Characterization of peripartum rumination and activity of cows diagnosed with health disorders postpartum

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CHAPTER I

A Literature Review

Physiological Changes during the Transition Period

The transition from late pregnancy to early lactation, traditionally known as the transition period, has been recognized as a challenging period on a cow's life. Recently, it has received much attention due to the recognition that the success of this period determines the profitability of the cow during the following lactation (Drackley, 1999). Grummer (1995) described the transition period as the period from 3 weeks pre-calving until 3 weeks post-calving. Although some authors define it slightly differently, this definition has been widely accepted.

The hallmark of the transition period is the paradoxical increase in nutrient requirements at a time when nutrient intake is compromised (Overton and Waldron, 2004). In the final trimester of pregnancy, nutrient requirement increases as a result of fetal development calculated to be at 2.3 Mcal of net energy on day 250 of pregnancy in Holstein dairy cows (Bell, 1995). However, the metabolic demands imposed on the cow by colostrogenesis far exceed the demands of the fetus (Goff and Horst, 1997). Goff and Horst (1997) observed that for the production of 10 kg of colostrum on the day of calving it is required that 11 Mcal of energy be supplied to the mammary gland. The most recent NRC (2001) did not include an energy requirement for mammary growth because insufficient research has been done to define its requirement; however, Grummer et al. (2004) indicated that it also increases substantially the energy expenditure during this period. As nutrient requirement increases, cows experience a decrease in voluntary dry matter intake (DMI) with nadir on the day of calving (Ingvartsen and Andersen, 2000). Bertics et al. (1992) reported a DMI decrease of 28% on the week before calving compared with 3 weeks before calving. Similarly, Hayirli et al. (2002) reported a decrease in DMI from 2% of the body weight (BW) in the beginning of the dry period to 1.4% of BW in the last week before calving.

Although DMI increases immediately after parturition, nutrient requirements still cannot be met through feed intake alone because the rate of dry matter intake increase is slower than the rate of milk energy output and, in healthy cows, requirements for net energy exceed intake by 26% on day 4 postpartum (Drackley, 1999). The milk yield typically peaks between 5 to 7 weeks postpartum, while maximum intake is reached between 8 and 22 weeks after calving (Ingvartsen and Andersen, 2000). As a result, lactating dairy cows undergo a period of negative energy balance (NEB) during the first 4 to 8 weeks postpartum (Drackley, 1999). Dairy cows have been genetically selected to cope with this shortfall in nutrient supply by increasing the mobilization of body reserves and by shifting the profile of nutrients consumed by non-mammary tissues (Bell, 1995). These metabolic adaptations are coordinated by changes in the plasma concentration of key hormones and by tissue-specific variation in hormone sensitivity and responsiveness (Lucy et al., 2008). In 1980, Bauman and Currie coined the term homeorhesis to describe the orchestrated changes in metabolism to meet lactation requirements (Bauman and Currie, 1980). Among the hormones associated with homeorhetic changes, growth hormone (GH) has been shown to be an important actor of homeorhetic events in transition dairy cows (Bauman, 2000). A variety of tissues are affected by GH, but coordinated events in the liver and adipose tissue may be the most important (Bell, 1995). Growth hormone increases the responsiveness of adipose tissue to lipolytic signals induced by catecholamines (Drackley, 1999) and antagonizes lipogenesis and blocks insulin-dependent glucose uptake (Lucy et al., 2008). The increase in non-esterified fatty acids (NEFA) resulting from adipose tissue mobilization are used as alternate fuels for much of the rest of the body and are also converted by the liver to ketone bodies, mainly beta-hydroxybutyrate (BHBA). The ketone bodies serve as alternate source of energy that can replace glucose in many tissues, thus conserving glucose for milk synthesis (Drackley, 2001). In the liver GH increases gluconeogenesis through a direct effect on the gluconeogenic pathway or through an antagonism of insulin action (Etherton and Bauman, 1998). During early lactation, GH-induced hepatic secretion of insulin-like growth factor-1 (IGF-1), which depends on GH binding to GH receptor 1-A (GHR-1A; Lucy et al., 2001), is compromised (Vicini et al., 1991). Growth hormone receptor 1-A expression is related to nutrient intake (Lucy et al., 2001). Consequently, during the transition from late pregnancy to lactation, a period of negative energy balance, GHR-1A expression is reduced and IGF-1 concentration is reduced despite the increase in GH concentrations, characterizing the decoupling of the somatotropic axis. Hypoinsulinemia and decreased insulin responsiveness in the skeletal muscle and adipose tissue are observed simultaneously with the decoupling of the somatotropic axis (Bell and Bauman, 1997). As a result, there is an increase in production and availability of glucose for the mammary gland where uptake is not dependent of insulin (Bell and Bauman, 1997).

