CLINICAL KETOSIS AND STANDING BEHAVIOUR IN TRANSITION DAIRY COWS

by

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Ketosis is a common disease in dairy cattle, especially in the days after calving, and is often undiagnosed. The objective of this study was to compare the standing behaviour of dairy cows with and without ketosis during the days around calving to determine if changes in this behaviour could be useful in the early identification of sick cows. Serum beta-hydroxybutyrate (BHBA) was measured in 370 cows on three commercial dairy farms, twice weekly from 2 to 21 d after calving. Standing behaviour was measured from 7 d before calving to 21 d after calving using data loggers. Retrospectively, 19 cows with subclinical ketosis (BHBA ≥1.2 and ≤ 2.9 mmol/L) and 20 cows with clinical ketosis (BHBA >2.9 mmol/L) were matched by farm with 39 non-ketotic cows (BHBA <1.2 mmol/L). Five periods were defined for the statistical analyses: wk -1 (d -7 to d -1), d 0 (day of calving), wk +1 (d 1 to d 7), wk +2 (d 8 to d 14) and wk +3 (d 15 to d 21). The first signs of both subclinical and clinical ketosis occurred 4.5 ± 2.0 d after calving. The standing behaviour of cows diagnosed with subclinical ketosis was not different than non-ketotic cows during any period. Total daily standing time was longer for clinically ketotic cows relative to non-ketotic cows during wk +1 (14.4 ± 0.8 vs. 12.2 ± 0.7 h/d) and d 0 (17.6 ± 1.0 vs. 13.4 ± 0.7 h/d) but was not different during the other periods. Clinically ketotic cows exhibited fewer standing bouts compared to non-ketotic cows on d 0 only (12.8 ± 2.2 vs. 18.1 ± 1.6 bouts/d). Average standing bout duration was also longer for clinically ketotic cows on d 0 compared to non-ketotic cows (64.9 vs. 40.4 min/bout) but was not different during the other periods. These results suggest that differences in standing behaviour before calving may be useful for the early detection of clinical ketosis in dairy cows.
A version of Chapter 2 has been submitted for publication and is currently under review: A. J. Itle, J. M. Huzzey, D. M. Weary and M. A. G. von Keyserlingk, Clinical ketosis and standing behaviour in transition cows. Amber Itle and Drs. Marina von Keyserlingk, Julie Huzzey and Dan Weary designed the study collaboratively. Amber Itle executed the research trial and collected all data. The main ideas for the manuscript were developed and researched by Amber Itle and analyzed by Amber Itle and Julie Huzzey. The co-authors supervised and edited drafts.
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LIST OF ABBREVIATIONS

AVMA = American Veterinary Medical Association

B = bouts

CI = confidence interval

BCS = body condition score

BHBA = beta-hydroxybutyrate

DMI = dry matter intake

NEB = negative energy balance

NEFA = non-esterified fatty acid

SE = standard error of the difference between the means

TMR = total mixed ration
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1. INTRODUCTION

1.1 Defining Animal Welfare

The Veterinarian’s Oath

“Being admitted to the profession of veterinary medicine, I solemnly swear to use my scientific knowledge and skills for the benefit of society through the protection of animal health and welfare, the prevention and relief of animal suffering, the conservation of animal resources, the promotion of public health, and the advancement of medical knowledge. I will practice my profession conscientiously, with dignity, and in keeping with the principles of veterinary medical ethics. I accept as a lifelong obligation the continual improvement of my professional knowledge and competence (AVMA, 2010).”

Every year, all graduating veterinary students in the United States mark the conclusion of their studies by reciting the Veterinarian’s Oath; verbalizing this promise to themselves, their clients, the public and the animals they set out to help. In preparation for this moment students entering veterinary school are taught to assess an animal’s biological functioning and health by connecting complete physical examination findings with systematic diagnostics to generate differential diagnoses. As the acknowledged stewards of animal care, veterinarians have a long history with the term animal health; however, likely due in part to the changes in the veterinarians’ oath (AVMA, 2010), there is an increasing use of the term ‘animal welfare’ both in practice and within the veterinary literature. Despite being defined very differently in the scientific literature (see key concepts, Fraser et al., 1997; Five Freedoms, Fraser, 2003; World Organization for Animal Health, 2008), the term animal welfare is frequently used
interchangeably with the term animal health within the field of veterinary medicine. Furthermore, welfare audits have traditionally been established on health-based outcomes and farm records (Whay et al., 2003). The failure of many veterinarians to differentiate between health and welfare may be due to a simplistic view of animal welfare; namely good health equates to good animal welfare.

1.1.1 Animal Welfare as a Framework: the Concepts

Animal welfare is a more complex concept that involves different kinds of costs and benefits (Fraser, 2006). One framework, that has gained considerable traction within the scientific literature, proposes three overlapping conceptions (Fraser et al., 1997). Each of these conceptions addresses different views of what constitutes a good life for animals: one is based on the affective states of animals, one on natural living and one on basic health and functioning. The three concepts can overlap, but each must be addressed to achieve good welfare. In a recent review von Keyserlingk et al. (2009) provided several examples of how this framework can be applied to dairy cattle production and explained how some attempts to address one type of concern may create or accentuate another. For example, housing dairy calves in groups allows them to engage in natural social interactions, but poorly managed groups can increase the risk of disease.

Each of the three concepts can be studied scientifically but there is no value-free way to establish their relative importance (Fraser, 2008a, von Keyserlingk et al., 2009). Producers and veterinarians may emphasize the health aspect of animal welfare but the lay public may emphasize affective state and natural living (see Spooner et al., 2013, Ventura et al., 2013). Individuals who rely on a single aspect may draw different conclusions about the welfare
compared to individuals who also consider other aspects. For example, two international teams of animal scientists reported opposing conclusions about the welfare of sows housed in gestation stalls (Scientific Veterinary Committee, 1997; Barnett et al. 2001) not because of disagreements about the scientific evidence but because of differences in the value the authors placed on each of the dimensions (see Fraser, 2003).

**1.1.2 The Production Medicine Approach to Veterinary Medicine**

Veterinary medicine typically focuses on caring for individual animals, but the profession has gone through many changes over the last 50 years, particularly in the case of food production animals. Intensification of animal agriculture since the 1950s (see Fraser, 2005) has resulted in a paradigm shift in the profession as veterinarians began embracing animal medicine targeted at the herd rather than the individual. Officially coined “Production Medicine” this form of animal medicine encompasses group (or herd) measures, nutritional analyses of the food provided to the animals and analysis of production and health records (see Radostits et al., 1994). Production Medicine emphasizes economics and has been a key driving force for veterinarians and producers making decisions about animal health on farms (Mulligan and Doherty, 2008). For example, veterinarians typically argue for solutions to health disorders that improve profitability of a herd or flock by considering the relationship of the disease to production, reproductive performance, and culling rates (Grohn et al., 2003; Ospina et al., 2010b). Furthermore, with the implementation of health monitoring tools and treatment protocols (LeBlanc et al., 2006), there is a growing disconnect between the veterinarian and the diseased animal. Despite the dramatic changes to the profession, farm animal veterinarians have continued to be considered animal husbandry experts relying on the scientific field of animal science and its associated disciplines.
including nutrition, reproductive physiology, and genetics to improve feeding, breeding, and health care on farms.

Prior to the last decade, aspects of animal husbandry research pertaining to animal handling, management and housing had been scant in the veterinary literature and educational curriculum. The scientific and veterinary communities have, however, become increasingly aware that integrating methodologies of traditional animal science (focusing on productivity, efficiency and profitability of the herd) with animal welfare science (considering the behavioural, biological and emotional needs of individual animals) can promote a multidisciplinary approach to research in the area of animal care and management (Fraser et al., 2013). For example, despite the recognition that behavioural changes are associated with clinical illness or health in the veterinary profession, little effort had focused on the use of behavioural changes as an early indicator of disease until recent years (Weary et al., 2009). Much work has been done, often by animal welfare scientists, on how changes in behaviour may be useful in identifying animals at risk of illness. Much of this work has focused on the dairy cow, particularly around the time of parturition, given the high incidence of disease. Research focusing on “transition” dairy cows has demonstrated the potential for using feeding, social and standing behaviours to predict disease in the days to weeks after calving (see review by Sepúlveda-Varas et al., 2013).

