCK in early lactation through an intensive monitoring program from 3 to 16 DIM and to determine the association of (1) DIM at onset of SCK, and (2) BHBA concentration at onset of SCK with development of DA and removal from herd in the first 30 DIM, conception to first service, days to conception within 150 DIM, and milk yield in the first 30 DIM.

MATERIALS AND METHODS

Study Population

Data were collected from 2 dairy farms (farms A and B) in New York State from May 18 until September 8, 2010, and from 2 dairy farms (farms C and D) in Wisconsin from June 11 until

August 30, 2010. To be selected, farms had to meet the following criteria: milk at least 1,500 cows, have headlocks in fresh cow pens, use the farm management program Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and be willing to participate in the proposed ketosis testing protocol. In-depth information concerning farm management, nutrition, and reproductive and disease events was published previously (McArt et al., 2011).

Data Collection and Study Design

Enrollment into the study occurred at calving. Cows were tested from 3 to 16 DIM on Mondays, Wednesdays, and Fridays for ketosis using a Precision Xtra meter. The Precision Xtra meter is a hand-held device used to test blood BHBA concentrations; sensitivity and specificity compared with serum BHBA concentrations determined photometrically are 88 to 96% and 96 to 98%, respectively, when using a cut-off value of ≥1.2 mmol/L (Iwersen et al., 2009; Konkol et al., 2009). Given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. Subclinical ketosis was defined as a BHBA concentration of 1.2 to 2.9 mmol/L; clinical ketosis was defined as BHBA ≥3.0 mmol/L (Oetzel, 2004). All testing of cows for SCK from 3 to 16 DIM was completed by the research team during the study. Detailed blood collection and testing information has been reported previously (McArt et al., 2011).

This observational study was part of a larger randomized field trial in which cows with BHBA concentrations of 1.2 to 2.9 mmol/L were sequentially randomized to treatment group [oral propylene glycol (PG) drench] or control group (no PG) after their first SCK-positive test. For the study, cows assigned to the PG treatment group were removed from analysis after their first positive BHBA test, at which point they began receiving PG. Remaining cows were excluded from the study if their previous gestation length was <260 d, if they died or were sold

before their first BHBA test, if they were diagnosed and treated by the farm for ketosis before their first BHBA test, or for lack of proper identification. Additionally, cows remaining in the herd through 16 DIM were excluded if they had fewer than 5 BHBA tests. Further data collected included parity, DA, metritis, sold and died events, conception to first service, and DIM at conception. Displaced abomasa, cows died, cows sold, and pregnancy outcomes were exported throughout the study period from each farm's Dairy Comp 305 program. In addition, milk weights were exported for the 3 herds that recorded milk production on per milking basis (farms A, B, and D).

A proposal was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (#2008-0099) and the University of Wisconsin-Madison School of Veterinary Medicine Animal Care and Use Committee (#V01479-0-05-10). All farmers were asked to sign a consent form agreeing to the proposed testing and treatment protocol and were given a document containing information on disease definitions including clinical milk fever, retained placenta, metritis, displaced abomasum, and clinical ketosis.

Statistical Analysis

Descriptive statistics were generated with the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC). Incidence and prevalence histograms were prepared in Excel (Microsoft Corp., Redmond, WA). Daily incidence of SCK was calculated by dividing the number of cows that tested BHBA positive for the first time on each DIM by the total number of cows at risk for that DIM. A cow was determined to be at risk at any given DIM if she had a recorded BHBA value for that DIM and had not previously had a BHBA test $\geq$1.2 mmol/L. Data from all eligible cows were used to develop the incidence histogram. Daily prevalence of SCK was calculated by dividing the number of cows with a positive BHBA test on each DIM by the total number of

cows tested for that DIM. To calculate prevalence for 3 and 4 DIM, data from all eligible cows were used because PG-treated cows were removed from the analysis after their first positive BHBA test. Prevalence for 5 to 16 DIM was calculated using only data from SCK-positive control cows and half of the nonketotic cows. One-half of the nonketotic cows were randomly removed to adjust for the fact that half of the SCK-positive cows were removed because they were assigned to the PG treatment group. If the prevalence denominator had not been adjusted in this manner, the calculated prevalence would have been falsely decreased by a factor of 2. Daily incidence of clinical ketosis included only data from SCK-positive control cows and nonketotic cows. As SCK-positive PG-treated cows were less likely to develop clinical ketosis than control cows (McArt et al., 2011), their data were excluded when developing the histogram to prevent bias. Similar to the SCK prevalence histogram, because half of the SCK-positive cows were removed due to PG treatment, half of the nonketotic cows were removed to appropriately adjust the denominator to calculate the incidence.

Time to resolution of SCK (BHBA <1.2 mmol/L) was analyzed with a Kaplan-Meier (Kaplan and Meier, 1958) model using the LIFETEST procedure of SAS. The time-series variables were defined as time from first BHBA test of 1.2 to 2.9 mmol/L until either 1 or 2 continuous subsequent BHBA tests of <1.2 mmol/L. A censoring variable was used to differentiate cows whose blood BHBA decreased to <1.2 mmol/L from cows that were removed from the herd, removed from the study, or did not have a BHBA <1.2 mmol/L by 16 DIM. Kaplan-Meier graphs were produced using MedCalc (MedCalc Software, Mariakerke, Belgium).

Five outcomes (development of a DA within 30 DIM, removal from herd within 30 DIM, conception to first service, time to conception within 150 DIM, and milk yield in the first 30 DIM) were analyzed for each of 3 conditions: SCK status, DIM at first positive SCK test, and BHBA concentration at first positive SCK test. The associations of the conditions with DA

development, removal from herd, and conception to first service were analyzed using mixed-effects multivariable Poisson regression with the GENMOD procedure of SAS (Frome and Checkoway, 1985; Spiegelman and Hertzmark, 2005; Ospina et al., 2012). For the model evaluating DA development, the 2 cows that developed a DA before their first positive BHBA test were removed from the analysis. The potential confounding variables parity (lactation 1, lactation 2, and lactation ≥3) and metritis were offered to the models (the variable DA was also offered to the model concerning removal from herd) as independent variables, in addition to the model-respective variables on SCK status (SCK), DIM at first positive SCK test (DIMPOS), and BHBA concentration at first positive SCK test (BHBAPOS). The variable SCK was entered into the SCK status models as a dichotomous variable.

For all outcomes except DA development, the variable DIMPOS was entered into the DIM at first positive SCK test models as a dichotomous variable comparing cows with their first positive SCK test at 3 to 7 DIM with those having a first positive SCK test at 8 to 16 DIM. Because all cows that developed a DA had their first positive test ≤6 DIM, the variable DIMPOS was entered as a dichotomous variable comparing cows with their first positive SCK test at 3 to 5 DIM with those having their first positive SCK test at 6 to 16 DIM. This change in grouping was performed because at least one event in each category is needed to compute a ratio statistic; grouping cows for this outcome into 3 to 7 DIM and 8 to 16 DIM as for the other outcomes would produce a statistically impossible calculation. The variable BHBAPOS was entered into the BHBA at first positive SCK test models as a continuous variable.

The variable herd was entered as a random effect in the DA and removal models. Because of large differences in voluntary waiting period (VWP), conception to first service, and breeding strategy (inseminating many cows twice) on farm D, the variable herd was tested as a fixed effect in the conception to first service model; an offset term was used to adjust for the difference in

VWP for each herd.

For all models, independent variables were removed by manual backward stepwise elimination if their contrast estimate was considered statistically nonsignificant ($P > 0.10$) and biologically not important. As only one fixed effect variable remained in all models after stepwise elimination, no interaction terms were tested. Statistical significance of the variable herd in the conception to first service model led to further analysis of conception by herd. Due to biologically plausible explanations for a change in outcome direction compared with the other 3 farms, farm D was excluded from both conception to first service and time to conception analyses for all conditions. The variable herd was then re-entered into the conception to first service model as a random effect to account for the unmeasured variations between the remaining 3 herds. Analyses of days to conception was completed by semiparametric proportional hazards model (Cox, 1972) using the PHREG procedure of SAS. The time-series variable for the model was the number of days from calving until conception within 150 DIM. Censoring variables were used to identify cows that conceived from cows that were removed from the herd or did not conceive by 150 DIM. Independent variables offered to the model included model respective variables (SCK, DIMPOS, and BHBAPOS), parity, and herd. Independent variables and their respective interaction terms were manually removed by backward stepwise elimination if considered statistically nonsignificant ($P > 0.10$) and biologically not important. Proportional hazards assumptions were verified by evaluating the time-dependent covariates (Allison, 1995); noninformative censoring was evaluated using sensitivity analysis. Kaplan-Meier analyses using only the respective model variables SCK, DIMPOS, and BHBAPOS were completed using the LIFETEST procedure of SAS to determine median days from calving until conception.

Differences between groups in milk yield for individual milk weights until 30 DIM was analyzed using repeated-measures ANOVA with first-order autoregressive covariance using the

MIXED procedure of SAS (Littell et al., 1998, 2000). Results were analyzed using different covariance structures; a first-order autoregressive covariance structure was chosen as it produced the lowest Akaike information criterion, a measure of the relative goodness-of-fit. Variables offered to the models included model-respective variables (SCK, DIMPOS, and BHBAPOS), parity (lactations 1, 2, ≥3), DIM, and herd as a random effect. Independent variables and their respective interaction terms were considered statistically significant if $P \leq 0.05$. A scatterplot, best-fit line, and R2 statistic were produced from the ANOVA model with the mean predicted milk yield output for each concentration of BHBA from 1.2 to 2.9 mmol/L using Excel (Microsoft Corp.).

RESULTS

Descriptive Statistics

Of the 1,717 eligible cows, 741 (43.2%) were diagnosed with SCK at least once and randomized, with 372 cows receiving PG treatment and 369 control cows. Cows in the PG treatment group were removed from further analysis after their first positive BHBA test, which left 369 cows that had at least one positive test for SCK and 976 cows that never tested positive. The SCK group was composed of 106 (28.7%), 97 (26.3%), and 166 (45.0%) cows in parity 1, 2, and ≥3, respectively (median = 2); the nonketotic group contained 398 cows (40.8%) in parity 1, 327 (33.5%) in parity 2, and 251 (25.7%) in parity ≥3 (median = 2). A chi-squared test showed a difference in parity between the 2 groups ($P < 0.001$).

Displaced Abomasum and Removal from Herd. In the first 30 DIM, 3 of the 976 nonketotic cows (0.3%) developed a DA and 24 of 367 cows (6.5%) developed a DA after testing positive for SCK (2 cows developed a DA before testing positive for SCK and were

excluded from the analysis). Cows testing positive for SCK were 19.3 times more likely [risk ratio (RR) 95% CI = 13.8 to 27.0, P < 0.001] to develop a DA than nonketotic cows. In the first 30 DIM, 18 of 976 (1.8%) nonketotic cows and 20 of 369 cows (5.4%) that tested positive for SCK were removed from the herd. Cows with SCK were 3.0 times more likely (RR 95% CI = 2.2 to 4.2, P < 0.001) to die or be culled than nonketotic cows. The final regression models for both DA and removal from herd included only SCK as a statistically meaningful predictor variable with herd as a random effect; the final model estimates are in Table 1.

Conception to First Service. Of the 3 farms used in the reproductive analyses (farms A, B, and C), 751 cows had data concerning conception at first service, of which 241 of 603 (40.0%) nonketotic cows and 52 of 148 (35.1%) SCK-positive cows conceived. Conception to first service did not differ between the 2 groups, with SCK cows equally likely (RR = 0.9, 95% CI = 0.8 to 1.2, P = 0.55) to conceive as nonketotic cows. The final regression model included only the SCK treatment variable with herd as a random effect; the estimate for the final conception to first service model is in Table 1.

Time to Conception. Of the 749 cows on farms A, B, and C with data on pregnancy status at 150 DIM, 496 of 601 (82.5%) nonketotic cows and 115 of 148 (77.7%) SCK-positive cows were pregnant. Days to conception within 150 DIM did not differ between the 2 groups [hazard ratio (HR) for SCK cows = 0.9, 95% CI = 0.7 to 1.1, P = 0.40], with a median time to conception of 96 d (95% CI = 92 to 101) and 104 d (95% CI = 95 to 114) for nonketotic and SCK cows, respectively. The final model concerning the association of SCK on time to pregnancy included the variables SCK, herd, and lactation group.

Milk Yield. In total, 1,115 cows from farms A, B, and D were used in the milk yield analysis: 804 nonketotic cows and 311 SCK-positive cows. The fixed-effect variables SCK, parity group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk

weights with the variable herd as a random effect. Nonketotic cows produced 0.4 kg more milk per milking in the first 30 d of lactation than SCK-positive cows, at 11.7 and 11.3 kg, respectively (P = 0.006), for a total difference of 1.2 kg/cow per day.

Epidemiology of SCK

Histograms of SCK incidence and prevalence by DIM are given in Figures 1 and 2, respectively. Figure 3 is a histogram of incidence of clinical ketosis by DIM. A Kaplan-Meier curve describing time from first positive BHBA test to 1 negative BHBA test for the SCK group is shown in Figure 4. Median time until 1 negative test was 5 d (95% CI = 4 to 5). Of the 369 SCK-positive cows, 163 (44.2%) did not test <1.2 mmol/L by 16 DIM (n = 35) or died (n = 14), developed clinical ketosis (n = 52), or were treated by the farm (n = 62) before testing <1.2 mmol/L.

Association of DIM at First Positive BHBA Test

Displaced Abomasum and Removal from Herd. Of the 24 SCK-positive cows that developed a DA in the first 30 DIM, 22 (91.7%) had their first positive BHBA test from 3 to 5 DIM and 2 (8.3%) at 6 DIM. The median time from first positive BHBA test to DA was 5 d (range = 1 to 24 d). Cows diagnosed with SCK for the first time from 3 to 5 DIM were 6.1 times more likely (RR 95% CI = 2.3 to 16.0, P < 0.001) to develop a DA than cows first testing SCK positive at 6 DIM or later. Of the 20 SCK-positive cows that were removed from the herd in the first 30 DIM, 19 (95.0%) had their first positive BHBA test from 3 to 7 DIM and 1 (5.0%) at 12 DIM. The median time from first positive BHBA test to removal from herd was 9 d (range = 2 to 24 d). Cows diagnosed with SCK for the first time from 3 to 7 DIM were 4.5 times more likely (RR

95% CI = 1.7 to 11.7, P = 0.002) to be removed from the herd than cows first testing SCK positive at 8 DIM or later. The final regression models for both DA and removal from herd included only the variable DIMPOS with herd as a random effect; the final model estimates are in Table 2.

Conception to First Service. In total, 148 SCK cows from farms A, B, and C had data concerning conception to first service, of which 52 (35.1%) conceived. Of these 52 cows, 35 (67.3%) had their first positive BHBA test from 3 to 7 DIM and 17 (32.7%) from 8 and 16 DIM. Cows diagnosed with SCK for the first time from 3 to 7 DIM were 0.7 times as likely (RR 95% CI = 0.6 to 0.8, P < 0.001) to conceive to first service than cows first testing SCK positive at 8 DIM or later. The final regression model included only the variable DIMPOS with herd as a random effect; the final model estimates are in Table 2.

Time to Conception. Of the 148 cows on farms A, B, and C with data concerning pregnancy status at 150 DIM, 115 (77.7%) were pregnant. Of these 115 cows, 82 (71.3%) had their first positive BHBA test between 3 to 7 DIM and 33 (28.7%) between 8 and 16 DIM. Cows diagnosed with SCK for the first time from 3 to 7 DIM were 0.7 times as likely (HR 95% CI = 0.5 to 1.1, P = 0.13) to conceive by 150 DIM than cows first testing SCK positive at 8 DIM or later, with a median time to conception of 107 d (95% CI = 95 to 119) and 98 d (95% CI = 78 to 117) for cows first testing SCK positive at 3 to 7 DIM and 8 to 16 DIM, respectively. The final model included the variables DIMPOS and herd.

Milk Yield. In total, 311 cows from farms A, B, and D were used in the milk yield analysis. The fixed effect variables DIMPOS, lactation group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk weights with the variable herd as a random effect. Cows diagnosed with SCK for the first time from 3 to 7 DIM produced 0.7 kg less milk per milking in the first 30 d of lactation than cows diagnosed with SCK for the first time

between 8 and 16 DIM, at 11.2 kg and 11.9 kg, respectively (P = 0.04). Given a 3-times-daily milking routine, the total difference in milk between the 2 groups was 2.1 kg per cow per day.

Association of Concentration at First Positive BHBA Test

Displaced Abomasum and Removal from Herd. Each 0.1 mmol/L increase in BHBA increased the risk of developing a DA by 30 DIM by a factor of 1.1 (95% CI = 1.0 to 1.2, P = 0.002). For example, cows whose first positive BHBA concentration was 1.3 mmol/L were 1.1 times more likely to develop a DA than cows whose first positive BHBA concentration was 1.2 mmol/L; cows whose first positive BHBA concentration was 2.4 mmol/L were 3.1 (1.112) times more likely to develop a DA than cows whose first positive BHBA concentration was 1.2 mmol/L. Each 0.1 mmol/L increase in BHBA increased the risk of removal from the herd in the first 30 DIM by a factor of 1.4 (95% CI = 1.1 to 1.8, P = 0.01). For example, cows whose first positive BHBA concentration was 1.3 mmol/L were 1.4 times more likely to be removed from the herd than cows whose first positive BHBA concentration was 1.2 mmol/L; cows whose first positive BHBA concentration was 2.4 mmol/L were 56.7 (1.412) times more likely be removed from the herd than cows whose first positive BHBA concentration was 1.2 mmol/L. The final regression models for both DA and removal from herd included only the continuous variable BHBAPOS with herd as a random effect; the final model estimates are in Table 3.

Conception to First Service. We observed no difference in BHBA concentration at first positive test and conception to first service (0.1 mmol/L increase RR = 1.1, 95% CI = 0.5 to 2.3, P = 0.84). Thus, cows with a BHBA concentration of 1.2 mmol/L at first positive test had the same risk of conception as cows with a BHBA concentration at first positive test of 1.3 or 2.4 mmol/L. The final regression model included only the continuous variable BHBAPOS with herd as a random effect; the final model estimates are in Table 3.

Time to Conception. We observed no difference in BHBA concentration at first positive test and time to conception (0.1 mmol/L increase HR = 1.2, 95% CI = 0.8 to 1.9, P = 0.38). Thus, cows with a BHBA concentration of 1.2 mmol/L at first positive test had the same hazard of conception as cows with a BHBA concentration at first positive test of 1.3 or 2.4 mmol/L. The final model included the variables BHBAPOS and herd.

Milk Yield. The fixed-effect variables BHBA concentration at first positive test, parity group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk weights with the variable herd as a random effect. The milk yield per milking decreased as BHBA concentration at first positive test increased (P < 0.001). Predicted mean milk yield per milking at each subclinical BHBA concentration is shown in Figure 5 and can be estimated from the following formula:

Predicted milk per day (kg) = 42.6 – 5.1 (BHBA concentration at first positive test).

Thus, for each 0.1 mmol/L increase in BHBA concentration at first positive test, the predicted milk produced for the first 30 d of lactation is estimated to decrease by 0.5 kg/d. The coefficient of determination for the model (R2) was 0.4.

Table 2.1. Estimates for 3 final Poisson regression models showing risk ratios (RR) comparing cows diagnosed with subclinical ketosis to nonketotic cows for 1,345 Holstein cows following intensive testing for subclinical ketosis from 3 to 16 DIM[1]

Model[1]	Estimate	SE[2]	*P*-value[3]	RR	95% CI[4]
DA	2.96	0.17	<0.001	19.3	13.8 to 27.0
Removal	1.10	0.17	<0.001	3.0	2.2 to 4.2
Conception	−0.06	0.11	0.55	0.9	0.8 to 1.2

[1]The 3 outcomes modeled were (1) development of a displaced abomasum (DA) within 30 DIM, (2) removal from herd within 30 DIM, and (3) conception to first service.

[1]SE = standard error for estimate.

[3]P-value reported for estimate.

[4]CI for risk or hazard ratio.

Table 2.2. Estimates for 3 final Poisson regression models showing risk ratios (RR) comparing cows diagnosed with subclinical ketosis (SCK) from 3 to 7 DIM with cows diagnosed with SCK from 8 to 16 DIM for 369 Holstein cows with at least one positive test for SCK from 3 to 16 DIM[1]

Model[1]	Estimate	SE[2]	*P*-value[3]	RR	95% CI[4]
DA	1.81	0.49	<0.001	6.1	2.3 to 16.0
Removal	1.51	0.49	0.002	4.5	1.7 to 11.7
Conception	−0.35	0.08	<0.001	0.7	0.6 to 0.8

[1]The 3 outcomes modeled were (1) development of a displaced abomasum (DA) within 30 DIM, (2) removal from herd within 30 DIM, and (3) conception to first service. For the DA outcome, cows were dichotomized into first positive test at 3 to 5 DIM or 6 to 16 DIM; for the remaining outcomes, cows were dichotomized into first positive test at 3 to 7 DIM or 8 to 16 DIM.

[2]SE = standard error for estimate.

[3]P-value reported for estimate.

[4]CI for risk or hazard ratio.

Table 2.3. Estimates for 3 final Poisson regression models showing risk ratios (RR) as BHBA concentration at first positive test increased for 369 Holstein cows with at least one positive test for subclinical ketosis from 3 to 16 DIM[1]

Model[1]	Estimate	SE[2]	*P*-value[3]	RR	95% CI[4]
DA	0.09	0.03	0.002	1.1	1.0 to 1.2
Removal	0.35	0.13	0.01	1.4	1.1 to 1.8
Conception	0.08	0.38	0.84	1.1	0.5 to 2.3

[1]The 3 outcomes modeled were (1) development of a displaced abomasum (DA) within 30 DIM, (2) removal from herd within 30 DIM, and (3) conception to first service.

[2]SE = standard error for estimate.

[3]P-value reported for estimate.

[4]CI for risk or hazard ratio.

Figure 2.1. Histogram of incidence of subclinical ketosis (SCK) in 1,717 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L.

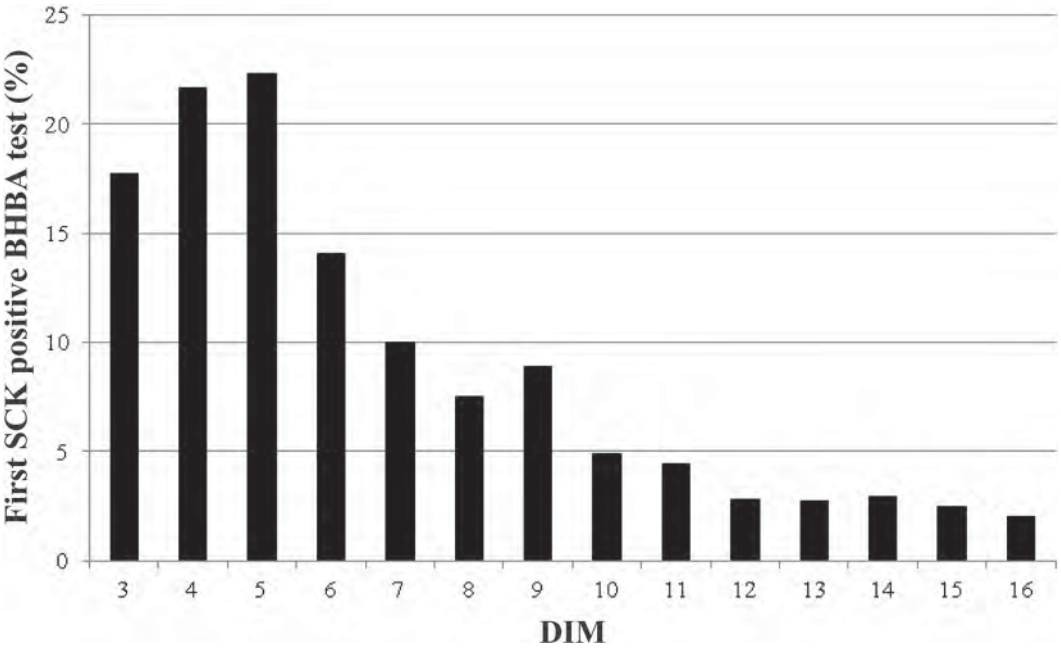


Figure 2.2. Histogram of prevalence of subclinical ketosis (SCK) in 1,717 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L.

