Monitoring transition dairy cow behaviour for the detection of subclinical ketosis

by
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ABSTRACT

MONITORING TRANSITION DAIRY COW BEHAVIOUR FOR THE DETECTION OF SUBCLINICAL KETOSIS

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An observational study of 4 free-stall farms was conducted to investigate the associations of cow behaviour and cow-level factors with subclinical ketosis (SCK) in transition dairy cows. Rumination time, lying behaviour, SCK, and other peripartum disorders were monitored from 2 wk before until 4 wk after calving for 339 cows. Lower rumination times and higher lying times in multiparous cows during the wk after calving (wk+1) were associated with increased odds of SCK with another health problem. Factors associated with lower odds of SCK in multiparous cows included: lower stall stocking density during wk+1, shorter dry period, lower milk yield during the previous lactation, and smaller BCS loss over transition. These results suggest monitoring behaviour may be useful in identifying multiparous cows with SCK and another health problem in wk+1; monitoring rumination behaviour, specifically, may aid in the early identification of multiparous cows at higher risk for developing SCK post-calving.

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CHAPTER 1: INTRODUCTION

The transition period has been defined as 3 wk before calving until 3 wk after calving (Drackley, 1999). This is a period of high energy demand where cows are going through many physiological and hormonal changes. Not only must cows support their calf during the final stages of development, but their bodies are getting ready to initiate lactation. While these cows are pressured with increased energy requirements, they have a depressed DMI in the days leading up to calving (Grant and Albright, 1995; Herdt, 2000). If these cows are not consuming enough feed to sustain energy demands they will reach a state of negative energy balance (NEB). As milk production peaks earlier in lactation than DMI, essentially all cows experience NEB at the beginning of their lactation (Herdt, 2000). As milk production increases and cows continue in a state of NEB they will begin to rely on fat stores to support their energy needs. Cows with subclinical ketosis (SCK) mobilize body reserves releasing ketone bodies in the blood (Baumen and Currie, 1980; Goldhawk et al., 2009; LeBlanc, 2010). This metabolic disorder is very prevalent in high-producing dairy herds, affecting an average of 43% of cows during the first 2 wk of lactation (McArt et al., 2012).

Many researchers have identified the risk factors for SCK, and there is much information on management approaches to help mitigate illness during the peripartum period (Duffield, 2000; Ingvartsen, 2006). To reduce the risk of SCK cows must be provided with the proper nutrition during the close-up period (Overton and Waldron, 2004), competition at the feed bunk should be reduced by providing adequate feed bunk space, management should aim to reduce the amount of over conditioning during the prepartum period (Ingvartsen, 2006), and producers should implement monitoring programs to identify subclinically sick cows.

There are many methods available to diagnose SCK including milk and urine tests, as well as blood tests. Some of these methods are more subjective than others, testing must be done on a regular basis during the first few weeks after calving, and testing may become quite costly for producers. Even with these methods available, it is very difficult to diagnose ketosis in the very early stages. In recent years, there is a growing amount of information of monitoring animal behaviour for the detecting illnesses (Weary et al., 2009). This review will outline management factors associated with SCK, while addressing current methods for detecting SCK and upcoming uses of automated behavioural monitoring systems, specifically for rumination and lying behaviour, for the detection of subclinical illness.

1.1 SUBCLINICAL KETOSIS

Dairy cows have a multitude of complex pathways to successfully adapt to milk production (Bauman and Currie, 1980). To meet the high energy demand of lactation in early lactation, while DMI intake is low, cows rely on body reserves (Ingvartsen, 2006). Fat reserves are mobilized throughout the body releasing non-esterified fatty acids (NEFA) that are converted to ketone bodies, i.e. acetone, acetate and beta-hydroxy butyrate (BHBA) in the liver. Production of ketone bodies supplies an alternative fuel source for tissues allowing glucose to be conserved for milk production (Ingvartsen, 2006). However, an accumulation of ketone bodies in the blood can lead to decreased appetite, which can make overcoming the illness even more difficult. Cows showing clinical signs of ketosis may have decreased appetite, increased lying time, and weight loss (Andersson, 1988); however, cows with SCK may not show any of these symptoms and only have high levels of circulating BHBA. This metabolic condition may decrease milk production (Duffield, 2009; McArt et al., 2012), reduce probability of pregnancy at first artificial insemination (Walsh et al., 2007; Ospina et al., 2010) and increase the risk of other illnesses including fatty liver, displaced abomasum, and metritis (LeBlanc, 2005; Suthar et al., 2013) which has a large impact on dairy cow welfare.

There are many studies that have assessed cow-level and farm-level factors associated with SCK. Cow-level factors, such as breed (Andersson and Emanuelson, 1985; Bendixen et al., 1987), parity (Suthar et al., 2013; Berge and Vertenten, 2014; Vanholder et al., 2015), and milk production (Baumen and Currie, 1980; Gröhn et al, 1989; Fleischer et al., 2001) may aid in understanding ketosis; however, these factors can not readily be changed on farm. Many farm-level factors associated with ketosis are management-related and may be modified to reduce the risk of ketosis in dairy herds. These include characteristics of the close-up diet (Overton and Waldron, 2004; VanSaun et al., 2014), as well as body condition score (BCS) of dry cows (Gillund et al., 2001; McArt et al., 2013; Vanholder et al., 2015), dry period length (Rastani et al., 2005; Santschi et al., 2011; Vanholder et al., 2015), and close-up pen characteristics (i.e. housing design, stocking density, and feed bunk space).

The NRC (2001) recommends that close-up dry cows be given an energy rich (1.54 to 1.62 Mcal/kg of NE_L) ration that provides the necessary nutrients required to advance metabolic and physiological adaptations necessary for the onset of lactation. Gustafsson et al. (1995) found that herds that fed fewer meals throughout the day, and those that had higher levels of concentrate, were at an increased risk of SCK. Cows fed higher levels of concentrate may have experienced periods of acidosis and decreased overall feed intake (Richert et al., 2013). More recent work has suggested that controlling energy consumption in late gestation may improve DMI in early lactation (Douglas et al., 2006; Janovick et al., 2011; Vickers et al., 2013). Vickers et al. (2013) found close-up cows fed an 87% forage diet had a lower incidence of ketosis in the first 10 d post-calving compared to close-up cows fed a 77% forage diet. Janovick and Drackley (2010) compared 3 close-up diets fed for 28 d before calving: the first provided 150% of NRC (2001) energy requirements (OVR), the second 80% (RES), and the third 100% (CON). The CON diet restricted energy intake by adding chopped wheat straw to the ration. Cows fed chopped wheat straw had little reduction in DMI. Cows on the CON and RES diets showed little NEB, however NEB was 55% greater 3 wk after parturition in MP cows on the OVR diet. Cows on the OVR diet not only had greater reductions in DMI, but also gained more weight prepartum and had greater weight loss postpartum.

Body condition score, and changes in BCS through the transition period, also have an impact on risk of SCK. Vanholder et al. (2015) found that cows in both the moderate ($3.5 \leq$ BCS ≤ 3.75) and fat BCS category (BCS ≥ 4) had an increased risk of SCK compared to cows with BCS ≤ 3. Cows with greater BCS pre-calving experience a greater decrease in DMI prior to calving (Hayirli et al., 2002), which is a major contributing factor for developing SCK postpartum (Goldhawk et al., 2009). Gillund et al. (2001) actually found the loss of weight over the transition period to be of greater importance rather than pre-calving BCS itself. Cows in a state of NEB will deplete fat stores to compensate for the high energy demands of lactation (Goldhawk et al., 2009). Researchers have found that a shorter dry period, 34 d or less versus the traditional 60 d, may improved NEB due to greater DMI (Rastani et al., 2005) and slightly lower milk production (Rastani et al., 2005; Watters et al., 2008). Rastani et al. (2005) also found all cows had a similar BCS pre-calving; however, cows on the traditional dry period length had a greater loss in BCS over the transition period compared to those with the shorter dry period. Vanholder et al. (2015) found that cows with shorter dry periods actually had lower odds of SCK. It is possible that longer dry periods are associated with greater late lactation pregnancies that result in higher BCS at dry off, which then is carried through the dry period. It is also possible that higher BCS associated with longer dry periods may be the result of overconsumption of nutrients during that time period, particularly if cows spend excess time feeding on the close-up ration.

The ration during the dry period and length of time these animals are dry are not the only factors affecting ketosis in early lactation. Access to feed and stocking density are also major factors that affect ketosis (Munksgaard et al., 2005; Proudfoot et al., 2009). Cows prefer to eat collectively as a group; however, they also readily form a dominance hierarchy which may influence their behavioural patterns. In close-up bedded packs, it is recommended that there is 11m^2 of space per cow (Nordlund, 2008) and in free stall barns the number of cows should not exceed the number of stalls provided (Fregonesi et al., 2007). A minimum of 0.76 m of feed bunk space per cow is also recommended for close-up dry cows (Nordlund et al., 2006).
Overcrowding can limit the ability of cows to access their desired resources, whether that be lying areas, feed, or water, at the times they would prefer (Munksgaard et al., 2005). When stalls are limited per cow, cows may be compelled to lay down right after milking (Fregonesi et al., 2007), rather than consume feed at the feed bunk, potentially limiting DMI. In a study conducted by Proudfoot et al. (2009), feeding behaviour of transition cows housed in a competitive group (2:1 cows per feed bin) were compared to cows in a non-competitive group (1:1 cows per feed bin). Researchers found that MP cows in the competitive environment showed a decrease in DMI 1 wk before calving. Other studies with lactating cows have shown that decreasing stocking density at the feed bunk increases feeding time, especially in subordinate cows (DeVries et al., 2004; Huzzey et al., 2006). Thus, to encourage DMI in late gestation, and limit the risk of SCK, it is important that producers ensure enough feeding space for dry cows.

Many of the factors discussed here have been associated with SCK, but more work needs to be done to understand their interactions. Multiple factors combine to alter the risk of SCK and these factors will vary both between farms and studies (McArt et al., 2013). Further research should focus on understanding how these factors come together to affect nutrient consumption, energy balance, physiological changes in dairy cows at transition and subsequent risk of SCK.

Many methods are available to identify cows with SCK. The gold standard diagnostic test is measuring BHBA in blood serum or plasma (Duffield et al., 2009). Depending on the outcome, thresholds of blood BHBA between 1.0 and 1.4 mmol/L have been used to define SCK (Duffield et al., 1998; Iwersen et al., 2009; Rollin et al., 2010). When evaluating diagnostic tests, they are compared to the gold standard and their accuracy is measured by sensitivity (the proportion of diseased animals that test positive) and specificity (the proportion of non-diseased animals that test negative). Measuring blood serum BHBA may be done by sending serum samples to a diagnostic laboratory, which is time consuming and requires spinning down a larger sample of blood. However, there are a number of cow-side tests available for detection of SCK (Geishauser et al., 2000; Carrier et al., 2004; Iwersen et al., 2009). Iwerson et al. (2009) validated the use of a handheld combined glucose and BHBA meter (Precision Xtra Abbott Diabetes Care, Saint Laurent, QC, Canada) in cows. The handheld meter requires only a drop of blood and will measure the concentration of blood BHBA on farm in 10s. The Precision Xtra meter had 88% and 96% sensitivity and specificity for determining ketosis at the 1.2mmol/L cut-off (Iwerson et al., 2009), making it a very accurate and reliable test. Another method for evaluating ketosis on-farm is the use of test strips that indentify the presence of ketone bodies in either urine or milk (Geishauser et al., 2000; Carrier et al., 2004). These test strips contain nitroprusside which reacts with ketone bodies causing a color change in the test strip - a greater concentration of ketone bodies creates a darker purple color. The strips have a reference color chart to indicate a range of the level of ketone bodies for each shade. Strips testing BHBA in urine had a sensitivity of 97% and 60% specificity (Osborne et al., 2002). Multiple studies have analyzed the reliability of test strips measuring BHBA in milk. When comparing a cut-off of 1.0mmol/L of BHBA in milk to the cut-off of 1.2mmol/L in blood serum, this test had sensitivity and specificity reported as: 72% and 89% (Geishauser et al., 1998) and 96% and 63% (Enjalbert et al., 2001), respectively. Milk and urine tests are economical tools useful for identifying ketosis in cows, even though they do yield more false positive readings than the Precision Xtra meter. These tests are also much more subjective as they only provide a semi quantitative diagnosis, unlike the Precision Xtra meter which displays the concentration of BHBA in the blood so a cow may easily be identified as above or below the threshold.

Even with the availability of numerous cowside tests for SCK, this metabolic disease remains highly prevalent in dairy herds. McArt et al. (2012) reported an average cumulative SCK incidence of 43% among cows tested thrice weekly from 3 to 16 DIM, with the peak incidence at 5 DIM. Duffield (2000) monitored ketosis in 25 Canadian herds and found the peak incidence of ketosis to be 30% in the first week of lactation; when the time frame was extended to 9 wk, cumulative incidence increased to 43%, with farm-level incidence ranging from 8-80%. The tests described above are mainly used during the first 2 wk after calving, which is the most optimal time to test for ketosis as many studies have found this disorder is most prevalent at this time (Duffield, 2000; Leblanc, 2010; McArt et al., 2012). A more recent study by Tatone et al. (2015) measured BHBA 3 to 9 d before the expected calving date with the Precision Xtra meter. These researchers found cows with BHBA ≥ 0.6 mmol/L during the prepartum period were 2.2 times likely to develop ketosis in the wk after calving compared to cows with a BHBA reading < 0.6

mmol/L. This is, thus, the first cowside test that has also been validated for use in the pre-calving period and may aid in detecting cows at risk for SCK and improve the timeliness of treating these cows.

1.2 BEHAVIOUR MONITORING

Monitoring animal behaviour may be another useful tool in identifying cows at risk for subclinical illness (Weary et al., 2009). For example, one study found that transition cows with decreased DMI spent less time feeding pre-calving and were at an increased risk of developing metritis post-calving (Huzzey et al., 2007). It has also been estimated that for every 1 kg decrease in DMI, or 10 min decrease in feeding time, during the wk prior to calving, the odds of developing SCK postpartum increased by 2.2 and 1.9 times, respectively (Goldhawk et al., 2009). Cows with dystocia were more likely to switch between standing up and lying down in the 24 h leading up to calving (Proudfoot et al., 2009b). Further, in a study by Calamari et al. (2014) it was suggested that a slower increase in rumination time post-calving may be associated with systemic inflammation. There is much recent work in evaluating feeding, rumination, and lying behaviour throughout the transition period and there is growing evidence that monitoring these behaviours may aid in identifying subclinical illness (Edwards and Tozer, 2004; Huzzey et al., 2007; Jawor et al., 2012; Soriani et al., 2012; Calamari et al., 2014).