1.2. THE TRANSITION PERIOD

1.2.1 Physiological, Nutritional and Management Challenges

The time around calving is one of the most challenging periods in the life cycle of a dairy cow. Nutrient demands increase and dramatic changes in the immune, endocrine and digestive
systems occur to accommodate fetal growth in late pregnancy and support the onset of lactogenesis (Bauman and Currie, 1980). Regulating mechanisms may be unable to adequately compensate to ensure optimal functioning, resulting in physiologic imbalance and in many cases disease (Ingvartsen, 2006). In addition to physiologic stressors, transition cows are exposed to several environmental challenges such as adapting to housing and milking parlor facilities (Cook and Norland, 2004, von Keyserlingk and Weary, 2007), feeding management systems (DeVries et al., 2005, von Keyserlingk et al., 2010), and frequent regrouping and new social structures (von Keyserlingk et al., 2008) that also likely contribute to the high incidence of disease observed during the weeks following calving (Ingvartsen 2006).

The “transition period”, which is typically defined as the period from 3 weeks before to 3 weeks after calving (Grummer, 1995), is associated with serious welfare implications for dairy cows. Previous work has shown that 75% of all health disorders occur during the transition period. Furthermore, 30 to 50% of dairy cows are affected by some form of metabolic or infectious disease during this time (LeBlanc et al., 2006). More than half of all culling events are associated with health disorders occurring within the first 60 DIM regardless of parity or production level (Beaudeau et al., 1993, Grohn et al., 2003). Despite attempts to genetically select conformational traits to improve longevity in dairy cows, 35 to 40% of cows within the herd are culled each year in Canada, resulting in an average lifespan of 5 to 6 years (CDIC, 2012). This lifespan is relatively short compared to a potentially 12 to 15 year natural lifespan of cattle. Clearly, research on the period around calving is important to cow welfare.

All three components of welfare outlined by Fraser et al. (1997) may be compromised during the time around calving. Maladies including infectious diseases (i.e. metritis, mastitis), metabolic diseases (i.e. ketosis, milk fever) and other disorders such as displaced abomasum and
retained placenta (Mulligan and Doherty, 2008) all compromise health and biological functioning (milk production). Transition cows also endure painful procedures such as caesarean section and dystocia correction and diseases such as mastitis and lameness are likely associated with negative affective states (Huxley and Whay, 2006). Furthermore, farm management practices or calving pen design may prevent transition cows from performing natural maternal behaviours associated with parturition (e.g. Proudfoot et al., 2014).

Although milk cow numbers in Canada have declined by 60% since the 1970’s (2.5 million cows in 1970 to under 1 million today), overall milk production (78,923,012 kg) has remained stable during the same time period indicating improvement in per cow milk production (Statistics of Canada, 2012, CDIC, 2013). The intense genetic selection for increased milk production in dairy cows has been paralleled by high nutritional and metabolic demands on the cow (Mulligan and Doherty, 2008, Zwald et al., 2004). All cows at this stage of lactation must rely to some degree on body reserves to meet the demands of lactation; however, prolonged negative energy balance is associated with increasing incidence of metabolic disorders (Ingvartsen, 2006) decreased milk production (Duffield et al., 2009, Ospina et al., 2010b), compromised reproductive performance (Walsh et al., 2007, Ospina et al., 2010b, McArt et al., 2013) and higher risk of culling (McArt et al., 2013, Seifi et al., 2011). Given these negative consequences associated with prolonged or excessive negative energy balance, there is much interest in determining effective ways in identifying cows at risk for ketosis.

1.2.2 Ketosis

The incidence of ketosis has been estimated to range from 26-60% for subclinical ketosis (Duffield et al., 1998, Simensen et al., 1990, McArt et al., 2012) and 2-20% for clinical ketosis.
Ketosis is a costly disease that negatively impacts well-being, productivity and reproductive performance (see review by Bobe et al., 2004). Costs associated with diagnosing and treating clinical ketosis are estimated to be $145/case (Guard, 1994) and estimated to cost the US dairy industry over $60 million per year (Bobe et al., 2004).

Despite the negative energy balance cows experience in early lactation, milk production often remains high, indicating that the nutritional requirements are being met through energy sources other than feed (Grummer, 1995; Overton et al., 2004). Ketosis is closely associated with fatty liver or hepatic lipidosis that occurs when the uptake of lipids exceeds the oxidation and secretion of lipids by the liver (Bobe et al., 2004). Excess lipids stored as triacylglycerol accumulate in the liver resulting in metabolic dysfunction (Grummer, 1993) and accumulation of non-esterified fatty acids (NEFA) and ketones [acetoacetate, acetone and beta-hydroxybutyrate (BHBA)] in the blood. Although there is a continued debate about what level of ketones indicates a risk to normal biological functioning (Duffield, 2000), it is now generally accepted that ketosis refers to the overproduction of ketones often in association with other conditions or health parameters (serum calcium metabolites; Seifi et al., 2011, milk production and health; Duffield et al., 2009, Ospina et al., 2010, reproductive performance; Walsh et al., 2007, Ospina et al., 2010, McArt et al., 2013, displaced abomasum; McArt et al., 2012, LeBlanc et al., 2005, Seifi et al., 2011).

Negative energy balance can be assessed by quantifying the concentration of NEFA in the blood of cows in the weeks before and after calving and the concentration of BHBA in the blood, milk or urine of cows after calving (5 to 50 DIM). Despite the availability of affordable cow side tests such as the Precision Xtra TM Blood Glucose and Ketone Monitoring System (validated by Iwerson et al., 2009), individual animal testing on a herd-wide scale can be time
consuming and expensive. Many veterinarians and producers implement cut-points for monitoring herd-level prevalence of ketosis, for example, randomly selecting 12 cows in the first 60 days of lactation (see Oetzel, 2004); if >10% are subclinically ketotic, then the herd is considered at high risk and nutritional management is reviewed. Although known calving period risk factors have been established, including precalving elevated NEFA, advanced parity and increased body condition score (McArt et al., 2013), the lack of information at the level of the individual cow likely results in many animals with subclinical or clinical ketosis being undiagnosed. Clearly, ketosis is an important under diagnosed disease on dairy farms with serious economic and welfare implications.

Although clinical signs associated with ketosis such as lethargy, decreased milk production, dry feces or empty rumen palpation have been used to diagnose individual animals, the reliance on these signs is also challenging given that many are nonspecific and difficult to detect. For instance, lethargy and anorexia associated with disease are common to many infectious and metabolic disease processes.

Behavioural changes associated with infectious disease typically divert energy towards an immune mediated response (Hart, 1988), but a metabolic disease such as ketosis dampens the immune system (Bauman and Currie, 1980). Immune responses require resources such as energy, nutrients and time and these are often inversely related to reproductive effort (Adelman and Martin, 2013). In late gestation cows, adaptive immune responses are dampened and metabolic processes are stressed to preserve the pregnancy and milk production (Bauman and Currie, 1980). Ketosis negatively impacts the immune system through several pathways: impaired leukocyte response to chemical factors released by microorganisms (Suriyasathaporn et al., 1999), a decrease in specific cytokines including interferon and tumor necrosis factor
(Zdzisinska et al., 2000) and depressed lymphocyte production (Sato et al., 1995). High concentrations of BHBA combined with immuno-suppression associated with hormonal changes around the time of calving likely accounts for the increased risk of concurrent disease processes such as displaced abomasum or metritis (Le Blanc et al., 2006, McArt et al., 2012, Garro et al., 2013).

Transition cow research has focused on nutrition (Overton et al., 2004), physiology and metabolism (Invartsen, 2006) and minimizing negative energy balance (Grummer, 1995) to minimize the incidence of disease (Mulligan and Doherty, 2008, Overton et al., 2004). For example, feeding a ration specifically formulated for the cows prior to calving has been shown to reduce culling (Bach et al., 2008) and hypocalcemia postpartum (Lean et al., 2006). Despite the known relationship between nutrition and disease, the incidence of most transition cow diseases continues to rise (Mulligan and Doherty, 2008). Much research has focused on increasing DMI (Grummer et al., 2004, Grant and Albright, 1995), varying energy density in the ration (see Eastridge, 2006, Rabelo et al., 2003) or modifying the amount of dietary forage (Vickers et al., 2013) to improve lactational performance and prevent disease. Only recently has research attempted to use behavioural indicators to identify, predict and assess health problems in dairy cows.