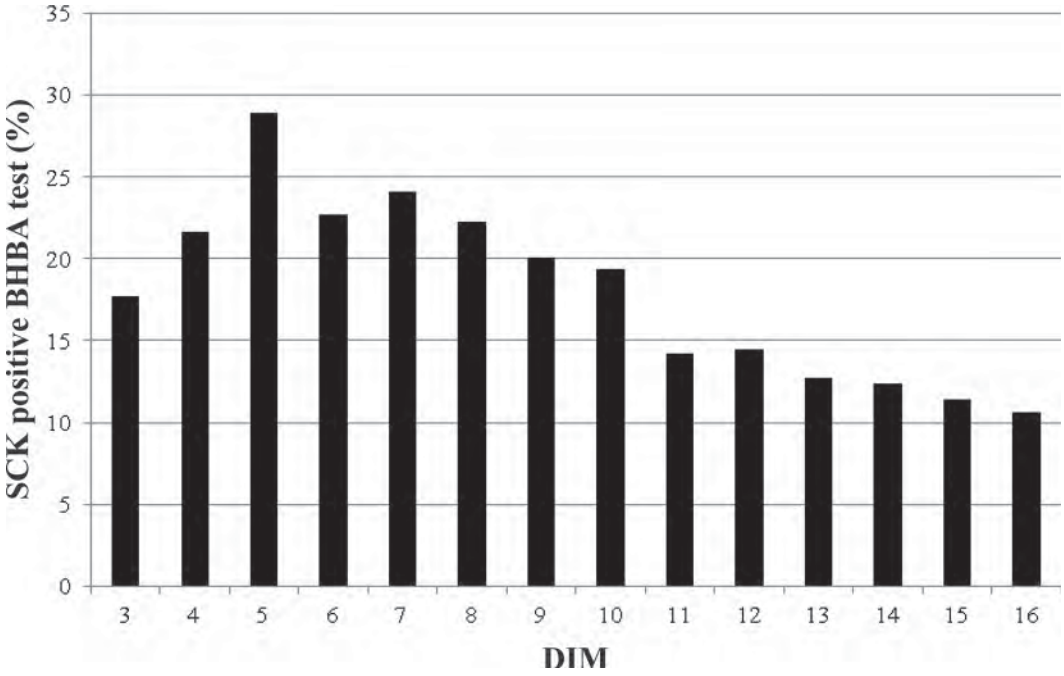


Figure 2.3. Histogram of incidence of clinical ketosis (CK) in 856 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. A positive test was defined as a blood BHBA concentration of ≥3.0 mmol/L.

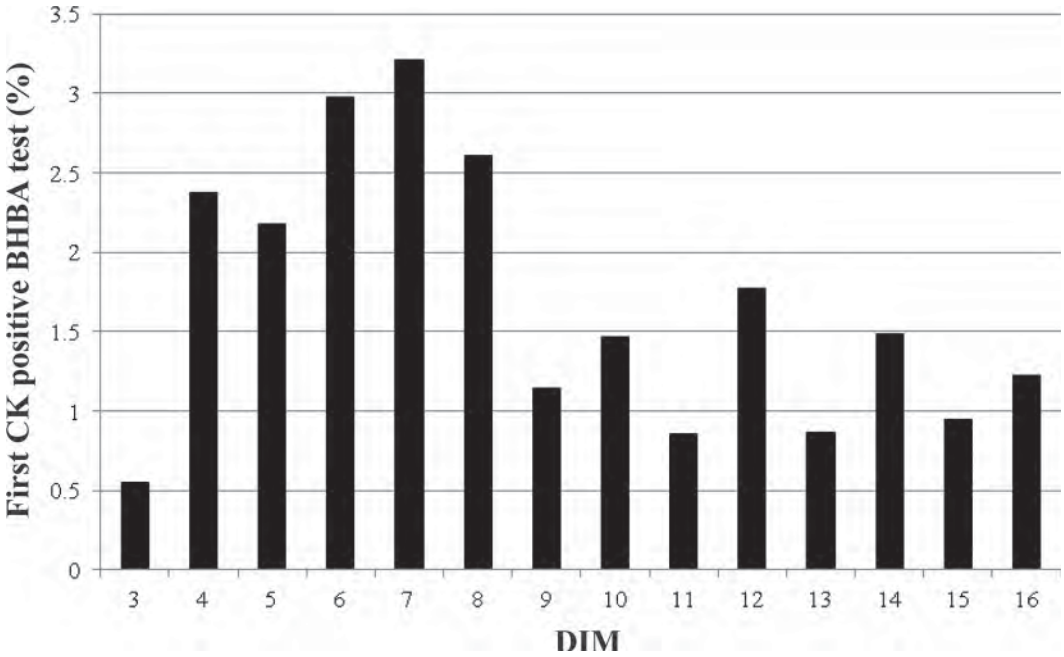


Figure 2.4. Kaplan-Meier curves of time from first positive test for subclinical ketosis (SCK; BHBA concentration of 1.2 to 2.9 mmol/L) to one blood BHBA concentration of <1.2 mmol/L in 369 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. Because of a Monday, Wednesday, Friday testing scheme, cows were not able to cure on d 0, 1, 6, or 8 after first positive BHBA test.

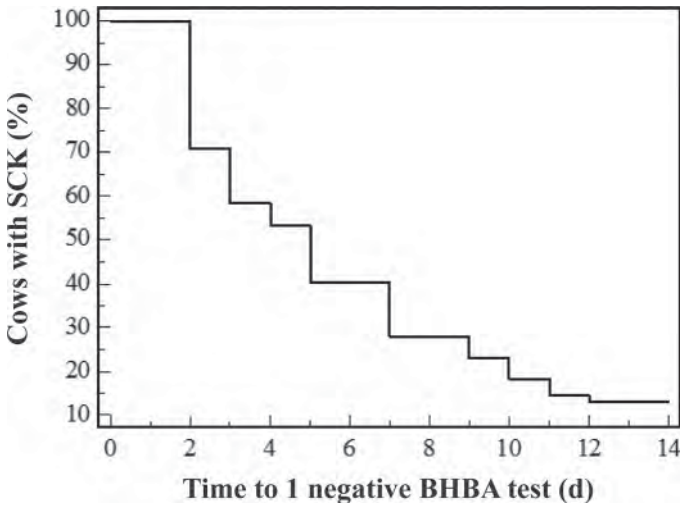
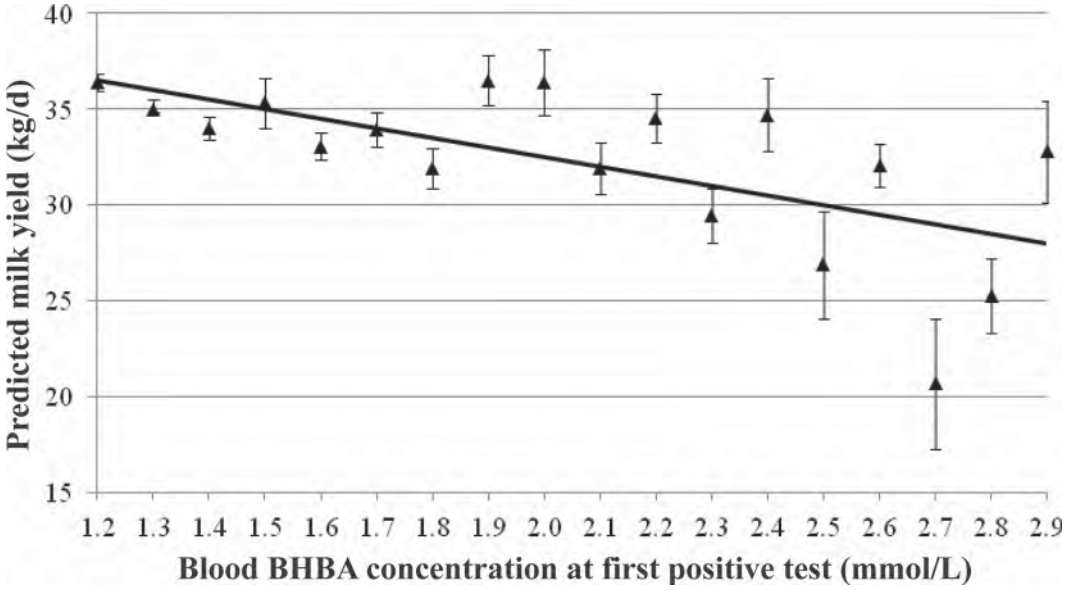


Figure 2.5. Regression plot of mean predicted milk yield per milking for the first 30 DIM by blood BHBA concentration of first positive BHBA test (1.2 to 2.9 mmol/L) for 369 Holstein dairy cows undergoing repeated testing for ketosis from 3 to 16 DIM. The solid line represents the best linear fit; 95% CI are shown for each predicted milk yield by BHBA concentration.



DISCUSSION

This observational study was conducted to describe the epidemiology of SCK in cows in early lactation and to determine the association of (1) DIM at onset of SCK and (2) blood BHBA concentration at onset of SCK with development of DA and removal from herd in the first 30 DIM, conception to first service, days to conception within 150 DIM, and early lactation milk yield. Our results indicated that cows develop SCK very early in lactation and that cows developing SCK within the first week postpartum were more likely to have adverse health events and produce less milk than cows developing SCK after the first week of lactation. In addition, as the concentration of BHBA at first positive SCK test increased, the risk of adverse events increased and milk production decreased.

The average incidence of SCK from 3 to 16 DIM in the reported study herds was 43% and ranged from 26 to 56% (McArt et al., 2011), with peak SCK incidence occurring at 5 DIM. Although cows were sampled beginning at 3 DIM, the shape of the incidence curve suggests that the incidence of SCK at 1 and 2 DIM is less than that at 5 DIM; however, the design of this study did not allow confirmation of this. No other studies have reported the incidence and prevalence of SCK in dairy cows undergoing BHBA testing more frequently than once a week in early lactation. Although previous studies from Emery et al. (1964), Simensen et al. (1990), and Duffield et al. (1998) had similar findings and reported cumulative SCK incidences of 49, 46, and 59%, respectively, these numbers most likely underestimate the true incidence as cows were tested once weekly. As shown in the current study, the median time from first SCK-positive BHBA test to first test <1.2 mmol/L was 5 d, which would allow cows to develop and resolve SCK between testing sessions if testing occurred only once weekly. In addition to underestimation due to the frequency of testing, previously reported SCK incidences may be falsely decreased due to timing of weekly testing. For example, Duffield et al. (2009) found a 24

and 25% incidence of SCK (BHBA concentration ≥1.2 mmol/L) for cows tested in the first and second weeks of lactation, respectively. Depending on what DIM cows were tested within the first week of lactation; for example, 4 versus 7 DIM, the true incidence could be falsely decreased by a factor of 2.

Both incidence (number of new cases divided by the number of cows at risk) and prevalence (number of existing cases divided by the number of cows sampled) of SCK are important measurements in herd settings. Incidence describes how quickly new cases develop, and it can be higher than the prevalence depending on the frequency of testing and the duration of disease. As a measure of SCK, incidence is a useful tool for individual animal treatment decisions. However, to get an accurate measure of incidence, frequent testing is necessary. The prevalence of SCK describes what proportion of the animals tested have SCK at the time of testing and can be used for herd monitoring over time as well as an outcome indicator for changes in dry or fresh cow management.

Results on the associations of SCK with adverse events are similar to those found in other studies concerning development of DA (Duffield et al., 2009; Ospina et al., 2010a) and early lactation milk yield (Dohoo and Martin, 1984; Ospina et al., 2010b), showing that the population of dairy cattle used in this study has good external validity with other published trials. Although no difference was found in conception to first service, similar to results found by Walsh et al. (2007) and Kessel et al. (2008), it is possible that the power of the reported study was too small to find a difference. The calculated power to find a difference in conception risk between SCK-positive and nonketotic cows was only 17%, most likely because of the smaller subset of SCK-positive cows used in the analysis. Thus, the findings concerning conception to first service should be interpreted with this in mind.

Whereas the studies mentioned above regarding the development of DA, early lactation

milk yield, and reproduction have detailed the negative associations of SCK on health events, reproduction, and milk production in fresh cows, no studies have reported the effects of DIM or blood BHBA concentration at onset of SCK. Data from this study clearly show that cows developing SCK within the first week postpartum are at a much higher risk for DA development, removal from herd, and poor milk production than cows developing SCK after 1 wk postpartum. It can be postulated that cows developing SCK within 1 wk postpartum have experienced extremely poor adaptation to negative energy balance through calving and into lactation, whereas cows developing SCK after the first week postpartum may have better adapted to the effects of decreased DMI during and immediately post calving but are not able to sustain energy stores for the increase in milk production in early lactation.

Blood BHBA concentration at first SCK-positive test also has an effect on health events and milk production. Extrapolating from this study's findings, a cow with a BHBA concentration at the high end of the SCK range (2.4 mmol/L) is 3 times more likely to develop a DA, is >50 times more likely to be removed from the herd, and is expected to produce 180 kg less milk in the first 30 d of lactation than a cow with a BHBA concentration on the low end of the SCK range (1.2 mmol/L). Intuitively, cows with a more severe ketosis have less energy available to make milk; thus, cows with a poorer adaptation to negative energy balance (higher mobilization of NEFA leading to a more severe ketosis) may be at greater risk for adverse health events.

CONCLUSIONS

Cows that developed SCK within the first week postpartum were more likely to develop a DA or be removed from the herd within the first 30 DIM, were less likely to conceive to first service, and produced less milk in the first 30 DIM than cows developing SCK after the first

week of lactation. In addition, as the concentration of BHBA at first positive SCK test increased, the risk of adverse events increased and milk production decreased. As 75% of all cows that developed SCK tested positive within 1 wk postpartum (with a peak at 5 DIM), it may be important to modify farm management protocols to maximize detection during this time. Cows that begin lactation with high BHBA concentrations may require special attention to decrease their risk of adverse events in early lactation.

ACKNOWLEDGMENTS

This study was funded in part by the US Department of Agriculture National Research Initiative (USDA-NRI no. 2008-55204-19393) and Boehringer Ingelheim Vetmedica Inc. (St. Joseph, MO). The authors thank Judith Alishauskas, Stephanie Brittin, Emily Bold, Mark Fagan, and Caitlin Jeffrey (Cornell University, Ithaca, NY), and Lindley Reilly, Eric Rooker, Angela Niles, Tyler Franks, Catherine Bargren, Diego Calderon, Cristian Vergara, and Loïc Commun (University of Wisconsin, Madison) for their invaluable assistance with data collection as well as the owners of the collaborating dairies for allowing us access to their cows and facilities on which to conduct this research.

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CHAPTER THREE

DRY PERIOD AND PARTURIENT PREDICTORS OF EARLY LACTATION HYPERKETONEMIA IN DAIRY CATTLE

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Journal of Dairy Science
http://dx.doi.org/10.3168/jds.2012-5681

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ABSTRACT

The purpose was to determine important dry and calving period predictors of (1) a cow developing hyperketonemia at any time between 3 and 16 d in milk (DIM) and (2) a cow having hyperketonemia at her first β-hydroxybutyrate (BHBA) test after calving (between 3 and 5 DIM). Cows from 4 freestall dairy herds [2 in New York (NY) and 2 in Wisconsin] were enrolled at 266 d carried calf. Precalving data included body condition score, locomotion score, and blood nonesterified fatty acids (NEFA) concentration; calving-associated data included previous days carried calf, calving ease, calf sex, twins, stillbirth, and parity. Cows were each tested 6 times for hyperketonemia from 3 to 16 DIM on Mondays, Wednesdays, and Fridays using the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). Hyperketonemia was defined as a blood BHBA concentration of ≥1.2 mmol/L. Multivariable fixed-effects Poisson regression models were developed to predict the probability of a cow developing hyperketonemia between either 3 and 16 DIM or at her first BHBA test. As only the NY herds had precalving NEFA data, each prediction model was developed twice: once with data from all 4 herds (n = 1,672) and once with data from only the NY herds (n = 544). For the models with data from all 4 herds, increased body condition score group and an interaction between advanced parity and herd were important predictors of hyperketonemia development at any time from 3 to 16 DIM; calf sex (male), herd, and an advanced parity by increased body condition score group interaction were important predictors of hyperketonemia development between 3 and 5 DIM. The 4-herd models had a 64 and 78% predictive concordance for hyperketonemia between 3 and 16 DIM and at first BHBA test, respectively. For the models with data from the NY herds only, increased NEFA, calf sex (male), advanced parity, and herd were found to be important predictors of hyperketonemia development at any time from 3 to 16 DIM; increased NEFA, calf sex (male), decreased calving ease, stillbirth, and advanced parity were important

predictors of having hyperketonemia at first BHBA test. The NY models had a 69 and 87% predictive concordance, respectively. These results may help identify at-risk animals and improve dry-cow management strategies before hyperketonemia develops.

Key words: dairy cow, hyperketonemia, nonesterified fatty acid, prediction

INTRODUCTION

All dairy cows transition through a state of negative energy balance during early lactation when the energy demands of milk production cannot be met by feed intake alone (Bauman and Currie, 1980; Baird, 1982; Herdt, 2000). Cows unable to adequately transition through this period often develop hyperketonemia, an excessive elevation of ketone bodies in the blood (Herdt, 2000). The negative effects of hyperketonemia on individual cow health and dairy economics are well reported. Hyperketonemic cows are more likely to develop displaced abomasa (LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010a) and be culled than nonhyperketonemic cows (Gröhn et al., 1998; McArt et al., 2012). In addition, hyperketonemia has been found to result in a substantial loss in milk yield during early lactation (Dohoo and Martin, 1984; Duffield et al., 2009; Ospina et al., 2010b). Economic losses due to treatment costs, increased culling, and decreased milk production have been estimated at $211 per case of clinical hyperketonemia (C. Guard, Cornell University, Ithaca, NY, personal communication, 2012) and $78 per case of subclinical hyperketonemia (Geishauser et al., 2001). McLaren et al. (2006) found that an economic index of return over feed (milk income minus feed cost) in Ontario (Canada) dairy herds decreased by $0.015 per cow per day for every 1% increase in the herd incidence of subclinical hyperketonemia.

Although the development of hyperketonemia is multifactorial, it has been suggested that body condition at calving and proper nutrition during the transition period can affect negative energy balance during early lactation and, thus, development of hyperketonemia. A review by Overton and Waldron (2004) concluded that cows with a moderately lower BCS that are well managed during the transition period are more likely to adequately transition into lactation than cows of higher BCS, possibly due to their ability to increase DMI in early lactation. Multiple studies have indicated that nutritional management during the dry period can affect postpartum health and production, and that overfeeding increases prepartum NEFA levels, decreases DMI before calving, and increases blood BHBA after calving (Dann et al., 2006; Douglas et al., 2006; Janovick et al., 2011).

The time of hyperketonemia development is an important prognostic indicator of downstream events. A recent observational study on large confinement dairies showed that the incidence of hyperketonemia, defined as a blood BHBA concentration of ≥1.2 mmol/L, peaks at 5 DIM, with 75% of cows that ever develop hyperketonemia first testing positive between 3 and 7 DIM. Cows first testing positive between 3 to 7 DIM were more likely to develop a displaced abomasum or leave the herd in the first 30 DIM, less likely to conceive to first service, and produced less milk in the first 30 DIM than cows testing positive from 8 to 16 DIM (McArt et al., 2012).

These reasons underscore the importance of identification of factors during the transition period that may affect the development of hyperketonemia in early lactation. Knowledge of these factors may improve the ability to predict which cows will develop hyperketonemia, allowing preventative measures and more focused testing for certain groups of animals. The objectives of this study were to determine important dry and calving period risk factors of (1) a cow developing hyperketonemia at any time between 3 and 16 DIM and (2) a cow having

hyperketonemia at her first BHBA test (between 3 and 5 DIM).

MATERIALS AND METHODS

Study Population

Data were collected from 2 dairy farms (farms A and B) in New York (NY) from May 18, 2010, to September 8, 2010, and from 2 dairy farms (farms C and D) in Wisconsin from June 11, 2010, to August 30, 2010. To be selected, farms had to meet the following criteria: milk at least 1,500 cows, have headlocks in fresh cow pens, use the farm management program Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and be willing to participate in the proposed data collection and hyperketonemia testing protocol. In-depth information concerning farm management structure, nutrition, and reproductive and disease events has been previously published (McArt et al., 2011).

Data Collection and Study Design

Precalving data was collected twice weekly on cows starting at approximately 266 d carried calf, and included BCS (Ferguson et al., 1994), locomotion score (LS; a modification of the system proposed by Nordlund et al., 2004), and blood collection for NEFA concentration (NY herds only). Body condition was assessed on a 5-point scale with 0.25-unit increments, with a higher score representing a greater body condition. Locomotion score was assessed on a 4-point scale with scores assigned from 1 to 4, representing non-lame, slightly lame, moderately lame, and severely lames cows, respectively. In the NY herds, blood was collected from the coccygeal vessels of each cow using a tube without anticoagulant and a 20-gauge × 2.54-cm blood-collection needle. Samples were immediately placed on ice and serum separated in a

centrifuge at 2,000 × g for 10 min within 1 h of collection. Serum samples were kept on ice until laboratory analysis (Stokol and Nydam, 2005). Body condition and lameness were scored by one individual in each region throughout the study. Additional postcalving data exported throughout the study period from each farm's Dairy Comp 305 program included previous days carried calf (PDCC), calving ease (CEASE; using a 1 to 5 scale, with 1 representing no assistance and 5 representing extreme assistance), calf sex (CSEX), twins, stillbirth, and parity.

To determine an outcome of hyperketonemia, cows were tested from 3 to 16 DIM on Mondays, Wednesdays, and Fridays for hyperketonemia using a Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). The Precision Xtra meter is a hand-held device used to test blood BHBA concentrations; sensitivity and specificity compared with serum BHBA concentrations determined photometrically are 88 to 96% and 96 to 98%, respectively, when using a cut-off value of ≥1.2 mmol/L (Iwersen et al., 2009; Konkol et al., 2009). Given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. Hyperketonemia was defined as a BHBA concentration of ≥1.2 mmol/L. All hyperketonemia testing of cows from 3 to 16 DIM was completed by the research team during the study.

Cows were excluded from the analysis if their PDCC was less than 260, if they died or were sold before their first BHBA test, or for lack of proper identification. Additionally, for the outcome concerning the development of hyperketonemia at any time between 3 and 16 DIM, cows remaining in the herd through 16 DIM were excluded if they had fewer than 5 BHBA tests. For the outcome concerning hyperketonemia at first BHBA test, cows without a recorded BHBA concentration at first test were excluded from the analysis.

A proposal was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (no. 2008–0099) and the University of Wisconsin-Madison School of

Veterinary Medicine Animal Care and Use Committee (no. V01479–0-05–10). All farms were asked to sign a consent form agreeing to the proposed data collection and testing protocol.

Statistical Analysis

Two predictive models were developed: (1) hyperketonemia at any time between 3 and 16 DIM (HYPKTN), and (2) hyperketonemia at first BHBA test (HYPKTN1). Each predictive model was developed using 2 differing data sets: (1) data from all herds that excluded blood NEFA concentration from the NY herds or (2) data from the NY herds that included blood NEFA concentration. For the subjective measurements of BCS and LS, the weighted kappa values (a measure of agreement; Landis and Koch, 1977) between the raters were 0.19 (slight) and 0.49 (moderate), respectively. Given only a slight agreement between raters for BCS, a within-rater adjustment was performed. The BCS of cows by each rater was grouped into 3 categories (1, 2, and 3) based on cows with a BCS less than the median, the median score, or a BCS greater than the median, respectively. The weighted kappa value comparing BCS group (BCSG) between raters was 0.60 (moderate). Variables used for analysis were categorical and included herd, BCSG (1, 2, and 3), LS (1 or 2, 3 or 4), NEFA (<0.30 mEq/L and ≥0.30 mEq/L), CEASE (1, 2, and ≥3), CSEX [female(s) and at least 1 male], twins, stillbirth (live calf or calves and at least 1 dead calf), PDCC (<272 and ≥272), and parity (lactation 1, lactation 2, and lactation ≥3). Cows with prepartum NEFA values were only included if blood was obtained between 14 and 3 d before calving and if the hemolytic index was <300 in the sample (Stokol and Nydam, 2006). Prepartum blood NEFA concentration was categorized based on studies showing the negative effects of prepartum NEFA ≥0.30 mEq/L on postpartum outcomes (Cameron et al., 1998; Ospina et al., 2010b). The continuous variable PDCC was categorized into early parturition (less than 1 standard deviation below the mean) or normal to prolonged

gestation (equal to or greater than 1 standard deviation above the mean). Not all data were available for all eligible cows, and only cows with complete data were included in the analyses.

Descriptive statistics (chi-squared tests and ANOVA) were generated with PROC FREQ and PROC GLM of SAS (SAS Institute Inc., Cary, NC). The assumptions of normality and homoscedasticity were tested and upheld for BHBA concentration at first positive test for all risk factors before performing ANOVA. Correlation between variables was determined using PROC CORR of SAS, with variables considered to be associated if their correlation coefficient was ≥0.3; no variables met this criteria.

The prediction models were produced using fixed effects multivariable Poisson regression with the PROC GENMOD of SAS (Frome and Checkoway, 1985; Spiegelman and Hertzmark, 2005; Ospina et al., 2012), with an adjustment for overdispersion. All putative risk factors were explored for their univariate association with the outcome of interest using a chi-squared test. Any variable with $P < 0.20$ was offered to a multivariable model. Multivariable models were then formed manually via backward stepwise elimination if $P > 0.05$. Any remaining variables were offered to the model in pairwise interactions if biologically important; the interactions were then removed manually via backward stepwise elimination if $P > 0.05$. Predictive concordance of the final model and actual outcomes were calculated to assess model fit (Steyerberg et al., 2010).