Feed Intake Regulation during the Transition Period

Feed intake regulation is a complex biological phenomenon (Ingvartsen and Andersen, 2000). The reasons for the decline in feed intake before parturition have not been elucidated but potential mechanisms have been reviewed (Ingvartsen and Andersen, 2000). Forbes (1977) suggested that the decrease in intake prepartum could be a result of the physical compression of the rumen from the growing fetus. However, the decrease in intake due to a decrease in rumen volume is compensated in sheep, at least in part, by an increase in rate of passage of particles out of the reticulo-rumen (Kaske and Groth, 1997). Moreover, growth of the fetus is more gradual during late gestation, whereas a more pronounced decrease in dry matter intake does not occur until the last week of parturition (Grummer et al., 2004). Additionally, at parturition the abdominal cavity is relieved of the fetus and fetal membranes and the disappearance of such mass from the abdominal cavity should allow a rapid increase in feed intake. However, DMI does not increase rapidly after calving, suggesting that factors other than rumen volume are involved in the regulation of feed intake in periparturient cows (Ingvartsen and Andersen, 2000).

Reproductive hormones are important in the regulation of appetite and energy metabolism in ruminants (Forbes, 1986). Changes in blood concentrations of steroid hormones before parturition are consistent with changes in DMI (Grummer, 2004). Grummer et al. (1993) speculated that the surge in blood estrogen might be responsible for the depression in feed intake around calving. Dewhurst et al. (2000) suggested that other factors rather than estrogen must be involved in intake regulation because the decrease in DMI occurs weeks before calving whereas major increases in plasma estrogen concentration do not occur until day 5 prior to parturition.

Metabolites are also thought to be involved in feed intake regulation. Grummer et al. (1993) observed a negative relationship between plasma concentration of NEFA and feed intake in dairy cattle. In dairy cows, a short-term (4 h) intravenous lipid infusion also resulted in a reduced DMI (Bareille and Faverdin, 1996). As the concentration of NEFA in the blood increases during the periparturient period, more NEFA are taken up by the liver (Emery et al. 1992). Non-esterified fatty acids may be completely beta-oxidized to carbon dioxide and partially oxidized to produce ketone bodies that are released into the blood or reconverted to triacylglycerol for storage (Drackley, 1999). The rate of oxidation of fatty acids in the liver alters signals generated by hepatic vagal afferent nerves to brain centers signaling satiety (Scharrer and Langhans, 1986).

Factors Associated with Feed Intake among Transition Cows

Animal Factors

Dry matter intake is considerably higher in multiparous cows compared with primiparous (Ingvartsen and Andersen, 2000). Kristensen and Ingvartsen (1985) demonstrated that the intake capacity of primiparous cows is only approximately 80% of multiparous after parturition. Hayirli et al. (2002) demonstrated that average daily DMI expressed as % of body weight (BW) in the final 3 weeks of gestation was 1.88% for multiparous cows and 1.69% for primiparous cows Moreover, the DMI of multiparous cows gradually decreased from 2.06 to 1.36% of BW, whereas the DMI of primiparous cows remained at 1.8 to 1.7% of BW until the final week of gestation (Hayirli et al., 2002). Similarly, Moodie and Robertson (1974) reported that DMI was reduced by 4% for primiparous cows between day 4 and 0 pre-calving compared with day 14 pre-calving, whereas multiparous experienced a drop of 50% of DMI during the same period. Marquardt et al. (1977) reported a 25 and 52% decreases in DMI for nulliparous and parous animals, respectively, during the final 2 weeks of gestation.