1.3 BEHAVIOUR AND DISEASE

1.3.1 Sickness Behaviour

The most common behavioural patterns associated with disease include lethargy, depression, decreased motivation to groom and anorexia (Hart, 1988). Sickness behaviour has been described as a highly organized, adaptive behavioural strategy that diverts the animal’s resources to fighting the invading pathogen or disease process that is critical for survival (Hart,
Physiological responses such as vasoconstriction conserve heat and promote thermoregulation, while behavioural responses such as increased rest conserve energy for mounting a febrile immune response (Hart, 1988). Cytokines act as signaling molecules responsible for a cascade of immune system mediators that produce the clinical signs of disease such as general malaise and declines in feeding behaviours. Sickness behaviour not only allows us to recognize animals when they are ill but a growing body of evidence now indicates the potential for recognizing behavioural changes that precede clinical disease (Weary et al., 2009).

1.3.2 Feeding Behaviour and Disease

Research with steers was the first to show that changes in feeding behaviour can be used to predict morbidity in cattle (Sowell et al., 1998, 1999). These authors showed that sick steers in a commercial feedlot spent less time at the feed bunk and had fewer feeding bouts than healthy steers. Furthermore, Quimby et al. (2001) showed that electronic monitoring of bunk attendance to measure reduced feeding behaviour can be used to detect illness in feedlot steers 4 days earlier than pen riders. Urton et al. (2005) was the first to describe a similar relationship between feeding time and metritis, a common disease in transition cows.

A deviation from normal feeding behaviour before calving can be used to predict disease postpartum. Huzzey et al. (2007) reported that for every 10-minute reduction in daily feeding time in pre-partum cows, the risk of developing acute metritis postpartum doubles. Furthermore, these authors showed that changes in feeding behaviour could be used to identify cows up to 3 weeks before metritis was diagnosed. Goldhawk et al. (2009) found a similar relationship between lower pre-partum DMI and increased risk for subclinical ketosis.
The relationship between reduced DMI and disease is now well established (Hammon et al., 2006, Huzzey et al., 2007, Goldhawk et al., 2009). Changes in intake may be due to competition and social behaviour around the time of feeding (Huzzey et al., 2007, Goldhawk et al., 2009, Proudfoot et al., 2010). Intensively housed dairy cows display diurnal feeding patterns and tend to consume the majority of their food during the day (DeVries et al., 2003). Motivation to feed is greatest immediately following the delivery of fresh feed (DeVries et al., 2005) and competition between lactating cows is greatest at this time emphasizing the importance of adequate bunk space (DeVries et al., 2004). By increasing feeding space and decreasing competition, subordinate cows are better able to engage in feeding activity throughout the day including during the hour and a half after fresh feed delivery (DeVries et al., 2004). When feeding space becomes limited the number of displacements at the feed bins increases regardless of parity or social status (Proudfoot et al., 2009), emphasizing the importance of management strategies that prevent overstocking. Increased stocking density and increased competition decrease feeding time, and increase the time subordinate cows stand waiting to gain access to the feed bunk (Huzzey et al., 2006).

Diurnal feeding patterns have been shown to vary between metritic and healthy cows especially during times of highest bunk attendance (Huzzey et al., 2007). This change in behaviour was likely influenced by socially subordinate behaviour as cows that developed disease engaged in fewer aggressive social interactions at the feedbunk during the week prior to calving (Huzzey et al., 2007, Goldhawk et al., 2009). Proudfoot et al. (2010) also showed that social behaviour during the transition period plays a role in susceptibility to lameness and the cows that later became lame consumed feed more rapidly especially around peak feeding times than cows that remained healthy.
1.3.3 Standing Behaviour and Disease

Cows appear to vary little in the amount of time spent standing before and after calving, standing approximately 13 h per day (Huzzey et al., 2005). However, there are differences in the number of standing bouts on the day of calving (Huzzey et al., 2005). The increased number of bouts may be associated with pain during calving, as the increase is more pronounced in the case of dystocia (Proudfoot et al., 2009). Differences in standing behaviour have also been shown in metabolic disease and lameness. In addition to changes in feeding behaviour, Proudfoot et al. (2010) showed that cows that developed claw horn lesions in mid lactation had increased standing times and increased the time spent perching (2 front feet in the stall) in the 2 weeks before calving. Jawor et al. (2012) showed that cows with subclinical hypocalcemia spent, on average, 2.5 h more time standing suggesting that this measure could be used to identify cows at risk for disease.

Deviation in standing times could suggest a problem with the cow’s environment, including social competition resulting in increased wait times for access to feed (Huzzey et al., 2007, Goldhawk et al., 2009), or poor stall design (Tucker and Weary, 2004; Fregonesi et al., 2009) or stall maintenance (Drissler et al., 2007). Collectively this body of research indicates that variation in standing behaviour may be related to pain (Proudfoot et al., 2009), sickness behaviour (hypocalcemia, Jawor et al., 2012) and increased risk for lameness (Proudfoot et al., 2010).

1.4 OBJECTIVE

A better understanding of subclinical sickness behaviour in cattle shows promise for the early detection of illness. Recent research has shown that monitoring feeding behaviour changes
through an automated system shows promise in providing information for early detection of transition cow diseases such as metritis and ketosis (Gonzalez et al., 2008), but monitoring of individual feeding behaviour on commercial farms is expensive and impractical. Technologies such as electronic data loggers are far more practical and can accurately monitor standing behaviour of cows on commercial farms (e.g. O’Driscoll et al., 2008; Ledgerwood et al., 2010), but to date there has been little work investigating whether this technology can be used to identify cows at risk for illness. The objective of this study was to describe the standing behaviours of cows with and without ketosis to assess the potential of using changes in standing behaviour for the early detection of ketosis in dairy cattle.
2. CLINICAL KETOSIS AND STANDING BEHAVIOUR IN TRANSITION COWS

2.1 Introduction

The transition from late gestation to early lactation has been associated with a high incidence of production diseases (Mulligan and Doherty, 2008) often related to the cows inability to overcome negative energy balance. Cows compensate for rapid fetal growth in the final weeks of gestation and the onset of lactogenesis by mobilizing fat stores. All cows at this stage of lactation must rely to some degree on body reserves to meet the demands of lactation but prolonged negative energy balance is often associated with ketosis (Ingvarsten, 2006). A poor adaptive response to negative energy balance and rapid lipolysis results in hyperketonemia defined by overproduction of non-esterified fatty acids (NEFA) and inadequate hepatic metabolism resulting in accumulation of ketone bodies (Grummer, 1993, Drackley, 1999, Andersson, 1988). Despite the benefits of NEFA analysis for identifying prepartum cows at risk for ketosis (e.g. Ospina et al., 2010a, Seifi et al., 2011), there is no cow-side test available for this metabolite, therefore limiting its’ practical usefulness. Beta-hydroxybutyrate (BHBA) is the predominate ketone produced by ruminants, and can be readily identified by an inexpensive cow-side test that facilitates the identification of cows at risk for ketosis (Iwerson et al, 2009). However, BHBA analysis is not routinely incorporated into herd health management practices and the early detection of ketosis remains a challenge.

Cows with high plasma concentrations of BHBA (≥1.2 mmol/L) in the first week after calving are at increased risk of disease (Duffield et al., 2009, Ospina et al., 2010a, Seifi et al., 2011), and are more likely to be removed from the herd (McArt et al., 2012), less likely to conceive at first service (McArt et al., 2012, Ospina et al., 2010b), and have lower milk production (McArt et al., 2012, Ospina et al., 2010b). The incidence of ketosis has been
estimated to range from 26-60% for subclinical ketosis (Duffield et al., 1998, Simensen et al., 1990, McArt et al., 2012) and 2-15% for clinical ketosis (Duffield, 2000).