RESULTS

Descriptive Statistics

In total, 1,955 cows began the study during their prefreshening period. The 4-herd HYPRKTN model had 1,618 cows eligible for analysis, with data recorded for all variables. The 4 herd HYPRKTN1 model had 1,672 cows eligible for analysis, with data recorded for all

variables. The NY HYPRKTN and HYPRKTN1 models had 767 cows eligible for analysis. Of these cows, 544 had data recorded for all variables (233 cows did not have a NEFA value obtained between 14 and 3 d precalving due to variability in actual calving dates). Histograms showing the distributions of precalving LS and BCSG, blood NEFA concentrations (for the NY herds), and PDCC are in Figure 1. Descriptive statistics for the HYPRKTN prediction models for all 4 herds and the 2 NY herds are in Table 1 and Table 2, respectively. Descriptive statistics for the HYPRKTN1 prediction models for all 4 herds and the 2 NY herds are in Table 3 and Table 4, respectively. The incidence of hyperketonemia in cows repeatedly tested from 3 to 16 DIM was 45.7%; the incidence of hyperketonemia in cows at their first BHBA test at 3 to 5 DIM was 23.0%.

Prediction of Hyperketonemia at Any Time from 3 to 16 DIM

All 4 Herds. The final model for prediction of hyperketonemia at any time from 3 to 16 DIM was as follows:

$$\log_e(\text{probability of HYPRKTN}) = \text{BCSG} + \text{parity} + \text{herd} + \text{parity} \times \text{herd} + \varepsilon,$$

where ε is a residual error term. Estimated Poisson regression coefficients and risk ratios for the variables included in the model are in Table 5. A 64% predictive concordance was found with the observed results using this model.

NY Herds. The final model for prediction of hyperketonemia at any time from 3 to 16 DIM was as follows:

$$\log_e(\text{probability of HYPRKTN}) = \text{NEFA} + \text{CSEX} + \text{parity} + \text{herd} + \varepsilon.$$

Estimated Poisson regression coefficients and risk ratios for the variables included in the model are in Table 6. No interactions were found between variables. A 69% predictive concordance was found with the observed results using this model.

Prediction of Hyperketonemia at First BHBA Test

All 4 Herds. The final model for prediction of hyperketonemia at first test (3, 4, or 5 DIM) was as follows:

$$\log_e(\text{probability of HYPRKTN1}) = \text{BCSG} + \text{CSEX} + \text{parity} + \text{herd} + \text{parity} \times \text{BCSG} + \varepsilon.$$

Estimated Poisson regression coefficients and risk ratios for the variables included in the model are in Table 6. A 78% predictive concordance was found with the observed results using this model.

NY Herds. The final model for prediction of hyperketonemia at first test (3, 4, or 5 DIM) was as follows:

$$\log_e(\text{probability of HYPRKTN1}) = \text{NEFA} + \text{CSEX} + \text{CEASE} + \text{stillbirth} + \text{parity} + \varepsilon.$$

Estimated Poisson regression coefficients and risk ratios for the variables included in the model

are in Table 8. No interactions were found between variables. An 87% predictive concordance was found with the observed results using this model.

Table 3.1. Descriptive statistics and chi-squared analysis of 1,618 Holstein cows from 4 herds undergoing repeated testing for hyperketonemia from 3 to 16 DIM[1]

Variable	Hyperketonemic [no. (%)]	Non-hyperketonemic [no. (%)]	*P*-value[2]
Herd			
Farm A	143 (42.7)	192 (57.3)	< 0.001
Farm B	95 (27.1)	256 (72.9)	
Farm C	120 (43.5)	156 (56.5)	
Farm D	381 (58.1)	275 (41.9)	
BCSG			
1	215 (41.5)	303 (58.5)	< 0.001
2	293 (41.5)	413 (58.5)	
3	231 (58.6)	163 (41.4)	
LS			
1 or 2	667 (45.0)	815 (55.0)	0.08
3 or 4	72 (52.9)	64 (47.1)	
CEASE			
1	596 (46.1)	697 (53.9)	0.75
2	98 (43.4)	128 (56.6)	
≥ 3	45 (45.5)	54 (54.5)	
CSEX			
Female	347 (44.4)	435 (55.6)	0.31
Male	392 (46.9)	444 (53.1)	
Twins			
0	703 (45.7)	835 (54.3)	0.90
1	36 (45.0)	44 (55.0)	
Stillbirth			
0	702 (45.8)	832 (54.2)	0.76
1	37 (44.0)	47 (56.0)	
PDCC			
< 272	96 (36.6)	166 (63.4)	0.001
≥ 272	643 (47.4)	713 (52.6)	
Parity			
1	206 (37.4)	345 (62.6)	< 0.001
2	182 (37.4)	305 (62.6)	
≥ 3	351 (60.5)	229 (39.5)	

[1]Cows were categorized as hyperketonemic if at any time between 3 and 16 DIM their blood BHBA concentration was ≥1.2 mmol/L. Analyzed variables included herd, BCS group (BCSG), locomotion score (LS), calving ease (CEASE), calf sex (CSEX: female or females only, at least 1 male), twins, stillbirth (at least 1 dead calf), previous days carried calf (PDCC), and parity.

[2]P-value reported for $\chi 2$ statistic.

Table 3.2. Descriptive statistics and chi-squared analysis of 544 Holstein cows from 2 New York herds undergoing repeated testing for hyperketonemia from 3 to 16 DIM[1]

Variable	Hyperketonemic [no. (%)]	Non-hyperketonemic [no. (%)]	*P*-value[2]
Herd			
Farm A	105 (41.0)	151 (59.0)	< 0.001
Farm B	79 (27.4)	209 (72.6)	
BCSG			
1	75 (34.6)	142 (65.4)	
2	93 (33.7)	183 (66.3)	0.91
3	16 (31.4)	35 (68.6)	
LS			
1 or 2	173 (34.2)	333 (65.8)	0.51
3 or 4	11 (29.0)	27 (71.0)	
NEFA			
< 0.30 mEq/L	142 (31.3)	311 (68.7)	0.006
≥ 0.30 mEq/L	42 (46.1)	49 (53.9)	
CEASE			
1	143 (33.4)	285 (66.6)	
2	31 (36.9)	53 (63.1)	0.79
≥ 3	10 (31.2)	22 (68.8)	
CSEX			
Female	83 (29.9)	195 (70.1)	0.05
Male	101 (38.0)	165 (62.0)	
Twins			
0	178 (34.2)	343 (65.8)	0.42
1	6 (26.1)	17 (73.9)	
Stillbirth			
0	169 (33.2)	340 (66.8)	0.24
1	15 (42.9)	20 (57.1)	
PDCC			
< 272	11 (22.4)	38 (77.6)	0.08
≥ 272	173 (35.0)	322 (65.0)	
Parity			
1	64 (30.3)	147 (69.7)	
2	31 (19.6)	127 (80.4)	< 0.001
≥ 3	89 (50.9)	86 (49.1)	

[1]Cows were categorized as hyperketonemic if at any time between 3 and 16 DIM their blood BHBA concentration was ≥1.2 mmol/L. Analyzed variables included herd, BCS group (BCSG), locomotion score (LS), NEFA, calving ease (CEASE), calf sex [CSEX: female(s), at least 1 male], twins, stillbirth (at least 1 dead calf), previous days carried calf (PDCC), and parity.

[2]P-value reported for $\chi 2$ statistic.

Table 3.3. Descriptive statistics, chi-squared analyses, and ANOVA for 1,672 Holstein cows with a blood BHBA concentration (mmol/L) recorded at first BHBA test (between 3 and 5 DIM)[1]

Variable	Hyperketonemic [no. (%)]	Non-hyperketonemic [no. (%)]	*P*-value[2]	Mean BHBA (SD)	*P*-value[4]
Herd					
Farm A	58 (17.3)	277 (82.7)		0.80 (0.48)	
Farm B	45 (12.8)	307 (87.2)	0.57	0.73 (0.55)	< 0.001
Farm C	69 (20.0)	276 (80.0)		0.88 (0.55)	
Farm D	213 (33.3)	427 (66.7)		1.07 (0.77)	
BCSG					
1	102 (19.0)	434 (81.0)		0.82 (0.55)	
2	144 (19.9)	578 (80.1)	< 0.001	0.86 (0.59)	< 0.001
3	139 (33.6)	275 (66.4)		1.09 (0.75)	
LS					
1 or 2	344 (22.6)	1181 (77.4)	0.14	0.90 (0.62)	0.02
3 or 4	41 (27.9)	106 (72.1)		1.03 (0.76)	
CEASE					
1	305 (22.9)	1028 (77.1)		0.89 (0.62)	
2	50 (21.1)	187 (78.9)	0.24	0.87 (0.56)	0.10
≥ 3	30 (29.4)	72 (70.6)		1.02 (0.84)	
CSEX					
Female	165 (20.6)	637 (79.4)	0.02	0.87 (0.61)	0.10
Male	220 (25.3)	650 (74.7)		0.92 (0.64)	
Twins					
0	358 (22.5)	1232 (77.5)	0.10	0.89 (0.60)	0.008
1	27 (32.9)	55 (67.1)		1.06 (0.89)	
Stillbirth					
0	360 (22.7)	1224 (77.3)	0.22	0.90 (0.62)	0.44
1	25 (28.4)	63 (71.6)		0.95 (0.76)	
PDCC					
< 272	48 (17.2)	231 (82.8)	0.01	0.81 (0.64)	0.002
≥ 272	337 (24.2)	1056 (75.8)		0.92 (0.64)	
Parity					
1	87 (15.2)	487 (84.8)		0.76 (0.52)	
2	95 (18.9)	408 (81.1)	< 0.001	0.84 (0.53)	< 0.001
≥ 3	203 (34.1)	392 (65.9)		1.09 (0.53)	

[1]Cows were categorized as hyperketonemic if their BHBA concentration was ≥1.2 mmol/L. Analyzed variables included herd, BCS group (BCSG), locomotion score (LS), calving ease (CEASE), calf sex [CSEX: female(s), at least 1 male], twins, stillbirth (at least 1 dead calf), previous days carried calf (PDCC), and parity.

[2]P-value reported for $\chi 2$ statistic.

[3]P-value reported for F-test statistic.

Table 3.4. Descriptive statistics, chi-squared analyses, and ANOVA of 544 Holstein cows in New York State with a blood BHBA concentration (mmol/L) recorded between 3 and 5 DIM[1]

Variable	Hyperketonemic [no. (%)]	Non-hyperketonemic [no. (%)]	*P*-value[2]	Mean BHBA (SD)	*P*-value[3]
Herd					
Farm A	39 (15.2)	217 (84.8)	0.57	0.79 (0.47)	0.52
Farm B	39 (13.5)	249 (86.5)		0.76 (0.62)	
BCSG					
1	29 (13.4)	187 (86.6)		0.77 (0.59)	
2	40 (14.4)	237 (85.6)	0.74	0.77 (0.53)	0.86
3	9 (17.6)	42 (82.4)		0.81 (0.53)	
LS					
1 or 2	71 (14.0)	435 (86.0)	0.46	0.76 (0.54)	0.10
3 or 4	7 (18.4)	31 (81.6)		0.91 (0.71)	
NEFA					
< 0.30 mEq/L	54 (11.9)	399 (88.1)	< 0.001	0.72 (0.42)	< 0.001
> 0.30 mEq/L	24 (26.4)	67 (73.6)		1.03 (0.94)	
CEASE					
1	57 (13.3)	372 (86.7)		0.75 (0.50)	
2	11 (13.1)	73 (86.9)	0.01	0.78 (0.48)	0.03
≥ 3	10 (32.3)	21 (67.7)		1.02 (1.09)	
CSEX					
Female	29 (10.5)	248 (89.5)	0.009	0.73 (0.54)	0.10
Male	49 (18.3)	218 (81.7)		0.81 (0.56)	
Twins					
0	72 (13.8)	449 (86.2)	0.10	0.77 (0.55)	0.41
1	6 (26.1)	17 (73.9)		0.87 (0.62)	
Stillbirth					
0	67 (13.2)	441 (86.8)	0.004	0.76 (0.55)	0.09
1	11 (30.6)	25 (69.4)		0.93 (0.53)	
PDCC					
< 272	4 (8.2)	45 (91.8)	0.20	0.63 (0.33)	0.05
≥ 272	74 (15.9)	421 (85.1)		0.79 (0.57)	
Parity					
1	22 (10.4)	190 (89.6)		0.70 (0.48)	
2	11 (7.1)	145 (92.9)	< 0.001	0.64 (0.33)	< 0.001
≥ 3	45 (25.6)	131 (74.4)		0.98 (0.71)	

[1]Cows were categorized as hyperketonemic if their BHBA concentration was ≥1.2 mmol/L. Analyzed variables included herd, BCS group (BCSG), locomotion score (LS), NEFA, calving ease (CEASE), calf sex [CSEX: female(s), at least 1 male], twins, stillbirth (at least 1 dead calf), previous days carried calf (PDCC), and parity.

[2]P-value reported for $\chi 2$ statistic.

[3]P-value reported for F-test statistic.

Table 3.5. Maximum likelihood estimates of Poisson regression coefficients (β), standard errors, risk ratios (RR), and 95% confidence intervals for model variables used in the prediction of hyperketonemia (blood BHBA concentration ≥1.2 mmol/L) at any time from 3 to 16 DIM in 1,618 Holstein cows from all 4 herds undergoing repeated monitoring for hyperketonemia[1]

Parameter	β	SE	*P*-value[2]	RR (95% CI)
Intercept	-0.85	0.10	< 0.001	-
BCSG				
1	-[3]	-		-
2	0.05	0.07	0.003	1.1 (0.9 to 1.2)
3	0.25	0.08		1.2 (1.1 to 1.5)
Parity*Herd				
1, Farm A	-[3]	-		-
1, Farm B	-0.20	0.17		0.8 (0.6 to 1.1)
1, Farm C	-0.03	0.18		1.0 (0.7 to 1.4)
1, Farm D	0.36	0.14		1.4 (1.1 to 2.0)
2, Farm A	0.15	0.18		1.2 (0.8 to 1.7)
2, Farm B	-0.88	0.22	0.001	0.4 (0.3 to 0.6)
2, Farm C	0.25	0.16		1.3 (0.9 to 1.8)
2, Farm D	0.47	0.14		1.6 (1.2 to 2.1)
≥ 3, Farm A	0.69	0.15		2.0 (1.5 to 2.6)
≥ 3, Farm B	0.28	0.16		1.3 (1.0 to 1.8)
≥ 3, Farm C	0.50	0.16		1.6 (1.2 to 2.3)
≥ 3, Farm D	0.72	0.13		2.0 (1.6 to 2.7)

[1]Variables retained in the final model included parity, BCS group (BCSG), and herd. As an interaction was found between parity and herd, the reported variables only include BSCG and the parity × herd interaction.

[2]P-value reported for coefficient estimate of entire variable.

[3]Reference group.

Table 3.6. Maximum likelihood estimates of Poisson regression coefficients (β), standard errors, risk ratios (RR), and 95% confidence intervals for model variables used in the prediction of hyperketonemia (blood BHBA concentration ≥1.2 mmol/L) at any time from 3 to 16 DIM in 544 Holstein cows from New York State undergoing repeated monitoring for hyperketonemia[1]

Parameter	β	SE	*P*-value[2]	RR (95% CI)
Intercept	-1.26	0.14	< 0.001	-
NEFA				
< 0.30 mEq/L	-[3]	-	0.05	-
≥ 0.30 mEq/L	0.28	0.14		1.3 (1.0 to 1.8)
CSEX				
F	-[3]	-	0.01	-
M	0.30	0.12		1.4 (1.1 to 1.7)
Parity				
1	-[3]	-	< 0.001	-
2	-0.29	0.18		0.7 (0.5 to 1.0)
≥ 3	0.60	0.13		1.7 (1.3 to 2.2)
Herd				
Farm A	-[3]	-	0.005	-
Farm B	-0.34	0.12		0.7 (0.6 to 0.9)

[1]Variables retained in the final model included NEFA, calf sex [CSEX: female(s), at least 1 male], parity, and herd.

[2]P-value reported for coefficient estimate of entire variable.

[3]Reference group.

Table 3.7. Maximum likelihood estimates of Poisson regression coefficients (β), standard errors, adjusted risk ratios (RR), and 95% confidence intervals for model variables used in the prediction of hyperketonemia (blood BHBA concentration ≥1.2 mmol/L) on the first test after parturition (3, 4, or 5 DIM) in 1,672 Holstein cows from all 4 herds[1]

Parameter	β	SE	*P*-value[2]	RR (95% CI)
Intercept	-4.48	0.58	< 0.001	-
CSEX				
F	-[3]	-	0.03	-
M	0.20	0.09		1.2 (1.0 to 1.5)
Herd				
Farm A	-[3]	-	< 0.001	-
Farm B	-0.32	0.18		0.7 (0.5 to 1.0)
Farm C	-0.01	0.16		1.0 (0.7 to 1.4)
Farm D	0.48	0.14		1.6 (1.2 to 2.1)
Parity x BCSG				
1[3], 1[4]	-[3]	-	0.004	-
1, 2	-0.19	0.28		0.8 (0.5 to 1.4)
1, 3	-0.17	0.30		0.8 (0.5 to 1.5)
2, 1	-0.24	0.29		0.8 (0.4 to 1.4)
2, 2	-0.16	0.30		0.9 (0.5 to 1.5)
2, 3	0.82	0.29		2.2 (1.3 to 4.0)
≥ 3, 1	0.42	0.27		1.5 (0.9 to 2.6)
≥ 3, 2	0.58	0.27		1.8 (1.1 to 3.0)
≥ 3, 3	0.80	0.27		2.2 (1.3 to 3.8)

[1]Variables retained in the final model included herd, calf sex [CSEX: female(s), at least 1 male], parity, and BCS group (BCSG). As an interaction was found between parity and BCSG, the reported variables include CSEX, herd, and the parity × BCSG interaction.

[2]P-value reported for coefficient estimate for entire variable.

[3]Reference group.

[4]Parity.

[5]BCSG.

Table 3.8. Maximum likelihood estimates of Poisson regression coefficients (β), standard errors, adjusted risk ratios (RR), and 95% confidence intervals for model variables used in the prediction of hyperketonemia (blood BHBA concentration ≥1.2 mmol/L) on the first test after parturition (3, 4, or 5 DIM) in 544 Holstein cows from New York State[1]

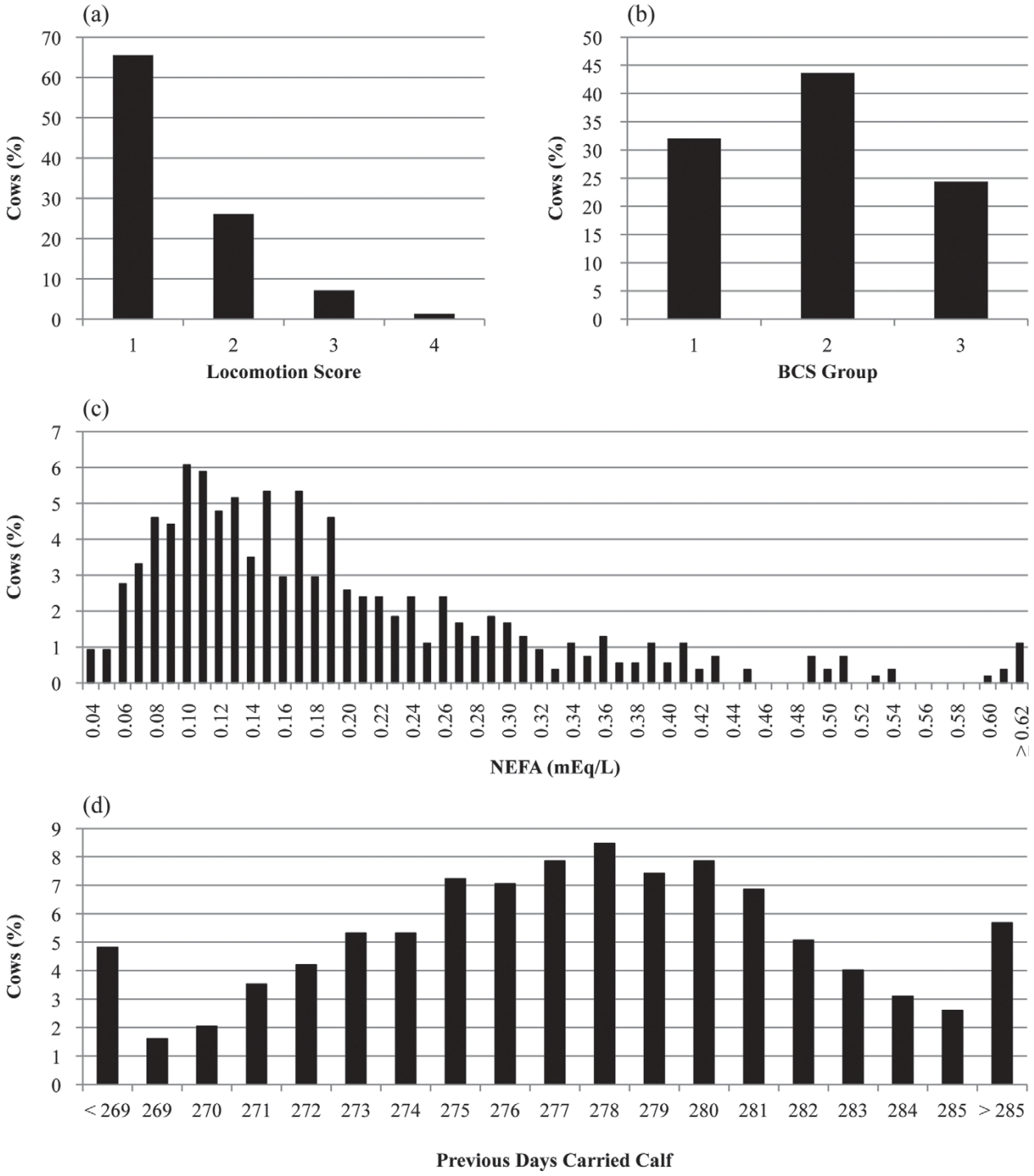
Parameter	β	SE	*P*-value[1]	RR (95% CI)
Intercept	-3.06	0.28	< 0.001	-
NEFA				
< 0.30 mEq/L	-[2]	-	0.008	-
≥ 0.30 mEq/L	0.62	0.23		1.9 (1.2 to 2.9)
CSEX				
F	-[2]	-	0.007	-
M	0.59	0.22		1.8 (1.2 to 2.7)
CEASE				
1	-[2]	-	0.04	-
2	0.13	0.31		1.1 (0.6 to 2.1)
≥ 3	0.95	0.33		2.6 (1.4 to 5.0)
Stillbirth				
0	-[2]	-	0.01	-
1	0.78	0.31		2.2 (1.2 to 4.0)
Parity				
1	-[2]	-	< 0.001	-
2	-0.17	0.35		0.8 (0.4 to 1.7)
≥ 3	1.11	0.26		3.0 (1.8 to 5.0)

[1]Variables retained in the final model included NEFA, calf sex [CSEX: female(s), at least 1 male], calving ease (CEASE), stillbirth, and parity.

[2]P-value reported for coefficient estimate for entire variable.

[3]Reference group.

Figure 3.1. Histograms showing data for 1,618 Holstein cows undergoing repeated testing for hyperketonemia from 3 to 16 DIM on (a) precalving locomotion score, (b) precalving BCS group, (c) precalving blood NEFA concentrations (544 cows), and (d) previous days carried calf.



DISCUSSION

The reported models were developed to determine important dry and calving period risk factors of (1) a cow developing hyperketonemia at any time between 3 and 16 DIM and (2) a cow having hyperketonemia at her first BHBA test. The results suggested that, in addition to variations in incidence of hyperketonemia between herds, advanced parity may be one of the biggest predictors of hyperketonemia development in early lactation. Moreover, increased BCS precalving, increased precalving blood NEFA concentration, and variables related to the calving process may help to identify at risk animals before hyperketonemia develops.

The important predictors in the model determining the probability of hyperketonemia development at any time between 3 and 16 DIM in all 4 herds included the variables BCSG, parity, and herd. A parity by herd interaction was also present, which precludes interpretation of the parity and herd variables individually. The model determined that cows in BCSG 1 and 2 were less likely to develop hyperketonemia than cows in BCSG 3; thus, an increased risk of hyperketonemia development existed in overweight cows with a BCS greater than the median score of cows in the herd. These results are in agreement with Gillund et al. (2001) who found that cows with a BCS of ≥3.5 at calving were associated with increased odds of hyperketonemia in early lactation. Multiple studies have reported on the association between cows overfed during the dry-off period and a greater negative energy balance during their subsequent lactation (Contreras et al., 2004; Dann et al., 2006; Janovick et al., 2011). In addition, Hayirli et al. (2002) found that DMI in the final 3 wk of gestation decreased linearly as BCS increased. From these studies, it follows that cows with an increased BCS at calving have an increased risk of periparturient diseases associated with a greater negative energy balance such as hyperketonemia.