1.2.1 Rumination and Feeding Behaviour

As a ruminant species, dairy cows rely on the process of rumination to fully digest their food. Microbes present in the rumen break down cellulose, allowing cows to digest grasses and plant matter. Larger food particles in the rumen are regurgitated, re-masticated, and re-swallowed to increase the surface area for microbes to attach and breakdown the food particles

(Welch, 1982). During the breakdown process, microbes release volatile fatty acids into the rumen which may be absorbed through rumen epithelium and used for energy. The large production of volatile fatty acids in the rumen may cause the pH of the rumen to drop. Rumination stimulates saliva production which aides in buffering the rumen (Erdman, 1988). Total mixed rations with greater amounts of concentrate are digested much faster than diets high in long fibrous particles, which may cause a depression in the pH of the rumen. Thus, it is important to supply large particle, neutral detergent fiber in the ration to stimulate rumination (Kononoff et al., 2003; Beauchemin and Yang, 2005), and in turn, saliva production to maintain stable rumen conditions for microbes.

Daily rumination time is highly variable within individual cows (Pederson, 2010; Sorinaini et al., 2012), but also between herds (Reith and Hoy, 2012). This variation may be due to differences in the ration fed. Work by Dado and Allen (1995) showed that rumination time in early lactation dairy cows increased from 380 to 500 min/d when NDF content of the ration was increased from 25 to 35%. Variation in rumination time between cows may be due to sorting, as well as intake levels. Cows consuming greater quantities of long ration particles may have longer rumination times compared to cows that sort out a higher percentage of long particles higher in NDF (Maulfair et al., 2010). Rumination time is more consistently associated with dietary NDF intake (Welch and Smith, 1970; Beauchemin et al., 1994), whereas its association with DMI varies in the literature (Hasegawa et al., 1997; Schirmann et al.; 2012). While some studies have suggested rumination time may be indicative of DMI (Hasegawa et al., 1997), Clement et al. (2014) recently found that rumination time was a significant, but small contributor in a DMI prediction model. This may possibly be due to the variability of rumination time within weeks and cows, making it difficult to predict DMI from rumination time. Schirmann et al. (2012)

found a negative correlation between periods of DMI and rumination time in dry cows throughout the day. These researchers hypothesized this was due to the fact that cows cannot feed and ruminate at the same time. They did find, however, that cows spend more time ruminating about 4 h after periods of high feed intake (Schirmann et al., 2012). This indicates that within-cow variations in rumination data may be used to indicate changes in feeding behaviour and intake, but may not be consistent in estimating DMI.

The average daily rumination time for close-up cows has been reported in multiple studies: 400 to 450 min/d during the pre-calving period (Adin et al., 2009); 463 min/d for PP cows and 522 min/d for MP cows from 10 to 2 d prior to calving (Soriani et al., 2012); and 477 min/d during 2 to 5 wk before calving (Aikman et al., 2008). Rumination time reaches its nadir at the time of calving (Schirmann et al., 2007; Soriani et al., 2012; Calamari et al., 2014). Schirmann et al. (2007) found that feeding time began to decrease 8 h before calving and rumination time was quick to follow, 4 h before the onset of calving. Feeding time and rumination time both began to increase at about 4 to 6 h post-calving. Rumination time increases after calving and begins to plateau to an average of 452 min/d at around 15 DIM (Calamari et al., 2014). The literature suggests lactating cows ruminate between 340 and 540 min/d (Kononoff and Heinrichs, 2003; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006). Soriani et al. (2012) reported daily rumination times from 15 to 40 DIM were at the higher end of the range compared to previous studies: 504 min/d for PP cows and 562 min/d for MP cows. Other studies have also found that PP cows ruminate less each day compared to MP cows (Beauchemin and Rode, 1994; Maekawa et al., 2002). Beauchemin and Rode (1994) also observed that PP and MP cows regurgitated a similar number of boluses, however, MP spent more time chewing each bolus. Cows that are regrouped show a decrease in rumination time the day after regrouping

(Schirmann et al., 2011). Soriani et al. (2012) suggested that PP cows suffer more from the stress of environmental changes at the initiation of lactation, and thus show a slower increase in rumination time after calving compared to MP cows.

Cows with decreased DMI in the pre-calving period have much higher odds for experiencing SCK post-calving (Goldhawk et al., 2009). Cows with SCK within the first few days postpartum have been observed to have lower rumination times than healthy cows (Soriani et al. 2012), and rumination time has been shown to have a negative association with blood BHBA concentration in lactating dairy cows (Soriani et al., 2013). Rumination behaviour may be a promising indicator of metabolic conditions (Soriani et al., 2012), particularly during the post-partum period as it is likely affected by changes in feeding behaviour and DMI (Okine and Mathison, 1991). Although a few studies have observed how rumination behaviour changes over the transition period and have identified multiple factors that affect rumination and SCK, there is little information on how rumination time and cow- and farm -level factors interact in their associations with subclinical illness.

1.2.2 Lying Activity

Lying time is also associated with a number of cow- and herd-level factors. Factors at the farm level that influence lying time include housing system (Haley et al., 2000; Sepúlveda-Varas et al., 2014), stall dimensions (Haley et al., 2001; Tucker et al., 2004), bedding (Tucker et al. 2003; Fregonesi et al., 2007; Norring et al., 2008), stocking density (Fregonesi et al., 2007), and season (Arazi et al., 2010; Steensels et al., 2012). Cows that were restricted from both lying down and feeding spent more time lying down than feeding when given access to both resources (Munksgaard et al., 2005). This research demonstrates the high priority for lying behaviour for dairy cows. If cows are deprived from lying down for more than 2 h, they will later spend more time lying and reduce feeding time to try to compensate for lost time. Even after 40 h of unrestricted access to lying down, cows restricted from lying down longer than 2 h could not recuperate normal levels of lying time (Cooper et al,. 2007). Normal lying behaviour has been associated with cow comfort (Cook et al., 2005), wellbeing (Haley et al., 2001; Fisher et al., 2003) and production (Fregonesi and Leaver, 2001; Bewley et al., 2010).

Lying time is highly variable within cows, as well as within farms (Ito et al., 2009). At the cow level, higher lying times are seen in cows with increased parity (Steensels et al., 2012; Sepúlveda-Varas et al., 2014), greater DIM (Nielson et al., 2000; Bewley et al., 2010) and lower production level (Bewley, et al. 2010; DeVries et al, 2011; Deming et al., 2013).

There are a number of studies that have specifically tried to understand the changes in lying time throughout transition. Huzzey et al. (2005) found that cows spent around 702 min/d lying down in the 10 d leading up to calving. Multiple studies have found that lying time, similar to rumination time, reaches its nadir during calving and then begins to rapidly increase 4 to 5 d post-calving (Arazi et al., 2010; Steensels et al., 2012). Blackie et al. (2006) also found that cows take a greater number of steps/h during the wk after calving, possibly due to increased inflammation or pain associated with calving (Proudfoot et al., 2009a). Cows are regularly regrouped after calving, and a change in pens may reduce lying time and number of lying bouts during the day after regrouping (von Keyserlingk et al., 2008). Less dominant cows may spend more time standing at the feed bunk waiting to eat and are less apt to displace cows in stalls to lie down. Researchers have found lying time stabilized after calving at: 636 min/d (Huzzey et al., 2005); 590 to 650 min/d (Calderon and Cook, 2011); 491 to 578 min/d (Steensels et al., 2012). Cows were found to spend more time standing post-calving mainly because they are dedicating more of their time to milking, as well as feeding, to support milk production (Goff and Horst, 1997; Huzzey et al., 2005; Gomez and Cook, 2010).

Although cows in NEB should spend more time feeding to compensate for their high energy demands, cows with SCK may lie down for longer periods of time to conserve energy (Hart, 1988) needed for milk production. Goldhawk et al. (2009) found that cows with SCK post-calving spent less time at the feeder and visited the feeder less during the wk before calving. Itle et al. (2015) found cows with clinical ketosis post-calving stood longer throughout the day in the week before calving than healthy cows, but saw no difference in standing time post-calving. Those researchers suggested that the cows that were later ketotic may have been more subordinate and, therefore, spent more time standing waiting to feed rather than competing for a spot at the feed bunk. A study that looked at standing behaviour of hypocalcaemic cows found these cows lay down less during the 24 h before calving, but lay down longer in the wk after calving (Jawor et al., 2012). Sepúlveda-Varas et al. (2014) looked at the post-calving differences in lying time between cows with no health issues and compared them to cows with one, and cows with greater than one, clinical postpartum disease. Primiparous cows with multiple illnesses showed greater change in lying time than those with only one illness. Thus, lying behaviour may be a promising indicator of metabolic conditions, particularly during the peripartum period.

1.2.3 Technologies for Behaviour Monitoring

With a growing number of technologies available to producers, monitoring individual animal behaviour on-farm is becoming much easier. In 2007, SCR Engineers Ltd. introduced an automating rumination monitoring system (Hi-Tag, SCR Engineers Ltd., Netanya, Israel). The data logger contains a small microphone located on a collar that detects the time each bolus is regurgitated and swallowed by the animal. These actions are recorded 24 h/d. Identification units are necessary to upload collected rumination data from each data logger to the control unit at least once every 23 h. Newer systems use an ID unit with radio technology to continuously upload recordings from data loggers. All uploaded information is sent to the control box where data from each cow can be read off the screen or sent to the producer via an internet connection. Schirmann et al. (2009) validated this system, indicating it could be an accurate tool for monitoring rumination behaviour in dairy cows in both commercial and research settings. In a commercial setting, the system may be set up to continuously record rumination and activity data for any cow equipped with a collar. Over time the system recognizes patterns in the data to determine each individual cow's normal rumination cycle. When the data deviates from the cow's normal pattern, the control box sends a message to the producer, notifying them to check that cow.

More recently another system has become available that monitors ear temperature, rumination and feeding behaviour, as well as activity using an ear tag monitor (CowManager SensOor ear tag, Agis Automatisering BV, Harmelen, The Netherlands). In this system, a microchip that attaches to the ear tag contains an accelerometer that detects changes in ear movement. Each minute the tag records 1 of 4 behaviours the cow may be expressing: "ruminating", "feeding", "resting" or "active". Each of these behaviours are expressed as a percentage of behaviour per hour as well as per day and are uploaded to a computer via routers. This system has been validated as another useful tool to monitor rumination and resting behaviours and found it may be quite promising in monitoring feeding behaviour (Bikker et al., 2014; Wolfger et al., 2015). Rumination and feeding times are compared to the previous day's values, and cows that experience a drop in these behaviours are flagged by the system as possibly sick.

There are two main ways to assess activity in dairy cows. Pedometers, which have been in use since the 1970's, measure the number of steps taken throughout the day, whereas accelerometers measures the acceleration the device receives in proportion to free fall (MacKay et al., 2012). Accelerometers do not count steps, but are able to quantify movement depending on where the device is placed on the cow (Rutten et al., 2013). Many of these systems use algorithms to identify spikes in movement that are characteristic of estrus behaviour and can identify cows in heat. Many of these types of technologies have been validated and are used on commercial farms, including the Afi Pedometer Plus leg tag (Afimilk, S.A.E. AFIKIM, Kibbutz Afikim, Israel; Mattachini et al., 2013), Rumiwatch Pedometer (GmbH, Switzerland; Zehner et al., 2012), and the IceQube activity monitor (IceRobotics, Scotland; McGowan et al., 2007). Some accelerometers, normally placed on the hind leg, can measure total daily lying and standing time. There are also a wide range of accelerometers used mainly in research settings such as the HOBO Data Logger (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA; Legerwood et al., 2010), the Tinytag Plus (Tinytag Plus, Re-Ed volt, Gemini Dataloggers (UK) Ltd., Chichester, UK; O'Driscoll et al., 2008), the IceTag Activity Monitor (IceRobotics, Scotland); McGowan et al., 2007). All of these devices that have the ability to measure lying time may be used to flag cows with low activity, or cows that spend more time lying down that may possibly be sick.

These technologies may be very useful if they can accurately describe behaviours on a continuous basis (Berckmans, 2004). If they are able to do this, dairy producers may be able to spend less time observing the behaviour of all cows in the herd, which may be very difficult on large scale farms, and instead spend more time with individual cows that have deviated from their normal behaviour.

1.3 OBJECTIVES AND HYPOTHESES

The overall objective of this thesis was to investigate rumination and lying behaviour of dairy cows, using automated technologies, over the transition period and explore the relationship among behaviour, management factors, and subclinical illness in high-producing, transition dairy cows. A cross-sectional study of commercial free-stall farms was conducted to describe animal behaviour and risk factors for subclinical illness at the cow-level, as well as to associate these factors with the incidence of SCK. Our first objective (Chapter 2) was to characterize changes in rumination behaviour across the transition period and determine if rumination behaviour may be used to identify cows at risk for SCK. We hypothesized that dairy cows with reduced rumination activity, both pre- and post-calving, would be at higher risk of experiencing SCK during early lactation.

The second objective (Chapter 3) focused on understanding changes in lying behaviours throughout transition and determined if daily lying time, frequency of lying bouts, and bout duration may be used to identify cows at risk for SCK. We hypothesized that dairy cows with increased lying activity, both pre- and post-calving, would be at higher risk of experiencing SCK during early lactation.

CHAPTER 2: Monitoring rumination in transition dairy cows for early detection of subclinical ketosis

2.1 INTRODUCTION

The transition period commences 3 wk prior to calving and lasts until 3 wk after calving (Drackley, 1999). It is both a critical and vulnerable time period for the dairy cow. Essentially all dairy cows experience a negative energy balance (NEB) in early lactation (Sovani et al., 2000), due to decreased DMI around calving and slower acceleration of DMI than of milk production (Grant and Albright, 1995; Schirmann et al., 2013). An excessive or prolonged drop in DMI around calving may result in non-adaptive NEB which may lead to subclinical ketosis (SCK) (Grummer, 1995), also referred to as hyperketonemia (McArt et al., 2012).