Recent work has demonstrated that changes in feeding and social behaviour can be used for the early identification of cows at risk for ketosis. Goldhawk et al. (2009) reported that ketotic cows spent less time at the feed bunk and visited the feeder less often during the week before calving; for every 1 kg decrease in DMI cows were 2.2 times more likely to develop ketosis. These authors also found that cows that developed subclinical ketosis after calving had fewer displacements at the feed bunk during peak feeding periods (Goldhawk et al. 2009). Although these behaviours show promise in identifying cows at risk for disease to date there are no technologies available that allow for the practical monitoring of individual feeding and social behaviour on commercial farms. Edwards and Tozer (2004) were the first to investigate the use of pedometers for the early detection of illness showing lower walking activity in ketotic cows on the day of calving. Electronic data loggers can be used to accurately and inexpensively monitor standing and lying behaviour of cows on farms (e.g. O’Driscoll et al., 2008; Ledgerwood et al., 2010), but no researchers have attempted to determine whether standing behaviour could be used to predict ketosis in dairy cattle. Therefore, the objective of this study was to describe the standing behaviour of dairy cows with and without ketosis during the period around calving to determine whether changes in this behaviour precede clinical signs of disease.

2.2 Materials and Methods

Farm Selection, Animals, Housing and Diet

This study was conducted on 2 commercial dairy farms in Washington State (Farms A and B; both assessed between May 2012 to September 2012). In addition, we performed
A retrospective analysis on data from the University of British Columbia Dairy Education and Research Center (Farm UBC) collected as part of another study (Vickers et al., 2013) from July 2009 to March 2010. Climatic conditions averaged from 10.5 to 21° C with an average total rainfall of 2.1 mm/d during the test period. All animal use was approved by the University of British Columbia’s Animal Care Committee, according to the guidelines outlined by the Canadian Council of Animal Care (CCAC 2009).

**Farm A.** A total of 120 Holstein dairy cows (38 primiparous and 67 multiparous cows) were monitored beginning 1 d before calving until 3 wk after calving. During the prepartum period cows were housed in a single group pen fitted with 34 headlocks (72 cm center to center) and 8.8 m post-and-rail, 1.5 m of linear water space, and a 236 m² dry-manure deep-bedded pack, bedded with approximately 10.6 ± 3.9 cm (mean ± SD) layer of sawdust twice per week. Cows were also provided free access to a dirt exercise lot located adjacent to the pen. Stocking ranged from 27-59 cows per group (40 ± 10 cows; mean ± SD), which equated to stocking rate of 60 - 131% at the feed bunk and 4 to 8.7 m²/cow on the pack. When signs of imminent calving were observed cows were moved to a deep straw bedded freshening pen where they calved and remained for the first 24 h post-partum. Cows were then moved to the postpartum pen where they remained until they were 16 to 21 DIM. The pen contained 31 headlocks (72 cm center to center) and 11 m of post-and-rail, 3.8 m of linear water space, and 37 stalls (1.2 x 2.5m) with a deep bedded dry manure base topped with 10 ± 2.3 cm (mean ± SD) layer of sawdust added twice per week and groomed daily; these cows also had free access to a pasture 24 h a day. Stocking ranged from 31-45 cows per group (36 ± 5; mean ± S.D.), which equated to an average stocking rate of 68-100% at the feed bunk and 100-145% in the freestalls. Cows were removed
from the pen for approximately 1 h for milking 3 times per d at approximately 1000, 1800 and 0200 h. Fresh TMR was fed once per d at approximately 1000 h to the fresh cows and 1100 h to the close up cows and feed was pushed closer to the cows 3 to 4 times per d. Three weeks prior to calving cows were fed a TMR consisting of 86 % forage (corn silage, alfalfa hay, straw) and 16 % concentrate mix with a standard vitamin and mineral pack. Lactating cows were fed a TMR consisting of approximately 70 % forage (grass silage, corn silage, alfalfa) and 30 % concentrate (corn/barley, distillers, canola, whey, bakery and molasses) with a standard vitamin and mineral pack.

**Farm B.** A total of 184 Holstein dairy cows (62 primiparous and 122 multiparous cows) were monitored beginning approximately 7 d before calving until 21 d after calving. Cows were housed in 3 separate identical prepartum pens, each equipped with 118 headlocks (76 cm center to center), 75 m of feed bunk space, 9 m of linear water space, and 100 stalls (1.3 x 2.4 m) bedded with an average of 4.6 ± 2.5 cm (mean ± SD) layer of sawdust over a tire and concrete base once per week. Stocking ranged from 50-70 cows per pen (61 ± 10; mean ± SD), which equated to an average stocking rate of 42-59 % at the feed bunk and 50-70 % in the freestalls. Animals were moved to a freshening pen bedded with deep straw when signs of imminent calving were visible and remained there for the first 24 h post-partum. After calving all cows were moved to a single postpartum pen that contained 180 headlocks (76 cm center to center), 6.8 m of post-and-rail with hay access, 5 m of linear water space, and 173 stalls (1.2 x 2.2 m) bedded with an average of 8.9 ± 2.5 cm (mean ± SD) of sawdust over a tire and concrete base twice per week. The stocking rate ranged from 136-163 cows per group (146 ± 8 cows; (mean ± SD)), which equated to an average stocking rate of 75-90 % at the feed bunk and 78-94 % in the
freestalls. Cows remained in the postpartum pen until they were approximately 21 to 25 DIM. Cows were removed from the pen for approximately 2 h for milking 3 times per d at approximately 0700, 1400 and 2100 h and fed a TMR once per d at approximately 0700 h with 5-6 push-ups per d. Three weeks prior to calving cows were fed a TMR consisting of 70 % forage (corn silage, alfalfa hay, oat hay, straw) and 30 % concentrate mix (canola, distillers, ground corn) with a standard vitamin and mineral pack. Lactating cows were fed a TMR consisting of approximately 50 % forage (corn silage, alfalfa hay, and oat hay) and 50 % concentrate (canola, cotton seed, distillers, and ground corn) with a standard vitamin and mineral pack and additional niacin, choline, yeast and sodium bicarbonate.

**Farm UBC.** A total of 91 multiparous Holstein cows were monitored beginning approximately 14 d before calving until 14 d after calving. During the prepartum period cows were housed in a freestall pen equipped with 12 Insentec feed bins and 2 Insentec water troughs (see Chapinal et al., 2007 for description), and 24 stalls fitted with a mattress (Pasture Mat, Promat Inc. Woodstock, Ontario, Canada) covered with approximately 5 cm of sand bedding. Stocking remained constant at 12 cows, which equated to a 100 % stocking rate at the feed bunk and 50 % in the freestalls. Animals were moved to a sawdust bedded freshening pen when signs of imminent calving were identified and remained there for the first 24 h post-partum. After calving all cows were moved to a single postpartum pen that contained 12 stalls identical to those described above, including 6 electronic feed bins and a single electronic water trough. Stocking rate remained constant at 12 cows per group equating to a 200 % stocking rate at the feed bunk and 100 % in the freestalls. Cows remained in the postpartum pen until they were approximately 15 DIM. Cows in the postpartum pen were milked twice a day at approximately 0630 and 1700 h.
and were away from the pen for approximately 30 minutes at each milking. Cows in the postpartum group were fed a TMR twice per d at approximately 0700 and 1600h. Three weeks prior to calving cows were fed a TMR consisting of 77 % forage (corn silage, alfalfa hay) and 23 % concentrate mix (rolled barley, distillers corn wheat, soybean-based supplement, molasses) with a standard vitamin and mineral pack. Lactating cows were fed a TMR consisting of approximately 50 % forage (corn silage, alfalfa hay, and grass silage) and 50 % concentrate (barley, soybean meal, canola meal, distillers, rolled corn, soybean bypass protein supplements, molasses) with a standard vitamin and mineral pack and additional yeast, methionine, and sodium bicarbonate.

*Standing Behaviour Data Collection*

Each cow enrolled in the study was fitted with a data logger within a week of her expected calving date (d -7 to d -3) on Farm B and Farm UBC. Data loggers were not attached to cows on Farm A until the day of calving (d 0). Standing behaviour was recorded using a data logger (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA) as described by Ito et al. (2009) and validated by Ledgerwood et al. (2010). The logger was wrapped in foam padding and attached to the lateral aspect of the distal hind cannon proximal to the fetlock with elastic wrap (Co-Flex, Andover Coated Products Inc., Salisbury, MA) and oriented so that the x-axis was parallel, the y-axis was perpendicular, and the z-axes pointed away from the sagittal plane but was parallel to the ground. At 1-min intervals, the logger recorded position relative to the axes orientation; this information was used to determine standing and lying times, number of bouts and the duration of each bout. After calving the
loggers were removed and replaced between d 0 and d 3 after calving and then every 10 d thereafter until 21 DIM.