The presence of a parity by herd interaction shows that the risk of hyperketonemia

development for each parity group varied depending from which herd they originated. For all herds except farm B, cows in parity 1 and parity 2 were less likely to develop hyperketonemia than cows in parity ≥3. Specifically, cows in parity ≥3 were 2.0 (95% CI = 1.5 to 2.6) times more likely on farm A, 1.7 (95% CI = 1.2 to 2.4) times more likely on farm C, and 1.4 (95% CI = 1.2 to 1.7) times more likely on farm D to develop hyperketonemia than cows in parity 1 in each herd, respectively. Cows on farm B had the same risk of hyperketonemia development no matter their parity. This may be due to increased monitoring of older cows or the fact that farm B had the lowest incidence of hyperketonemia among the 4 farms at 27.1 versus 42.6, 43.5, and 58.1% on farms A, C, and D, respectively. From these data, it can be suggested that older cows (parity ≥3) are at a higher risk of developing hyperketonemia than younger cows. These results are in agreement with many authors who have reported an increase in the prevalence or odds of hyperketonemia in early lactation with increasing parity (Kauppinen, 1983; Gröhn et al., 1989; Duffield et al., 1997).

For the model determining the probability of hyperketonemia development at any time between 3 and 16 DIM in the NY herds, the important predictor variables were precalving NEFA, CSEX, parity, and herd. It is well known that herd management factors are important predictors of hyperketonemia development in cows, and it is therefore likely that a cow's risk of hyperketonemia may be greater solely due to the fact that she resides in a certain herd. For example, cows in this study from farm B were 0.7 times as likely to develop hyperketonemia as cows from farm A. Similar to the model with data from all 4 herds, cows in parity 1 and 2 were less likely to develop hyperketonemia than cows in parity ≥3. Additionally, cows that gave birth to male calves were 1.4 times more likely to develop hyperketonemia than cows birthing female calves. Although the larger size of male calves has been associated with increased calving difficulty and stillbirth (Correa et al., 1993; Johanson and Berger, 2003; Bicalho et al., 2007),

CEASE and stillbirth were not important predictors of hyperketonemia development in these studies. Male calves may require more energy in late gestation due to their larger size and this increased energy requirement may result in a larger negative energy balance in early lactation, leading to an increased risk of hyperketonemia development. However, CSEX was not an important predictor of hyperketonemia in the model with data from all 4 herds suggesting that herd and parity may be more important variables to monitor.

Cows from NY with precalving blood NEFA concentrations ≥0.30 mEq/L were 1.3 times more likely to develop hyperketonemia between 3 and 16 DIM than cows with NEFA concentrations <0.30 mEq/L. As BCSG was not an important predictor of hyperketonemia in this model, it follows that precalving blood NEFA concentrations were a better indicator of hyperketonemia development between 3 and 16 DIM than precalving body condition. Although precalving blood NEFA concentrations are a less subjective measure than BCS, the cost of labor to collect blood and process samples as well as the expense to run the test in a diagnostic laboratory, currently $11.00 per sample at the New York State Animal Health Diagnostic Center (http://ahdc.vet.cornell.edu/test), makes collection of precalving NEFA data less attractive. Based on the results of the prediction model using all 4 herds, if the cost of collection and processing prevent availability of precalving NEFA data, a simpler, though less predictive, measurement would include precalving BCS, assuming it is consistently scored on a farm by the same individual.

The important variables from the model using data from all 4 herds to predict hyperketonemia at first BHBA test were similar to those discussed above for predicting hyperketonemia at any time between 3 and 16 DIM, specifically CSEX, herd, and a parity by BCSG interaction. Additionally, cows birthing male calves were 1.2 times more likely to have hyperketonemia at first BHBA test than cows that delivered females. The parity by BCSG

interaction showed no increase in risk of hyperketonemia for parity 1 cows, regardless of their BCSG. However, parity 2 cows in BCSG 3 and parity 3 cows in BCSG 2 or 3 were approximately twice as likely to have hyperketonemia at first BHBA test than parity 1 cows in BCSG 1. These results suggest that older cows with greater body condition are at a higher risk of early lactation hyperketonemia development, and that parity 1 cows are somehow able to compensate for the increased risk associated with a greater body condition. It can be postulated that this compensation may be due to the fact that parity 1 cows make less milk than older animals and, thus, less energy is needed for milk production, decreasing the magnitude of negative energy balance in these young cows.

For the model predicting hyperketonemia development at first BHBA test using only data from the NY herds, the important predictor variables were precalving NEFA, CSEX, CEASE, stillbirth, and parity. As with the other models, cows birthing male calves were more likely to develop hyperketonemia than cows birthing females, and parity 3 cows had a higher risk of hyperketonemia development than parity 1 or 2 cows. Similarly to the NY model predicting hyperketonemia at any time between 3 and 16 DIM, cows with precalving NEFA ≥0.30 mEq/L were more likely to develop hyperketonemia at first BHBA test than cows with precalving NEFA <0.30 mEq/L. However, the magnitude of the NEFA effect was greater for the model predicting hyperketonemia at first BHBA test, with high NEFA cows almost twice as likely to have hyperketonemia at first BHBA test compared with low NEFA cows. In addition to the aforementioned variables, cows with a CEASE ≥3 and cows delivering at least 1 stillborn calf were 2.6 and 2.2 times more likely to develop hyperketonemia than cows with a CEASE of 1 or cows birthing live calves, respectively. It is likely that cows with difficult births have a decreased DMI in early lactation, and it is possible that this decrease in intake has a negative effect on energy balance in early lactation, which increases the risk of hyperketonemia.

The models developed to predict hyperketonemia at first BHBA test had a higher predictive concordance at 78% (all 4 herds) and 87% (NY herds) than the models predicting hyperketonemia at any time between 3 and 16 DIM at 64% (all 4 herds) and 69% (NY herds). Predictive concordance is a measure of model accuracy. For example, the NY model for hyperketonemia at first BHBA test correctly predicted 87% of cows that developed hyperketonemia during that time frame in the sampled NY herds. The models predicting hyperketonemia at first BHBA test may be more accurate than those predicting hyperketonemia at any time between 3 and 16 DIM because the effect of dry and calving period predictors may wane as time progresses. However, as 75% of cows hyperketonemia positive cows in this study first tested positive between 3 and 7 DIM (McArt et al., 2012), it is reasonable to suggest that the early predictor models may be of greater benefit than the models predicting hyperketonemia at any time between 3 and 16 DIM.

A cow's risk for developing hyperketonemia depends on in which herd she resides, with cows in herds with a high incidence of early lactation hyperketonemia at a greater risk of developing hyperketonemia themselves. Within herds, the best predictors of early lactation hyperketonemia development in this study included parity, precalving NEFA, precalving BCS, and birth of a male calf. From these results, it can be suggested that increased monitoring and testing of fresh cows be focused on parity ≥3 cows, cows entering lactation with a blood NEFA concentration ≥0.3 mEq/L or BCS greater than the median of the herd, and cows that had difficulty giving birth or birthed male calves. However, these results do not suggest that cows in parity 1 or thin cows do not develop hyperketonemia in early lactation, only that they are at a decreased risk of developing the disease.

CONCLUSIONS

Advanced parity was a major predictor of hyperketonemia development in early lactation after herd-level hyperketonemia incidence was taken into account. Additionally, increased precalving BCS, increased precalving blood NEFA concentration, and variables related to a more difficult calving process (high CEASE score or stillbirth) may help identify at risk animals before hyperketonemia develops.

ACKNOWLEDGMENTS

This study was funded in part by the US Department of Agriculture–National Research Initiative (USDA-NRI no. 2008-55204-19393) and Boehringer Ingelheim Vetmedica Inc. (St. Joseph, MO). The authors thank Judith Alishauskas, Stephanie Brittin, Emily Bold, Mark Fagan, and Caitlin Jeffrey (Cornell University, Ithaca, NY), and Lindley Reilly, Eric Rooker, Angela Niles, Tyler Franks, Catherine Bargren, Diego Calderon, Cristian Vergara, and Loïc Commun (University of Wisconsin, Madison) for their invaluable assistance with data collection, as well as the owners of the collaborating dairies for allowing us access to their facilities and cows on which to conduct this research.

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CHAPTER FOUR

A FIELD TRIAL ON THE EFFECT OF PROPYLENE GLYCOL ON MILK YIELD AND RESOLUTION OF KETOSIS IN FRESH COWS DIAGNOSED WITH SUBCLINICAL KETOSIS

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Journal of Dairy Science 94:6011-6020

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ABSTRACT

The purpose of this study was to determine the effect of oral propylene glycol (PG) administration on ketosis resolution and milk yield in cows diagnosed with subclinical ketosis (SCK). Cows from 4 freestall dairy herds (2 in New York and 2 in Wisconsin) were each tested 6 times for SCK from 3 to 16 d in milk on Mondays, Wednesdays, and Fridays. Subclinical ketosis was defined as a β-hydroxybutyrate (BHBA) concentration of 1.2 to 2.9 mmol/L, and clinical ketosis was defined as ≥3.0 mmol/L. Cows with SCK were randomized to the treatment group (oral PG) or control group (no PG); treatment cows were drenched with 300 mL of PG once daily from the day they tested 1.2 to 2.9 mmol/L until the day they tested <1.2 mmol/L. Outcomes evaluated for all farms included time from SCK until BHBA test <1.2 mmol/L or until BHBA test ≥3.0 mmol/L. Individual milk weights for the first 30 d of lactation were evaluated for the 3 farms monitoring daily milk. Semiparametric proportional hazards models were used to evaluate time to event outcomes; repeated-measures ANOVA was used to assess milk weights. A total of 741 of 1,717 (43.2%) eligible enrolled cows had at least one BHBA test of 1.2 to 2.9 mmol/L. Of these, 372 were assigned to the treatment group and 369 to the control group. Based on hazard ratios, PG-treated cows were 1.50 times more likely (95% confidence interval = 1.26 to 1.79) to resolve their SCK and 0.54 times less likely (95% confidence interval = 0.34 to 0.86) to develop clinical ketosis than control cows. Across the 3 herds measuring individual milk weights, treated cows produced 0.23 kg more milk per milking in the first 30 d of lactation than control cows, for a total difference of 0.69 kg/cow per day. After identification of a treatment by herd interaction, stratification by herd showed that treated cows produced more milk per milking on farm A (0.44 kg) and farm B (0.53 kg) in the first 30 d of lactation than control cows, for a total difference of 1.34 and 1.59 kg/d, respectively; milk production did not differ (0.02 kg per milking) between the 2 groups on farm D. These results show the positive

effects of oral PG administration in fresh cows with SCK by helping to resolve SCK and preventing clinical ketosis. In addition, oral PG improves milk yield during early lactation in cows diagnosed with SCK.

Key words: dairy cow, ketosis, propylene glycol, milk yield

INTRODUCTION

Early lactation is a difficult period for dairy cows, which must transition from the demands of late gestation to those of early lactation. Those unable to adapt to this period of negative energy balance are prone to metabolic disorders and decreased milk production (Cameron et al., 1998; Drackley, 1999; Herdt, 2000). Among the sequelae of a poor adaptive response is an excessive elevation of circulating ketone bodies in the blood (Herdt, 2000), which can present clinically as a decrease in appetite, weight loss, and a decrease in milk production. The economic losses due to clinical ketosis are not trivial because of treatment costs, decreased milk yield, increased culling, and decreased reproductive efficiency (Fourichon et al., 1999; Ostergaard and Gröhn, 1999; Gröhn et al., 2003). Cows also suffer from subclinical ketosis (SCK), defined as an excess of circulating ketone bodies without clinical signs of ketosis (Andersson, 1988), which places them at an increased risk of other parturient diseases such as displaced abomasum and metritis (Duffield et al., 2009; Ospina et al., 2010a,b). In addition to the effects on disease events, SCK has been found to decrease milk yield in early lactation (Dohoo and Martin, 1984; Ospina et al., 2010b). The lactational incidence of SCK, which can be as high as 80% in some herds, is much greater than the 2 to 15% found with clinical ketosis (Duffield, 2000).

Propylene glycol (PG) has long been used to treat clinical ketosis (Johnson, 1954; Maplesden, 1954) and is known to be antiketogenic by increasing plasma glucose concentrations through decreased peripheral tissue glucose demand (Kristensen and Raun, 2007) and lowering NEFA and liver triglyceride levels, resulting in a decrease of plasma BHBA (Sauer et al., 1973; Grummer et al., 1994; Chung et al., 2009). Although many trials have been conducted with PG using various dosages, lactational stages of administration, routes of delivery, and length of treatment (Studer et al., 1993; Miyoshi et al., 2001; Nielsen and Ingvartsen, 2004), the small scale of these trials may have resulted in a lack of significant findings or have poor external validity in relation to larger herds. In a review of 12 papers concerning the effect of PG on milk production in dairy cows (Nielsen and Ingvartsen, 2004), the general trend of PG administration was to increase milk production over control cows, although these trials used prophylactic dosing of PG rather than a treatment based on results of ketosis testing.

Interest exists in developing drenching programs on farms with intensive monitoring protocols to evaluate the effect of PG on milk production in early lactation and to determine whether PG administration decreases the incidence of metabolic diseases (Pickett et al., 2003). The recent identification and validation of the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL; Iwersen et al., 2009; Konkol et al., 2009), a rapid, accurate, and relatively inexpensive cow-side test for SCK, eases many of the previous difficulties associated with intensive monitoring programs.

The objective of this study was to determine the effect of oral PG administration on SCK resolution, clinical ketosis prevention, and milk yield in cows diagnosed with SCK in early lactation with an intensive monitoring program.

MATERIALS AND METHODS

Study Population

Data were collected from 2 dairy farms (farms A and B) in New York State from May 18 until September 8, 2010, and from 2 dairy farms (farms C and D) in Wisconsin, from June 11 until August 30, 2010. To be selected, farms had to meet the following criteria: milk at least 1,500 cows, have headlocks in fresh cow pens, use the farm management program Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and be willing to participate in the proposed ketosis testing and treatment protocol. Detailed information concerning farm management structure and nutrition can be found in the Appendix.

Data Collection and Study Design

Enrollment into the study occurred at calving (Figure 1). Cows were tested from 3 to 16 DIM on Mondays, Wednesdays, and Fridays for ketosis using a Precision Xtra meter. The Precision Xtra meter is a hand-held device used to test blood BHBA concentrations; sensitivity and specificity compared with serum BHBA concentrations determined photometrically are 96 to 100% and 98 to 100%, respectively, when using a cut-off value of ≥1.2 mmol/L (Iwersen et al., 2009; Konkol et al., 2009). Given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. Subclinical ketosis was defined as a BHBA concentration of 1.2 to 2.9 mmol/L; clinical ketosis was defined as ≥3.0 mmol/L (Oetzel, 2004).

On farms A and B, 10 mL of blood was collected from the coccygeal vessels of each cow using a tube without anticoagulant and a 20-gauge, 2.54-cm blood collection needle. On farms C and D, approximately 0.5 mL of blood was collected from the coccygeal vessels using a

22-gauge, 2.54-cm needle and a 1-mL syringe. Beta-hydroxybutyrate testing was completed according to Precision Xtra meter instructions and performed immediately after blood collection. A ketone strip was attached to the Precision Xtra meter until the "add blood" symbol appeared on the meter display. The lot number of the inserted ketone strip was then checked to ensure matching with the lot number displayed on the meter. For each cow test, a drop of blood was applied to the ketone test strip test chamber; the meter indicated when the chamber was full. After 10 s, the BHBA concentration was displayed on the meter and the value recorded.

All testing and treatment of cows for SCK from 3 to 16 DIM was completed by the research team during the study. Cows with BHBA concentrations of 1.2 to 2.9 mmol/L were sequentially randomized to treatment group (oral PG drench) or control group (no PG) after their first SCK positive test. Randomization to treatment group for the first cow on each farm was completed in Excel (Microsoft, Redmond, WA) using the random number function. Cows assigned to treatment were drenched with 300 mL of PG (E.H. Wolf & Sons, Green Bay, WI) once daily from the day they tested ≥1.2 mmol/L until the day they tested <1.2 mmol/L or reached 17 DIM. Administration of a 300-mL volume was chosen because it is a common dose used on farms and delivers approximately 310 g of PG. Drenching on farms A and B was completed by the research team; drenching on farms C and D was completed by on-farm personnel. Cows with BHBA concentrations of ≥3.0 mmol/L were treated by on-farm personnel per farm protocol for cows diagnosed with ketosis.

Cows were excluded from the study if their previous days carried calf was less than 260 d, if they died or were sold before their first BHBA test, if they were diagnosed and treated by the farm for ketosis before their first BHBA test, or for lack of proper identification. Additionally, cows remaining in the herd through 16 DIM were excluded if they had fewer than 5 BHBA tests. Further data collected included lactation number and individual milking weights

for the first 30 d of lactation (farms A, B, and D only). Individual milking weights were exported throughout the study period from each farm's Dairy Comp 305 program. All milking values recorded as "0" pounds of milk were reentered as missing data points.

The study aimed to enroll 2,400 cows. Based on previous research by Ospina et al. (2010c), with approximately 22% of cows testing positive for SCK, enrollment of 2,400 cows would render approximately 530 cows available for randomization, with 265 cows randomized to each treatment group. This sample size, assuming a desired type I error rate of 5%, a power of 80%, and a standard deviation of 1.8 kg of milk per milking, would allow detection of a 0.5-kg difference in individual milk yield. A proposal was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (#2008-0099) and the University of Wisconsin Institutional Animal Care and Use Committee (#V01479-0-05-10). All farms were asked to sign a consent form agreeing to the proposed testing and treatment protocol and were given a document containing information on disease definitions including clinical milk fever, retained placenta, metritis, displaced abomasum, and clinical ketosis.

Statistical Analysis

Descriptive statistics were generated with the FREQ and UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC). The effect of PG on time to resolution of SCK and time to diagnosis of clinical ketosis were analyzed by semiparametric proportional hazards (Cox, 1972) models using the PHREG procedure of SAS. The time-series variables for the models were first BHBA test of 1.2 to 2.9 mmol/L until BHBA test <1.2 mmol/L and first BHBA test of 1.2 to 2.9 mmol/L until BHBA test ≥3.0 mmol/L, respectively. Censoring variables were used to identify cows that had the event of interest from cows that either died (or were culled) or did not have the event by 16 DIM. In addition to the PG treatment variable, the potential confounding

variables lactation group (lactation 1, lactation 2, and lactation ≥3) and herd were offered to the model as independent variables. Independent variables and their respective interaction terms were manually removed by backward stepwise elimination if considered statistically nonsignificant ($P > 0.15$) or biologically not important. Proportional hazards assumptions were verified by evaluating the time-dependent covariates (Allison, 1995); noninformative censoring was evaluated using sensitivity analysis. Difference between treatment groups in milk yield for individual milk weights until 30 DIM was analyzed using repeated-measures ANOVA with first-order autoregressive covariance using the MIXED procedure of SAS (Littell et al., 1998, 2000). Results were analyzed using different covariance structures; the 3 covariance structures producing outcomes with the smallest Akaike information criterion were retained for further discussion. A first-order autoregressive covariance structure was chosen over compound symmetry and Toeplitz covariance structures for logical and model simplicity reasons. Compound symmetry covariance does not take into account the fact that individual milk weights are more likely to be correlated with weight measurements taken in close time proximity and less correlated with weight measurements taken farther away in time. The first-order covariance model was retained over the model using a Toeplitz covariance structure because a simpler model (fewer parameters) is more desirable. Variables offered to the model included PG treatment, lactation group (lactation 1, 2, ≥3), DIM, and herd as a random effect. A second model was developed using the variables lactation group, DIM, herd, and a treatment by herd interaction. If the interaction between treatment and herd was found to be significant, then the outcome would be stratified by herd. Independent variables and their respective interaction terms were considered statistically significant if $P \leq 0.05$.

RESULTS

Descriptive Statistics

Of the 2,115 cows enrolled in the trial, 741 were diagnosed with SCK and randomized, with 372 cows in the PG treatment group and 369 control cows (Figure 1). The control group was composed of 106, 97, and 166 cows in lactations 1, 2, and ≥3, respectively (median = 2); the treatment group contained 109 cows in lactation 1, 92 in lactation 2, and 171 in lactation ≥3 (median = 2). A Chi-squared test showed no difference in parity between the 2 groups (P = 0.89). The incidence of SCK was 40.4% on farm A, 26.4% on farm B, 40.9% on farm C, and 55.7% on farm D.

Time to Resolution of SCK and Time to Clinical Ketosis

Figure 2 shows the Cox proportional hazards curve for the effect of PG on time to resolution of SCK; the final model included only PG treatment as an independent variable. Based on hazard ratios, PG-treated cows were 1.50 times more likely (95% CI = 1.26 to 1.79; P < 0.001) to resolve their SCK than control cows. Figure 3 shows the Cox proportional hazards curve for the effect of PG on time to development of clinical ketosis; the final model included only PG treatment as an independent variable. Cows treated with PG were 0.54 times less likely (95% CI = 0.34 to 0.86; P = 0.009) to develop clinical ketosis than control cows. The final Cox proportional models can be seen in Table 1.

Milk Yield

In total, 622 cows from farms A, B, and D were used in the analysis to determine the

effect of PG treatment on early lactation cows diagnosed with SCK. The variables PG treatment, lactation group, and DIM were used in the final repeated-measures ANOVA model to assess individual milk weights. The first model using the variable herd as a random effect found that treated cows produced 0.23 kg more milk per milking in the first 30 d of lactation than control cows ($P < 0.001$), for a total difference of 0.68 kg/d. The second model, using herd as a fixed effect and including a treatment by herd interaction, was stratified by herd after a significant treatment by herd interaction was found. Treated cows produced more milk per milking on farm A (0.44 kg; $P < 0.001$) and farm B (0.53 kg; $P < 0.001$) in the first 30 d of lactation compared with control cows, for a total difference of 1.34 and 1.59 kg/d, respectively, based on 3 times a day milking. Milk production per milking did not differ (0.02 kg; $P = 0.70$) between the 2 groups on farm D, with treated cows making 0.074 kg more milk per day than control cows. Table 2 shows PG treatment results by herd.

Table 4.1. Cox proportional models showing hazard ratios (HR) for 741 Holstein cows with at least one positive test for subclinical ketosis from 3 to 16 DIM randomly assigned to propylene glycol treatment (n = 372) or control (n = 369)

Event[1]	Estimate	SE[2]	*P*-value[3]	HR	95% CI[4]
BHBA <1.2 mmol/L	0.41	0.091	<0.001	1.50	1.26 to 1.79
BHBA ≥3.0 mmol/L	0.62	0.24	0.009	0.54	0.34 to 0.86

[1]HR events include time from positive test for subclinical ketosis (BHBA concentration of 1.2 to 2.9 mmol/L) to either ketosis resolution (BHBA concentration <1.2 mmol/L) or clinical ketosis (BHBA concentration ≥3.0 mmol/L)
[2]SE = standard error for estimate.
[3]P-value reported for estimate.
[4]CI for hazard ratio.

Table 4.2. Individual milking yield stratified by herd for 622 Holstein cows randomly assigned to propylene glycol (PG) or control group with at least one positive blood test for subclinical ketosis (BHBA of 1.2 to 2.9 mmol/L) from 3 to 16 DIM

Herd[1]	Ketosis Incidence (%)	Yield Per Milking Per Cow (kg)	SE[2]	Milk Difference Per Day (kg)	*P*-value[3]
Farm A					
Control (n = 70)	40.4	12.41	0.089	1.32	< 0.001
PG (n = 73)		12.85	0.087		
Farm B					
Control (n = 52)	26.4	12.26	0.096	1.59	< 0.001
PG (n = 54)		12.79	0.091		
Farm D					
Control (n = 189)	55.7	10.97	0.047	0.06	0.70
PG (n = 184)		10.99	0.047		

[1]Of the 4 farms in the study, 3 recorded individual milk weights and were included in the analysis.
[2]SE = standard error for individual milk yield.
[3]P-value reported for difference between control and PG treatment individual milk yield.

Figure 4.1. Flowchart showing total enrollment, reasons for exclusion before testing, reasons for nonrandomization, reasons for removal from analysis, and final analysis by treatment group for 2,115 Holstein cows from 4 dairy farms in New York and Wisconsin undergoing repeated testing for subclinical ketosis from 3 to 16 DIM.