McArt et al. (2012) reported an average cumulative SCK incidence of 43% among cows tested thrice weekly from 3 to 16 DIM, with the peak incidence at 5 DIM. This condition can result in low milk production (McArt et al., 2012), reduced reproductive performance (Walsh et al., 2007), and increased risk of other illnesses including fatty liver, displaced abomasum, and metritis (Suthar et al., 2013). Technological improvements have improved detection of SCK. Cows in NEB begin to mobilize fat stores in an attempt to meet the high energy demand during early lactation, which releases ketone bodies (i.e. BHBA) into the blood (Baumen and Currie, 1980; Goldhawk et al., 2009; LeBlanc, 2010). An electronic cow-side test for the quantification of blood BHBA concentration (Precision Xtra Abbott Diabetes Care, Saint Laurent, QC, Canada), has been validated in dairy cows (Iwersen et al., 2009; Voyvoda and Erdogan, 2010).

The current challenge for producers is identifying SCK at an early stage. There is growing evidence that measurements of cow behaviour can be used to identify cows at risk for illness (Weary et al., 2009). Huzzey et al. (2007) found that transition cows with decreased feed intake spent less time feeding pre-calving and were at an increased risk of developing metritis. It has also been estimated that for every 1 kg decrease in DMI and 10 min decrease in feeding time during the week prior to calving, the odds of developing SCK increased by 2.2 and 1.9 times, respectively (Goldhawk et al., 2009). Another study by Calamari et al. (2014) suggested that a slower increase in rumination time post-calving may be associated with systemic inflammation. Cows with subclinical ketosis within the first few days postpartum have been observed to have lower rumination times than healthy cows (Soriani et al. 2012) and rumination time has been shown to have a negative association with blood BHBA concentration in lactating dairy cows (Soriani et al., 2013). Rumination behaviour may be a promising indicator of metabolic conditions (Soriani et al., 2012), particularly during the post-partum period as it is likely affected by changes in feeding behaviour and DMI (Okine and Mathison, 1991).

The objective of this study was to characterize changes in rumination behaviour across the transition period and determine if rumination behaviour might be used to identify cows at risk for SCK. We hypothesized that dairy cows with reduced rumination activity, both pre- and post-calving, would be at higher risk of experiencing SCK during early lactation.

2.2 MATERIALS AND METHODS

2.2.1 Herd Selection

This prospective observational study was conducted on 4 commercial dairy farms located in Eastern Ontario, Canada between March and October 2014. Herds were selected as a convenience sample according to proximity to the University of Guelph, Kemptville Campus (Kemptville, Ontario, Canada). Participating dairies milked between 125 and 400 Holstein cows (Table 2.1). All cows were housed in a free stall facility, fed a TMR 1x/d, and milked in a parlour 3x/d. Animal use, data collection, and study design were approved by the University of Guelph's Animal Care Committee and Research Ethics Board.

Researchers surveyed each participating producer during the first farm visit and recorded general farm information (herd size), as well as dry and fresh cow management practices (dry off protocol, ionophore usage, frequency of feed delivery and feed push up). At each weekly visit, the total number of cows in each pen were counted and recorded. At the end of the 7-mo research period, researchers measured stall and feed bunk dimensions for all dry and fresh cow pens to calculate stocking density and feed bunk space available during each week of the transition period. Management practices for lactating and dry cows are summarized in Tables 2.1 and 2.2, respectively.

2.2.2 Cow Enrollment

Researchers obtained a list of expected calving dates from each participating farm at the first farm visit. Each week, cows were systematically enrolled in the study based on the availability of rumination collars and parity (1:2 ratio of primiparous to multiparous cows). Cows were enrolled 2 to 3 wk before their expected calving date and at this time, individual animal information (cow identification number, parity, dry-off date, expected calving date) was recorded. We aimed to study each cow from 2 wk before calving until 4 wk after calving. In total, 346 cows were monitored from an average of -16 ± 5.4 d (mean ± SD; min = -34 d, max = -2 d) until +28 d relative to calving. This study aimed to screen a minimum of 300 cows; with an expected SCK incidence rate of 40%, this would yield 120 cows with SCK. Given 95% confidence and 80% power, this sample size was expected to allow for detection of a 50±10 min/d difference in rumination time between health categories.

2.2.3 Rumination Behaviour

An automated rumination monitoring system (Hi-Tag, SCR Engineers Ltd., Netanya, Israel) was installed at each participating dairy farm. Schirmann et al. (2009) validated the use of this automated monitoring system for recording daily rumination time in dairy cows. In this validation study, rumination time during a 2-hr interval was highly correlated (r = 0.93, R^2 = 0.87, n = 51) with rumination time recorded using direct human observation over the same time interval. In our study each cow was fitted with a SCR rumination collar at enrollment, which monitored rumination 24 h/d over the 6-wk study period. The collars contained a small microphone that recorded each time a bolus was regurgitated, re-masticated, and swallowed to determine total time spent ruminating during each 2-h interval throughout the day. This information was transferred to the control unit via radio frequency or when collars were scanned by identification units located in high traffic areas (e.g. parlour exits or above water troughs). Data were backed-up from the control unit and downloaded to the database on a weekly basis. The 12, 2-h intervals each day were summed to determine total time spent ruminating per day per cow.

2.2.4 Subclinical Ketosis Diagnosis

Each enrolled cow was assessed for SCK 1x/wk over the 6-wk study period for each cow. Cows were restrained within 2 to 6 h after feeding in a stall or headlock to obtain a small blood sample from the coccygeal vein using a vacuum-sealed blood collection tube (Blood Collection Tube Vacutainer Glass 10ml - Red, Becton Dickinson Canada Inc, Mississauga, Ontario, Canada) and 21G needle (Needle Vacutainer Multiple Sample 21G x 1 in, Becton Dickinson Canada Inc, Mississauga, Ontario, Canada). The concentration of BHBA in this whole blood sample was tested immediately using an electronic hand-held device (Precision Xtra meter, Abbott Diabetes Care, Saint Laurent, QC, Canada), as validated by Iwerson et al. (2009). The BHBA concentration of the blood was recorded on farm; cows with BHBA ≥ 1.2mmol/L at one or more of the 4 postpartum samples were classified as having SCK (Geishauser et al., 1998; McArt et al., 2012).

2.2.5 Determining Health Status

Body condition score (1 to 5, following Wildman et al., 1982) and locomotion score (1 to 5, following Flower and Weary, 2007) were assessed at enrollment, 2 to 3 wk before the expected calving date, and at the end of the study period, 4 wk after calving. Cows were scored by one of two individuals at the time of enrollment and removal; inter-observer reliability was determined between individuals to ensure validity of results (locomotion score, Kappa= 0.83; BCS, Kappa= 0.84).

Producers were asked to monitor and record the incidence of retained placenta, metritis, milk fever, displaced abomasum, and clinical mastitis. Occurrences of these conditions that occurred during the 6-wk study period for each cow were recorded. Cows were categorized into 1 of 4 groups: healthy (H) cows had no SCK or any other recorded health problem; healthy plus (H+) cows that did not have SCK but were treated for at least one other health problem; Cow with SCK (K) but with no other health problems during the observation period; or ketotic plus (K+) cows that had SCK and one or more other health problems during the observation period.

2.2.6 Ration Composition

Feed samples of the close-up dry cow ration and fresh cow ration were collected twice each month, 1 d before the weekly farm visit. At each sampling, individual samples were taken from 10 different areas of the feed bunk and combined into one sample of each diet per farm per sample day to ensure a representative sample. All samples were frozen at -20°C until nutrient analysis.

Samples for DM were weighed then dried at 55°C for 48 hours. After drying, each sample was weighed again to calculate the % DM of each close-up dry cow and fresh cow ration. After drying, samples were ground to fit through a 1 mm screen. Samples of each diet at each farm were pooled together into 3 samples (May-June, June-July, August-October). Pooled samples were sent to Cumberland Valley Analytical Services Inc. (Maugansville, USA) for analysis of DM (135°C; AOAC International, 2000: method 930.15), ash (535°C; AOAC International, 2000: method 942.05), ADF (AOAC International, 2000: method 973.18), NDF with heat-stable α-amylase and sodium sulfate (Van Soest et al, 1991), and CP (N x 6.25; AOAC International, 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Lecom St. Joseph, USA). Non-fiber carbohydrate content was also calculated as 100 – (% CP + % NDF + % fat + % ash) (NRC, 2001). Feed rations for each participating farm are summarized in Table 2.3.

2.2.7 Statistical Analyses

Cows that had aborted (n=2), were sold (n=2), or diagnosed with SCK before calving (n=3) were not included in the statistical analysis. Cows that were sold (n=22) or died (n=1) during the post-calving period with behavioural and health measurements recorded until the day they left the herd, were included in the analysis. The final dataset included 339 cows, (107 primiparous and 232 multiparous) categorized as H (n=139), H+ (n=50), K (n=97) and K+ (n=53).

For all further analyses described, comparisons were made between H and K cows and H and K+ cows, respectively. Statistical analyses were performed with SAS (version 9.4; SAS Institute, 2013) using cow within farm (n = 289) as the experimental unit. Daily rumination times (min/d) were summarized by cow and week such that these data aligned with the once weekly testing of SCK. These data were analyzed in a general linear mixed model (PROC MIXED in SAS), treating week as a repeated measure. The model for rumination activity included the random effects of farm and cow within farm (subject of repeated statement) and the fixed effects of health status, parity, and week, the interactions of health status by parity and health status by week, as well as the three-way interaction of health status, parity, and week. The covariance structure was heterogeneous compound symmetry, selected by best fit according to Schwarz's Bayesian information criterion. A three-way interaction was found between health status, parity, and week ($P < 0.01$); thus, data from first lactation (primiparous, PP) and multiparous (MP) cows were analyzed separately. These separate models included the fixed effects of health status, week and the interaction between health status by week, with farm and cow within farm included as random effects. Differences in rumination time between health categories and weeks were compared using the least-squares means procedure with the PDIFF option. Significance was declared at $P < 0.05$, and tendencies were reported if $0.05 < P < 0.10$.

In the analysis of the impact of health status on rumination time, as described above, differences were only found between health categories for MP cows. Thus multivariable logistic regression was only performed on data from MP cows and not on PP cows. This analysis was performed using the GLIMMIX procedure (distribution = binomial and link = logit) in SAS (version 9.4; SAS Institute, 2013) to model to effects of rumination time and other cow-level factors on the presence or absence of SCK. This was done using two models: one model compared H and K cows, while the other compared H and K+ cows. Parity and pre-calving BCS were both treated as categorical variables. Multiparous cows were characterized as second lactation (2; n = 99) or third lactation and greater (3+; n = 103). BCS pre-calving was categorized into three groups: underweight, BCS < 3; normal, BCS = 3 to 3.5; overweight, BCS > 3.5. Parity, pre-calving BCS category, change in BCS over the transition period, length of dry period, milk yield from the previous lactation, as well as rumination time and stall stocking density during the weeks prior to the mean day of diagnosis (wk -2, -1, and +1 relative to calving), were all assessed for an association with presence or absence of K and K+ using univariable logistic regression models. Variables with $P \leq 0.25$ were then used to construct a multivariable logistic regression model. The CORR procedure in SAS was used to check for correlations between the explanatory variables included in the multivariable model. If 2 variables were highly correlated (r > 0.8), the variable with the lowest *P*-value and most biological relevance was retained for the multivariable model. Manual backward elimination of variables with $P > 0.10$ was used to create the final models and from the resultant models, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those variables retained in the final multivariable model are presented.

2.3 RESULTS

A descriptive summary of cow-level variables, characterized by herd, is found in Table 2.4. Of the 339 cows, 139 (41%) did not have SCK or any other health problems. Table 2.5 describes the prevalence of ketosis. In total there were 150 cows with ketosis (44%) and of these, 53 were also treated for at least one other health problem (16% of all cows). The incidence risks for diseases other than SCK are described in Table 2.5, with metritis being most common treated illness, followed by retained placenta, mastitis, milk fever, foot problems, and displaced abomasum.

Among cows in their first lactation, from 2 wk prior to calving until 4 wk after calving, there were no differences (*P*= 0.5) in rumination time among H, K and K+ cows (Table 2.6). Rumination time in PP cows varied by week (*P*< 0.001). Primiparous cows ruminated less in wk -1 compared to wk -2 (*P*=0.001), and rumination time increased from wk +1 to wk +2 (*P*<0.001) and wk +3 to wk +4 (*P*=0.04) as seen in Figure 2.1.

For MP cows, an interaction was found between health status and week (*P*= 0.01; Table 2.6). Figure 2.2 illustrates how daily rumination time differed among H, K and K+ cows over the observation period. There was an effect of time (*P* < 0.001) across all health statuses: daily rumination time decreased in wk -1 compared to wk -2 but increased each week from wk -1 to +2. Multiparous K cows tended to ruminate less than multiparous H cows during wk -1 and during wk +1 (Table 2.6). The largest differences in rumination time between multiparous H and K+ cows were seen during wk -1, +1 and +2.

Table 2.7 shows the unconditional associations of the independent variables from the univariable analyses for H versus K cows prior to building the multivariable model. Increased odds of SCK with no other recorded health problems (K) were associated with higher parity (3+ compared to second lactation cows), greater milk yield during the previous lactation, longer dry period, cows being in the overweight category pre-calving, greater stall stocking density during wk -2, -1, and +1, and greater loss in BCS over the transition period. Decreased odds of SCK with no other health problems, relative to H, were associated with a greater stall stocking density during wk +1, and greater rumination time during wk -1. Four of these variables were retained in the final multivariable model (Table 2.8). Greater rumination time during the wk before calving was associated with decreased odds of K, whereas greater milk yield in the previous lactation, greater loss of BCS over the transition period, and greater stall stocking density in the week prior to calving were associated increased odds of K relative to H.

Unconditional associations of the independent variables for H versus K+ cows are shown in Table 2.9. There were increased odds of developing SCK combined with another health problem with higher parity (3+ compared to second lactation cows), greater milk yield during the previous lactation, longer dry period, higher BCS pre-calving, cows being in the overweight or underweight category pre-calving, greater change in BCS over the transition period, and increased stall stocking density during wk -2 and -1. There were decreased odds of SCK with another health problem (K+) with a greater daily rumination time during wk -1 and +1. Four of these variables were retained in the final multivariable model (Table 2.10). Greater rumination time during the wk after calving was associated with decreased risk of K+, whereas being in the 3rd parity or higher, having a longer dry period, and experiencing greater stall stocking density in the wk prior to calving were associated with increased risk of K+ relative to H.