**Determination of Ketosis Status and Cow Participation in Study**

Blood samples were obtained from cows twice per week (Monday and Thursday) following the morning milking. Testing started at 2 DIM and occurred at 3 to 4 d intervals until 21 DIM. The biweekly testing scheme was chosen based on the results of a recent epidemiology paper on subclinical ketosis that reported the first confirmed positive BHBA test between 3 to 5 DIM and the median time to subclinical ketosis resolution as 5 d (McArt et al., 2012). It is recognized that cases lasting 2 to 3 d that did not coincide with a day of testing would have been missed and underestimated ketosis prevalence.

Blood was collected from the coccygeal vessel using a 1-mL tuberculin syringe and 27-gauge, 2.54-cm needle within 3 h of the morning feeding as cows returned from milking. Approximately 0.25 ml of blood was collected and then immediately tested for BHBA concentration using the Precision Xtra meter (Abbott Laboratories, Alameda, CA; Iwersen et al., 2009). Additional detailed blood collection, calibration and testing information associated with this procedure can be found in McArt et al. (2011).

Ketosis was diagnosed by the research team based strictly on BHBA values. Cows with BHBA < 1.2 mmol/L throughout the entire 3 wk data collection period were considered non-ketotic. Cows with at least 3 consecutive samples of BHBA ≥ 1.2 and ≤ 2.9 mmol/L were considered subclinically ketotic. Cows with at least 3 consecutive samples of BHBA ≥ 1.2 mmol/L and at least one > 2.9 mmol/L were considered clinically ketotic. On all farms cows diagnosed with clinical ketosis were drenched orally with 240 ml of propylene glycol (VetOne,
distributed by MWI Veterinary Supply, Meridian, Idaho). The subclinically ketotic cows were rarely treated but occasionally would be administered propylene glycol based on the discretion of farm personnel. In addition, clinical ketosis cows were administered 1000 ml of dextrose 50% (VetOne, MWI Veterinary Supply, Meridian, Idaho) intravenously on the day of diagnosis by farm personnel; this treatment was only repeated if the cow tested positive on the following test day. Farm UBC had no clinically ketotic cows and treatment was rare.

**Data Analyses**

All statistical analyses were performed with SAS (Version 9.3) using cow (n = 78) as the experimental unit. Five experimental periods were defined for analysis: wk -1 (d-7 to d-1 before calving), d 0 (day of calving), wk +1 (d 1 to 7 after calving), wk +2 (d 8 to 14), and wk +3 (d 15 to 21). With the exception of d 0, at least 3 complete days of data were required within each defined experimental period to generate mean value for the period. Non-ketotic cows (n = 39) were matched with subclinical (n = 19) and clinical ketotic cows (n = 20) by farm and when possible by parity for the data analysis. Differences in standing time, standing bouts, and standing bout duration between health categories were analyzed using a mixed model which included the fixed effects of farm (A, B, and UBC), parity (primiparous or multiparous), period (wk -1, d 0, wk +1, wk +2, wk +3), health status (no-ketosis, subclinical ketosis, clinical ketosis), and the period x health status interaction. Period was considered a repeated measure and cow a random effect. An autoregressive covariance structure was used for these models based on best fit using the Bayesian information criteria in the mixed procedure of SAS (SAS Institute, 1999). When a period by health status interaction was detected ($P < 0.1$) the data were stratified by period. Differences in standing time, standing bouts, and standing bout duration between non-
ketotic and clinically ketotic cows, and between non-ketotic and subclinically ketotic cows, were analyzed by period using the contrast statement in a mixed model that included the fixed effects of farm, parity, and health status. A logarithmic transformation was used to normalize standing bout duration; for this variable least squared means and confidence intervals were back-transformed to obtain the geometric mean and 95% confidence interval on the original scale.

To further explore the effect of time of day on standing behaviour in ketotic and non-ketotic cows, average hourly standing time was determined based on data collected during the week before (wk – 1) and the week after calving (wk +1). Since the 24-h pattern of daily activity is known to be influenced by farm factors (i.e. timing and frequency of feed delivery; DeVries et al., 2005) this analysis was restricted to cows from Farm B, and utilized a subset of non-ketotic (n =15) and clinical ketosis cows (n=15), that were match paired by parity (average parity 2.6 ± 1.4 vs. 2.4 ± 1.0, respectively). Farm B was chosen due the high prevalence of clinical ketosis on that farm and the most complete and continuous standing logger data for the analysis.

Differences among health categories in the distribution of 24-h standing times were analyzed by period with a mixed model that used a heterogeneous autoregressive covariance structure. Cow was treated as a random effect and hour as a repeated measure. The model tested fixed effects of parity, hour, health status, and the health status by hour interaction.

2.3 Results

Based on the strict definition of ketosis used in this study, Farm A had 6 cases of subclinical ketosis and 5 cases of clinical ketosis, Farm B had 4 cases of subclinical ketosis and 15 cases of clinical ketosis and Farm UBC had 9 cases of subclinical ketosis cows and no cases of clinical ketosis, resulting in a total of 19 subclinical ketosis and 20 clinical ketosis cows.
Across all 3 farms, the average parity for each category was: non-ketotic 2.1 ± 1.3 (mean ± SD), subclinical ketosis 2.7 ± 1.0 and clinical ketosis 2.8 ± 1.3. There was no difference in day of diagnosis between the 2 illness categories with diagnosis taking place on average (± S.D.) at 4.5 ± 2.1 DIM.

Taking into consideration only the cows enrolled in the study on each of the 3 farms, the prevalence of subclinical ketosis and clinical ketosis, respectively, was 26 % and 22 % (Farm A), 44 % and 12 % (Farm B), and 33 % and 0 % (Farm UBC). When determining ketosis prevalence based on a single positive sample over a test period (described by Duffield et al., 1998; 1999) to identify ketosis [subclinical ketosis, BHBA ≥ 1.2 and ≤ 2.9; clinical ketosis, BHBA > 2.9 mmol/L (Andersson, 1988, Oetzel, 2004)], the overall herd prevalence was 39 % and 6 %, 44 % and 12 %, and 31 % and 0 % on Farm A, B, and UBC, respectively. Of the ketotic cows, 42 % of the subclinically ketotic and 15% of the clinically ketotic cows had BHBA < 1.2 mmol/L by the end of the test period. Of the cows that recovered from ketosis, the average days from first positive test until a BHBA test < 1.2 mmol/L was 9.3 ± 5.2 d for subclinical ketosis and 9.0 ± 4.4 d for clinical ketosis.

**Standing time.** During wk -1, d 0, and wk +3 there was no effect of farm on standing time (P ≥ 0.21); however during wk +1 and +2 standing time did differ between farms (wk +1, Farm A: 13.9 ± 0.4, B: 15.1 ± 0.4, UBC: 12.6 ± 0.6 h/d, P = 0.001; wk +2, Farm A: 14.9 ± 0.5, B: 14.8 ± 0.5, UBC: 13.2 ± 0.6, P = 0.07). Cows with clinical ketosis stood 2.2 h/d longer than non-ketotic cows the week before calving (14.4 ± 0.8 h/d vs. 12.2 ± 0.7 h/d, P < 0.03; Figure 1A) and 4.2 h/d longer on d 0 (17.6 ± 1.0 h/d vs. 13.4 ± 0.7 h/d, P < 0.001; Figure 1A). There were no differences in standing time between clinically ketotic cows and non-ketotic cows in all other periods (wk +1, P = 0.13; wk +2, P = 0.14; wk +3, P = 0.16). There was no difference in
standing time between subclinically ketotic cows and non-ketotic cows during any period ($P \geq 0.26$).

**Standing bouts.** There was no farm effect on standing bouts during any period ($P \geq 0.24$). On d 0 cows that were diagnosed with clinical ketosis had fewer standing bouts than their non-ketotic counterparts ($12.8 \pm 2.2$ vs. $18.1 \pm 1.6$ bouts/d, $P < 0.05$; Figure 1B). However, the number of standing bouts between these health categories did not differ during the other periods around calving ($P \geq 0.20$). There was no difference in the number of standing bouts between subclinically ketotic cows and non-ketotic cows at any period relative to calving ($P \geq 0.32$).