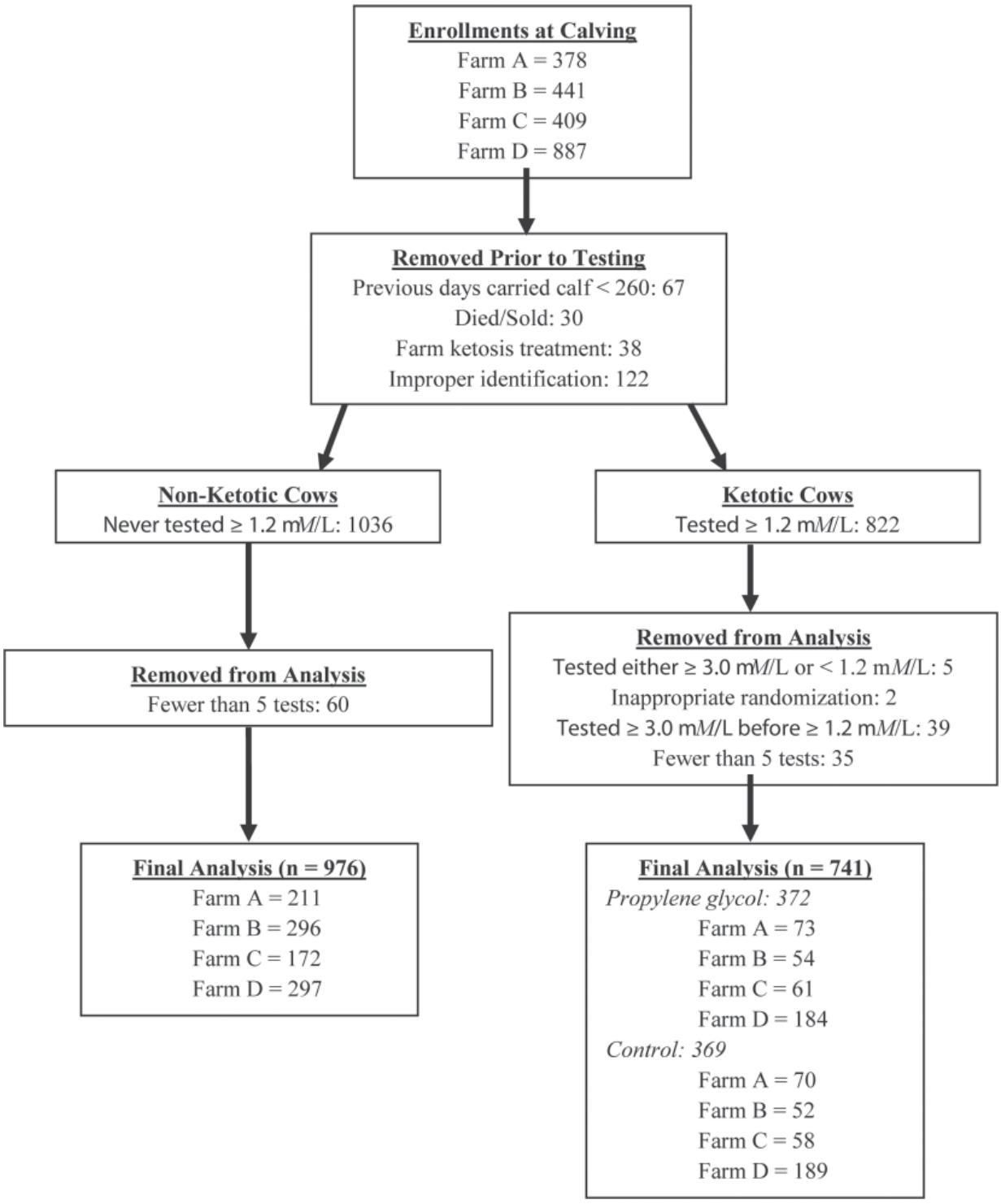


Figure 4.2. Cox proportional hazards analysis illustrating the time from diagnosis of subclinical ketosis (BHBA concentration of 1.2 to 2.9 mmol/L) until resolution of subclinical ketosis (BHBA concentration <1.2 mmol/L) for 741 Holstein cows from 4 dairy farms in New York and Wisconsin undergoing repeated testing for subclinical ketosis from 3 to 16 DIM. Cows treated with propylene glycol (PG; n = 372) were 1.50 (95% CI = 1.26 to 1.79) times more likely (P < 0.0001) to resolve their subclinical ketosis than control cows (n = 369).

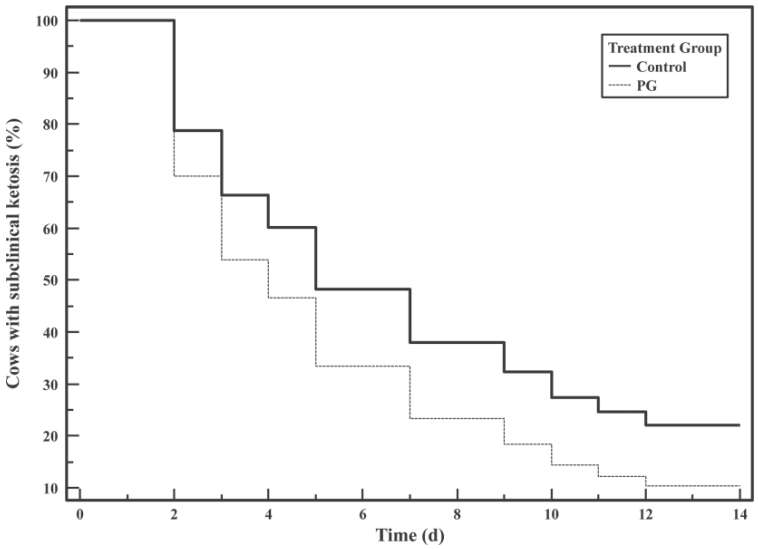
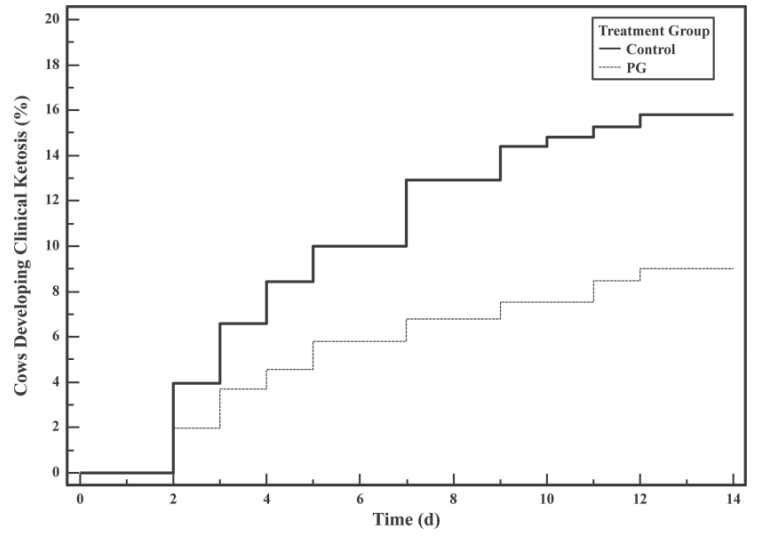


Figure 4.3. Cox proportional hazards analysis illustrating the time from diagnosis of subclinical ketosis (BHBA concentration ≥1.2 to 2.9 mmol/L) until diagnosis of clinical ketosis (BHBA concentration ≥3.0 mmol/L) for 741 Holstein cows from 4 dairy farms in New York and Wisconsin undergoing repeated testing for subclinical ketosis from 3 to 16 DIM. Cows treated with propylene glycol (n = 372) were 0.54 (95% CI = 0.34 to 0.86) times less likely (P = 0.009) to develop clinical ketosis than control cows (n = 369).



DISCUSSION

The reported study was conducted to determine the effects of oral PG on resolution of SCK, development of clinical ketosis, and milk yield in cows diagnosed with SCK. Results showed that cows treated with oral PG were more likely to resolve their ketosis, less likely to develop clinical ketosis, and, in some herds, produce more milk per milking in the first 30 d of lactation than control cows.

The wide range of SCK incidence found among the research farms (26.4 to 55.7%) illustrates the varying severity of common periparturient diseases between herds. These values are similar to those reviewed by Duffield (2000), who suggested a lactational incidence of SCK of approximately 54% with herds ranging from 8 to 80%. Other studies examining the prevalence of SCK have reported values at 2 wk postpartum of 33% (Duffield et al., 1998), within 2 wk postpartum of 22% (Ospina et al., 2010a), and within the first 2 mo of lactation of 0 to 33.9% (Dohoo and Martin, 1984). Whereas the variability of herd SCK found in the current study did not have an effect on the time to resolution of ketosis or time to diagnosis of clinical ketosis, it may have had an effect on subsequent milk production, as discussed below.

To preserve data quality, individual milking weights were used in the analysis rather than daily milk yields. All farms had cows with missing weights for a variety of reasons, including inability of the parlor system to read electronic identification numbers, temporary malfunctioning of the parlor recording system, or inclusion of sick cows in pens that were not milked. In addition, cows on farm A and farm B that were milked in the parlor reserved for colostrum collection and milk withholding did not have weights recorded for these milkings. Rather than determine daily milk yield for cows with missing weights by averaging surrounding values or by data imputation, the analysis was completed using the available individual milking

weight data (Gornbein et al., 1992).

Once an interaction between herd and treatment group was identified, milk weight analysis was stratified by herd. On farm A and farm B, SCK cows treated with PG made significantly more milk than control cows, amounting to 1.34 and 1.59 kg/d, respectively, for the first 30 DIM. To the authors' knowledge, this is the first study that examined the effect of PG on milk yield of cows diagnosed with SCK. Although multiple studies have found a positive effect on milk yield after prophylactic administration of PG during early lactation (Formigoni et al., 1996; Miyoshi et al., 2001; Lien et al., 2010), only the trial completed by Lien et al. (2010) showed a significant difference between groups, with PG-treated cows producing 0.61 kg more milk per day in the first 90 d than control cows. A few studies found a numerical decrease in milk yield after prophylactic administration of PG in early lactation (Emery et al., 1964; Fisher et al., 1973; Pickett et al., 2003), in which none of the results were statistically significant. One study evaluating the effect of PG on cows positive for clinical ketosis based on the milk ketone test failed to find a difference in milk production between the treated and control groups (Ruegsegger and Schultz, 1986). In the studies mentioned above that failed to detect a difference or that did not detect a significant difference, the small number of animals assigned to each treatment group (ranging from 7 to 22 cows per group) may have failed to provide enough power to determine if a true difference existed in milk yield between groups.

Considering the milk production differences between SCK cows treated with PG and control cows on farm A and farm B, the lack of difference found on farm D was unexpected. One explanation for this disparity in results is the higher incidence of ketosis on farm D. Although PG administration in SCK cows on farm D may have helped them resolve their ketosis faster and prevent clinical ketosis, the underlying metabolic challenges these cows faced to produce more milk may have been too large to improve with PG treatment alone. Changes in

dry cow management and nutrition may decrease the metabolic stresses of parturition and early lactation to a level at which PG treatment is effective in elevating milk production in this herd. Another possible explanation for the differences seen between study herds is the average milk yield of the herds; the average daily milk yield for all lactating cows during the study period for farm A, farm B, and farm D was 41.8, 41.8, and 35.3 kg, respectively. Similarly, the average daily milk produced by study cows in the first 30 DIM on farm A and farm B was higher during the study period than that by cows on farm D, at 37.5, 37.0, and 32.9 kg, respectively. There may be a limiting management or nutrient factor on farm D that did not allow SCK cows treated with PG to improve as seen in the other herds. Additionally, the on-farm protocol for treating cows with clinical ketosis differed slightly between the farms. On farm A and farm B, cows were tested daily either by the research group (on Mondays, Wednesdays, and Fridays) or by farm personnel (the remaining days of the week) and treated according to the ketosis diagnosis of the given test day. On farm D, cows diagnosed with clinical ketosis were given 4 d of oral PG regardless of their test results for the last 3 of the 4 treatment days. Given this on-farm treatment for clinical ketosis, of the SCK positive cows on farm D that subsequently developed clinical ketosis (6%, n = 48), 22 of 31 cows in the control group and 3 of 17 cows in the treatment group received repeated doses of PG that were unnecessary. Because more cows developed clinical ketosis in the control group than the treatment group, it is possible that the additional PG received by the control group cows may have increased milk production enough to inappropriately bias the finding toward the conclusion that milk yield did not differ between the 2 groups. Thus, the true difference in milk yield found between the 2 groups on farm D is most likely greater than 0.06 kg/d. Cows that developed clinical ketosis were included in the analysis. Because cows in the control group were more likely to develop clinical ketosis than cows in the PG treatment group, excluding cows with clinical ketosis from the milk yield analysis would

have removed more control cows with poor milk production than treatment cows, thus falsely inflating the milk yield of the control cows and further biasing the findings toward the conclusion that milk yield did not differ between the 2 groups.

The treatment effect of PG on resolution of SCK was the same for all herds, with PG-treated cows 1.50 times more likely to resolve their ketosis than control cows. Additionally, the PG treatment effect on prevention of clinical ketosis diagnosis was the same for all herds, with PG-treated cows 0.54 times less likely to be diagnosed with clinical ketosis than control cows. Only a few published studies have looked at the effect of PG on cows with clinical ketosis. In a study by Ruegsegger and Schultz (1986) involving PG treatment in cows testing positive for milk ketones, none of the treated or control cows developed clinical ketosis. In cows treated with prophylactic PG during the dry period or early lactation, 2 studies found a significant decrease in blood BHBA concentration (Grummer et al., 1994; Formigoni et al., 1996), and 1 found no difference between groups (Pickett et al., 2003). Miettinen (1995) showed that treatment with PG and a nicotinamide solution did not prevent clinical ketosis compared with control treatment; however, PG treatment started at 14 DIM. The discrepancies in these results may be due to the differences in PG dose, route of administration, or in the time, method, and frequency of BHBA or ketosis testing. In addition, all studies used a relatively small number of cows per group; thus, the studies without a significant finding may have lacked the necessary cow numbers for adequate statistical power. The randomization of 372 cows to PG treatment and 369 cows to the control group in the current study was more than enough to detect significant positive outcomes when using PG to treat cows with SCK.

In addition to the health benefits found with PG administration on all farms, the economic implications of increased milk production within the first 30 DIM on farm A and farm B are not trivial. Further investigation is needed to determine the best testing and dosing scheme

that is economical, beneficial to the cows, and practical for management and labor purposes.

CONCLUSIONS

These results show the positive effects of oral PG administration in fresh cows with SCK in helping resolve SCK as well as preventing clinical ketosis. In addition, cows diagnosed with SCK that received oral PG had improved milk yield during early lactation in some herds.

ACKNOWLEDGEMENTS

This study was funded in part by the USDA-NRI (Washington, DC) and Boehringer Ingelheim Vetmedica Inc. (St. Joseph, MO). The authors thank Judith Alishauskas, Stephanie Brittin, Emily Bold, Mark Fagan, and Caitlin Jeffrey of Cornell University (Ithaca, NY), and Lindley Reilly, Eric Rooker, Angela Niles, Tyler Franks, Catherine Bargren, Diego Calderon, Cristian Vergara, and Loïc Commun of the University of Wisconsin (Madison) for their invaluable assistance with data collection as well as the owners of the collaborating dairies for allowing us access to their cows and facilities on which to conduct this research.

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APPENDIX

Farm A was a 1,900-cow Holstein herd that milked 3 times daily in 1 of 2 parlors, a double-28 parallel parlor used for the majority of cows or a double-12 parallel parlor used for collection of colostrum and antibiotic-treated cow milk, and averaged 41.8 kg of milk per cow per day during the study period. Close-up dry heifers and cows were housed together in a 3-row freestall barn with an average of 44 cm of bunk space per cow and approximately 20% more stalls than cows. The close-up diet consisted of wheat straw and corn silage; both cows and heifers freshened in group pens bedded with straw. The fresh cow pen was located in a 3-row barn with an average of 52 cm of bunk space per cow and approximately 10% fewer stalls than cows; stalls were covered with water mattresses and bedded with recycled manure solids. Cows remained in the fresh pen until approximately 40 DIM. The farm's prestudy fresh cow management involved physical examination of all cows from 1 to 10 DIM using rectal temperature recording and urine ketosis dipsticks (Ketostix, Bayer Animal Health, Shawnee, KS). Cows with results of moderate or large on the urine dipsticks were recorded by the farm as having ketosis and were given 300 mL of oral PG and 500 mL of 50% dextrose intravenously. Lactating cows were fed a TMR consisting of approximately 60% forage (corn silage, alfalfa and grass haylage, alfalfa silage, alfalfa hay, and wheat straw) and 40% concentrate (cornmeal, soybean meal, and canola meal) with a standard vitamin and mineral pack that contained 16 g of monensin per tonne of diet DM.

Farm B was a 1,800-cow Holstein herd that milked 3 times daily in 1 of 2 parlors, a double-22 parallel parlor used for the majority of cows or a single-10 parallel parlor used for collection of colostrum and antibiotic-treated cow milk, and averaged 41.8 kg milk per cow per day during the study period. Close-up dry heifers and cows were housed separately in a 2-row freestall barn with an average of 50 cm of bunk space per animal in each pen. Both pens

contained 30 to 40% more cows than stalls and cows freshened in a group pen bedded with straw. The close-up diet consisted of wheat straw, canola meal, and corn silage. The fresh pens were located in a 6-row barn with a separate pen for heifers and cows, and contained an average of 56 and 62 cm of bunk space per animal, respectively. Stalls were covered with mattresses and bedded with recycled manure solids, and both pens were maintained with equal stall and cow numbers. Heifers remained in their pen until approximately 60 DIM and cows until approximately 40 DIM. The farm's prestudy fresh cow management involved physical examination of all animals listed as "deviation" on the farm's Dairy Comp 305 program; ketosis status was tested using Ketostix urine dipsticks. Cows with results of trace or small on the urine dipstick received 300 mL of oral PG, those with a result of moderate received 300 mL of PG orally and 500 mL of dextrose and 10 mL of vitamin B12 intravenously, and those with a result of large received 300 mL of PG orally and 500 mL of 50% dextrose, 10 mL of vitamin B complex, and 10 mL of dexamethasone intravenously. Both fresh pens were fed a TMR consisting of approximately 53% forage (corn silage, and alfalfa and grass haylage) and 47% concentrate (cornmeal, canola meal, and high-moisture shelled corn) with a standard vitamin and mineral pack that contained 12 g of monensin per tonne of diet DM.

Farm C was a 2,800-cow predominantly Holstein herd that milked cows 3 times daily in a double-20 parallel parlor and averaged 39.4 kg of milk per cow per day during the study period. Cows and heifers were housed separately during the close-up dry period. Heifers were kept in a 3-row freestall pen that provided an average of 72 cm of bunk space per animal and contained 40% more stalls than heifers in the pen. Cows were housed in a 3-row freestall pen that provided an average of 61 cm of bunk space per cow and 25% more stalls than the number of cows in the pen. All stalls were deeply bedded with recycled sand over a packed gravel base. Close-up heifers and cows were fed a TMR consisting of approximately 71% forage (alfalfa

hay, corn silage, grass hay, and wheat straw) and 29% concentrate (grain, protein supplements) with trace minerals, supplemental anions, and vitamins. Cows and heifers were moved to individual maternity pens deeply bedded with straw when they exhibited signs of active labor. After calving, both cows and heifers were moved to a 2-row hospital pen occupied by other recently fresh cows as well as sick cows with nonsaleable milk. This pen provided an average of 78 cm of bunk space per cow and 17% more stalls than the number of animals in the pen. After determining that the milk was saleable, heifers were moved to a 2-row fresh pen for first-lactation animals that provided 90 cm of bunk space per cow and 28% more stalls than the number of cows in the pen. Cows were moved to a different 2-row pen that provided an average of 65 cm of bunk space per cow and the same number of stalls as cows. After approximately 11 d in either fresh pen, healthy animals were moved to a large early lactation pen that housed both lactating cows and heifers. This pen provided 58 cm of eating space per cow and 7% fewer stalls than the number of cows in the pen. Lactating cows were fed a TMR consisting of approximately 68% forage (alfalfa hay, alfalfa silage, corn silage, and wheat straw), 32% concentrate (ground shelled corn and corn gluten feed), and whey permeate with a mix containing additional protein supplements, minerals, vitamins, and 12 g of monensin per tonne of diet DM. Early lactation cows were monitored daily for attitude, appetite, and rumen fill. Animals suspected of having ketosis were tested using a nitroprusside milk powder (KetoCheck, Great States Animal Health, Lenexa, KS). Positive animals were treated with 335 mL of oral PG for 3 or more days. A small proportion of ketotic cows with strongly positive milk ketones or additional clinical signs were also treated with 500 mL of 50% dextrose intravenously.

Farm D was a 4,100-cow Holstein herd that milked cows 3 times daily on an 80-cow rotary parlor and averaged 35.3 kg of milk per cow per day during the study period. Cows and heifers were housed together before calving in a 2-row freestall pen that provided an average of

86 cm of bunk space per cow and 15% more stalls than the number of cows in the pen. All freestalls were deeply bedded with partially dried solids from an anaerobic digester over a packed clay base. Close-up animals were fed a TMR consisting of approximately 57% forage (alfalfa silage, corn silage, grass hay, and wheat straw) and 43% concentrate (ground shelled corn, corn gluten feed, dried distillers grains, and wet brewers grains) and a mix containing minerals, supplemental anions, and vitamins. Cows and heifers were moved to one side of a straw-bedded group maternity pen when they exhibited active signs of labor. Cows were kept on the other side of the maternity pen until their milk was determined to be saleable. Both fresh cows and heifers were then moved to a 2-row post-fresh pen that provided an average of 73 cm of bunk space per cow and 8% more stalls than cows in the pen. Healthy animals remained in the pen until approximately 30 DIM. Cows in early lactation were fed a TMR consisting of approximately 67% forage (alfalfa silage and corn silage) and 33% concentrate (ground shelled corn, corn hominy feed, corn gluten feed, dried distillers grains, wet distillers grains, wet brewers grains, blood meal, barley malt sprouts, and corn starch) with a mineral and vitamin mix not supplemented with monensin. Early lactation cows were monitored daily for attitude, appetite, rumen fill, and milk weights. Animals suspected of having ketosis were tested using KetoCheck nitroprusside milk powder. Positive animals were treated with 335 mL of oral PG for 4 or more days and given a 10-mL intramuscular injection of vitamin B complex once daily until the milk ketone test was negative.

CHAPTER FIVE

A FIELD TRIAL ON THE EFFECT OF PROPYLENE GLYCOL ON DISPLACED ABOMASUM, REMOVAL FROM HERD, AND REPRODUCTION IN FRESH COWS DIAGNOSED WITH SUBCLINICAL KETOSIS

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Journal of Dairy Science 95:2505-2512

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ABSTRACT

The purpose was to determine the effect of oral propylene glycol (PG) administration in fresh cows diagnosed with subclinical ketosis (SCK). Measured outcomes were development of displaced abomasum (DA) and removal from herd in the first 30 d in milk (DIM), conception to first service, and time to conception within 150 DIM. Cows from 4 freestall dairy herds (2 in New York and 2 in Wisconsin) were each tested 6 times for SCK from 3 to 16 DIM on Mondays, Wednesdays, and Fridays using the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). Subclinical ketosis was defined as a blood β-hydroxybutyrate concentration of 1.2 to 2.9 mmol/L. Cows with SCK were randomized to treatment group (oral PG) or untreated control group (no PG); treatment cows were orally drenched with 300 mL of PG once daily from the day they tested 1.2 to 2.9 mmol/L until the day they tested <1.2 mmol/L. Mixed effects multivariable Poisson regression was used to assess the effect of PG on DA, removal from herd, and conception to first service; a semiparametric proportional hazards model was used to evaluate the days-to-conception outcome. A total of 741 of 1,717 (43.2%) eligible enrolled cows had at least 1 β-hydroxybutyrate test of 1.2 to 2.9 mmol/L. Of these, 372 were assigned to the PG treatment group and 369 to the control group. Thirty-nine cows (5.3%) developed a DA after testing positive for SCK and 30 cows (4.0%) died or were sold within the first 30 DIM. Based on risk ratios, control cows were 1.6 times more likely [95% confidence interval (CI) = 1.3 to 2.0] to develop a DA and 2.1 times more likely (95% CI = 1.2 to 3.6) to die or be sold than cows treated with PG. In addition, PG-treated cows were 1.3 times more likely (risk ratio 95% CI = 1.1 to 1.5) to conceive at first insemination than control cows in 3 of the herds. No difference was observed in days to conception within 150 DIM between treatment groups (hazard ratio for PG cows = 1.1, 95% CI = 0.8 to 1.4), with a median time to conception of 100 d (95% CI = 93 to 111) and 104 d (95% CI = 95 to 114) for PG-treated and control cows, respectively. These results show that

intensive detection of SCK, followed by treatment of positive cows with oral PG decreased the risk of developing a DA or leaving the herd within the first 30 DIM and increased the risk of conception to first service.