Body condition score (BCS) is considered another animal characteristic that influences DMI (Grummer et al., 2004). Forbes (1986) postulated that intake capacity of ruminants is influenced by their body composition or fatness accumulation. Garnsworthy and Topps (1982) observed that cows that were thinner at calving were able to increase their DMI at a faster rate than fatter cows postpartum. Moreover, thinner cows had a shorter delay between peak in milk yield and peak in DMI after parturition (Garnsworthy and Topps, 1982). Hayirli et al. (2002) observed that DMI during the final 3 weeks of gestation decreased linearly as BCS increased and the lowest DMI, expressed as % of BW, was observed among obese cows (obese = 1.68 %, moderate = 1.83 %, and thin = 1.84 %).

Breed has also been reported as an animal factor influencing feed intake. French (2006) demonstrated that DMI decreased 35% among Holstein cows in the last 3 weeks of gestation, whereas the reduction in DMI for Jersey cows was only 17% during the same period. The author also reported that the magnitude of intake depression in late gestation was less for Jerseys compared with Holsteins (French, 2006). Mendonça et al. (2014) demonstrated that multiparous Holsteins cows tended to have a greater DMI than multiparous Montbéliarde-sired crossbred cows from 6 weeks before to 6 weeks after calving. The DMI of Holstein cows, however, decreased from 1.9 to 1.4% of BW from day 15 to day 1 before calving, whereas DMI of crossbred cows was 1.5 and 1.4% f BW on days 15 and 1 before calving, respectively (Mendonça et al., 2014).

Dietary Factors

Physical and chemical characteristics as well as energy density of transition diets influence DMI of dairy cows (Allen, 2000). Physical regulation is thought to be the primary mechanism regulating DMI as the animal's requirement increases (Allen, 2000). In fact, Allen (2000) demonstrated that diets high in neutral detergent fiber (NDF) may limit DMI due to rumen fill when NDF content exceed 25% of DM of the diets and DMI increases with decreasing NDF. Grummer (1995) suggested that increasing energy density of the diets by substituting forages for non-fiber carbohydrates (NFC) would improve total DMI, offsetting a negative energy balance before parturition, especially when feed intake is greatly depressed. Results from Rabelo et al. (2003) indicated that increasing the energy density of the prepartum diet (32% versus 40% NDF) in the last 4 weeks prior to parturition increased DMI. Feeding high energy density diets during prepartum, however, was associated with a greater decline of DMI as parturition approached (Hayirli et al., 2002). There is considerable evidence indicating that the extent of DMI depression immediately before parturition is positively associated to the level of feed intake (Rabelo et al., 2003; Mashek and Grummer, 2003) and that changes in DMI around calving rather than absolute DMI are more important for performance and health after calving (Drackley, 1999).