**Standing bout duration.** During wk +1 there was an effect of farm on standing bout duration whereby Farm B had longer bout duration than Farm A and Farm UBC (wk +1, Farm A: $94.9$ min/b (CI: 76.6 to 117.4), B: $99.7$ min/b (CI: 82.1 to 117.8), UBC: $67.3$ (51.3 to 82.2), $P < 0.05$); but there were no differences between farms during any other period ($P \geq 0.33$). Standing bout duration was $62\%$ longer for clinically ketotic cows on d 0 compared to non-ketotic cows ($64.9$ min/b (CI: 55.6 to 75.9) vs. $40.4$ min/b (CI: 36.4 to 44.8), $P=0.003$; Figure 2). However, the standing bout duration between other health categories did not differ for any other period for clinically ketotic cows ($P \geq 0.11$) or during any period for subclinically ketotic cows ($P \geq 0.25$).

**Hourly standing time.** The 24-h prepartum analysis (wk -1) revealed an effect of health status ($P = 0.002$) whereby clinically ketotic cows had longer average hourly standing times than non-ketotic cows ($36.1 \pm 1.3$ min vs. $30.4 \pm 1.5$ min). However, no standing time by hour interaction was detected ($P = 0.20$), indicating that across all hours of the day ketotic cows stood for longer compared to non-ketotic cows. During the 24-h postpartum analysis (wk +1) there was no effect of health status ($P = 0.52$). However, a health status by hour interaction was noted ($P =$
suggesting that standing time differed between health categories at certain periods of the day, in particular the evening hours between 1700 and 2100 h (Figure 3).

2.4 Discussion

Although the majority of studies have used a single measure of BHBA to evaluate ketosis (Goldhawk et al., 2009; Seifi et al., 2011, McArt et al., 2012), others have incorporated factors such as data distribution (Neilen et al., 1994), serum calcium metabolites (Seifi et al., 2011), milk production and health (Duffield et al., 2009, Ospina et al. 2010b), and reproductive performance (Walsh et al, 2007, Ospina et al., 2010b, McArt et al., 2013) in their assessment. Previous research has shown a relationship between a single value above a predetermined concentration of BHBA and increased risk of negative outcomes. The purpose of the current study was not to determine the optimum threshold for ketosis diagnosis, but rather to describe changes in standing behaviour between cows that were ketotic and cows that were not. A conservative case definition (3 consecutive positive ketosis diagnoses) was chosen to increase our confidence that cows were indeed ketotic. This definition eliminated cows that spontaneously recovered after a single ketosis event. Future work may wish to also consider other case definitions of ketosis. Cows eligible for analysis required both 3 consecutive positive ketosis diagnoses (BHBA testing interval every 3 to 4 d) and a minimum of 3 continuous days of prepartum and 10 continuous days of postpartum standing data to be enrolled. This conservative approach greatly reduced the number of cows available from each farm for the final analyses and thus sample size was improved by combining the 3 farms. Future work should look at more conservative definitions of ketosis to identify predictive thresholds that are practical, but this will require more statistical power than what we were able to achieve in the present study.
To our knowledge this is the first paper to describe the standing behaviour of cows afflicted with subclinical or clinical ketosis. Our results show that differences in standing behaviour before calving can be useful for the early detection of clinical ketosis, but not subclinical ketosis. Thus, standing behaviour is likely not a sensitive indicator of subclinical disease.

**Prepartum Standing Behaviour**

Variation in standing times by clinically ketotic cows in the first wk before calving may be a consequence of differences in social rank or competition. Previous work has shown that subordinate cows are more likely to engage in avoidance behaviour in response to social confrontations (Huzzey et al., 2006; Proudfoot et al., 2009a; Goldhawk et al., 2009). This may have profound effects when cows are housed in freestall barns where they are usually required to compete for access to resources (Val-Lailet et al., 2008). Cows that are provided inadequate bunk space have been shown to exhibit increased standing times likely due to waiting to access feed (Huzzey et al., 2006). Cows in this study were not consistently overcrowded on Farm B and Farm UBC in the freestalls. However, social status and avoidance behaviour may have influenced standing times on Farm A, and on Farm UBC given that the stocking rate exceeded 100% at the feedbunk (Fregonosi et al., 2007).

Cows that are unable to maintain sufficient DMI are at higher risk of impaired immune systems (Mallard et al., 1998) and increased susceptibility to disease (Huzzey, et al., 2007, Goldhawk et al., 2009). The presence of elevated circulating BHBA concentration in ketotic cows negatively impacts the immune system through several pathways (impaired leukocyte response; Suriyasathaporn et al., 1999, decreased cytokine activity; Zdzisinska et al., 2000,
lymphocyte suppression; Sato et al., 1995). BHBA ≥1.2 mmol/L combined with immuno-
suppression associated with calving likely accounts for the increased risk of concurrent disease
processes (metritis; Ospina et al., 2010, Garro et al 2013). The ketotic and non-ketotic
populations of cows enrolled in the current study were classified strictly based on BHBA levels
and animals in each group may have had other conditions. It is unknown if other subclinical
concurrent disease processes may have influenced the behaviour of clinically ketotic or non-
ketotic cows.

**Calving Day Standing Behaviour**

In addition to a 31% increase in standing times, cows that developed clinical ketosis in
the days following calving had fewer standing bouts and longer bout duration on d 0 compared
with their non-ketotic counterparts. Normal standing behaviour for cows around the time of
calving has previously been established (Huzzey et al., 2005; Jawor et al., 2012); identifying
variation from this baseline may predict cows at risk for disease. Previous work has shown that
on d 0 cows stand longer and increase the number of standing bouts (Huzzey et al., 2005). This
change in standing behaviour on d 0 may reflect pain associated with parturition (von
Keyserlingk and Weary, 2007) or lameness (Calderon and Cook, 2011). Moreover, cows with
dystocia have been shown to increase the number of standing bouts compared to eutocia cows
(Proudfoot et al., 2009b).

In the current study, on the day of calving clinical ketosis cows engaged in fewer
standing bouts than non-ketotic cows. According to Huzzey et al. (2005) normal variation in
standing day behaviour from the prepartum period to calving day included an increase in
standing time by approximately 1 hour and bouts increased by approximately 5.6 b/d, which is
very similar to the 1 hour difference relative to the period before calving and 5 bout increase observed in the present study. Interestingly, despite differences in how cows were managed on each farm, our results on standing behaviour are similar to Huzzey et al. (2005), which may indicate that changes in calving day standing time and bouts may be a consistent measure across farms. It is unlikely that the calving event itself differed from that experienced by cows on other farms, but rather this difference may suggest that ketotic cows were less able or motivated to change position. There is some energy cost associated with changing from the lying to standing position (see Susanbeth et al. 2004); cows at risk for ketosis may already be less willing to engage in any energetically expensive behaviour. Previous research shows that cows diagnosed with ketosis have lower walking activity during the first 14 DIM and in the days around disease diagnosis (Edwards and Tozer, 2004); these authors speculated that the difference was likely due to sick cows having a loss of appetite, spending less time at the feed bunk, and spending more time lying down. Previous work shows that ketosis is a progressive disease associated with gradual changes in NEFA and blood glucose starting in the prepartum period with cows initially becoming hypoglycemic which may present clinically as weakness and/or lethargy and later progressing to varying degrees of hyperketonemia (subclinical and clinical ketosis) (Bobe et al., 2004). The severity and display of sickness behaviour associated with ketosis likely depends on the degree and duration of reduced DMI associated with the disease. Goldhawk et al., (2009) reported that for every 1 kg decline in prepartum DMI the risk of developing ketosis postpartum doubles.

Although a study by Calderon and Cook (2011) showed an increased risk of subclinical ketosis in cows classified as moderate/severely lame in the prepartum period, no relationship between lameness and prepartum NEFA was identified which may suggest that excessive fat
mobilization was not occurring prior to calving. The authors suggest that the higher number of bouts on the day of calving was attributed to pain and not the disease conditions (Calderon and Cook, 2011). Similar to our results, the calving day behaviour was not different between cows with and without subclinical ketosis. Although there does not appear to be a difference with subclinical disease. We encourage further research that concurrently monitors prepartum physiological changes highly predictive of ketosis, as well as standing and social behaviour on and before the day of calving, to discern whether the clinically ketotic cows are in fact feeling the effects of the illness prior to the development of overt clinical signs.