2.4 DISCUSSION

In this study we characterized the changes in rumination behaviour across the transition period. Both PP and MP cows experienced a reduction in daily rumination time from wk -2 to -1 pre-partum, which may be associated with the common reduction in DMI leading up to calving. Similarly, the daily rumination time of PP and MP cows began to increase from wk +1 to +2, again potentially reflective of changes in DMI. Dry matter intake typically decreases as the cow approaches calving and begins to increase rapidly after calving (Grant and Albright, 1995). While an association of rumination time and DMI is not consistently reported in the literature, there are examples of these being positively associated. Cows have been found to spend more time ruminating about 4 h after periods of high feed intake (Schirmann et al., 2012), however, there was no correlation between periods of DMI and rumination time in that study, possibly due to large variations of these variables both between and within cows. Clement et al. (2014) recently found that rumination time was a significant but small contributor in a DMI prediction model. These researchers suggested that the variability of rumination time within weeks and cows makes it difficult to predict DMI from rumination time.

Rumination time is more consistently associated with dietary NDF intake (Welch and Smith, 1970; Beauchemin et al., 1994) and particle size (Kononoff et al., 2003; Beauchemin and Yang, 2005). Rumination time increases as particle size increases (Beauchemin et al., 1994), unfortunately, we were unable to measure particle size in this study, which may have provided greater insight into differences in rumination times observed. Even though the close-up dry cow diets were greater in NDF than the fresh cow diets in the study herds, the expected changes in DMI across this time period would result in much greater intake of total NDF in the post-partum period. Thus, it is possible that the changes in rumination time were reflective of the changes in DMI across this time period. More research on the association of DMI and rumination of during the transition period is needed, particularly accounting for changes in physical and chemical composition of diets from pre- to post-calving.

In this study, PP cows ruminated 61 min/d less than MP cows during the post-calving period. Maekawa et al. (2002) found PP cows ruminated 52 min/d less than MP cows; this difference was attributed to the greater DMI of MP cows, which also had greater BW and higher milk yields than PP cows. Beauchemin and Rode (1994) also observed lactating MP cows to have a longer daily rumination time; PP and MP cows regurgitated a similar number of boluses, however, MP spent more time chewing each bolus. Soriani et al. (2012) suggested that PP cows suffer more from the stress of environmental changes at the initiation of lactation, and thus show a slower increase in rumination time after calving compared to MP cows. Other researchers have measured rumination time over the transition period and found no difference between PP and MP cows (Soriani et al., 2013; Calamari et al., 2014), but no discussion of this lack of difference was presented in those studies.

Daily rumination time for H, MP cows during the dry period (408 min/d) is within the range of 400 to 450 min/d, reported by Adin et al. (2009) for close-up cows fed the same diet. Soriani et al. (2012) found that daily rumination time averaged 522 min/d during d -10 to -2 pre-calving, which was higher than what was observed in this study for H cows during the same time period. This difference in rumination time is probably due to the greater amount of NDF in their dry cow diet, which was 56% of DM (Soriani et al., 2012), compared to an average of 37% of DM in this study.

The NDF content in the fresh cow diets ranged from 28 to 32% of DM across the 4 commercial dairy farms in this study. Work by Dado and Allen (1995) showed that rumination time in early lactation dairy cows increased from 380 to 500 min/d when NDF content of the ration was increased from 25 to 35%. Daily rumination time averaged 418 min/d and 481 min/d for healthy PP and MP cows respectively, which is comparable to that reported Dado and Allen (1995). These averages are also within the range of 340 to 540 min/d for lactating cows found in the literature (Kononoff and Heinrichs, 2003; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006).

The cumulative incidence of SCK across 25 Ontario farms ranged from 8 to 80% during the first 9 wk postpartum, with a mean of 43% of cows that experienced SCK (Duffield, 2000). McArt et al. (2012) also found a 43% cumulative incidence of ketosis with thrice weekly testing between 3 and 16 DIM, with the peak incidence of ketosis occurring at 5 DIM. These estimates are in line with the 44% cumulative incidence of SCK within the first 4 wk postpartum observed in the present study. It is apparent that SCK is common in commercial dairy herds, but the causes of SCK are not always apparent as there are numerous factors, including parity, breed, BCS, milk yield, dry cow nutrition and management factors, which have been associated with risk of both SCK and clinical ketosis (Andersson, 1988; Duffield, 2000). Increasing parity is a known risk factor for SCK (Suthar et al., 2013; Berge and Vertenten, 2014; Vanholder et al., 2015), which was also found in the present study; the odds of SCK in K+ cows were 8 times higher in 3+ lactation cows compared to H cows in their 2nd lactation. Cows with higher milk production have higher nutrient demands, putting them at a higher risk of developing SCK (Bauman and Currie, 1980; Gröhn et al., 1989; Fleischer et al., 2001), which is why cows in the present study with greater 305 d milk yield in the previous lactation were at increased odds for having SCK with no other health issues. Vanholder et al. (2015) found cows in both the moderate ($3.5 \leq$ BCS ≤ 3.75) and fat BCS categories (BCS ≥ 4) had an increased risk of SCK compared to cows in the thin category (BCS ≤ 3). Cows with greater BCS pre-calving have a greater decrease in DMI prior to calving (Hayirli et al., 2002), which is a major contributing factor for developing SCK postpartum (Goldhawk et al., 2009). Cows in a state of NEB will deplete fat stores to compensate for the high energy demands of lactation (Goldhawk et al., 2009). Therefore, it is not surprising that in our study, a greater loss of BCS over the transition period was associated with increased risk of SCK in cows with no other health problems.

In the present study, each extra 5 d dry above the mean (59 d), increased the odds of developing SCK combined with another postpartum health disorder 1.3 fold. Vanholder et al. (2015) similarly observed this positive association between the length of the dry period and

SCK. It is possible that cows with a longer dry period become over conditioned. Cows consuming the close-up ration longer than the recommended 3 wk have been shown to have increased BCS and risk of metritis post-partum (Mashek and Beede, 2001). It could also be hypothesized that these cows with long dry periods became pregnant later in lactation and were already over conditioned prior to dry off.

Increasing stall stocking density by 5% during the wk prior to calving was found to increase the risk of ketosis by 10% in both K and K+ cows. Overcrowding can limit the ability of cows to access their desired resources, whether that be lying areas, feed, or water, at the times they would prefer. This has the potential to decrease lying time (Munksgaard et al., 2005) and may also impel cows to lay down sooner post-milking (Fregonesi et al., 2007), rather than consume feed at the feed bunk, potentially limiting DMI. Proudfoot et al. (2009b) demonstrated that when subjected to a competitive feeding environment, MP cows showed a decrease in DMI 1 wk before calving. It should be noted that both stall stocking density and feed bunk stocking density were highly variable among study farms. However, in general, more space was provided on these farms than typically seen on commercial dairy farms for transition cows (vonKeyserlingk et al., 2012). In any case, these results suggest that dry cow management should aim to reduce competition for resources by reducing stocking density in close-up dry cow pens.

There is much evidence in the literature supporting the notion that severe NEB in the transition period increases the risk for postpartum diseases such as RP, MF, metritis, mastitis, DA and SCK (Dohoo et al., 1983; Duffield et al., 2009; LeBlanc, 2010). LeBlanc (2010) estimated that 30 to 50% of cows experience some form of health problem around the time of calving. Similar to that, in the current study, 35% of cows diagnosed with SCK had at least one other recorded health problem during the first 4 wk postpartum.

Lower rumination times were observed in K+ cows during wk -1, +1, and +2 compared to H cows. Soriani et al. (2012) categorized cows into 3 groups based on rumination time before calving: longer rumination time, middle rumination time and shorter rumination time. Cows in the shorter group showed a higher incidence of clinical disease (including mastitis, lameness, ketosis and DA) and these cows had a decreased rumination time after calving, similar to what was seen in K+ cows in the current study. This also agrees with the observations made by Calamari et al. (2014) who found that 90% of cows in the low rumination group post-calving had a clinical health problem, compared to 45% cows categorized in the high rumination group.

The odds of developing SCK and another clinical disease were 1.2 times greater for every 20 min/d decrease in rumination time during the week after calving. Although there was also a difference in rumination during the wk prior to calving, the depression in rumination time was much greater in the wk after calving, possibly due to the combined effect of multiple transition disorders occurring post-calving, some of which may have preceded the diagnosis of SCK. In this study, SCK was only diagnosed once weekly, which was a limitation of the study. If cows were ketotic on the day of diagnosis it in unknown if that was the first day of SCK or if the cow had been ketotic for multiple days. This limited our ability to fully understand how rumination changes directly before and after the onset of SCK. Future studies monitoring this association should monitor SCK more frequently to understand the detailed changes in rumination around the onset of illness.

When comparing K to H cows, it was found that for every 20 min/d decrease in rumination time during the week prior to calving, the odds of becoming K post-calving increased 1.1-fold. Low DMI and reduced feeding time have been considered important risk factors for subclinical ketosis. Studies by Gonzalez et al. (2008) and Goldhawk et al. (2009) observed a 10 kg/d reduction in fresh feed intake and 3 kg reduction in daily DMI, respectively, during the week before being diagnosed ketotic. Shorter rumination times in the current study may be indicative of low DMI in the prepartum period (Clement et al., 2014); however, there are many cow-level and management related factors that vary between farms and have a great impact of rumination time.

2.5 CONCLUSIONS

Multiparous cows ruminate longer over the course of a day compared to PP cows during the transition period. Primiparous cows showed no difference in rumination time between health statuses, however K and K+ cows were found to ruminate less than H multiparous cows. Higher rumination times during the week prior to calving and the week after calving were associated with decreased odds of K and K+, respectively, in MP cows. Other factors that were found to decrease the odds of SCK in MP cows included lower stall stocking density (less than 80%) during the week before calving, lower parity, shorter dry period, lower milk yield during the previous lactation, and smaller loss of BCS over the transition period. Rumination monitoring across the transition period may contribute to the identification of MP cows either at risk for developing SCK or those that have SCK in combination with another health problem. To identify MP cows at risk for developing ketosis post-calving, it is important for farms to begin monitoring rumination during the dry period to establish a baseline for each cow.

2.6 ACKNOWLEDGEMENTS

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Table 2.1 Descriptive summary of farm-level variables for lactating cows in an observational study of the associations of rumination time from 2 wk before to 4 wk after calving and subclinical ketosis.

Variable	Herd 1	Herd 2	Herd 3	Herd 4
Number of milking cows	400	145	250	125
Fresh Cows				
Fresh period (DIM)	1 to 11-14	1 to14-21	1 to 28	1 to 21-28
Stall base	Mats and waterbeds	Bedded pack	Rubber mats	Deep bedding
Bedding type[1]	Shavings	Straw	Compost	Sand
Stocking density (%)[2]	62	134	73	99
Stall length (cm)	165	--	178	178
Stall width (cm)	118	--	116	132
Feed bunk design	Post/rail	Headgates	Post/rail	Headgates
Feed bunk space (cm/cow)[3]	84	45	99	44
Use of ionophore in TMR	no	no	yes	No
Lactating Cows[2]				
Lactating period (DIM)	11-14 to 28	14-21 to 28	--[4]	21 to 28
Stall base	Mats and waterbeds	Deep bedding	--	Deep bedding
Bedding type[1]	Shavings	Sand	--	Sand
Stocking density (%)[2]	97	94	--	100
Stall length (cm)	161	160	--	174
Stall width (cm)	121	127	--	117
Feed bunk design	Post/rail	Headgates	--	Headgates
Feed bunk space (cm/cow)[3]	43	25	--	39
Use of ionophore in TMR	no	no	no	No

[1]Surface of stall base in freestall pens

[2]Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack]

[3]Feed bunk space = [length of feed bunk (cm)] / [no. of cows in the pen]

[4]Sample cows did not occupy this pen during the sample period, they remained in the fresh pen up to 4 wk post-calving

Table 2.2 Descriptive summary of farm-level variables for far-off and close-up dry cows in an observational study of the associations of rumination time from 2 weeks before to 4 weeks after calving and subclinical ketosis.

Variable	Herd 1	Herd 2	Herd 3	Herd 4
Far- off Dry Cows				
Far-off period (d before expected calving date)	60 to 21-7	60 to 14	60 to 21	60 to 21-14
Stall base[1]	Rubber mats	Deep bedding	Rubber mats	Deep bedding
Bedding type	Shavings	Sand	Compost	Sand
Stocking density (%)[2]	109	58	106	91
Stall length (cm)	164	156	177	178
Stall width (cm)	119	130	119	125
Feed bunk design	Post/rail	Headgates	Post/rail	Headgates
Feed bunk space (cm/cow)[3]	47	42	83	54
Fresh feed delivery (no./d)	1	1	1	1
Feed push-up frequency (no./d)	6	6	5	5
Use of ionophore in TMR	no	no	yes	no
Close-up Dry Cows				
Close-up period (d before expected calving date)	21-7 to calving	14 to calving	21 to calving	21-14 to calving
Stall base[1]	Bedded pack	Bedded pack	Rubber mats	Bedded pack
Bedding type	Straw	Straw	Compost	Straw
Stocking density (%)[2]	138	58	75	115
Stall length (cm)	--	--	177	--
Stall width (cm)	--	--	119	--
Feed bunk design	Headgates	Headgates	Post/rail	Headgates
Feed bunk space (cm/cow)[3]	29	96	171	130
Fresh feed delivery (no./d)	1	1	1	1
Feed push-up frequency (no./d)	0	6	5	5
Use of ionophore in TMR	no	no	yes	no
Use of choline in TMR	no	yes	no	yes
Use of rumensin bolus pre-calving	no	yes	yes	yes

[1]Surface of stall base in freestall pens

[2]Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack]
[3]Feed bunk space = [length of feed bunk (cm)] / [no. of cows in the pen]

Table 2.3 Feed analysis summary for close-up dry cow and fresh cow feed rations at each participating dairy farm[3] in an observational study of the associations of rumination time and subclinical ketosis over the transition period.

Ration Component	Herd 1	Herd 2	Herd 3	Herd 4
Close-up Dry Cow Ration				
DM (%)	46.6	46.6	43.1	45.7
NDF (% of DM)	38.4	37.5	33.3	41.1
ADF (% of DM)	26.2	23.9	23.0	25.9
NFC (% of DM)	30.0	32.1	34.5	31.8
CP (% of DM)	15.4	14.6	15.8	12.5
ME (Mcal/kg)[1]	2.4	2.4	2.5	2.4
NE_L(Mcal/kg)[2]	1.5	1.4	1.5	1.4
Fresh Cow Ration				
DM (%)	47.8	45.9	48.6	44.7
NDF (% of DM)	32.2	27.8	27.6	29.2
ADF (% of DM)	21.8	19.2	18.6	19.7
NFC (% of DM)	36.7	38.1	39.4	40.3
CP (% of DM)	15.0	17.9	16.6	14.7
ME (Mcal/kg)[1]	2.6	2.7	2.7	2.7
NE_L (Mcal/kg)[2]	1.6	1.6	1.6	1.6

[1]Metabolizable energy (ME)
[2]Net energy for lactation (NE_L)
[3]All numbers presented in the table were determined using NRC (2001) guidelines

Table 2.4 Descriptive summary (± SD) of focal cows sampled in each herd during an observational study of the associations of rumination time and subclinical ketosis over the transition period.