Psychogenic Factors

Grouping Strategies

The industry-standard management of dairy cows during the dry period consists of a 2-group nutritional strategy (Overton and Waldron, 2004). The NRC (2001) recommends that a diet containing approximately 1.25 Mcal/Kg of energy should be fed from dry off until approximately 21 days before calving and a diet containing 1.54 to 1.62 Mcal/Kg of energy should be fed during the last 3 weeks before parturition (Overton and Waldron, 2004). The primary rationale for feeding a lower energy diet during the early dry period is to minimize BCS gain, whereas increasing energy density of the diet as parturition approaches is to enhance energy intake, adapt rumen microorganisms to high concentrate lactating diets, and to promote the development of ruminal papillae (Grummer et al., 2004; Overton and Waldron, 2004). Diet changes, however, are usually only possible when cows are moved to different pens and regrouped. Cattle are social animals and readily establish dominance hierarchies (Friend and Polan, 1974) by physical and non-physical interactions (von Keyserlingk et al., 2008). Research from Schein and Fohrman (1955) indicated that a week was required for dominance to be reestablished and stabilized after new cows were introduced into a new group. Hasegawa et al. (1997) suggested that social behavior returns to baseline levels between 5 to 15 days after regrouping. von Keyserlingk et al. (2008) demonstrated that aggressive interactions were more frequent on the day of regrouping, with regrouped cows being displaced more often from the feed bunk, and that regrouped animals had decreased feeding and lying times. Schirmann et al. (2011) found that cows that were moved to a new pen had decreased DMI on the day of regrouping but no differences in DMI were observed for the cows that were already in the pen. Much of the social competition in a group occurs in the feeding area immediately after feed delivery (DeVries et al., 2004) and socially subordinate animals may be less able to cope with competitive displacements, which could result in lesser feeding time and reduced DMI (DeVries et al., 2004). In fact, Friend and Polan (1974) observed that dominant cows spent more time eating than cows of lower social rank, resulting in greater DMI. Social dominance is correlated with body weight, chest girth and age of cattle (Dickson et al., 1970), but not with milk yield (Grant and Albright, 1995), suggesting that a more pronounced negative energy balance can be observed for high producing submissive cows.

Stocking Density

The best practice with regard to space allowance in prepartum dry cow pens is to provide 1 lying stall for every cow and a minimum of 76 cm of linear bunk space per animal (NFACC, 2009). According to the USDA (2010), however, 58% of farms in the United States have less than 76 cm of feeding space and 43% have less than the recommended lying stall availability. The relationship between stocking density and DMI is not well understood (Huzzey et al., 2012). Friend et al. (1977) concluded that cows can be kept with 20 cm of feeding space per cow without adverse effects on DMI or milk production. Proudfoot et al. (2009) reported no effects of overstocking on DMI when feeding space was reduced from 60 to 30 cm/cow. Olofsson (1999) reported an increase in DMI when the number of cows per feeding bin was changed from 1 to 4. Similarly, Friend et al. (1977) observed a 16% increase of DMI when the length of the feed bunk was decreased from 50 to 25 cm per cow. Competitive social environments, however, are known to alter feeding behavior (Huzzey et al., 2005; Proudfoot et al., 2009; Lobeck-Luchterhand et al., 2015). Overstocked cows have decreased feeding time, increased time standing and increased aggressive displacements (Huzzey et al., 2005). Lobeck-Luchterhand et al. (2015) observed that feeding time was greater for prepartum Jersey nulliparous animals housed at 100% stocking density compared with Jersey nulliparous animals housed at 80% stocking density. On the other hand, Jersey multiparous animals housed at 100% stocking density tended to spend fewer minutes per day feeding than Jersey multiparous animals housed at 80% stocking density. Olofsson (1999) concluded that when the competition level increased, cows of low social rank tended to adjust behavior to a greater extent than dominant cows by spending less time eating and more time spend standing and feeding at less preferred hours of the day.

Importance of Feed Intake to Transition Cow Health

Zamet et al. (1979) suggested that health disorders that occur early postpartum might be signaled by a greater than normal depression of voluntary feed intake. Drackley (1999) demonstrated that coefficients of variation for DMI during the first week after parturition vary from 30 to 40%, whereas the coefficients of variation for DMI after peak of lactation range from 6 to 10%. The greater variation in DMI early postpartum coincides with the development of metabolic and infectious disorders, suggesting that DMI may be an important contributor to disease susceptibility. Zamet et al. (1979) observed that cows that developed illness, of both metabolic and infectious nature, had a 25% lower DMI than healthy cows on day 1 prepartum and on day 30 postpartum. Proudfoot et al. (2009) demonstrated that cows that had dystocia consumed 1.9 kg less DM on day 2 before calving than healthy cows and the difference increased to 2.6 kg on day 1 before calving. Goldhawk et al. (2009) observed that, from week -1 to week +2 relative to calving, the DMI of cows with subclinical ketosis (SCK) was lower than the DMI of cows that remained healthy after calving. Similarly, Huzzey et al. (2007) observed that DMI of healthy cows was greater than the DMI of cows diagnosed with acute metritis from 2 weeks before calving until 4 weeks after parturition. Additionally, cows that remained healthy after parturition had little decline in DMI before calving, whereas cows diagnosed with acute metritis had a notable decline in daily DMI 1 week before calving (Huzzey et al., 2007). Similarly, Hammon et al. (2006) also reported a lower DMI during the 2 weeks before calving for cows diagnosed with acute metritis early postpartum compared with healthy cows.