**Postpartum Standing Behaviour**

During the 24-h postpartum analysis (wk +1) the effect of health status was not significant, however there was a health status by hour interaction suggesting that standing time differed between health categories at certain periods of the day. All three farms had similar management practices during the prepartum period but varied considerably in how they managed their groups of cows during the postpartum period. For instance, Farm B had the largest pen size and therefore cows likely spent more time waiting in a holding area to be milked. On Farm B cows were also routinely locked up during the morning for health exams. These factors may have contributed to the longer standing times observed on Farm B.

Fresh feed delivery is an important driver stimulating cattle to feed (DeVries et al., 2003). The hourly analysis showed the pattern of standing behaviour and its relationship to the time of fresh feed delivery. However, contrary to our prediction, cows with clinical ketosis appeared to engage in normal herd diurnal feeding patterns (DeVries et al., 2003). Our analysis showed that differences in standing times between non-ketotic and clinically ketotic cows did not coincide
with fresh feed delivery and only peaked between specific hours of the day (1700 to 2100) in the postpartum period. Specifically, the differences appeared 2 h after the second feeding when cows with ketosis spent less time standing compared to the healthy cows. These differences may be the result of farm management practices that likely influence and override natural standing patterns of all cows in the group (milking at 700, 1300, 2100; lock up for morning health exams at 700 to 900) and ultimately have impacted our results away from standing behaviour significance in the postpartum periods for clinically ketotic cows (wk +1, \( P = 0.13 \); wk +2, \( P = 0.14 \); wk +3, \( P = 0.16 \)). To our knowledge no known management factors influenced standing behaviour after the second milking (1700 to 2100); this may indicate that classic sickness behaviour (Hart, 1988) can be overcome by herd activities such as movement to the parlor and milking. However, when cows were uninhibited and allowed to choose between standing and lying, the ketotic cows chose to lie down perhaps indicative of lethargy and depression classically associated with ketosis. Research on cows in robotic milking systems may give some insight on their motivation to be milked as forced management likely influenced our results towards behavioural tendencies rather than significance in the post partum period.

The research described herein is the first to show the potential for employing activity meters for the early detection of ketosis. Similar to other work that showed lower walking activity in ketotic cows on the day of calving (Edwards and Tozer, 2004), this study identified differences in standing bouts and time on calving day that shows considerable promise to identify clinical ketosis.
2.5 Conclusion

Cows that developed clinical ketosis during the week after calving spent more time standing in the week prior to, and on d 0, and engaged in fewer standing bouts on d 0; cows with subclinical ketosis did not differ from non-ketotic animals. Given the current availability of practical and cost effective technologies for monitoring standing and lying activity in cattle, these results suggest that there may be an opportunity to use changes in standing behaviour to improve the early identification of ketosis in cows. Future studies that make use of larger sample sizes that are able to control for management differences are warranted to identify practical thresholds for the use of standing behaviour to predict ketosis.
2.6 Figures

Figure 1. Least squares mean (± SE) for A) standing time and B) number of standing bouts for Holstein dairy cows that were non-ketotic (♦, n = 39) subclinically ketotic (■, n = 19) and clinically ketotic (▲, n = 20) during the transition period. Subclinical cows were not significantly different than non-ketotic cows (SE bars were not included for clarity), however averages for this group are provided in the figure for a reference. Clinically ketotic cows were significantly different. *$P < 0.05$; **$P < 0.001$. 
Figure 2. Geometric mean for standing bout duration (± SE) for Holstein dairy cows that were non-ketotic (◇, n = 39) subclinically ketotic (■, n = 19) and clinically ketotic (▲, n = 20) during the transition period. Subclinical cows were not significantly different than non-ketotic cows (SE bars were not included for clarity), however averages for this group are provided in the figure for a reference. Clinically ketotic cows were significantly different. ** $P < 0.01$. 
Figure 3. Least square means (±SE) for standing time for Holstein dairy cows from Farm B that were non-ketotic (♦, n=15) or that had clinical ketosis (▲, n=15) during the periods around fresh feed delivery (↓, 0700) and feed push up (↑, 1400; 2100) during wk +1. Subclinical cows (n=19) were not significantly different than non-ketotic cows and were not included in this figure. * $P < 0.003$. 
3. GENERAL DISCUSSION

Production medicine focuses on the herd. This means that on many farms the individual animal is overlooked, and some sick cows are never diagnosed or receive treatment. Veterinarians have typically focused on nutritional and genetic advances to improve health on farms, but recent research demonstrates that behavioural assessments can also provide valuable insights, particularly in identifying cases before clinical signs are evident (Weary et al., 2009). Identifying cows at risk of disease rather than identifying cows after the onset of clinical disease is a transformational shift from the traditional veterinary approach.

3.1 Using automated sensor technology to identify illness

The growing availability of affordable sensor technologies (e.g. data loggers; Ledgerwood et al., 2010) for use on farms shows much promise (Rutten et al., 2013). Automated systems enable dairy producers to manage larger herds with lower labour costs by providing risk assessment information for individual animals that improve cow health management (Rutten et al., 2013). Favourable results presented in the scientific literature on the automated detection of feeding behaviour to identify cows at risk for infectious or metabolic disease (e.g. Huzzey et al., 2007; Gonzalez et al., 2008; Goldhawk et al., 2009) have received much interest but unfortunately broad uptake has been limited due to economic constraints associated with quantifying individual feeding behaviour on commercial farms (reviewed by Rutten et al., 2013). In contrast, the technologies available to objectively measure standing behaviour are practical and affordable. Although pedometer technology is typically used for heat detection on farms (Firk et al., 2002), one study investigated the use of pedometer technology to identify illness. Edwards and Tozer (2004) showed that sick dairy cows walked less than
healthy cows regardless of parity, season, and farm. Most of the work to date on the use of automated monitoring of standing behaviour has been focused on lameness detection (e.g. Ito et al., 2010). Calderon and Cook (2011) recently used activity meters to show the effect of lameness on standing behaviour during the transition period. Surprisingly, very few researchers have looked at the use of standing or activity behaviour to detect metabolic or infectious disease.

Traditionally, identification of cows with ketosis has made use of direct animal based measures that report raw sensor data to the producer (e.g. rumen pH, body temperature measured by a radiotelemetric rumen bolus) or indirect, nonspecific indicators (e.g. milk fat percentage, milk ketones measured by spectrophotometry). However, reliance on these types of factors has been cautioned as additional information to accurately predict ketosis is needed (de Roos et al., 2007), given the weak direct link between the sensor information and the specified health outcome (Rutten et al., 2013). Arguably, these limitations also apply to standing behaviour measures. However, unlike the sensors previously used to detect ketosis, activity monitors are inexpensive and widely used on farms (Rutten et al., 2013). Thus, there appears to be considerable merit to further investigate the use of this technology as an alternative method to identifying cows at risk for disease.

Research on commercial farms has several strengths and benefits. Farms vary greatly in their management, and even within a farm, management can vary day-to-day depending on weather, feed quality changes, regrouping demands, and changes in availability of bedding, feed and labour resources. In the current study it was impossible to achieve consistency between groups within and between farms. Farms varied in facility design, for example, stalls, bedding, stocking rates, pasture access and total mixed ration (TMR) formulations. Moreover, there were inconsistencies in milk testing practices, record systems, and health protocols. The limitation to
our analysis of clinically ketotic cows is that it is based on only a small number of cases from one farm only, and that to add external validity this analysis should be conducted on a larger sample size and on different farms with different management systems.