Herd	Number of cows	Mean Parity	Mean 305-d milk production (kg)	Mean length of dry period (d)	Mean pre-calving BCS[1]	Mean post-calving BCS[2]	Change in BCS[3]	% Lame pre-calving[4]	% Lame post-calving[5]
1	79	2.5 ± 1.35	10,710 ± 1,458.1	59 ± 27.7	3.6 ± 0.46	2.9 ± 0.49	-0.6 ± 0.33	7	14
2	98	2.2 ± 1.45	11,205 ± 2,229.5	61 ± 4.9	3.4 ± 0.39	2.9 ± 0.40	-0.5 ± 0.36	2	4
3	91	2.2 ± 1.11	11,294 ± 1,610.5	60 ± 18.7	3.6 ± 0.46	3.0 ± 0.41	-0.6 ± 0.39	6	9
4	71	2.1 ± 1.01	11,016 ± 1,704.5	58 ± 16.2	3.4 ± 0.36	2.9 ± 0.41	-0.5 ± 0.34	1	4
All	339	2.3 ± 1.26	11,066 ± 1,781.1	59 ± 18.7	3.5 ± 0.43	3.0 ± 0.43	-0.5 ± 0.37	4	8

[1]Pre-calving BCS was recorded at the time of enrollment in the study, 2 to 3 wk prior to the expected calving date
[2]Post-calving BCS was recorded at the time of removal from the study, 4 wk after the calving date
[3] Change in BCS = BCS at enrollment - BCS at time of removal of study
[4] Pre-calving lameness score was recorded at the time of enrollment in the study, 2 to 3 wk prior to the expected calving date; % Lame pre-calving = [(no. of cows with a lameness score ≥ 3 pre-calving)/(total number of cows scored pre-calving)]*100
[5]Post-calving lameness score was recorded at the end of the study, 4 wk after the calving date; % Lame post-calving = [(no. of cows with a lameness score ≥ 3 post-calving)/(total number of cows scored post-calving)]*100

Table 2.5 Health status summary of focal cows sampled in each herd during an observational study of the associations of rumination time and subclinical ketosis over the transition period.

Herd	Mean ± SD d diagnosed ketotic (DIM)	% Ketotic[1]	% treated for RP[2]	% treated for metritis	% treated for DA[3]	% treated for MF[4]	% treated for foot problems	% treated for mastitis	% K[5]	% K+[6]	% H+[6]
1	6 ± 6.3	56	9	11	0	4	0	5	39	17	6
2	6 ± 7.1	27	11	32	1	2	0	5	12	14	24
3	5 ± 6.4	51	9	13	3	0	4	4	35	15	10
4	11 ± 7.6	48	3	27	0	3	3	0	31	17	10
All	7 ± 7.1	44	8	21	1	2	2	4	29	16	

[1]Cumulative incidence over 4 tests, once weekly in the first 4 weeks postpartum
[2]Percentage of cows with retained placenta (RP)
[3]Percentage of cows treated for displaced abomasum (DA)
[4]Percentage of cows treated for milk fever (MF)
[5]Percentage of cows with ketosis and no other health issue (K)
[6]Percentage of cows with ketosis and at least one other health issue (K+)
[7]Percentage of cows that were not subclinically ketotic but had at least one other health issue (H+)

Table 2.6 Least squares means (± SE) for daily rumination time (min/d) for healthy cows without subclinical ketosis or other recorded illnesses (H), subclinically ketotic cows with no other health problems (K), and subclinically ketotic cows with other health problems (K+) during each week of the study period.[1]

		Period (relative to calving)					
Health Status	n	wk -2	wk -1	wk +1	wk +2	wk +3	wk +4
Primiparous							
H	52	407.1 ± 13.73	376.1 ± 11.81	375.0 ± 10.61	438.6 ± 12.50	439.0 ± 14.24	421.3 ± 17.00
K	21	421.0 ± 25.15	373.5 ± 21.34	392.8 ± 16.70	464.9 ± 19.66	460.0 ± 22.31	434.4 ± 26.71
K+	14	380.0 ± 25.79	342.3 ± 22.38	365.9 ± 20.45	450.9 ± 24.08	427.8 ± 27.33	395.1 ± 32.87
Multiparous							
H	87	420.0 ± 12.07	401.6 ± 11.99	429.6 ± 11.54	509.1 ± 13.02	503.8 ± 14.11	488.7 ± 14.83
K	76	406.4 ± 12.65	374.4 ± 12.90†	407.9 ± 12.08†	483.5 ± 13.71	477.3 ± 15.05	453.2 ± 15.84†
K+	39	405.4 ± 16.62	353.5 ± 16.04**	356.4 ± 15.19***	444.2 ± 17.67**	463.2 ± 19.98†	468.1 ± 21.71

[1]Significance level for difference between K and H cows and K+ and H cows within weeks: †$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Table 2.7 Unconditional estimates for factors associated with the incidence of subclinical ketosis with no recorded clinical disease (K; n = 76) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Percentage or Mean (±SD)[1]	Odds ratio (95% CI)[2]	*P*-value
Parity	--	--	0.002
2	51%	Ref[7]	--
3+	49%	2.9 (1.49 to 5.65)	--
305 d milk yield (kg)	11,060 (1,785.0)	1.2 (0.88 to 1.73)	0.21
Length of dry period (d)	59 (19.0)	1.7 (1.00 to 2.86)	0.049
BCS pre-calving	3.4 (0.44)	1.3 (0.96 to 1.88)	0.083
BCS category pre-calving[3]	--	--	0.17
Normal	70%	0.9 (0.20 to 4.32)	--
Underweight	5%	Ref	--
Overweight	25%	2.1 (0.95 to 4.54)	--
Change in BCS[4]	0.5 (0.37)	1.6 (1.13 to 2.29)	0.0081
Stocking density (%)[5]			
w -2	84 (23.6)	1.6 (1.15 to 2.29)	0.0063
w -1	79 (24.5)	1.6 (1.15 to 2.16)	0.0053
w +1	81 (14.8)	0.6 (0.36 to 0.87)	0.011
Rumination time (min/d)			
w -1	382 (85.6)	0.7 (0.46,0.97)	0.036

[1] Proportion of animals for categorical variables or mean and standard deviation for continuous variables.

[2] Odds ratio and 95% CI for 1 SD increase in the variable presented

[3] Cows were placed into 1 of 3 categories based on their body condition score pre-calving: normal (BCS 3 - 3.5), underweight (BCS 1 - 2.5), overweight (BCS 4 - 5)

[4] Change in BCS = BCS at enrollment - BCS at time of removal of study

[5] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11 m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack]

[6] Feed bunk space = length of feed bunk (cm) / no. of cows in the pen

[7] Ref = reference category

Table 2.8 Final logistic regression model for factors associated with the incidence of subclinical ketosis with no other health issues (K; n = 76) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Coefficient	SE	Odds ratio (95% CI)[1]	*P*-value
Intercept	-3.27	2.042	--	0.21
305 d milk yield (kg)	0.00024	0.000133	1.5 (0.96 to 2.47)	0.073
Change in BCS[2]	1.68	0.622	1.9 (1.18 to 2.94)	0.0083
Stocking density (%)[3]				
w -1	0.02	0.009	1.7 (1.10 to 2.58)	0.018
Rumination time (min/d)				
w -1	-0.01	0.003	0.6 (0.38 to 0.97)	0.037

[1]Adjusted odds- ratio and 95% CI for 1 SD increase in each variable in the model. The mean ± SD for each variable are as follows: 11,060 ± 1,785.0 kg, 305 d milk yield; 0.5 ± 0.37, change in BCS; 80 ± 24.8 %, stocking density (wk -1); 382 ± 85.6 min/d, rumination time (wk -1).

[2] Change in BCS = BCS at enrollment - BCS at time of removal of study.

[3] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack].

Table 2.9 Unconditional estimates for factors associated with the incidence of subclinical ketosis with other health problems (K+; n = 39) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Percentage or Mean (±SD)[1]	Odds ratio (95% CI)[2]	*P*-value
Parity	--	--	<0.001
2	51%	Ref[6]	--
3+	49%	5.5 (2.35 to 12.92)	--
305 d milk yield (kg)	11,061 (1,785.0)	1.5 (1.02 to 2.18)	0.039
Length of dry period (d)	59 (19.0)	1.9 (1.05 to 3.27)	0.034
BCS pre-calving	3.4 (0.44)	1.3 (0.90 to 1.94)	0.15
BCS category pre-calving[3]	--	--	0.14
Normal	70%	Ref	--
Underweight	5%	1.1 (0.20 to 6.25)	--
Overweight	25%	2.4 (1.00 to 5.87)	--
Change in BCS[4]	0.5 (0.37)	1.5 (0.97 to 2.24)	0.068
Stocking density (%)[5]			
w -2	84 (23.6)	1.4 (0.97 to 2.13)	0.071
w -1	79 (24.5)	1.9 (1.22 to 2.92)	0.0048
Rumination time (min/d)			
w -1	382 (85.6)	0.6 (0.40 to 0.92)	0.019
w +1	407 (87.4)	0.4 (0.25 to 0.63)	<0.001

[1] Proportion of observations for categorical variables or mean and standard deviation for continuous variables.
[2]Odds- ratio and 95% CI for 1 SD in variable presented
[3] Cows were placed into 1 of 3 categories based on their body condition score pre-calving: normal (BCS 3 - 3.5), underweight (BCS 1 - 2.5), overweight (BCS 4 - 5)
[4]Change in BCS = BCS at enrollment - BCS at time of removal of study
[5] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack].
[6] Ref = reference category

Table 2.10 Final logistic regression model for factors associated with the incidence of subclinical ketosis with other health problems (K+; n = 39) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Coefficient	SE	Odds ratio (95% CI)[1]	*P*-value
Intercept	-3.67	2.750	--	0.27
Parity				<0.001
2	Ref[3]	--	--	--
3+	2.09	0.580	8.1 (2.55 to 25.43)	--
Length of dry period (d)	0.06	0.032	2.9 (0.87 to 9.56)	0.083
Stocking density (%)[2]				
w -1	0.02	0.012	1.8 (1.01 to 3.27)	0.046
Rumination time (min/d)				
w +1	-0.01	0.003	0.5 (0.27 to 0.80)	0.0063

[1]Adjusted odds- ratio and 95% CI for 1 SD increase in each variable in the model. The mean ± SD for each variable are as follows: 59 ± 19.0 d, length of dry period; 80 ± 24.8%, stocking density (wk -1); 382 ± 85.6 min/d, rumination time (wk -1).

[2] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack].

[3] Ref = reference category.

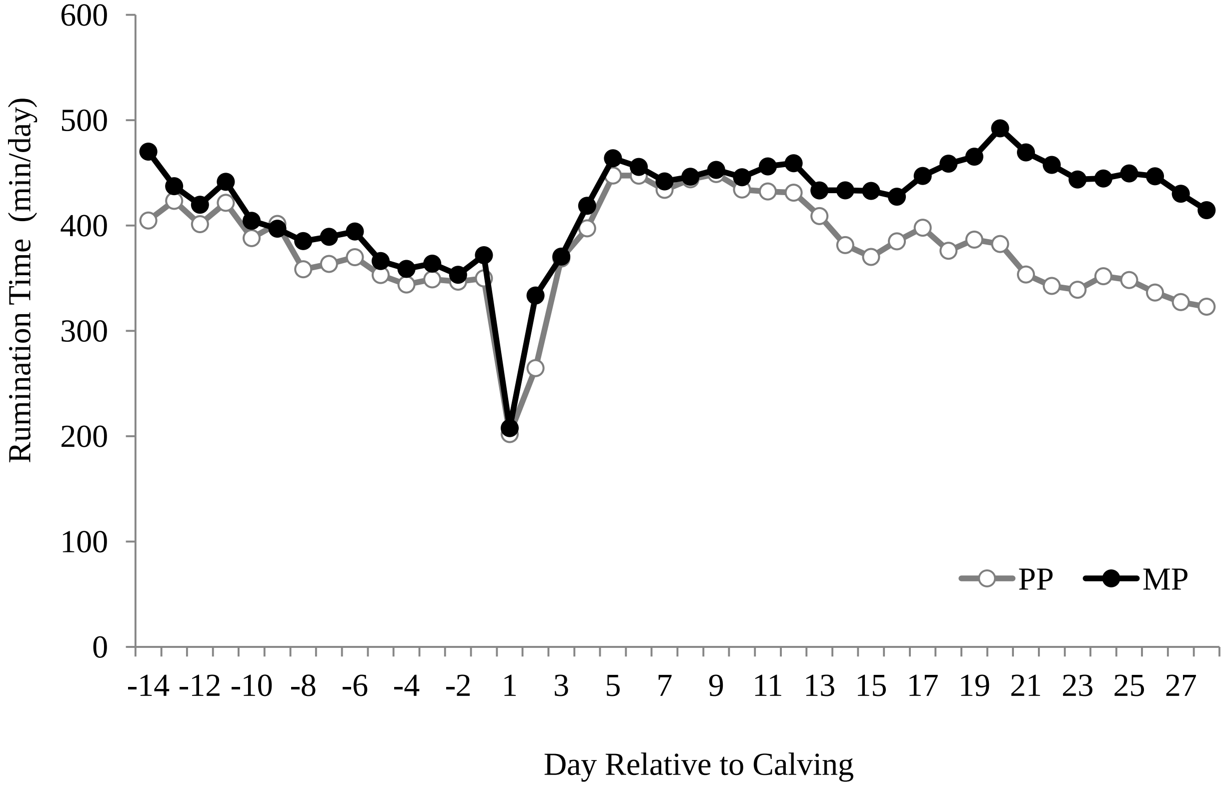

Figure 2.1 Daily rumination time (min/d) over the transition period (-14 to 28d) for primiparous (PP, n = 107) and multiparous (MP, n = 232) cows during an observational study of the associations of rumination time and subclinical ketosis over the transition period.