Key words: dairy cow, ketosis, propylene glycol, cull

INTRODUCTION

During the transition from late gestation to early lactation, dairy cattle undergo a period of negative energy balance as the demands for milk synthesis cannot be met by feed intake (Bauman and Currie, 1980; Baird, 1982; Herdt, 2000). To adapt to this negative energy balance, among other mechanisms, cows mobilize lipid reserves, which circulate in the blood as NEFA. Circulating NEFA can then be used directly as a fuel source, metabolized in the liver to ketone bodies, or converted back into triglycerides. When the liver is overwhelmed by NEFA, ketone bodies are produced in excess and the cow becomes hyperketonemic (Herdt, 2000). The clinical signs associated with hyperketonemia may include a decrease in appetite, weight loss, and a decrease in milk production. In addition to clinical hyperketonemia, cows also suffer from subclinical ketosis (SCK), defined as an excess of circulating ketone bodies without clinical signs of ketosis (Andersson, 1988).

Cows that develop SCK are at an increased risk of additional postpartum diseases such as displaced abomasum (DA) and metritis (Duffield et al., 2009; Ospina et al., 2010a), which may increase their risk of removal from the herd during early lactation. Studies evaluating the effect of SCK on reproductive performance have provided differing results. In large studies by Walsh et al. (2007) and Ospina et al. (2010b), the concentration of blood BHBA and the duration of

elevation were negatively associated with pregnancy at first service, whereas a smaller study by Kessel et al. (2008) showed no difference in days from calving to conception or first-service conception rate between cows diagnosed with and without elevated blood BHBA.

The use of propylene glycol (PG) to treat clinical ketosis is not novel (Johnson, 1954; Maplesden, 1954); it is known to be anti-ketogenic by increasing plasma glucose concentrations through decreased peripheral tissue glucose demand (Kristensen and Raun, 2007) and lowering NEFA and liver triglyceride levels, resulting in a decrease in plasma BHBA concentrations (Sauer et al., 1973; Grummer et al., 1994; Chung et al., 2009). Although numerous trials have been conducted using various PG dosages, lactational stages of administration, routes of delivery, and length of treatment to test the effect of prophylactic PG administration (Studer et al., 1993; Miyoshi et al., 2001; Nielsen and Ingvartsen, 2004), no studies have reported the effect of PG on DA development or removal from herd in early lactation. However, the use of PG in decreasing the incidence of DA has been suggested as potentially beneficial (LeBlanc et al., 2005). It has also been postulated that the decrease in fat mobilization and hepatic ketogenesis after PG administration may have beneficial effects on reproduction (Nielsen and Ingvartsen, 2004). No studies have reported the effect of PG on cows with SCK except McArt et al. (2011).

The objective of this study was to determine the effect of oral PG administration on development of DA and removal from the herd in the first 30 DIM, conception to first service, and days to conception within 150 DIM in cows intensively monitored and diagnosed with SCK.

MATERIALS AND METHODS

Study Population

Data were collected from 2 dairy farms (farms A and B) in New York from May 18, 2010 until September 8, 2010 and from 2 dairy farms (farms C and D) in Wisconsin from June 11,

2010 until August 30, 2010. To be selected, farms had to meet the following criteria: milk at least 1,500 cows, have headlocks in fresh cow pens, use the farm management program Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and be willing to participate in the proposed ketosis testing and treatment protocol. Detailed information concerning farm management structure and nutrition has been reported previously (McArt et al., 2011); an overview of herd size, milk production, and reproductive and disease events can be found in Table 1.

Data Collection and Study Design

Enrollment into the study occurred at calving as described previously (McArt et al., 2011). Cows were tested from 3 to 16 DIM on Mondays, Wednesdays, and Fridays for ketosis using a Precision Xtra meter (Abbott Laboratories, Abbott Park, IL). The Precision Xtra meter is a hand-held device used to test blood BHBA concentrations; sensitivity and specificity compared with serum BHBA concentrations determined photometrically are 96 to 100% and 98 to 100%, respectively, when using a cut-off value of ≥1.2 mmol/L (Iwersen et al., 2009; Konkol et al., 2009). Given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. Subclinical ketosis was defined as a BHBA concentration of 1.2 to 2.9 mmol/L; clinical ketosis was defined as ≥3.0 mmol/L (Oetzel, 2004).

On farms A and B, 10 mL of blood was collected from the coccygeal vessels of each cow using a tube without anticoagulant and a 20-gauge × 2.54-cm blood collection needle. On farms C and D, approximately 0.5 mL of blood was collected from the coccygeal vessels using a 22-gauge × 2.54-cm needle and a 1-mL syringe. Beta-hydroxybutyrate testing was completed according to Precision Xtra meter instructions and performed immediately after blood collection. A ketone strip was attached to the Precision Xtra meter until the add blood symbol appeared on

the meter display. The lot number of the inserted ketone strip was then checked to ensure matching with the lot number displayed on the meter. For each cow test, a drop of blood was applied to the ketone test strip test chamber; the meter indicated when the chamber was full. After 10 s, the BHBA concentration was displayed on the meter and the value recorded.

All testing of cows for SCK from 3 to 16 DIM was completed by the research team during the study. Cows with BHBA concentrations of 1.2 to 2.9 mmol/L were sequentially randomized to treatment group (oral PG drench) or untreated control group (no PG) after their first SCK-positive test. Randomization to treatment group for the first cow on each farm was completed in Excel (Microsoft Corp., Redmond, WA) using the random number function. Cows assigned to treatment were orally drenched with 300 mL PG (E. H. Wolf & Sons Inc., Green Bay, WI) once daily from the day they tested ≥1.2 mmol/L until the day they tested <1.2 mmol/L or reached 17 DIM. Administration of a 300 mL volume was chosen, as it is a common dose used on farms and delivers approximately 310 g of PG. Drenching on farms A and B was completed by the research team; drenching on farms C and D was completed by on-farm personnel. Cows with BHBA concentrations of ≥3.0 mmol/L were treated by on-farm personnel per farm protocol for cows diagnosed with ketosis.

Cows were excluded from the study if their previous days carried calf was less than 260 d, if they died or were sold before their first BHBA test, if they were diagnosed and treated by the farm for ketosis before their first BHBA test, if they tested ≥3.0 mmol/L before testing 1.2 to 2.9 mmol/L, or for lack of proper identification. Additionally, cows remaining in the herd through 16 DIM were excluded if they had fewer than 5 BHBA tests. Further data collected included parity, DA, metritis, sold and died events, conception to first service, and DIM at conception. Displaced abomasa, cows died, cows sold, and pregnancy outcomes were exported throughout the study period from each farm's Dairy Comp 305 program.

A proposal was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (Ithaca, NY; #2008–0099) and the University of Wisconsin Institutional Animal Care and Use Committee (Madison; #V01479–0-05–10). All farms were asked to sign a consent form agreeing to the proposed testing and treatment protocol and were given a document containing information on disease definitions, including clinical milk fever, retained placenta, metritis, displaced abomasum, and clinical ketosis.

Statistical Analysis

Descriptive statistics were generated with the FREQ procedure of SAS (SAS Institute Inc., Cary, NC); difference in parity between the 2 treatment groups was analyzed using a chi-squared test. The effect of PG in SCK-positive cows on DA development, removal from herd, and conception to first service was analyzed using mixed effects multivariable Poisson regression with the GENMOD procedure of SAS (Frome and Checkoway, 1985; Spiegelman and Hertzmark, 2005). For the model evaluating DA development, SCK-positive cows developing a DA before their first SCK-positive test were removed from the analysis. The potential confounding variables lactation group (lactation 1, lactation 2, and lactation ≥3) and metritis were offered to the models (the variable DA was also offered to the model concerning removal from herd) as independent variables in addition to PG treatment. The variable herd was entered as a random effect in the DA and removal models. Due to large differences in voluntary waiting period (VWP), conception to first service, and breeding strategy (inseminating many cows twice) on farm D, the variable herd was tested as a fixed effect in the conception-to-first-service model; an offset term was used to adjust for the difference in VWP for each herd. Independent variables were removed by manual backward stepwise elimination if their contrast estimate was considered statistically nonsignificant ($P > 0.10$) and biologically not important. Statistical

significance of the variable herd in the conception-to-first-service model led to further analysis of conception by herd. Due to biologically plausible explanations for a change in outcome direction compared with the other 3 farms, farm D was excluded from both conception-to-first-service and time-to-conception analyses. The variable herd was then re-entered into the conception-to-first-service model as a random effect to account for the unmeasured variations between the remaining 3 herds. The effect of PG on time to conception was analyzed by a semiparametric proportional hazards model (Cox, 1972) using the PHREG procedure of SAS. The time series variable for the model was the number of days from calving until conception within 150 DIM. Censoring variables were used to identify cows that conceived from cows that were either removed from the herd or did not conceive by 150 DIM. Independent variables offered to the model included PG treatment, lactation group, and herd. Independent variables and their respective interaction terms were manually removed by backward stepwise elimination if considered statistically nonsignificant ($P > 0.10$) and biologically not important. Proportional hazards assumptions were verified by evaluating the time-dependent covariates (Allison, 1995); noninformative censoring was evaluated using sensitivity analysis. A Kaplan-Meier analysis (Kaplan and Meier, 1958) using only the PG treatment variable was completed using the LIFETEST procedure of SAS to determine median days from calving to conception for both treatment groups.

RESULTS

Descriptive Statistics

Of the 1,717 eligible enrolled cows, 741 (43.2%) were diagnosed with SCK and randomized, with 372 cows receiving PG treatment and 369 control cows. The control group was composed of 106, 97, and 166 cows in lactation 1, 2, and ≥3, respectively (median = 2); the

treatment group contained 109 cows in lactation 1, 92 in lactation 2, and 171 in lactation ≥3 (median = 2). No difference in parity between the 2 groups (P = 0.89) was observed. Table 2 shows the incidence of SCK, DA, and early lactation removals by herd.

Displaced Abomasum

In the first 30 DIM, 3 of 976 SCK-negative cows (0.3%) developed a DA and 39 of 739 cows (5.3%) developed a DA after testing positive for SCK (2 cows developed a DA before testing positive for SCK and were excluded from the analysis). For the SCK-positive cows, 24 (6.5%) of the control cows developed a DA compared with 15 (4.0%) of the PG-treated cows. Table 3 shows DA incidence in SCK-positive cows by herd and treatment group. Control cows were 1.6 times more likely [risk ratio (RR) 95% CI = 1.3 to 2.0, P < 0.001] to develop a DA than cows treated with PG. The final regression model included only the PG treatment variable with herd as a random effect. The final model estimate is in Table 4.

Early Lactation Removals

In the first 30 DIM, 18 of 976 (1.8%) SCK-negative cows and 30 of 741 cows (4.1%) that tested positive for SCK were removed from the herd. For the SCK-positive cows, 20 (5.4%) of the control cows were removed before 30 DIM compared with 10 (2.7%) of the PG-treated cows. Table 3 shows the incidence of removal from the herd in the first 30 d by herd and treatment group. Control cows were 2.1 times more likely (RR 95% CI = 1.2 to 3.6, P = 0.01) to die or be culled than cows treated with PG. The final regression model included only the PG treatment variable with herd as a random effect. The final model estimate is in Table 4.

Conception to First Service and Time to Conception

Table 5 shows conception to first service and median days to conception by herd and treatment group. Cows in farm D were removed from analysis of reproductive outcomes due to major differences in VWP and first-service breeding protocols. Of the remaining 3 herds, 907 cows had data concerning conception at first service; 241 of 603 (40.0%) SCK-negative cows and 123 of 304 (40.5%) SCK-positive cows conceived to first service. Of the SCK-positive cows, 52 of 148 (35.1%) control cows conceived at their first service compared with 71 of 156 (45.5%) PG-treated cows. Cows treated with PG were 1.3 times more likely (RR 95% CI = 1.1 to 1.5, P = 0.002) to conceive than control cows. The final regression model included only the PG treatment variable with herd as a random effect. The estimate for the final conception-to-first-service model is in Table 4. Farms A, B, and C had 904 cows with data concerning pregnancy status at 150 DIM; 496 of 601 (82.5%) SCK-negative cows and 237 of 303 (78.2%) SCK-positive cows were pregnant. Of the SCK-positive cows, 115 of 148 (77.7%) control cows and 122 of 155 (78.7%) PG-treated cows were pregnant. No difference was observed in days to conception within 150 DIM between treatment groups (hazard ratio for PG cows = 1.1, 95% CI = 0.8 to 1.4, P = 0.70), with a median time to conception of 100 d (95% CI = 93 to 111) and 104 d (95% CI = 95 to 114) for PG-treated and control cows, respectively. The final model concerning the effect of PG on time to pregnancy included the variables PG treatment, herd, and lactation group. The final model estimates are in Table 6.

Table 5.1. Farm management features

	Herd			
Feature	A	B	C	D
Size (cows)	1,900	1,800	2,800	4,100
Avg. milk/cow per day (kg)	41.8	41.8	39.4	35.3
Feeding system	TMR	TMR	TMR	TMR
Monensin in transition diet (g/t)	16	12	12	—
VWP[1] (d)	50	55 for lactation ≥2; 67 for lactation 1	65	80
Pregnancy rate[2]	21	24	19	19
DA risk[3] (%)	3.2	2.7	2.6	2.9
Early removal risk[4] (%)	3.3	3.5	4.1	7.3
Late removal risk[5] (%)	42.9	41.9	42.0	46.3

[1]Voluntary waiting period.

[2]Pregnancy rate = number of cows confirmed pregnant/total number of eligible cow 21-d risk periods.

[3]DA risk = number of cows diagnosed with a displaced abomasum (DA) within 30 DIM/number of fresh cows.

[4]Early removal risk = cows that died or were sold within 30 DIM/number of fresh cows.

[5]Late removal risk = cows that died or were sold >30 DIM/average number of lactating and dry cows.

Table 5.2. Herd incidence during the study period of subclinical ketosis (SCK) from 3 to 16 DIM, displaced abomasum (DA) in the first 30 DIM, and removal from herd in the first 30 DIM for all 1,717 Holstein cows

Herd	SCK (%)	DA (%)	Early Removal (%)
Farm A	40.4 (n = 143)	2.0 (n = 7)	2.3 (n = 8)
Farm B	26.4 (n = 106)	2.0 (n = 8)	3.0 (n = 12)
Farm C	40.9 (n = 119)	1.7 (n = 5)	7.2 (n = 21)
Farm D	55.7 (n = 373)	3.3 (n = 22)	8.8 (n = 59)

Table 5.3. Herd incidence of displaced abomasum (DA) and removal from herd in the first 30 DIM for 741 Holstein cows diagnosed with subclinical ketosis between 3 and 16 DIM and randomized to treatment with propylene glycol (PG) or without PG (control)

	Herd			
Item	A	B	C	D
DA (%)				
Control	4.3	9.3	5.2	7.1
PG	4.1	3.8	3.3	4.2
Removal (%)				
Control	5.7	3.7	3.4	6.5
PG	1.4	0.0	1.6	4.2

Table 5.4. Estimates for 3 final Poisson regression models showing risk ratios (RR) for 741 Holstein cows with at least 1 positive test for subclinical ketosis from 3 to 16 DIM randomly assigned to propylene glycol treatment (n = 372) or control (n = 369)[1]

Model	Estimate	SE[2]	*P*-value[3]	RR	95% CI[4]
DA	0.47	0.11	<0.0001	1.6	1.3 to 2.0
CULL	0.72	0.29	0.01	2.1	1.2 to 3.6
PREG	0.27	0.09	0.002	1.3	1.1 to 1.5

[1]The 3 outcomes modeled were 1) development of a displaced abomasum (DA) within 30 DIM, 2) removal from herd (CULL) within 30 DIM, and 3) conception to first service (PREG).

[2]SE = standard error for estimate.

[3]P-value reported for estimate.

[4]Confidence interval for risk or hazard ratio.

Table 5.5. Conception risk and median time to conception (95% CI) for 741 Holstein cows with at least 1 positive test for subclinical ketosis from 3 to 16 DIM randomly assigned to propylene glycol (PG) treatment (n = 372) or control (n = 369)

	Herd			
Item	A	B	C	D
Conception risk (%)				
Control	32.8	43.2	32.0	80.4
PG	42.9	44.2	50.0	76.6
Days to conception				
Control	106 (94 to 123)	95 (82 to 104)	118 (96 to 146)	112 (103 to 123)
PG	100 (83 to 121)	101 (95 to 111)	100 (78 to 134)	105 (92 to 111)

Table 5.6. Covariate estimates and hazard ratios (HR) for Cox proportional analysis of time to conception within 150 DIM for 303 Holstein cows with at least 1 positive test for subclinical ketosis from 3 to 16 DIM randomly assigned to propylene glycol treatment (n = 372) or control (n = 369)[1]

Covariate	Estimate	SE[2]	*P*-value[3]	HR	95% CI[5]
PG	0.05	0.13	0.70	1.1	0.8 to 1.4
LACT					
1	0.27	0.15	0.08	1.3	1.0 to 1.8
2	-0.04	0.17	0.83	1.0	0.7 to 1.3
≥ 3[5]	-	-	-	-	-
HERD					
Farm A	0.24	0.16	0.14	1.3	0.9 to 1.7
Farm B	0.54	0.18	0.002	1.7	1.2 to 2.4
Farm C[5]	-	-	-	-	-

[1]Independent variables offered to the model included propylene glycol (PG) treatment, lactation (LACT), and herd.

[2]SE = standard error for estimate.

[3]P-value reported for estimate.

[4]Confidence interval for hazard or risk ratio.

[5]Reference category.

DISCUSSION

The reported study was conducted to determine the effects of oral PG administration on development of DA and removal from the herd in the first 30 DIM, conception to first service, and days to conception within 150 DIM in cows diagnosed with SCK. Results show that cows treated with oral PG were less likely to develop a DA, less likely to be removed from the herd in the first 30 DIM, and more likely to conceive to first service than control cows; no difference existed in time to conception within 150 DIM between the 2 groups. Whereas these results derive from large, freestall, TMR-fed dairies and are, thus, more likely to have external validity under these conditions, the PG treatment was on an individual-cow basis. It is, therefore, biologically plausible that the benefits of PG pertain to cows managed in a variety of environments; however, the physiology and ecology of ketosis may be different in component-fed herds. In these herds, ketogenesis may be more related to a mismatch between energy expenditure and consumption slightly later in lactation than to events associated with lipolysis in the dry period. It remains undetermined from this study if PG would be beneficial to cows managed in these conditions.

The choice to compare PG-treated cows to an untreated control group rather than a group receiving a placebo solution was made to include the stress of handling and dosing in the PG response. On farms A and B, because the treatments were given by the research team, no potential existed for management bias from farm employees, as they were not aware of which cows received treatment. Although PG-treated cows on farms C and D were dosed by farm personnel, they were dosed under the direction and supervision of the research team at the time of testing. Although not blinded to treatment, it is unlikely that any management bias subsequently occurred. In addition, all measured outcomes were objective and numerical. Sequential randomization was chosen for allocation to treatment group over other randomization methods to ensure an almost equal number of cows assigned to the PG and control group by day.

In the studied on-farm settings, where environmental factors such as heat index and delayed feedings could potentially affect the outcome, it was deemed important to have cows in both the PG treatment and control groups be equally exposed to these daily variations.

Administration of oral PG to SCK-positive cows decreased the risk of developing a DA, as control cows were 1.6 times more likely to develop a DA than cows treated with PG. No other studies have analyzed the effect of PG on DA development. Pickett et al. (2003) reported the number of cows developing a DA when administered water, PG, or a combination of PG and fat during the first 3 d postpartum, but acknowledged that the sample size was too small to draw conclusions on an overall treatment effect.

In addition to having an increased risk of developing a DA, SCK-positive control cows were 2.1 times more likely to be removed from the herd within the first 30 DIM than PG-treated cows, at a 5.4 and 2.7% early removal rate, respectively. In fact, administration of PG to SCK-positive cows decreased the rate of removal to that of their non-ketotic herdmates at 2.7 and 1.8%, respectively ($P = 0.20$). Possible reasons that PG administration decreases removal risk in cows with SCK include its ability to assist in resolution of ketosis, to help prevent clinical ketosis, and to improve milk yield in cows diagnosed with SCK (McArt et al., 2011) as well as its ability to help prevent DA development. No other studies have examined the effect of PG in cows with SCK on removal of cows from the herd.

During analysis of conception to first service, it was noted that the variable herd was very important with a low type 1 error risk. However, no important herd by treatment interaction occurred. Upon further stratification and examination by herd, it was discovered that the first-service conception risk on farm D was of a different magnitude and direction than that of the other 3 farms. Compared with these herds, the reproductive management on farm D was quite different, giving a plausible biological explanation for the difference in conception to first

service. The cows on farm D had an extended VWP (80 d versus 50 to 67 d for the other 3 farms), and approximately 20% of cows were bred a second time, within 24 h of first insemination. Farm D was subsequently excluded from the analysis of both reproductive outcomes. Analyzing only the data from farms A, B, and C, a beneficial effect of PG was found concerning conception to first service, with PG-treated cows 1.3 times more likely to conceive to first service than control cows. However, no difference was found in time to conception within 150 DIM. It is hypothesized that the effect of PG on reproduction may be time dependent in that it affects cows earlier in lactation but its benefits diminish over time.

Although no studies have examined the effect of PG on reproduction in cows diagnosed with SCK, multiple studies report on the reproductive effects of PG when given prophylactically in early lactation, although with differing results. Hoedemaker et al. (2004) found no difference in first-service conception, pregnancy rate, time from first AI to conception, or days open for cows supplemented with PG in the feed from 13 d before expected calving until 12 d postpartum. Similarly, Castañeda-Gutiérrez et al. (2009) found no difference in time to first ovulation in multiparous cows after daily topdressing with PG from 21 d before expected calving until 21 d postpartum, nor did Lien et al. (2010) after oral administration of 500 mL of PG from 7 d prepartum to 30 d postpartum. Although no difference was found in days to first service, days open, or services per conception in both primiparous and multiparous cows by Miyoshi et al. (2001), oral administration of 500 mL of PG from 7 to 42 DIM did decrease the interval from calving until first ovulation from 44.5 to 32.3 d. Chagas et al. (2007) also found a decreased interval from calving to first ovulation in heifers with poor body condition after being drenched with 250 mL of PG twice daily for 16 wk after parturition. However, these studies are difficult to compare with the current study because they either did not determine the ketosis status of the cows before giving PG or they administered PG in the diet instead of oral drench. No

comparison can be made concerning time to first ovulation, as it was not measured in this study.

It is important to note that all final Poisson models, in addition to the random effect variable (herd) contained only the PG treatment variable. This suggests that PG treatment alone, across multiple outcomes, is an important factor in determining the risk of DA development and early removal from the herd in cows with SCK. The administration of PG to SCK-positive cows carries potential economic implications resulting from the decreased risk of DA development and removal from the herd in the first 30 DIM, as well as the increased risk of conception to first insemination. Further investigation is needed to determine the best testing and dosing scheme that is both economical and practical for management and labor purposes.

CONCLUSIONS

Intensive detection of SCK, followed by treatment of positive cows with oral PG, decreased the risk of developing a DA or leaving the herd within the first 30 DIM and had a positive effect on conception to first service in some herds.

ACKNOWLEDGMENTS

This study was funded, in part, by the US Department of Agriculture-National Research Initiative (USDA-NRI) and Boehringer Ingelheim Vetmedica Inc. (St. Joseph, MO). The authors thank Judith Alishauskas, Stephanie Brittin, Emily Bold, Mark Fagan, and Caitlin Jeffrey (Cornell University, Ithaca, NY), and Lindley Reilly, Eric Rooker, Angela Niles, Tyler Franks, Catherine Bargren, Diego Calderon, Cristian Vergara, and Loïc Commun (University of Wisconsin, Madison) for their invaluable assistance with data collection, as well as the owners of the collaborating dairies for allowing us access to their cows and facilities on which to conduct

this research.