Although additional research may help to better understand the relationship between standing behaviour and ketosis, identifying a meaningful number of clinical ketosis cases to undertake this type of analysis remains a challenge. The incidence of clinical ketosis varies between farms (2-15%; Duffield, 2000, 0-12% in the current study). The production medicine approach to diagnosing ketosis on commercial dairy farms arguably results in gross under-diagnosis of ill cows. For example, farm records for 2013 for Farm A where routine BHBA testing was not incorporated for disease diagnosis, reported only 20 cases of clinical ketosis based on 750 calvings that year. On Farm A case diagnosis was limited to visual observations, concurrent disease, or identification by the herd veterinarian. In contrast, with biweekly testing during the 2 months of my study, the overall prevalence of clinical ketosis was determined to be 6%, double the prevalence reported from farm records (<3%). In the case of Farm B visual observations or concurrent disease or low milk production was used to select cows for BHBA testing. Although Farm B routinely implemented an aggressive weekly testing of 12-15 cows to determine herd based ketosis monitoring through herd cutoffs points (described by Oetzel, 2004) only 105 cases of clinical ketosis following 2041 calvings were reported in 2013. During the course of the current study, clinical ketosis prevalence was found to be 12%, but Farm B only reported 5% indicating that potentially 143 cases of ketosis were left undiagnosed in a single year. This demonstrates that even aggressive programs are grossly underestimating the true prevalence of clinical ketosis thus compromising individual cow welfare. Even though our sample size was small, the research presented in this thesis shows that activity meter technology
could potentially identify a significant number of clinically ketotic cows that would have previously gone undiagnosed. Although this technology is limited in its ability to detect subclinical disease, integrating activity meter technology has the potential to improve diagnosis of ketosis in the most severely affected cows on farms where this type of technology infrastructure already exists.

3.2 Using standing behaviour as an indicator of poor health

Decreases in DMI before calving have been shown to be a risk factor for postpartum disease; however, the mechanism is not well understood. It is unknown whether the disease is a result of decreased feeding behaviour or if there is another unknown confounding factor or subclinical disease condition that influences the abnormal behaviour (Proudfoot et al., 2010, Huzzey et al., 2007, Goldhawk et al., 2009). The work described by Edwards and Tozer (2004) reported that cows diagnosed with metabolic or gastrointestinal disease had higher activity than healthy cows 9 d prior to veterinary diagnosis, supporting the idea that the behaviour (increased walking activity) was a risk factor for disease similar to increasing standing behaviour in the current study. However, it remains unclear whether the increase in standing or walking behaviour was related to a physiologic (discomfort associated with calf position), social (displacement), management (overcrowding) or cow comfort (perching) risk factor or if the activity was associated with early sickness behaviour (self-isolation). Future research should include continuous video footage to better describe how and where cows are spending the additional time. For example, if cows are spending time perching in the stall, the increases in standing time may be related to cow comfort whereas if the cows are standing in the feed alley,
they may be unsuccessfully competing for feed or protecting a valuable resource (grooming brush).

Previous research focusing on behaviour and disease suggests that ketotic cows have lower DMI and more displacements at the feedback before calving. We also know that ketosis is strongly correlated to elevated NEFA and hypoglycemia in the prepartum period (Bobe et al., 2004). Concurrently monitoring these individual physiological parameters with feeding and standing behaviour on and before the day of calving may help discern whether the ketotic cows are in fact feeling the effects of the illness prior to the development of overt clinical signs. I predict that cows that develop clinical ketosis, have longer standing times and decreased standing bouts on the day of calving and also have elevated NEFA concentrations compared to cows that develop subclinical or no ketosis. The progression of change of physiological parameters, such as NEFA, may also play a key role in determining the impact of social status and bunk displacement on feeding behaviours. Discerning when the biological intermediaries associated with ketosis change may clarify whether the behaviour is the result of social status or the result of sickness.

Regrouping is a common management practice on farms and may increase the risk of disease (Cook and Norlund, 2004, Ingvartsen, 2006); often cows are regrouped 4-5 times during the transition period and are forced to cope with changes in social structures. In order to better discern if behaviour precedes the disease process or if the animal is already feeling ill, NEFA could be assessed before, during and after regrouping. If NEFA is normal prior to regrouping and then differs dramatically afterwards, I would predict that social status impacted the behaviour. However, if NEFA changed over time without a change in social groups, I would predict that the behaviour was influenced by the illness. Further research that considers attributes
associated with different coping styles (e.g. active vs. passive) or personalities may also be useful in identifying cows at higher risk for ketosis (see Proudfoot et al., 2012). Frequent regrouping may be associated with chronic stress response or immune dysfunction especially in subordinate animals (Burdick et al., 2011). This physiological response varies between individual animals and may be associated with social status, personality traits, and coping strategies. Cows with metabolic disorders are known to have reactive coping styles that are often associated with lower social status (Korte et al., 2005, Proudfoot et al., 2012). I would predict that cows that are feeling “ill” on the day of calving are socially subordinate or became socially subordinate as a result of illness and that this coincides with changes in NEFA in the weeks prior to calving.

Physiological changes may help account for the changes in behaviour, however whether ketotic cows are less motivated or less able to change positions remains unclear. A recent paper describing the relationship between metabolic status and standing behaviour in lame cows around transition may begin to shed some light on this question. Contrary to other reports that demonstrated both decreases in number of bouts per day (Gomez and Cook, 2010) and increased bout duration (Chapinal et al., 2009) usually associated with lameness, Calderon and Cook (2011) reported lame cows increased the number and duration of bouts on calving day and speculated that this was in response to pain associated with parturition. Although these authors reported lame cows were more likely to be ketotic within a few days after calving, there was no relationship between lameness and NEFA indicating that fat mobilization did not begin before calving and could not be used to predict cows at risk of ketosis. It is unknown if the standing and bout behaviour associated with a metabolic disease condition could be overcome by a painful disease condition such as lameness. However, the physiological changes in Calderon and Cook’s
(2011) study suggest early signs of subclinical ketosis were not evident on calving day and their findings support the prediction that these changes were more indicative of pain than ketosis. Work in rats and humans have shown that ketogenic diets reduce inflammation and pain responses (Kawamura and Masino, 2009) indicating that ketosis is unlikely a painful condition. However, this does not fully explain why the clinically ketotic cows in the current study did not exhibit normal pain affiliated behaviour on calving day. We encourage further research that concurrently monitors prepartum physiological changes highly predictive of ketosis with standing behaviour on and before the day of calving to discern how different disease conditions influence the ability or motivation to change positions.

New behavioural research focusing on exploratory, social and play behaviour may help to shed some light on why behavioural differences occur when animals are afflicted with illness. Weary et al. (2009) suggested that behaviours associated with longer-term fitness may be most likely to decline with illness in order to divert energy sources to critical functions; e.g. grooming (Hart, 1988), libido (Adelman and Martin, 2009), play (Spinka et al., 2001), and environmental sampling (Stephens and Krebs, 1986). Svensson and Jensen (2007) reported that diseased calves remained motivated to access the feeder when they actually received milk but reduced the number of nonnutritive visits compared with healthy calves. Nonnutritive visits, argued to be a form of sampling behaviour, are an indication that the calves are testing the feeder for availability of milk (Weary et al., 2009). In the current context, I predict that clinically ketotic cows will be less motivated to expend energy performing exploratory, grooming or social behaviours. I speculate that non-ketotic cows were more likely to be engaged in exploratory or social behaviour during this period, and encourage future work to test this prediction.
In the current study, the lack of significant differences in standing behaviour between the clinically ketotic and non-ketotic cows described in the first two weeks following calving (wk +1, $P = 0.13$; wk +2, $P = 0.16$) may be the result of farm management practices that likely influence and override natural standing patterns. When no management factors influenced standing behaviour after the second milking in our study, ketotic cows chose to lie down perhaps indicative of lethargy and depression classically associated with ketosis. Ketosis also has been described as an appetite suppressant in the human literature (Zupec-Kania and Spellman, 2008), so it may be speculated that cows would be less motivated to compete for food resources. However, our results suggest that sickness behaviour can be suppressed by herd activities such as movement to the parlor and milking. It is unknown if cows only accessed the bunk during fresh feed delivery because the management practices encouraged it or if the cows would chose to access feed with the group without intervention. Duplicating the current study with cows in a robotic milking system may give some insight on the ketotic cows’ motivation to be milked, feed or remain lying during different time periods compared with her non-ketotic counterparts and I encourage further research to test this prediction.

3.3 Conclusion

The work in this dissertation shows that monitoring of standing behaviour has potential to provide veterinarians and producers with a useful tool for early identification of cows with clinical ketosis. This and other use of sensors can be applied to improve the health, management, and welfare of dairy cows.
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