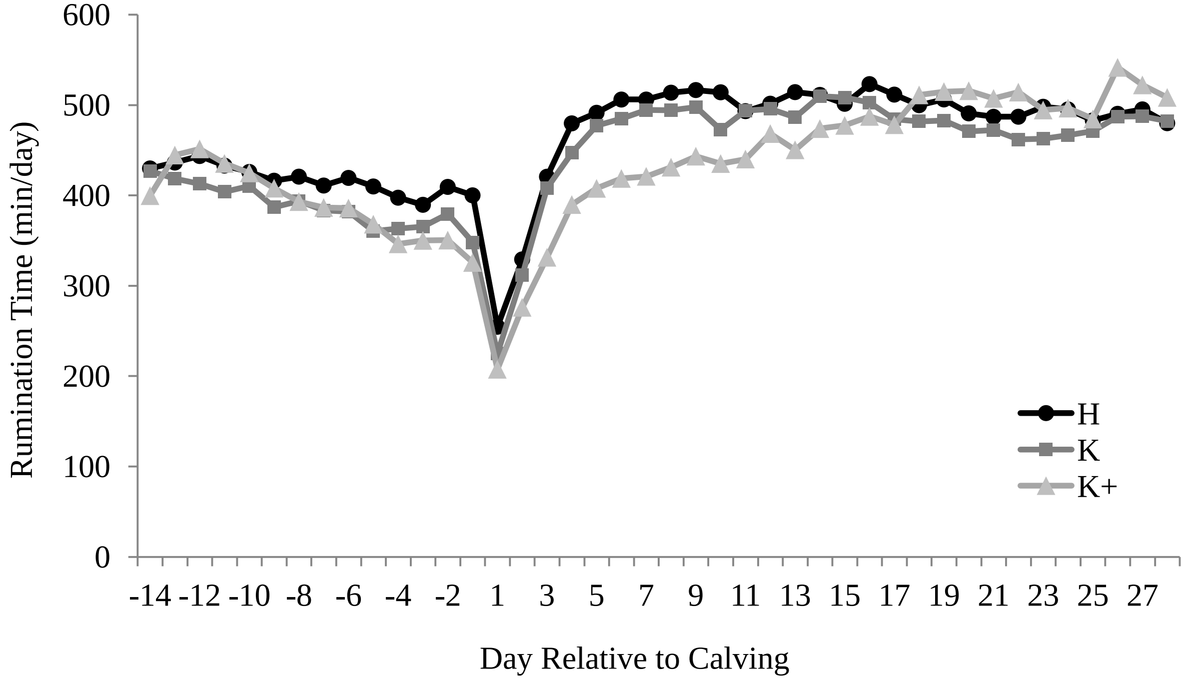

Figure 2.2 Daily rumination time (min/d) over the transition period (-14 to 28d) for healthy multiparous (MP) cows with no other recorded illnesses (H; n = 87), ketotic MP cows with no other health problems (K; n = 76) and ketotic MP cows with other health problems (K+; n = 39).

CHAPTER 3: The association between lying behaviour and subclinical ketosis in transition dairy cows

3.1 INTRODUCTION

High-producing dairy cows experience negative energy balance (NEB) during the transition period (Ingvartsen, 2006) due to a decrease in DMI intake in the days leading up to calving (Grant and Albright, 1995; Schirmann et al., 2013) accompanied by high energy requirements for lactation. These cows undergo many physiological changes to cope with the high energy demands of lactogenesis. An excessive drop in DMI around calving combined with prolonged NEB may lead to sub-clinical ketosis (SCK) (Grummer, 1995).

Technological improvements have enhanced on-farm detection of SCK. Cows in NEB begin to mobilize fat stores in an attempt to meet their high energy needs during early lactation, which releases ketone bodies (i.e. BHBA) into the blood (Baumen and Currie, 1980; Goldhawk et al., 2009; LeBlanc, 2010). An electronic cow-side test for the quantification of blood BHBA concentration (Precision Xtra Abbott Diabetes Care, Saint Laurent, QC, Canada), has been validated in dairy cows (Iwersen et al., 2009; Voyvoda and Erdogan, 2010).

Even with various methods for detecting ketosis available, it is still challenging to for producers to identify SCK at an early stage. There is growing evidence that measurements of activity and feeding behaviour may be used to pre-emptively identify cows at risk for subclinical illness (Weary et al., 2009). In addition to feeding behaviour (Goldhawk et al., 2009), rumination behaviour (Chapter 2), and walking activity (Edwards and Tozer, 2004), there is potential for SCK to be identified through changes in lying behaviour. Lying behaviour may be a promising indicator of metabolic conditions, particularly during the peripartum period. Itle et al (2015) recently found cows with SCK post calving spent more time standing in the week before calving.

It may be possible that SCK cows in this study were subordinate cows that spent more time standing, waiting to access the feed bunk. Cows with SCK are in a state of excessive NEB, as ketone bodies in the blood rise with low glucose availability, cow may spend more time lying down to decrease energy expenditure. Increased lying time in sick cows agrees with the concept that sick animals become less active in attempt to conserve energy needed to facilitate recovery (Hart, 1988; Dantzer and Kelley, 2007).

The objective of this study was to characterize changes in lying behaviours across the transition period and determine if daily lying time, frequency of lying bouts and bout duration may be used to identify cows at risk for SCK. We hypothesized that early lactation dairy cows with increased lying activity, both pre- and post-calving, would be at higher risk of experiencing SCK in early lactation.

3.2 MATERIALS AND METHODS

This research is part of a larger study aimed at evaluating the usefulness of rumination monitoring for the early detection of SCK. As such, detailed descriptions of the methodology are presented in Chapter 2.

3.2.1 ***Animals and Disease Diagnosis***

A total of 339 dairy cows (107 primiparous and 232 multiparous) on 4 commercial dairy farms were monitored for lying behaviour and SCK from 14 d prior to calving until 28 d after calving. A blood sample was taken from the coccygeal vein of each cow for measurement of BHBA 1x/wk. Cows with BHBA $\geq$1.2mmol/L at one or more weekly sample postpartum were considered to have SCK. Cases of retained placenta, metritis, milk fever, or mastitis during the study period were also recorded. Cows were categorized into 1 of 4 groups: healthy (H) cows had no SCK or any other recorded clinical disease (n=139); healthy plus (H+) cows were not diagnosed with SCK but were treated for at least one other health problem (n=50); SCK (K) cows with no other health problems during transition (n=97); or ketotic plus (K+) cows that had SCK and one or more other clinical diseases (n=53). Animal use, data collection, and study design were approved by the University of Guelph's Animal Care Committee and Research Ethics Board, respectively.

3.2.2 Lying Behaviour

All enrolled cows were affixed weekly with a data logger (Onset HOBO Pendant G data loggers; Onset Computer Corporation, Bourne, MA) to record standing and lying behaviour for 7 d, as validated by Ledgerwood et al. (2010). Individual data loggers were placed on the medial side of a hind leg, and secured with bandaging wrap (Vetrap Bandaging Tape, 3M, London, Ontario, Canada). To ensure accurate and consistent collection of data, each data logger was positioned identically on each cow as described by Ledgerwood et al. (2010). During each weekly farm visit, another data logger was attached to the cow's opposite hind leg and the data logger that had been recording data for the past week was removed. Data were downloaded weekly to the database; recordings were used to calculate daily measurements of lying time (min/d), frequency of lying bouts (no. of bouts/d), and average lying bout length (min; UBC AWP, 2013).

3.2.3 Statistical Analysis

Cows that had aborted (n=2), were culled (n=2), or diagnosed ketotic (n=3) before calving were not included in the statistical analysis. Cows that were sold (n=22) or died (n=1) during the post-calving period, with behavioural and health measurements recorded until the day they left the herd, were included in the analysis. Descriptive statistics were performed on a total of 339 cows, (107 primiparous and 232 multiparous) categorized as H (n=139), H+ (n=50), K (n=97) and K+ (n=53). The linear and logistic models compared the measures of lying behaviour between K cows and H cows and K+ cows and H cows; H+ cows were not included in this analysis (n=50).

Statistical analyses were performed with SAS (version 9.4; SAS Institute, 2013) using cow (n = 289) as the experimental unit. Daily lying times (min/d), average frequency of lying bouts per day (bouts/d) and average lying bout lengths (min) were each summarized by cow and week such that these data aligned with the once weekly testing of SCK. These data were analyzed in a general linear mixed model (PROC MIXED in SAS), treating week as a repeated measure. Separate models were used to analyze lying time, bout frequency, and bout length. Each model included the random effect of farm and cow within farm (subject of repeated statement) and the fixed effects of health status, parity, and week, the interactions of health status by parity and health status by week, as well as the three-way interaction of health status, parity, and week. The covariance structure was heterogeneous compound symmetry, selected by best fit according to Schwarz's Bayesian information criterion. A three-way interaction was found between health status, parity, and week ($P < 0.001$) when analyzing daily lying time and average number of lying bouts; thus, first lactation and multiparous (MP) cows were analyzed separately. These separate models included the fixed effects of health status, week and the interaction between health status by week, with farm included as a random effect. Differences in lying behaviour between health categories and weeks were compared using the least-squares means procedure with the PDIFF function. Significance was declared at $P < 0.05$, and tendencies were reported if $0.05 < P < 0.10$.

In the analysis of the impact on health status on lying behaviours, as described above, differences were only found between health categories for MP cows. Thus, multivariable logistic regression was only performed on data from MP cows and not on PP cows. The analysis was performed using the GLIMMIX procedure (distribution = binomial and link = logit) in SAS (version 9.4; SAS Institute, 2013) to model to effects of lying behaviour and other cow-level factors on the presence or absence of SCK. This was done using two different models: one model compared K to H cows while the other compared K+ to H cows. Parity and pre-calving BCS were both treated as categorical variables. Multiparous cows were characterized as second lactation (2; n= 99) or third lactation and greater (3+; n= 103). Body condition pre-calving was categorized into three groups: underweight, BCS < 3; normal, BCS = 3 to 3.5; overweight, BCS > 3.5. Parity, pre-calving BCS category, change in BCS over the transition period, length of dry period, milk yield from the previous lactation, as well as lying time, number of lying bouts per day, average bout length, and stall stocking density during the weeks prior to the mean day of diagnosis (wk -2, -1, and +1), were all assessed for an association with presence or absence of K and K+ using univariable logistic regression models. Variables with $P \leq 0.25$ were then used to construct a multivariable logistic regression model. The CORR procedure in SAS was used to check for correlations between the explanatory variables included in the multivariable model. If 2 variables were highly correlated ($r > 0.8$), the variable with the lowest *P*-value and most biological relevance was retained for the multivariable model. Manual backward elimination of nonsignificant and non-trending ($P > 0.10$) variables was used to create the final models and from the resultant models, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those variables retained in the final multivariable models are presented.

3.3 RESULTS AND DISCUSSION

From 2 wk prior to calving until 4 wk after calving, H, K and K+ cows in their first lactation showed no difference (*P* = 0.4) in daily lying time, however there was an effect of week (*P* < 0.001; Table 1). The lying time of PP cows decreased each week from wk -2 to +1. PP cows also had lower daily lying times compared to MP cows (Figure 1a). PP cows were lying on average 385.5 ± 11.17 min/d (mean ± SD) during the pre-calving period and 424.0 ± 15.52 min/d during the post-calving periods (Table 3.1) compared to 394.5 ± 6.32 min/d and 456.4 ± 12.67 min/d for MP cows, respectively (Table 3.2). Steensels et al. (2012) reported that that MP cows, milked 3x/d, lay down between 491 and 578 min/d in the first 28 d after calving. The difference between parities observed in the present study agrees with that reported by Sepúlveda-Varas et al. (2014), who found that PP cows spent less time lying down than MP cows on pasture during the transition period. Steensels et al. (2012) hypothesized that higher lying times in later parities may be due to increased BW with age.

Lying time also decreased for all MP cows moving from wk -1 to +1 (*P* < 0.001; Table 3.2; Figure 3.1a). Calderon and Cook (2011) saw lying time decrease for both PP and MP cows from d -16 until calving, and post-calving lying time re-stabilize at roughly 590 - 650 min/d. Another study monitoring change in standing behaviour from -10 d to +10 d observed lying time to be 702 min/d pre-calving and 636 min/d post-calving, similar to the trend in the current study (Huzzey et al., 2005). We hypothesize that cows spend more time standing post-calving because they are dedicating more of their time to milking, as well as feeding, to support milk production (Huzzey et al., 2005; Gomez and Cook, 2010).

The PP cow model for frequency of lying bouts showed no effects of health status (*P* = 0.3), week (*P* = 0.7), or interaction between these variables (*P* = 0.9; Table 3.1). Table 3.2

shows the frequency of lying bouts for MP cows; health status did not show an effect (P = 0.3), but week did have an effect on frequency of lying bouts (P < 0.001). MP cows had fewer lying bouts in wk -2 compared to wk -1, but frequency of lying bouts increased each wk moving from wk +1 to +3 (P < 0.01). The difference between parities in the number of lying bouts over the transition period (Figure 3.1b; PP: 10.4 ± 0.53 bouts/d vs. MP: 9.6 ± 0.46 bouts/d) is similar to that reported by a study on cows that had access to pasture (PP: 9.7 ± 0.54 vs. MP: 8.4 ± 0.26 bouts/d; Sepúlveda-Varas et al., 2014). Other studies in the literature performed in freestall facilities reported similar frequencies of lying bouts for transition cows: approx. 10.5 bouts/d (Calderon and Cook, 2011); 11.1 ± 0.4 (Steensels et al., 2012).

Lying bout length differed between parities (P < 0.001) and changed by week (P < 0.001), but was not impacted by health status (P=0.28; Tables 3.1 and 3.2). All cows showed a decrease in average lying bout length each week from wk -2 to +1, but moving into wk +2, bout length increased. Over the transition period, PP cows had shorter lying bout durations compared to MP cows (Figure 3.1c). Sepúlveda-Varas et al. (2014) also found PP cows to have shorter lying bouts, however, bout duration for PP and MP cows did not change over the transition period in their study.

Figure 3.2 shows the difference in daily lying time between H, K and K+ multiparous cows over the transition period. An interaction of health status and week was detected (P < 0.001) when comparing the daily lying time of MP cows, as seen in Table 3.2. Differences in lying time were seen for MP cows in wk +3 and +4, where K cows spent more time lying down than H cows. K cows tended to lie down longer throughout the day during wk -1 and +1. As the major difference in lying time was seen during wk 3 and 4 and the average day of SCK diagnosis was 7 DIM, we hypothesize that because these cows were sick, they spent more time lying down

(Hart, 1988; Dantzer and Kelley, 2007). Interestingly, another study found that cows that were clinically ketotic postpartum stood longer per day during the week before calving (Itle et al., 2015), which was quite different from the tendency of K cows to lying down longer in the current study. Those researchers suggested that the cows that later developed ketosis may have been more subordinate and, therefore, spent more time standing waiting to feed rather than competing for a spot at the feed bunk.