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CHAPTER SIX

COST OF EARLY LACTATION SUBCLINICAL KETOSIS IN DAIRY CATTLE: AN ECONOMIC ANALYSIS OF TESTING AND TREATMENT WITH PROPYLENE GLYCOL

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Journal of Dairy Science: in revision

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ABSTRACT

The purpose was to develop stochastic economic models to: 1) estimate the per case cost of subclinical ketosis (SCK) in the first 30 d in milk (DIM) and 2) evaluate different on-farm testing and treatment strategies based on herd SCK incidence. Data used in model development was from a large field trial in which cows from 4 free-stall dairy herds (2 in New York and 2 in Wisconsin) were each tested 6 times for SCK from 3 to 16 DIM using the Precision Xtra meter. Subclinical ketosis was defined as a β-hydroxybutyrate concentration of 1.2 to 2.9 mmol/L. Data from 741 SCK positive cows and 976 non-ketotic cows were used in model development. The per case cost of SCK in the first 30 DIM was calculated based on the loss in milk production and increase in DA and early removal risks of SCK positive cows compared with non-ketotic cows. Four on-farm testing and treatment strategies were analyzed at herd SCK incidences ranging from 5 to 80% and included: 1) treating all cows with 5 d of propylene glycol (PG) starting at 5 DIM, 2) testing all cows for SCK 1 day per week (e.g. Mondays) from 3 through 16 DIM and treating all positive cows with 5 d of oral PG, 3) testing all cows for SCK 2 days per week (e.g. Mondays and Thursdays) from 3 through 9 DIM and treating all positive cows with 5 d of oral PG, and 4) testing all cows for SCK 3 days per week (e.g. Mondays, Wednesdays, and Fridays) from 3 through 16 DIM and treating all positive cows with 5 d of oral PG. Cost-benefit analysis included the costs associated with labor to test cows, β-hydroxybutyrate test strips, labor to treat cows, PG, and the associated gain in milk production, decrease in DA and early removal risks of PG treated SCK positive cows compared to non-PG treated SCK positive cows. Stochastic models were developed to account for variability in the distribution of input variables. The mean cost per case of SCK in the first 30 DIM was $67. Per 100 fresh cows in a herd with an SCK incidence of 40%, the mean economic benefits of the 4 different strategies were $910, $598, $943, and $541, respectively. Testing cows 2 days per week from 3 through 9 DIM was the most

cost-effective strategy for herds with SCK incidences between 15 and 50%; above 50%, treating all fresh cows with 5 d of PG was the most cost-effective strategy. These results show that when herd SCK incidences rise above 25%, almost any SCK testing and treatment protocol will be economically beneficial for the farm.

Keywords: dairy cow, ketosis, economics, propylene glycol

INTRODUCTION

The negative associations of subclinical ketosis (SCK) with downstream health events and production have been well described in early lactation dairy cattle. Cows diagnosed with SCK have an increased risk of developing additional postpartum diseases such as displaced abomasum (DA) and metritis, are at an increased risk of leaving the herd, and have decreased milk yield in early lactation (Duffield et al., 2009; Ospina et al., 2010a; McArt et al., 2012b). While early lactational incidence of SCK is widely variable, it was found to affect approximately 40 to 60% of cows in herds undergoing once weekly testing (Emery et al., 1964; Simensen et al., 1990; Duffield et al., 1998). This may be an underestimation of the true incidence as the median duration of SCK was found to be 5 d (McArt et al., 2012b), thus once weekly testing will fail to detect all SCK positive cows.

The cost per case of SCK depends on a variety of factors including increased occurrence of postpartum diseases, cost of herd replacements, and loss of milk production. The intensity and accuracy of diagnostic testing used to define a case of SCK then affects the reliability of the cost estimate. Duffield (2000) made 2 example herds using data from 25 herds that measured serum BHBA once weekly at 1, 2, 3, 6, and 9 weeks after calving (Duffield et al., 1998) and estimated the cost per case of SCK (defined as serum BHBA $\geq$ 1.4 mmol/L) to be CAN $50 to CAN $100,

which is approximately US $46 to US $92 after adjustment for today's inflation and exchange rate. Geishauser et al. (2001) synthesized data from multiple studies that diagnosed SCK by either a serum BHBA ≥ 1.4 mmol/L or milk acetoacetic acid and accounted for the loss of milk production, increased calving-to-conception interval, and increased risk of clinical ketosis and DA. The cost of a case of SCK was estimated to be CAN $78, which, after adjustment for today's inflation and exchange rate is approximately US $68. Both of these studies accounted for the costs associated with increased risk of diseases and loss of production, however neither accounted for the variability in risk and production costs that occur between farms and over time. In addition, both studies were conducted in herds with a variety of feeding systems and were not exclusively free-stall, TMR fed herds. Stochastic Monte Carlo modeling approaches are able to account not only for disease risks and production costs, but also their variability (for example, fluctuations in the price of milk, cost of a replacement cow, and feed prices); these approaches allow an estimation of per case cost as well as a range around the estimate (Metropolis and Ulam, 1949).

Overall herd costs of SCK vary based on herd SCK incidence, and a cost-treatment benefit ratio depends on the frequency and accuracy with which cows are tested. McArt et al. (2012b) showed that in 4 large dairies in New York and Wisconsin, 75% of cows that were detected with SCK from 3 to 16 DIM were first positive from 3 to 7 DIM; thus early and accurate identification of cows with SCK could be a crucial determinant of the economic benefits of an SCK testing and treatment program. Use of a Precision Xtra meter (Abbott Laboratories, Abbott Park, IL), a handheld device used to test blood BHBA concentrations, gives excellent cow-side accuracy when compared to serum BHBA concentrations determined photometrically (Iwersen et al., 2009; Konkol et al., 2009). Other cowside tests for ketosis use urine or milk as the diagnostic sample and have lower accuracy than the Precision Xtra meter (Oetzel, 2004).

Following accurate identification of SCK positive cows, individual cow treatment with propylene glycol (PG) has been found to be an effect means of reducing the risks associated with SCK and improving milk production (McArt et al., 2011; McArt et al., 2012a). Geishauser et al. (2001) calculated the cost-benefit ratio of testing each cow twice in the first 2 weeks of lactation followed by treatment with PG twice daily for 3 days to be 1 to 3.2. However the sensitivity and specificity of the testing for SCK was not included in the calculation, nor did the analysis consider variation in herd SCK incidence or variation in other inputs used to determine the cost-benefit ratio.

The objectives of this study were to develop stochastic models to: 1) estimate the per case cost of SCK in the first 30 DIM and 2) evaluate different on-farm testing and PG treatment strategies based on herd SCK incidence.

MATERIALS AND METHODS

Study Population

Data were collected from 2 dairy farms (farms A and B) in New York from 18 May 2010 until 8 September 2010 and from 2 dairy farms (farms C and D) in Wisconsin from 11 June 2010 until 30 August 2010. To be selected, farms had to meet the following criteria: milk at least 1,500 cows, have headlocks in fresh cow pens, use the farm management program Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and be willing to participate in the proposed ketosis testing protocol. Data from 741 SCK positive cows and 976 non-ketotic cows were used in model development, as described below. In-depth information concerning farm management, nutrition, and reproductive and disease events has previously been published (McArt et al., 2011).

Data Collection and Study Design

Enrollment into the study occurred at calving. Cows were tested from 3 to 16 DIM on Mondays, Wednesdays, and Fridays for SCK using a Precision Xtra meter. Given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. Subclinical ketosis was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L. All testing of cows for SCK from 3 to 16 DIM was completed by the research team during the study. Detailed blood collection and testing information has previously been reported (McArt et al., 2011).

Cows with BHBA concentrations of 1.2 to 2.9 mmol/L were sequentially randomized to treatment group (300 mL oral PG drench) or control group (no PG) after their first SCK positive test. Cows with blood BHBA concentrations ≥ 3.0 mmol/L were considered clinical cases, excluded from the study, and promptly treated according to each farm's ketosis protocols. Cows were also excluded from analysis if their previous gestation length was less than 260 d, if they died or were sold before their first BHBA test, if they were diagnosed and treated by the farm for ketosis before their first BHBA test, or for lack of proper identification. Additionally, cows remaining in the herd through 16 DIM were excluded if they had fewer than 5 BHBA tests. Further data collected included parity, DA, metritis, sold and died events, conception to first service, and DIM at conception. Displaced abomasa, cows died, cows sold, and pregnancy outcomes were exported throughout the study period from each farm's Dairy Comp 305 program. Milk weights were exported for the 3 herds that recorded milk production on per milking basis (farm A, farm B, and farm D). Collected data was used to determine the associated risks of SCK on development of a DA and removal from herd in the first 30 DIM, conception to first service, time to conception, and milk yield in the first 30 DIM (McArt et al., 2012b) as well as the benefits of administration of PG to cows diagnosed with SCK on these outcomes (McArt

et al., 2011; McArt et al., 2012a).

Research protocols were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (#2008-0099) and the University of Wisconsin-Madison School of Veterinary Medicine Animal Care and Use Committee (#V01479-0-05-10). All farms were asked to sign a consent form agreeing to the proposed testing and treatment protocol and were given a document containing information on disease definitions including clinical milk fever, retained placenta, metritis, DA, and clinical ketosis.

Model Development

Stochastic Monte Carlo partial budget models were developed using @Risk, version 5.7 (Palisade Corporation, Ithaca, NY). The first model developed analyzed the cost per case of SCK in the first 30 DIM. The second model analyzed testing and PG treatment strategies for 4 possible strategies: 1) treating all cows with 300 mL of oral PG for 5 d starting at 5 DIM (TREAT ALL), 2) testing all cows for SCK 1 day per week (e.g. Mondays) from 3 through 16 DIM and treating all positive cows with 300 mL of oral PG for 5 d (TEST1), 3) testing all cows for SCK 2 days per week (e.g. Mondays and Thursdays) from 3 through 9 DIM and treating all positive cows with 300 mL of oral PG for 5 d (TEST2), and 4) testing all cows for SCK 3 days per week (e.g. Mondays, Wednesdays, and Fridays) from 3 through 16 DIM and treating all positive cows with 300 mL of oral PG for 5 d (TEST3). For the TEST1, TEST2, and TEST3 strategies, once a cow is identified as SCK positive she is treated with 5 d of PG and not tested again.

Variables used in model development are in Table 1 and included: cost of labor per hour, cost per BHBA test, number of cows tested per hour, number of cows treated per hour, cost of PG, length of PG treatment, DA risk in the first 30 DIM, cost per case of DA, early removal risk

in the first 30 DIM, cost of a replacement cow, price of milk, feed cost, increased risk of DA and early removal and decreased milk production in SCK positive cows compared to non-ketotic cows (for the cost per case of SCK model), and decreased risk of DA and early removal and increased milk production in SCK cows treated with PG (for the testing and PG treatment models). National average dairy feed cost data were collected for 3 years from 2009 to 2012 from the University of Wisconsin (Gould, 2012) and ranged from $0.14 to $0.26 per kg of feed (dry matter). Mailbox milk prices were collected for the same time period from the United Stated Department of Agriculture (USDA) Agricultural Marketing Service (2012) and ranged from $0.25 to $0.49 per kg of milk. Cull and replacement cow prices were collected for the three years from 2009 to 2012 through the USDA Agricultural Statistics Service (2012); the exchange cost for replacement was calculated by subtracting the cull cow price (based on a 545 kg cow) from the replacement cow price and ranged from $421 to $775. Farm labor costs were based on information from the USDA Agricultural Marketing Service (2012) and estimated at $13.50 per hour after incorporating a 30% increase to account for employee benefits. Minimum, mean, and maximum risks for DA were estimated based on Shaver (1997) with the assumption that 90% of DA cases occur in the first 30 DIM. Cost of a DA was determined by economic analysis (Guard, 2008; Chuck Guard, personal communication, 2012). Minimum, mean, and maximum risks for culling were estimated based on Roberts et al. (2012) with the assumption that 75% of cows leaving the herd in the first 60 DIM do so within the first 30 DIM. Price per BHBA test was based on cost through a veterinary distributor (MWI Veterinary Supply, Boise, ID) with an additional 15% mark-up. The number of cows tested and treated per hour were approximated based on field trials previously completed by the research group. The cost of one dose of PG was estimated based on cost of a 208 L drum (E. H. Wolf & Sons, Green Bay, WI) assuming an oral drench of 300 mL per dose, and a five day length of PG treatment was based on the median time

to resolution of SCK (McArt et al., 2011). The minimum, mean, and maximum risk ratios associated with the use of PG on DA and early removal were determined by McArt et al. (2012a). The minimum, mean, and maximum risk ratios associated with ketosis status (SCK versus non-ketotic) were based on McArt et al. (2012b). The association of ketosis status with milk yield per milking and the association of milk yield per milking based on treatment of SCK cows with PG were determined by McArt et al. (2012b) and McArt et al. (2012a), respectively.

Distribution functions for the raw data for each variable were fitted by 1 of 2 methods. For variables with repeated measurements over time (e.g. monthly mailbox milk price for 3 years), distribution functions were fitted using @Risk's BestFit function that selects the best fitting distribution based on the selected data. For variables with recorded minimum, most likely, and maximum values (e.g. herd DA% in the first 30 DIM), a pert distribution (a special form of a beta distribution) was chosen as it emphasizes the most likely value over the minimum and maximum estimates, i.e. values around the mean are more likely to be chosen than extreme values. Variables and distribution functions used in model development are in Table 1. A correlation matrix was added to account for possible correlations in variations between cost of feed, price of milk, and exchange cost for a replacement cow and were calculated using the CORREL function in Excel (Microsoft, Redmond, WA). Correlations coefficients included in the models were: cost of feed versus price of milk (0.68), cost of feed versus exchange cost of a replacement cow (-0.63), and price of milk versus exchange cost of a replacement cow (-0.58).

The equation used to calculate cost per case was:

$$\begin{aligned}&\text{Cost per case of SCK in the first 30 DIM}\\&= \text{loss in milk revenue } [[(\text{fewer kg milk produced in the first 30 DIM by SCK vs non}\\&- \text{ketotic cows}) \; x \; (\text{price of milk per kg})]\\&- \text{ feed cost savings } [(\text{fewer kg milk produced in the first 30 DIM by SCK cows vs non}\\&- \text{ketotic cows}) \; x \; (1{:}2 \text{ feed to milk conversion ratio}) \; x \; (\text{feed cost per kg dry matter})]]\\&+ \text{ loss due to DA } \left[(\text{herd DA\% in the first 30 DIM}) \; x \; \left(1 \right.\right.\\&\left.\left. - \frac{1}{\text{DA risk ratio of SCK vs non} - \text{ketotic cows}}\right) \; x \; (\text{cost of DA})\right]\\&+ \text{ loss due to removal from herd } \left[(\text{herd removal\% in the first 30 DIM}) \; x \; \left(1 \right.\right.\\&\left.\left. - \frac{1}{\text{removal risk ratio of SCK vs non} - \text{ketotic cows}}\right) \; x \; (\text{exchange cost for a replacement cow})\right]\end{aligned}$$

The 3 strategies involving testing before treatment (TEST1, TEST2, and TEST3) assumed SCK positive cows were correctly identified based on frequency of testing and a 5 d median time to resolution. Table 2 shows the percentage of cows correctly identified for each testing strategy based on DIM at onset of SCK. The percentage of cows correctly identified at each DIM was then multiplied by the incidence of SCK for each respective DIM (McArt et al., 2012b) in order to determine the total percentage of SCK positive cows correctly identified for each testing strategy. TEST1, TEST2, and TEST3 correctly identified 69.2%, 79.7%, and 99.2% of the SCK positive cow, respectively. The positive benefits of treatment with PG were extended to the correctly identified SCK positive cows for each testing strategy. For the TREAT ALL strategy, positive benefits of treatment with PG were extended to all cows expected to develop SCK from 3 through 9 DIM; it was assumed that PG had no negative or positive effects on non-ketotic cows. Outcome benefits from PG treatment included increased milk production (McArt et al., 2011) and a decreased risk of DA development and early removal from herd (McArt et al., 2012a). As found by McArt et al. (2012b), the risk of developing a DA in SCK cows is entirely due to cows first SCK positive from 3 to 7 DIM, thus the benefit of a reduced risk of DA

development after treatment with PG was extended to all SCK positive treated cows from 3 through 7 DIM. Similarly, the risk of early removal from the herd was found to be greater in cows first SCK positive from 3 to 7 DIM, thus 90% of PG's association with reducing the risk of early removal from the herd was assumed to occur in cows positive from 3 through 7 DIM. For the model determining testing and treatment strategies, average herd SCK incidence was estimated to be 40% (Emery et al., 1964; Duffield, 2000; McArt et al., 2011). Testing strategies were based on sampling cows in the first 2 weeks (3 to 16 DIM) postpartum as most cows that develop SCK do so during this period (Duffield et al., 2009; McArt et al., 2012b).

The equation used to calculate the cost or benefit of each testing and treatment strategy was similar to that used to calculate the per case cost of SCK in the first 30 DIM, with the milk production difference and DA and removal risk ratios using data from both SCK positive PG treated cows versus SCK positive non-treated cows and SCK positive versus non-ketotic cows. Additional variables incorporated into the models were the labor costs to test and treat cows, the number of cows tested and treated per hour, cost of PG, cost of BHBA strip tests, number of days of treatment with PG, and herd SCK incidence. Model simulations were run using 10,000 iterations with replacement. Probability density and correlation coefficient graphs were produced using @Risk. Cost versus incidence graphs were produced using Excel after running the testing and treatment model at various herd SCK incidences; herd SCK incidence was entered manually into the testing and treatment models in increments of 5% from a 5 to 80% herd incidence.

RESULTS AND DISCUSSION

Stochastic modeling was conducted in order to: 1) estimate the per case cost of SCK in the first 30 DIM and 2) evaluate the best on-farm testing and PG treatment strategy based on herd SCK incidence.

Per case cost of SCK

The mean per case cost of SCK in the first 30 DIM was estimated to be $67. The relative frequency graph of the cost is in Figure 1. Accounting for variation in the input variables, 95% of the time a case of SCK will cost between $34 and $109 during the first 30 DIM. For example, a dairy that freshens 1,000 cows per year with a herd SCK incidence of 40% will lose, on average, $26,800 per year due to costs associated with SCK in the first 30 DIM.

The per case cost of SCK in the first 30 DIM estimated in this analysis was similar to that found by Duffield (2000) and Geishauser et al. (2001) after adjusting for inflation and the exchange rate. However, the current study only used data obtained from the research group's previous work (McArt et al., 2011; McArt et al., 2012a; McArt et al., 2012b). As milk production was only measured for the first 30 DIM in these studies, entire lactation milk production differences and reproductive repercussions between SCK positive and non-ketotic cows were not included in this analysis. Previous studies have shown a negative milk effect across the entire lactation between SCK positive and non-ketotic cows (Dohoo and Martin, 1984; Ospina et al., 2009). As only data from the first 30 DIM was used in model development, reproductive costs associated with SCK were not included even though studies have found a difference in conception to first service and time to conception between SCK positive and non-ketotic cows (Walsh et al., 2007; Ospina et al., 2010b). For these reasons, the calculated cost per case of SCK in the first 30 DIM is considered a conservative estimate of a lactational cost per case of SCK.

The percent contribution of each of the main costs per case (loss in milk revenue, loss due to DA, and loss due to removal from herd) were 17.9%, 40.6%, and 41.5%, respectively. A tornado graph showing the correlation coefficients of the input variables with cost per case in the

first 30 DIM is in Figure 2. It shows that a herd's underlying DA and early removal risks in the first 30 DIM have the highest impact on the cost per case with a larger cost per case of SCK in herds with a higher DA and early removal risks. Based on correlation coefficients, the herd DA and early removal risk explained 59% and 26% of the variation in the cost per case of SCK, respectively, with an increase in the herd DA or early removal risk increasing the cost of SCK. Exchange cost for a replacement cow explained 6% of the variation with the cost per case of SCK increasing as the exchange cost increased. When combined, the cost of a DA, difference in kg milk produced in the first 30 DIM between SCK and non-ketotic cows, and the price of milk accounted for a total of 6% of the variation, with a higher value of each increasing the cost per case of SCK. Variation in per case cost of SCK due to the DA and removal risk ratios between SCK and non-ketotic cows and the cost of feed were negligible. As 85% of the total variation in cost per case of SCK depends on a herd's DA and early removal risk, herds with high DA or early removal risks will have a greater economic benefit from controlling SCK than herds with low DA or early removal risks.

On-farm testing and treatment strategies

Per 100 fresh cows in a herd with an SCK incidence of 40%, the mean benefit of the TREAT ALL, TEST1, TEST2, and TEST3 models were $910, $598, $943, and $541, respectively. Table 3 shows the mean, standard deviation, and 95% cost-benefit range when accounting for variation in the input variables. The relative frequency graph for the most beneficial testing and treatment strategy at a 40% herd SCK incidence, TEST2, is in Figure 3. A graph showing the mean cost-benefit for each of the 4 strategies in herds with SCK incidences ranging from 5 to 80% is in Figure 4.

Blanket treatment of all cows at 5 DIM with PG was the most cost-effective strategy

when herd SCK incidences were ≥ 50%. Below this level, the cost of labor and supplies for treatment of all cows outweighed the positive benefits of PG treatment of SCK positive cows. Testing cows 1 day per week from 3 through 16 DIM has the benefit of being easy to implement, but has the drawback that some SCK positive cows will not be identified as having SCK due to the length between testing days. Testing cows 2 days per week from 3 through 9 DIM has the advantage of accurately identifying most SCK positive cows during that week of lactation, although cows that first test positive from 10 through 16 DIM will not be identified. However, as the negative associations of SCK on DA development and early removal from herd is limited solely to those cows first positive from 3 to 7 DIM for DA and the majority of cows first positive in the same time frame for early removal (McArt et al., 2012b), the main drawback of not testing cows from 10 through 16 DIM is in the loss of milk production in the first 30 DIM. As seen in the model analysis, this loss of milk production was less than the increased cost of labor for testing and treatment, thus testing cows twice per week from 3 through 9 DIM was, until high herd incidences of SCK, the most cost-effective testing and treatment strategy. Testing cows three times per week from 3 through 16 DIM, while accurately identifying almost all SCK positive cows during those 2 weeks of lactation, was not the most cost-effective strategy at any point between a 5 and 80% herd SCK incidence due to the large costs of testing and treatment. In fact, herd SCK incidence must be greater than 25% for this strategy to have a beneficial economic impact (Figure 4).

On average, per 100 fresh cows, a herd with a 40% SCK incidence will see an approximately $1,000 return (Figure 3) after testing cows 2 days per week (e.g. Mondays and Thursdays) from 3 through 9 DIM and treating SCK positive cows with 5 d of PG; 95% of the time, the benefit will be approximately $150 to $2,000. Thus a herd freshening 1,000 cows per year will see, on average, a $10,000 return, and 95% of the time will incur a $1,500 to $20,000

benefit. Alternatively explained, a herd freshening 1,000 cows per year that chooses the TEST2 strategy will see a benefit between $10,000 and $25,000 per year 50% of the time. As with the per case cost of SCK in the first 30 DIM, this estimate is conservative as lactational milk yield, improved detection of cows with very high BHBA concentrations, and reproductive measures were not taken into account. Lactational milk yield improvements due to PG treatment cannot be quantified as no studies have looked at the association of PG treatment of SCK positive cows on milk yield longer than the first 30 DIM. Although a beneficial association of PG treatment of SCK positive cows has been found on conception to first service (McArt et al., 2012a), since the objective of this research was to quantify the cost-effectiveness of testing and treatment strategies for the first 30 DIM only, reproductive measures were not included in the analysis. Thus, herds using this testing and treatment strategy may see a larger benefit. While testing 2 days per week was found to be the most beneficial strategy, Figure 4 shows that above a 25% herd SCK incidence, any method of testing and treatment was better than ignoring the problem.

It may be easier to first conduct a SCK prevalence test on a herd in order to approximate the herd incidence and determine the best testing and treatment plan. The incidence of SCK is approximately twice the prevalence (Duffield et al., 1999; McArt et al., 2012b). For those herds with an incidence greater than 50%, where blanket treatment with PG is initiated, repeated prevalence testing may be necessary after management changes to determine if treating all fresh cows remains the best option. For herds with an incidence from 15 to 50% (approximately 7.5 to 25% prevalence), any of the testing strategies will allow for repeated monitoring of herd incidence; however, it is important to remember that depending on which testing method is chosen, only a certain percentage of cows will be correctly identified (69.2%, 79.7%, and 99.2% for TEST1, TEST2, and TEST3, respectively). Of course, the goal of any SCK testing and treatment strategy is to optimize the economic return while making management changes that

decrease the incidence of SCK on the farm.

Table 6.1. Description and distribution of model input variables used to estimate the cost per case of subclinical ketosis (SCK) in the first 30 DIM and to evaluate different strategies for testing and treating SCK positive cows with propylene glycol (PG).

Variable	Distribution	Parameters	Model[1]
Fresh cows	Fixed	100	2
Labor ($/hr)	Fixed	13.50	2
BHBA ($/test)	Fixed	1.53	2
Test (cows/hr)	Fixed	45	2
Treat (cows/hr)	Fixed	60	2
Propylene glycol ($/dose)	Fixed	1.00	2
Length of treatment (days)	Fixed	5	2
DA[2] % (1st 30 DIM)	Pert[3]	0.0, 4.5, 18.0	1, 2
DA cost ($)	Pert	350, 500, 650	1, 2
Increased risk of DA in SCK cows	Pert	13.8, 19.3, 27.0	1, 2
Decreased risk of DA in SCK cows given PG	Pert	1.3, 1.6, 2.0	2
Early removal % (1st 30 DIM)	Pert	1.3, 6.1, 14.4	1, 2
Exchange cost for replacement cow ($)	Logistic[4]	623.70, 65.08	1, 2
Increased risk of early removal in SCK cows	Pert	2.2, 3.0, 4.2	1, 2
Decreased risk of early removal in SCK cows given PG	Pert	1.2, 2.1, 3.6	2
Price of milk ($/kg)	Normal[5]	0.38, 0.07	1, 2
Decrease in 30 d milk in SCK cows (kg)	Pert	0.5, 1.3, 2.2	1, 2
Increase in 30 d milk in SCK cows given PG (kg)	Pert	0.05, 1.3, 1.6	2
Feed ($/kg dry matter)	BetaGeneral[6]	0.48, 0.66, 0.14, 0.26	1, 2

[1]Model: 1 = cost per case of subclinical ketosis in the first 30 d in milk, 2 = risk assessment of testing and propylene glycol treatment strategies

[2]DA = displaced abomasum

[3]Pert distribution includes minimum, mean, and maximum parameters.