Differences in lying time were seen also for MP cows in wk +1 when K+ cows spent more time lying down than H cows, and during wk +2 when K+ cows tended to spend more time lying down than H cows (Table 3.2; Figure 3.2). Sepúlveda-Varas et al. (2014) looked at the post-calving differences in lying time between cows with no health issues or lameness and compared them to cows with one, and cows with greater than one, clinical postpartum disease (excluding lameness). Cows with multiple illnesses showed greater changes in lying time than those with only one illness, which is also observed in the current study. However, it was PP cows with more than one illness that had greater lying times during the first few days post-calving and no difference in lying time was seen between these groups in MP cows (Sepúlveda-Varas et al., 2014).

Table 3.3 shows the unconditional associations of the independent variables for H versus K multiparous cows. The final multivariable model, summarized in Table 3.4, found higher lactation (3+), longer dry period, greater loss in BCS over the transition period, and greater stall stocking density in wk -1 to be associated with increased odds of SCK, as was found in Chapter 2. Neither daily lying time nor lying bout length were retained in the final model comparing H and K cows. As there was only a tendency for K cows to lie down longer during the wk after calving (Table 2), this difference in lying time was not large enough to be associated with increased odds of SCK.

Unconditional associations of the independent variables for H versus K+ multiparous cows are shown in Table 3.5. Table 3.6 describes the 4 variables retained in the multivariable model for H versus K+ status. Cows with a higher parity, longer dry period, greater stall stocking density in wk -1 and longer daily lying time during wk +1 were associated with having increased odds of SCK with at least one other clinical disease.

Researchers have observed various associations between lying behaviour and postpartum illnesses. Proudfoot et al. (2009a) found cows with dystocia to have a greater number of lying bouts compared to cows without calving difficulty in the 48 h before calving which persisted until 48 h post-calving. A study that looked at standing behaviour of hypocalcaemic cows found these cows lay down less during the 24 h before calving, but they lay down longer in the wk after calving (Jawor et al., 2012). Itle et al. (2015) found clinically ketotic cows stood longer throughout the week before calving than healthy cows, but saw no difference in standing time post-calving. The current study found a 30 min increase in lying time per day during the wk after calving was associated with 1.2 times higher odds of being K+. The mean day of diagnosis for SCK in the current study was 7 DIM. Cows with SCK are in a state of NEB and may very well lie down for longer periods of time to conserve energy (Hart, 1988) needed for milk production. It may also be that cows that spend more time lying down are spending less time at the feed bunk and, therefore, consuming less feed. Goldhawk et al. (2009) found cows with SCK spent less time at the feeder and visited the feeder fewer times during the wk before calving. We cannot determine if SCK and other illnesses are directly causing the increase in lying behaviour or vice versa, however, future research that aims to understand the motivation behind lying behaviour in sick cows may be beneficial in indentifying subclinical illness pre-emptively.

3.4 CONCLUSIONS

Multiparous cows had a greater daily lying time, less lying bouts and, longer lying bout durations compared to PP cows during the transition period. Primiparous cows showed no difference in daily lying time or frequency or duration of lying bouts between health statuses, however K and K+ cows were found to lie down longer than H multiparous cow during the post-calving period. Increased odds of SCK occurring with another postpartum health issue (K+) was associated with longer daily lying time during the week after calving. Overall, these results suggest that monitoring lying behaviour across the transition period may not be useful for the early identification of SCK, but may contribute to the identification of MP cows that have SCK in combination with another health issue.

3.5 ACKNOWLEDGEMENTS

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Table 3.1 Least squares means (± SE) for primiparous cow lying behaviour for healthy cows with no other illnesses (H, n = 52), and subclinically ketotic cows with no other health issues (K, n = 21) and subclinically ketotic cows with other health issues (K+, n = 14) during each week over the transition period (wk -2, -1, +1, +2, +3, +4).[1]

	Period					
Health Status	wk -2	wk -1	wk +1	wk +2	wk +3	wk +4
Lying time (min/d)						
H	407.1 ± 13.73	376.1 ± 11.81	375.0 ± 10.61	438.6 ± 12.50	439.0 ± 14.24	421.3 ± 17.00
K	421.0 ± 25.15	373.5 ± 21.34	392.8 ± 16.70	464.9 ± 19.66	460.0 ± 22.31	434.4 ± 26.71
K+	380.0 ± 25.79	342.3 ± 22.38	365.9 ± 20.45	450.9 ± 24.08	427.8 ± 27.33	395.1 ± 32.87
Lying bouts (bouts/d)						
H	9.8 ± 0.82	10.4 ± 0.84	10.6 ± 0.78	10.8 ± 0.78	10.9 ± 0.80	10.6 ± 0.77
K	10.3 ± 1.08	10.9 ± 1.04	10.8 ± 0.93	10.5 ± 0.94	10.9 ± 0.98	11.2 ± 0.92
K+	9.5 ± 1.12	9.4 ± 1.19	10.1 ± 1.03	9.5 ± 1.04	9.3 ± 1.09	9.7 ± 1.00
Bout length (min)						
H	85.2 ± 5.66	69.7 ± 5.15	57.3 ± 4.57	57.5 ± 4.62	59.6 ± 4.70	61.2 ± 4.61
K	73.9 ± 8.06	60.5 ± 6.39	53.8 ± 5.23	55.6 ± 5.35	54.9 ± 5.52	52.3 ± 5.38
K+	84.6 ± 8.44	76.8 ± 7.33	61.3 ± 5.65	66.2 ± 5.81†	67.7 ± 6.04	62.1 ± 5.83*

[1]Significance level for difference between K and H cows and K+ and H cows within weeks: †P ≤ 0.10; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

Table 3.2 Least squares means (± SE) for multiparous cow lying behaviour for healthy cows with no other illnesses (H, n = 87), and subclinically ketotic cows with no other health issues (K, n = 76) and subclinically ketotic cows with other health issues (K+, n = 39) during each week over the transition period (wk -2, -1, +1, +2, +3, +4).[1]

	Period					
Health Status	wk -2	wk -1	wk +1	wk +2	wk +3	wk +4
Lying time (min/d)						
H	771.7 ± 22.25	741.9 ± 23.42	571.2 ± 21.77	591.2 ± 20.88	568.3 ± 20.53	552.1 ± 21.38
K	752.6 ± 22.83	764.7 ± 24.17	606.0 ± 22.38†	622.4 ± 21.43†	612.0 ± 21.09**	593.3 ± 22.07*
K+	742.9 ± 27.58	703.4 ± 29.38	663.1 ± 26.30***	629.3 ± 24.76†	589.5 ± 24.35	578.5 ± 26.40
Lying bouts (bouts/d)						
H	8.9 ± 0.48	10.3 ± 0.55	10.1 ± 0.47	9.1 ± 0.44	8.5 ± 0.43	8.5 ± 0.44
K	9.4 ± 0.49	11.3 ± 0.57	10.6 ± 0.49	9.3 ± 0.46	9.3 ± 0.45	9.3 ± 0.46
K+	8.9 ± 0.63	10.7 ± 0.74	11.2 ± 0.61	9.7 ± 0.56	8.6 ± 0.55	8.8 ± 0.57
Bout length (min)						
H	97.8 ± 4.78	80.8 ± 3.96	63.4 ± 3.21	71.9 ± 3.55	73.7 ± 3.53	71.7 ± 3.57
K	94.4 ± 5.00	78.3 ± 4.13	66.5 ± 3.31	76.0 ± 3.70	75.5 ± 3.69	73.7 ± 3.73
K+	94.8 ± 6.72	77.9 ± 5.27	69.8 ± 3.94	73.0 ± 4.58	75.8 ± 4.61	71.8 ± 4.75

[1]Significance level for difference between K and H cows and K+ and H cows within weeks: †$P \leq 0.10$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Table 3.3 Unconditional estimates for factors associated with the incidence of subclinical ketosis with no other health issues (K; n = 76) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Percentage or Mean (±SD)[1]	Odds ratio (95% CI)[2]	*P*-value
Parity			0.002
2	51%	Ref[6]	--
3+	49%	2.9 (1.49 to 5.65)	--
Milk yield at 305 DIM (kg)	11,061 (1,785.0)	1.2 (0.88 to 1.73)	0.21
Length of dry period (d)	59 (19.0)	1.7 (1.00 to 2.86)	0.049
BCS pre-calving	3.4 (0.4)	1.3 (0.96 to 1.88)	0.083
BCS category pre-calving[3]			0.17
Normal	70%	Ref	--
Underweight	5%	0.9 (0.20 to 4.32)	--
Overweight	25%	2.1 (0.95 to 4.54)	--
Change in BCS[4]	0.5 (0.4)	1.6 (1.13 to 2.29)	0.0081
Stocking density (%)[5]			
w -2	84 (23.6)	1.6 (1.15 to 2.29)	0.0063
w -1	79 (24.5)	1.6 (1.15 to 2.16)	0.0053
w +1	81 (14.8)	0.6 (0.36 to 0.87)	0.011
Daily lying time (min/d)			
w +1	601 (131.3)	1.3 (0.95 to 1.85)	0.09
Bout length (min)			
w +1	66 (19.4)	1.2 (0.88 to 1.71)	0.22

[1] Proportion of observations for categorical variables or mean and standard deviation for continuous variables.

[2]Odds- ratio and 95% CI for 1 SD in variable presented.

[3] Cows were placed into one of 3 categories based on their body condition score pre-calving: normal (BCS 3 - 3.5), underweight (BCS 1 - 2.5), overweight (BCS 4 - 5).

[4] Change in BCS = BCS at enrollment - BCS at time of removal of study.

[5] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11 m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack].

[6] Ref = reference category.

Table 3.4 Final logistic regression model for factors associated with the incidence of subclinical ketosis with no other health issues (K; n = 76) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Coefficient	SE	Odds ratio (95% CI)[1]	*P*-value
Intercept	-5.02	1.456	--	0.041
Parity				0.004
2	--	--	Ref[2]	--
3+	1.15	0.393	3.17 (1.46,6.90)	--
Length of dry period (d)	0.03	0.017	1.80 (0.95,3.40)	0.069
Change in BCS[2]	1.47	0.550	1.72 (1.15,2.58)	0.0086
Stocking density (%)[3]				
wk -1	0.02	0.009	1.75 (1.14, 2.67)	0.011

[1] Adjusted odds- ratio and 95% CI for 1 SD increase in each variable in the model. The mean ± SD for each variable are as follows: 59 ± 19 d, dry period length; 0.5 ± 0.37, change in BCS; 80 ± 24.8 %, stocking density (wk -1).
[2] Change in BCS = BCS at enrollment - BCS at time of removal of study.
[3] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack].

Table 3.5 Unconditional estimates for factors associated with the incidence of subclinical ketosis with other health problems (K+; n = 39) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Percentage or Mean (±SD)[1]	Odds ratio (95% CI)[2]	*P*-value
Parity			<0.001
2	51%	Ref[6]	--
3+	49%	5.5 (2.35 to 12.92)	--
Milk yield at 305 DIM (kg)	11,061 (1,785.0)	1.5 (1.02 to 2.18)	0.039
Length of dry period (d)	59 (19.0)	1.9 (1.05 to 3.27)	0.034
BCS pre-calving	3.4 (0.44)	1.3 (0.90 to 1.94)	0.15
BCS category pre-calving[3]			0.14
Normal	70%	Ref	--
Underweight	5%	1.1 (0.20 to 6.25)	--
Overweight	25%	2.4 (1.00 to 5.87)	--
Change in BCS[4]	0.5 (0.37)	1.5 (0.97 to 2.24)	0.068
Stocking density (%)[5]			
w -2	84 (23.6)	1.4 (0.97 to 2.13)	0.071
w -1	79 (24.5)	1.9 (1.22 to 2.92)	0.0048
Daily lying time (min/d)			
wk -1	741 (151.4)	0.8 (0.53 to 1.11)	0.16
wk +1	601 (131.3)	2.3 (1.42 to 3.63)	0.0007
Number of lying bouts (bouts/d)			
wk +1	11 (3.3)	1.4 (0.96 to 2.23)	0.077
Bout length (min)			
wk +1	66 (19.4)	1.4 (0.93 to 2.01)	0.11

[1] Proportion of observations for categorical variables or mean and standard deviation for continuous variables.
[2]Odds- ratio and 95% CI for 1 SD in variable presented.
[3] Cows were placed into one of 3 categories based on their body condition score pre-calving: normal (BCS 3 - 3.5), underweight (BCS 1 - 2.5), overweight (BCS 4 - 5).
[4]Change in BCS = BCS at enrollment - BCS at time of removal of study.
[5] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack].
[6] Ref = reference category.

Table 3.6 Final logistic regression model for factors associated with the incidence of subclinical ketosis with other health problems (K+; n = 39) relative to healthy animals (H; n = 87), in multiparous cows.

Variable	Coefficient	SE	Odds ratio (95% CI)[1]	*P*-value
Intercept	-9.06049	2.6301	--	0.035
Parity				0.0037
2	--	--	Ref[3]	--
3+	1.72	0.580	5.6 (1.78 to 1.80)	--
Length of dry period (d)	0.049	0.0291	2.5 (0.84 to 7.59)	0.097
Stocking density (%)[2]				
wk -1	0.026	0.0130	1.9 (1.00 to 3.60)	0.049
Daily lying time (min/d)				
wk +1	0.0046	0.00235	1.8 (1.00 to 3.39)	0.051

[1]Adjusted odds- ratio and 95% CI for 1 SD increase in each variable in the model. The mean ± SD for each variable are as follows: 59 ± 19.0 d, length of dry period; 80 ± 24.8%, stocking density (wk -1); 382 ± 85.6 min/d, rumination time (wk -1).

[2] Stocking density (ST) for freestall pens was calculated as ST = [no. of stalls] / [no. of cows in pen]. Bedded packs were calculated as: ST = [(dimensions, m^2) / (recommended space allowance, $11m^2$ (Nordlund, 2009))] / [no. of cows in bedded pack].

[3]Ref = reference category.

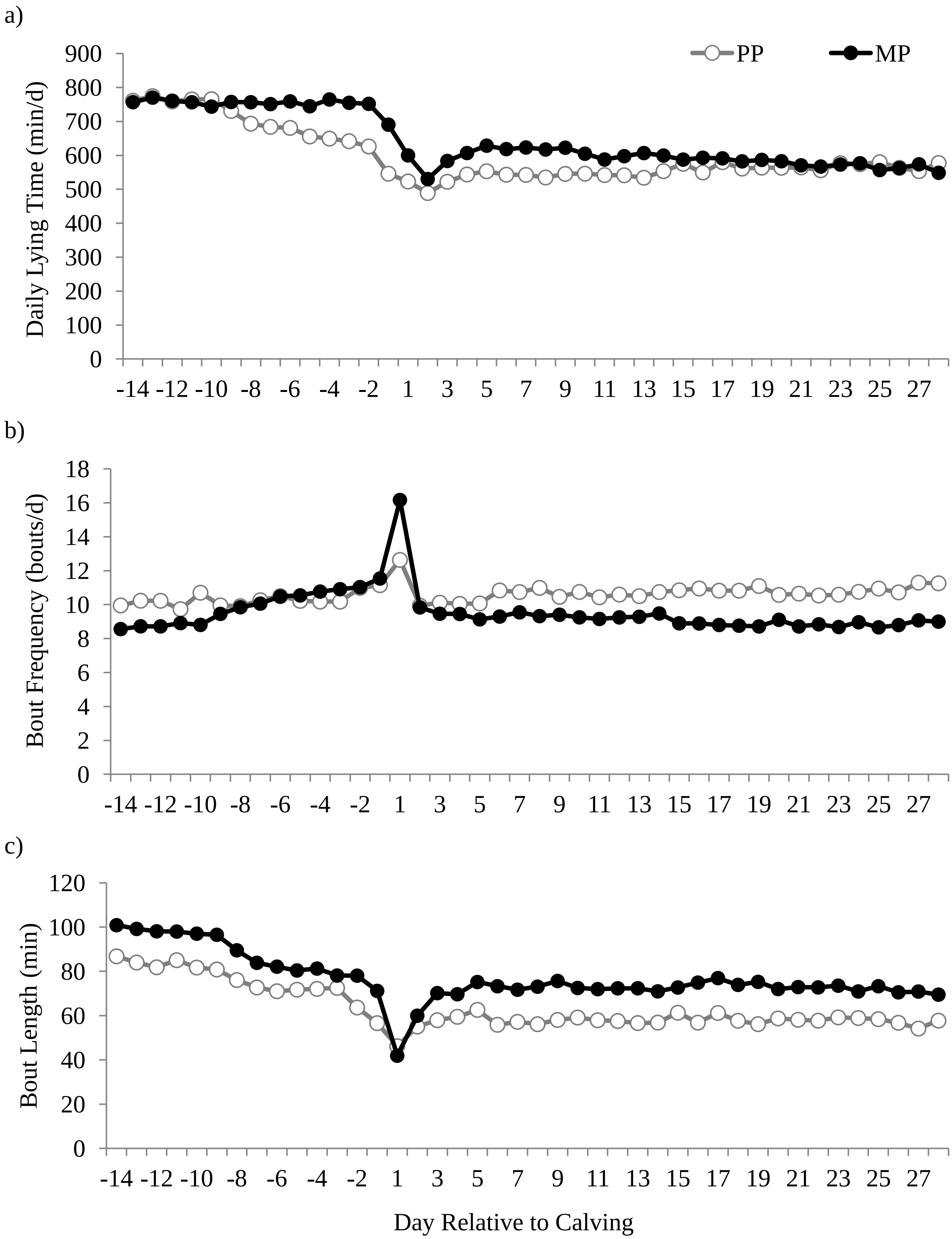

Figure 3.1 Daily a) lying time (min/d), b) bout frequency (no. of bouts/d), and c) average bout length (min) over the transition period (-14 to 28d) for multiparous (MP; n = 232) and primiparous (PP; n = 107) cows.

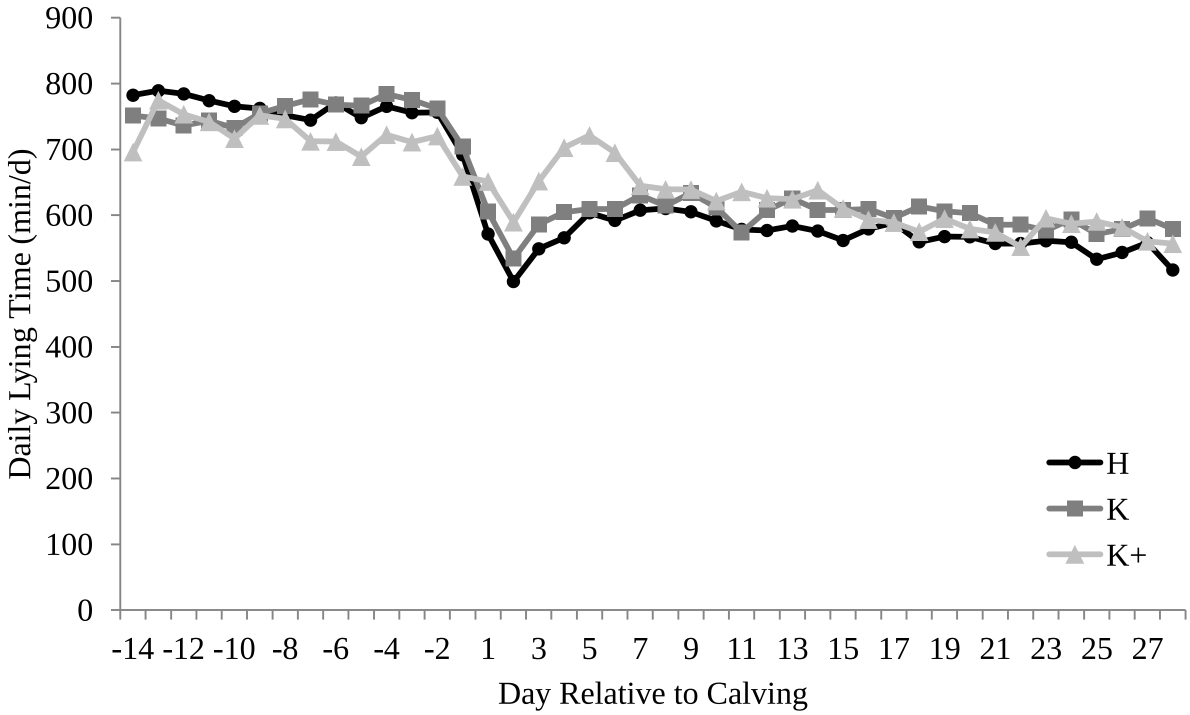

Figure 3.2 Average daily lying time (min/d) over the transition period (-14 to 28d) for healthy multiparous (MP) cows with no other illnesses (H; n = 87), subclinically ketotic MP cows with no other health issues (K; n = 76) and subclinically ketotic MP cows with other health issues (K+; n = 39).

CHAPTER 4: GENERAL DISCUSSION

4.1 IMPORTANT FINDINGS

Transition is a very vulnerable period for the high-producing dairy cow, as up to 50% of cows develop at least one metabolic and/or infectious disease cows during this time (LeBlanc, 2010). There is growing evidence in the literature that changes in time devoted to certain behaviours, including feeding and general activity, are associated with increased risk of certain peripartum illnesses (Huzzey et al. 2007; Goldhawk et al., 2009; Proudfoot et al. 2009a; Soriani et al., 2012). In Chapter 2, we sought to understand how rumination changes over the transition period for both healthy cows and those with SCK and to identify if monitoring this behaviour would be useful in the early detection of SCK. We hypothesized that early lactation dairy cows with reduced rumination activity, both pre- and post-calving, would be at higher risk of experiencing SCK. Our findings mostly supported this theory in MP cows. Multiparous cows actually spent more time ruminating during the day than PP cows throughout the transition period. For both healthy PP and MP cows, rumination time decreased in the week leading up to calving and began to rapidly increase to an even longer duration in the week after calving. These changes in time spent ruminating are likely associated with changes in DMI over that same time period. Primiparous cows showed no difference in rumination time between health statuses. However in MP cows, K cows ruminated less than H cows during the week before and after calving and K+ cows had even lower rumination times during these weeks.

With this information we determined the associations of herd- and cow-level factors, including rumination behaviour in the week preceding and following calving, with SCK (Chapter 2). Lower milk yield during the previous lactation, smaller loss of BCS over the transition period, decreased stall stocking density, and increased rumination during the week prior to calving were all associated with decreased odds of SCK in MP cows. There were decreased odds of SCK occurring with another postpartum health issue (K+) in cows with lower parity, shorter dry period, lower stocking density in the week prior to calving and greater rumination time in the week following calving. Although rumination time was also lower for K+ cows during the week prior to calving, this factor was not retained in the final model, as it was correlated with rumination time in wk +1. Thus, in addition to controlling for BCS loss, not overcrowding and ensuring sufficient dry period length, these results suggest that rumination monitoring systems may contribute to identifying MP cows at high risk for SCK, so preventative measures may be taken prior to development of the illness.

Pedometers and activity monitors are commonly used for estrus detection in many dairy herds, but recent research suggests they may be useful in detecting changes in lying behaviour around calving that are associated with subclinical illness (Edwards and Tozer, 2004; Jawor et al. 2012). Due to the association between lying time and rumination time (Philips and Leaver, 1986; Cooper et al., 2007), we decided to analyze these two behaviours separately over transition. Chapter 3 characterized changes in lying behaviours, specifically daily lying time, frequency of lying bouts and bout duration, across the transition period and determined if these factors were useful in identifying cows at risk for SCK. We hypothesized that dairy cows with increased lying activity, both before and after calving, would be at higher risk of experiencing SCK in early lactation.

In our study, PP cows had a shorter daily lying time, more lying bouts and, short bout durations compared to MP cows over the transition period (Chapter 3). Daily lying time and frequency of bouts were not different between PP cows of different health statuses. Frequency of lying bouts and bout duration were similar between the health categories of MP cows over the study period. No difference in daily lying time was found during the pre-calving period when comparing healthy MP cows to K or K+, however, K cows tended to and K+ cows did lie down longer than H multiparous cow during the week after calving. The increased odds of SCK occurring with another postpartum health issue (K+) was associated longer daily lying time during the week after calving (Chapter 3), in addition to those factors identified in our model of rumination and odds of SCK (Chapter 2). Thus, monitoring lying behaviour may contribute to the identification of MP cows that have SCK in combination with another health problem, but may not be as useful for the early detection of this subclinical illness.

When comparing rumination time of K to H cows in Chapter 2, it was found that for every 30 min/d decrease in rumination time during the week prior to calving, the odds of becoming K post-calving increased by 20%. The odds of developing SCK and another health problem was 1.31 times greater for every 30 min/d decrease in rumination time during the week after calving. In Chapter 3 we found that a 30 min increase in lying time throughout the day during the wk after calving was associated with a 1.15 times higher odds of being K+. Thus, these results suggest that for monitoring behaviour during the week prior to and after calving, rumination time may be a better predictor than lying time for identifying cows at risk for, or experiencing, SCK.

4.2 FUTURE RESEARCH

Animal behaviours are highly variable, not only within herds, but also within cows. It would be ideal if studies aiming to measure animal behaviours, such as lying time or rumination, employ a greater sample size to account for these large variations. A better understanding of trends in the behaviour of healthy cows over the transition period and even throughout lactation will aid in developing a better understanding of fluctuations over time. If we can determine a baseline for behaviours it will be easier to pinpoint where abnormal changes in behaviour occur that could identify cows at a higher risk for illness.

It is important to note that this research suggests automated behavioural monitoring systems may be useful in identifying high risk cows, but it is still necessary for these cows to be tested for illness prior to any sort of treatment. Testing for SCK only 1x/wk was definitely a limitation in this study. Repeated BHBA sampling during the pre- and post-partum period may have enhanced our understanding of the relationship between BHBA and both rumination and lying behaviour. Future studies that also measure particle size and sorting of the ration could grasp an even better understanding of the factors that not only affect illness, but also affect rumination over the transition period. With daily measurements of BHBA and individual dairy cow behaviour, future research may be able to determine how behaviour changes specifically at the onset of illness. More in depth studies like this may be able to determine the cause of this change in behaviour (rumination, lying etc.) in association with SCK. By measuring multiple behaviours over transition (including feeding behaviours) we may be able to differentiate whether rumination and lying time changes are simply related to DMI and, therefore, associated with ketosis, or if changes in these behaviours are related to sickness and may be considered a sickness behaviour.

This study was carried out on commercial dairy farms where we were unable to record DMI, particle size, sorting, social behaviour, competition at the feed bunk or timing regrouping of cows, which other studies (vonKeyserlingk et al. 2008; Goldhawk et al. 2009; Proudfoot et al, 2009; Schirmann et al. 2011) have associated with subclinical illness. Ideally, future studies will monitor multiple animal behaviours to create larger, more in depth multivariable models. With a better understanding of the numerous risk factors for ketosis, that include, but are not limited to, milk production, DMI, dry period length, ration composition, rumination, lying time, pre-calving BCS, stocking density, feed access, and grouping strategies, we can determine which of these variables are easiest to monitor on specific farms and develop tools that producers and veterinarian may use to target animals with subclinical illness.

4.3 IMPLICATIONS

From both an economic and welfare perspective, it is always within the dairy producer's best interest to ensure optimal health of their dairy cows over the transition period. Prevention is the best possible strategy in reducing incidence, and resultant prevalence, of illnesses and disease in herds. There are various management approaches to optimize DMI and encourage cow behaviour to reduce the risk of SCK, but this metabolic disorder continues to affect a large number of cows in the industry.

This research has provided a greater understanding of how rumination activity and lying behaviour change over transition for both PP and MP cows. Although we did not find any differences in the behaviour of PP cows with and without SCK, we did observe a difference in MP cows. Rumination time tended to be lower for cows with only SCK and was even lower for cows with SCK and at least one other health problem during the week preceding and the week following calving. Lying time was also longer in cows with SCK and another health problem during the week after calving.

Automated behavioural monitoring systems are used on many farms, especially for estrus detection, but this research supports a potential secondary use for the systems in reporting cows at an increased risk for having a subclinical illness. Of course, to use these systems to their full potential producers must begin monitoring rumination and lying behaviour during the dry period to establish a baseline for behaviours. Any changes in behaviour for each cow would be compared to that animal's normal levels of rumination or lying behaviour. Even with the use of automated systems, testing for ketosis is still necessary to confirm a SCK diagnosis. In the case of monitoring rumination, it may be possible to identify cows in the pre-calving period that are at an increased risk of developing SCK post-calving. With this information producers may be able to intervene, to ensure cows flagged as high risk are consuming sufficient nutrients to support their needs.

CHAPTER 5: REFERENCES

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