[4]Logistic distribution includes alpha and beta parameters with a minimum and maximum cost of $280 and $970.

[5]Normal distribution includes mean and standard deviation parameters with a minimum and maximum value of $0.20 and $0.55.

[6]BetaGeneral distribution includes alpha1, alpha2, minimum, and maximum.

Table 6.2. Percentage of cows correctly identified with subclinical ketosis (SCK) for each testing strategy based on DIM at onset of SCK and a 5 d median time to resolution. The testing strategies include: 1) testing all cows for SCK 1 day per week (e.g. Mondays) from 3 through 16 DIM (TEST1), 2) testing all cows for SCK 2 days per week (e.g. Mondays and Thursdays) from 3 through 9 DIM (TEST2), and 3) testing all cows for SCK 3 days per week (e.g. Mondays, Wednesdays, and Fridays) from 3 through 16 DIM (TEST3).

	DIM at Onset of Ketosis													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
TEST1	71.4	71.4	71.4	71.4	71.4	71.4	71.4	71.4	71.4	71.4	57.1	42.9	28.6	14.3
TEST2	100.0	100.0	100.0	100.0	85.7	57.1	28.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TEST3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	85.7	42.9

Table 6.3. Mean, 95% range, and standard deviation (SD) of cost-benefit analysis for 4 different testing and propylene glycol (PG) treatment strategies per 100 fresh cows with a herd subclinical ketosis incidence of 40%. The testing strategies include: 1) treating all cows with 300 mL of oral PG for 5 d starting at 5 DIM (TREAT ALL), 2) testing all cows for SCK 1 day per week (e.g. Mondays) from 3 through 16 DIM and treating all positive cows with 300 mL of oral PG for 5 d (TEST1), 3) testing all cows for SCK 2 days per week (e.g. Mondays and Thursdays) from 3 through 9 DIM and treating all positive cows with 300 mL of oral PG for 5 d (TEST2), and 4) testing all cows for SCK 3 days per week (e.g. Mondays, Wednesdays, and Fridays) from 3 through 16 DIM and treating all positive cows with 300 mL of oral PG for 5 d (TEST3). Numbers in parentheses denote a negative value.

Strategy	Mean	95% range			SD
TREAT ALL	$910	$94	to	$1986	492
TEST1	$598	$28	to	$1334	340
TEST2	$943	$157	to	$2014	478
TEST3	$541	($299)	to	$1644	506

Figure 6.1. Relative frequency graph showing 10,000 iterations of the cost of per case of subclinical ketosis (SCK) in the first 30 DIM. The mean cost was $67 and the standard deviation $19.

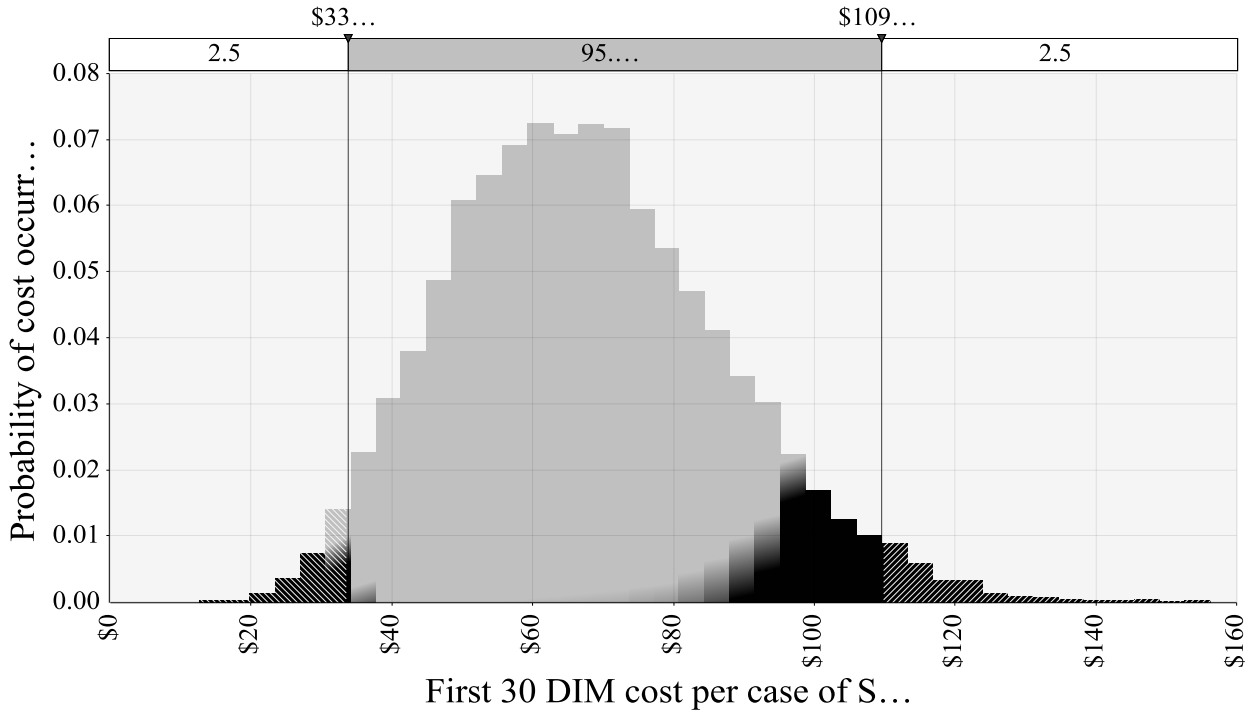


Figure 6.2. Tornado graph showing correlation coefficients between the model input variables and the cost per case of subclinical ketosis (SCK) in the first 30 DIM. Variables used in the model included displaced abomasum (DA) in the first 30 DIM, cows removed from the herd (cull) in the first 30 DIM, exchange cost for a replacement cow, cost of a DA, price of milk, difference in kg milk produced per cow per day between SCK and non-ketotic cows, culling risk ratio for SCK versus non-ketotic cows, DA risk ratio for SCK versus non-ketotic cows, and cost of feed.

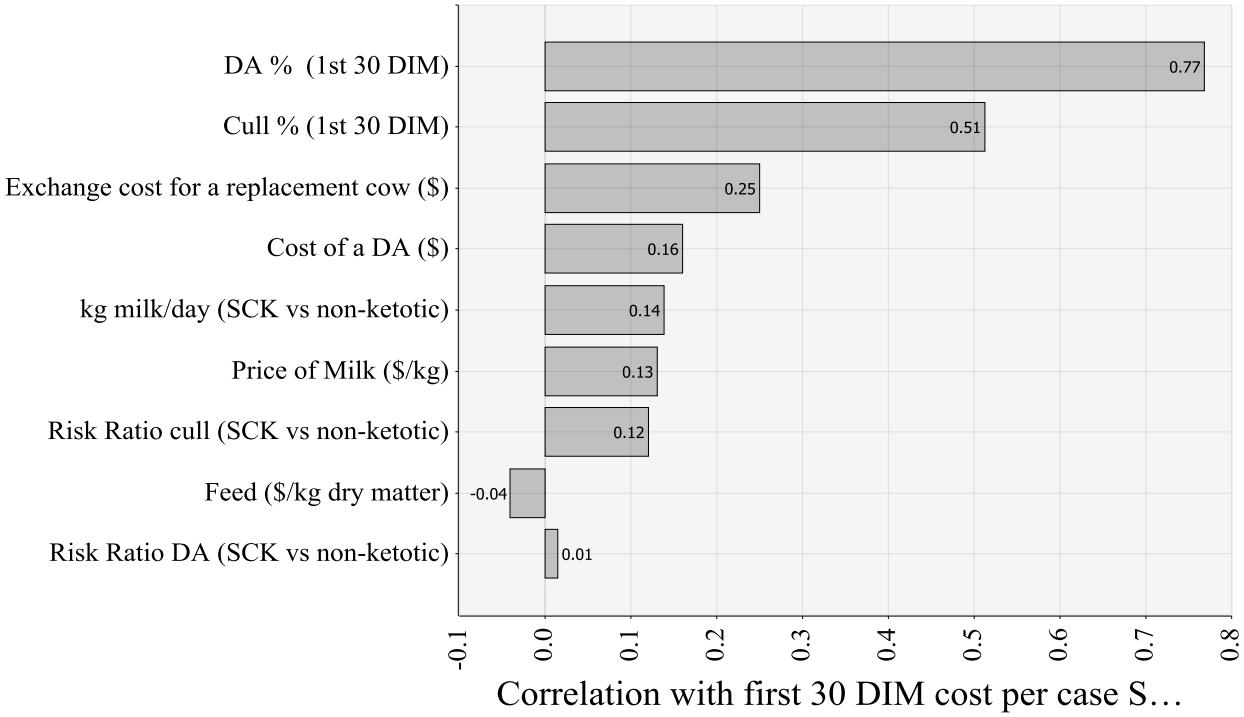


Figure 6.3. Relative frequency graph showing 10,000 iterations of the most profitable testing and treatment strategy per 100 fresh cows in a herd with a 40% incidence of subclinical ketosis by testing all cows for subclinical ketosis 2 times per week from 3 through 9 DIM and treating all cows with a blood β-hydroxybutyrate concentration of 1.2 to 2.9 mmol/L with 300 mL oral propylene glycol for 5 d. The mean benefit was $943 and the standard deviation $478.

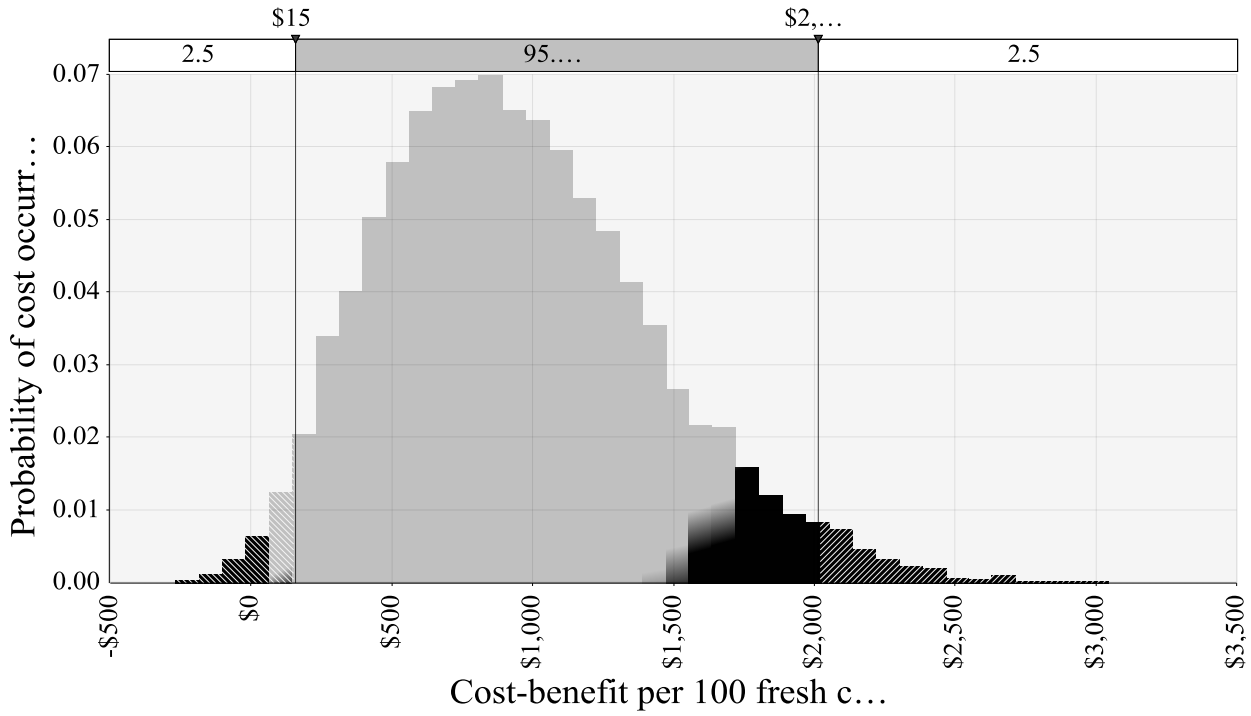
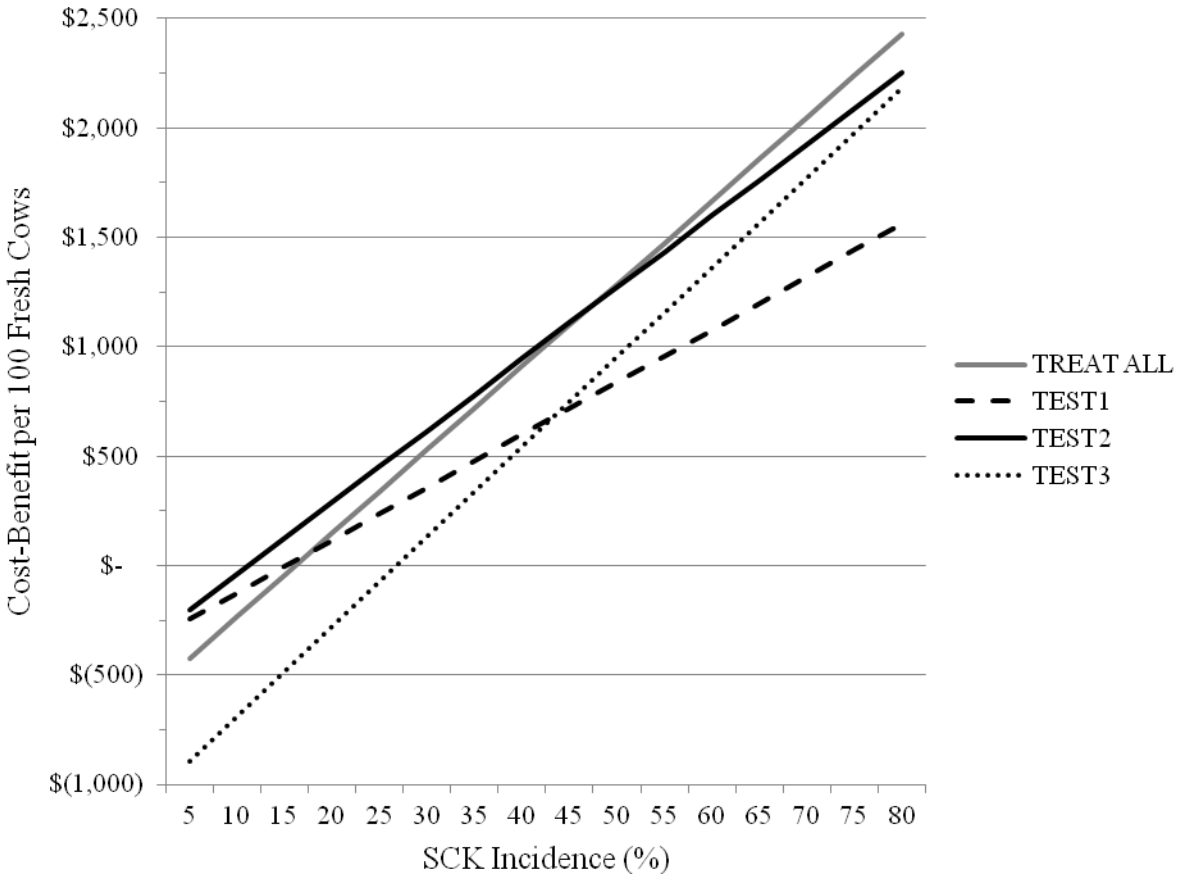


Figure 6.4. Line graph showing mean cost-benefit versus herd subclinical ketosis (SCK) incidence for 4 testing and propylene glycol (PG) treatment strategies, with herd SCK incidences ranging from 5 to 80%. The strategies include 1) treating all cows with 300 mL of oral PG for 5 d starting at 5 DIM (TREAT ALL), 2) testing all cows for SCK 1 day per week (e.g. Mondays) from 3 through 16 DIM (TEST1), 3) testing all cows for SCK 2 days per week (e.g. Mondays and Thursdays) from 3 through 9 DIM (TEST2), and 4) testing all cows for SCK 3 days per week (e.g. Mondays, Wednesdays, and Fridays) from 3 through 16 DIM (TEST3). The TEST1, TEST2, and TEST3 models assume all cows with a blood β-hydroxybutyrate concentration of 1.2 to 2.9 mmol/L are treated with 300 mL of oral PG for 5 d.



CONCLUSIONS

The estimated cost per case of SCK for the first 30 DIM was $67, and the benefit of testing and treatment with PG per 100 fresh cows in a herd with a 40% SCK incidence was $943 for the best testing and treatment strategy (2 days per week from 3 through 9 DIM). These are likely conservative estimates of lactational costs as they do not consider potential improvements in milk yield throughout lactation or potential improvements in reproductive performance. In herds with an SCK incidence of 15 to 50%, it is most economical to test cows 2 days per week from 3 through 9 DIM. Herds with SCK incidences over 50% should consider blanket treatment of all cows with 5 d of oral PG starting at 5 DIM to minimize the negative economic effects of SCK until management changes can be made that reduce the incidence of the disease. Repeated incidence or prevalence testing is recommended in order to determine which approach to testing and treatment is optimal for an individual herd and to evaluate changes in transition cow management that could justify adjustments in these protocols.

ACKNOWLEDGEMENTS

This study was funded in part by the US Department of Agriculture – National Research Initiative (USDA-NRI no. 2008-55204-19393) and Boehringer Ingelheim Vetmedica Inc. (St. Joseph, MO). The authors thank Judith Alishauskas, Stephanie Brittin, Emily Bold, Mark Fagan, and Caitlin Jeffrey (Cornell University, Ithaca, NY), and Lindley Reilly, Eric Rooker, Angela Niles, Tyler Franks, Catherine Bargren, Diego Calderon, Cristian Vergara, and Loïc Commun (University of Wisconsin, Madison) for their invaluable assistance with data collection as well as the owners of the collaborating dairies for allowing us access to their cows and facilities on which to conduct this research.

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CHAPTER SEVEN

OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

Negative energy balance is a normal occurrence in dairy cattle as they transition from late gestation to early lactation; this is a dynamic period for dairy cattle during which most infectious and metabolic diseases are likely to occur. Cows unable to appropriately adapt to this challenging time are more prone to negative downstream events, and the associations between excessive NEB and these detrimental health effects are well documented. The economic impacts of maladaptation are not trivial due to an increased risk of metabolic disease, reduced milk production, early removal from the herd, and poor reproductive performance. This research explored the epidemiology of SCK, risk factors of SCK development, health and production consequences of cows with SCK, treatment of SCK positive cows with PG, the economics of SCK in the first 30 DIM, and the cost-benefit ratio of different testing and treatment strategies based on herd SCK incidence.

Conclusions

The first objective of this research was to describe the epidemiology of SCK in cows diagnosed with SCK in early lactation through an intensive monitoring program from 3 to 16 DIM and to determine the association of DIM at onset of SCK and BHBA concentration at onset of SCK with downstream health and production measures. Peak incidence and prevalence of SCK was found to occur at 5 DIM with a median of 5 days to resolution of SCK. This research supported the conclusions of others that cows with SCK were more likely to have negative health events and produce less milk than non-ketotic cows. This research also showed that cows that developed SCK within the first week postpartum were more likely to develop a DA or be removed from the herd within the first 30 DIM, were less likely to conceive to first service, and produced less milk in the first 30 DIM than cows developing SCK after the first week of lactation. In addition, as the concentration of BHBA at first positive SCK test increased, the risk

of adverse events increased and milk production decreased. As 75% of all cows that developed SCK tested positive within 1 wk postpartum, it may be important to modify farm management protocols to maximize detection during this time. Cows that begin lactation with high BHBA concentrations may require special attention to decrease their risk of adverse events in early lactation.

Once the basic epidemiologic patterns of SCK were identified, risk factors for development of hyperketonemia were analyzed. Advanced parity was a major predictor of hyperketonemia development in early lactation after herd-level hyperketonemia incidence was taken into account. Additionally, increased precalving BCS, increased precalving blood NEFA concentration, and variables related to a more difficult calving process (high CEASE score or stillbirth) may help identify at risk animals before hyperketonemia develops.

The second major objective of this research was to determine the effects of oral PG administration in fresh cows diagnosed with SCK on resolution of ketosis, development of additional health disorders, and milk production. Results showed that administration of oral PG to cows diagnosed with SCK helped resolve SCK faster and prevent clinical ketosis. In addition, SCK positive cows given PG had a decreased risk of developing a DA or leaving the herd within the first 30 DIM, and in some herds had an increased risk of conception to first service and improved milk production.

Economic models produced from the data above estimated the cost per case of SCK for the first 30 DIM at $67. The benefit of testing and treatment with PG per 100 fresh cows in a herd with a 40% SCK incidence was $943 for the best testing and treatment strategy (testing 2 days per week from 3 through 9 DIM). These are likely conservative estimates of lactational costs as they do not consider potential improvements in milk yield throughout lactation or potential improvements in reproductive performance. Results showed that in herds with an SCK

incidence of 15 to 50%, it was most economical to test cows 2 days per week from 3 through 9 DIM. Herds with SCK incidences over 50% should consider blanket treatment of all cows with 5 d of oral PG starting at 5 DIM to minimize the negative economic effects of SCK until management changes can be made that reduce the incidence of the disease. Repeated incidence or prevalence testing is recommended in order to determine which approach to testing and treatment is optimal for an individual herd and to evaluate changes in transition cow management that could justify adjustments in these protocols.

Future directions

As has been shown in the above chapters as well as in other studies, inappropriate adaptation of dairy cows to the transitional period of negative energy can lead to postpartum elevation of NEFA and BHBA which are associated with negative downstream health events, poor reproductive measures, and a loss of milk production. However, these measures of NEB are not well correlated (i.e. some cows with high NEFA have low BHBA and vice versa), and it is still unknown why some cows with elevated NEFA transition well into early lactation while others do not. Identification in mice of fibroblast growth factor-21 (FGF21), a potent regulator of metabolism, has been suggested as a marker of oxidative capacity in liver and is induced in conditions associated with extensive lipid use. Thus FGF21 may play a role in the ability of dairy cattle to mobilize and effectively convert NEFA from adipose tissue to usable energy without an overproduction of ketone bodies (e.g. BHBA). In addition to the role of FGF21 in an individual cow, genetic evaluation of body condition score, NEFA, and BHBA have shown that significant genetic variance exists, especially during the first weeks of lactation. Although it is unknown whether FGF21 has a genetic component, the high-yielding dairy cow offers distinctive advantages for exploring the role of FGF21 in lactation as it depends more extensively on lipid

reserves for lactation success than either the mouse or rat. Collaborative work with Dr. Daryl Nydam and Dr. Yves Boisclar aims to describe the normal postpartum profile of NEFA, BHBA, and FGF21 concentrations and their ratios in dairy cows in early lactation and establish if there is an association between the ratios and downstream negative health events, reproduction, and milk production. In addition, we hope to determine if there is a heritability component to postpartum FGF21 concentrations in dairy cows and establish if this genetic component is associated with postpartum NEFA, BHBA, and FGF21 concentrations.

Current collaborative work includes preliminary organization of a field trial in Jersey herds to determine if pre- and postpartum NEFA and BHBA threshold concentrations for prediction of disease, reproduction, and milk production are similar to those reported above in Holsteins.

While NEB is a physiologically normal process, prevention of excessive NEB and improved identification of cows with poor adaptation should be the goal on any dairy farm. In the future, I hope to identify herd-level risk factors that identify management bottlenecks associated with an increase in the proportion of cows with elevated NEFA and BHBA in a herd. I also hope to assess how exercise during the dry period affects NEB in dairy cattle and examine postpartum health events and production measures. In addition, I would like to determine if pain relief immediately postpartum improves NEB due to an increase in DMI in the days following calving.

Final remarks

When I agreed to work with Dr. Daryl Nydam on dairy cow metabolism during the transition period, I had no idea how interested I would become in the topic or how it would affect me personally. Having gone through two pregnancies during my time as a graduate student and realizing the demands it puts on your body before and immediately after giving birth as well as while lactating, I now have much more empathy for what we ask from our dairy cows. As with many others involved in the dairy industry, I hope my current and future research helps make dairy cows as comfortable as possible and give them what they require not only to maximize their production but also to improve their quality of life.