Tools for Early Identification of Diseases in Transition Cows

There is much interest in finding ways to improve the early identification of health disorders and understand how the risk of such disorders can be predicted (Van Saun, 2001). Godden et al. (2003) demonstrated that approximately 25% of cows leave dairy herds during the first 60 DIM due in part to health disorders during the transition period. LeBlanc (2010) suggested that up to 50% of dairy cows in the transition period may be affected by infectious or metabolic diseases. Thus, it seems imperative to identify sick cows in a timely fashion to mitigate risk of culling and improve the profitability of dairy herds. Early identification of sick animals, however, is difficult and frequently depends on subjective identification by farm personnel (González et al., 2008). Changes in milk yield have been used as an indicator of health early postpartum (Fourichon et al.; 1999). The relationship between diseases early postpartum and changes in milk yield, however, are not consistent (reviewed by Erb,1987 and Fourichon et al., 1999). Jawor et al. (2012) demonstrated that cows identified with subclinical hypocalcemia had greater milk yield on weeks 2, 3, and 4 compared with healthy counterparts. Similarly, Goldhawk et al. (2009) observed that milk production of cows diagnosed with SCK did not differ from healthy cows. Urton et al. (2005) suggested that changes in milk yield might correspond poorly with recognition of mild or subclinical diseases.

Several studies (Huzzey et al., 2007; Urton et al., 2005; Goldhawk et al., 2005) have demonstrated that short-term changes in feeding behavior during the periparturient period can affect the health status of the cow early postpartum. Cows diagnosed with acute metritis spent less time feeding compared with healthy cows, with differences most pronounced during the week before and the week after calving (Urton et al., 2005; Huzzey et al., 2007). Moreover, Huzzey et al. (2007) demonstrated that cows diagnosed with acute metritis early postpartum engaged in fewer aggressive interactions at the feed bunk during the week before calving and avoided the feed bunk when feed competition was at the highest. Goldhawk et al. (2009) observed that cows diagnosed with SCK spent less time at the feed bunk and had fewer visits to the feed bins than cows that remained healthy early postpartum. Cows diagnosed with SCK initiated fewer displacements at the feed bunk during peak feeding periods compared with healthy animals, especially in the week before calving (Goldhawk et al., 2005). Although the current technology required to monitor feeding behavior and individual intakes is expensive and technically challenging for routine use in commercial farms, it has provided measures that may help identify animals at risk of developing diseases (Urton et al., 2005).

Increases in circulating concentrations of NEFA and BHBA are expected in the transition period as the energy intake lags far behind the energy demands for milk production (LeBlanc et al., 2005). Excessive plasma NEFA and BHBA concentrations, however, have been associated with immunosuppression in transition cows (Hammon et al., 2006). LeBlanc et al. (2005) and Ospina et al. (2010) evaluated NEFA and BHBA thresholds that may be used to predict diseases in transition dairy cows. Ospina et al. (2010) demonstrated that cows with serum NEFA concentrations above 0.29 mEq/L prepartum were 1.8 times more likely to develop displaced abomasum (DA), clinical ketosis and retained placenta postpartum. Likewise, cows with serum BHBA concentrations higher than 10 mg/dL postpartum were 2.3 times more likely to develop any of these three diseases (Ospina et al., 2010). Additionally, LeBlanc et al. (2005) observed that cows diagnosed with left displaced abomasum (LDA) had higher serum NEFA concentrations than healthy cows starting at day 14 before calving. Furthermore, cows with NEFA concentrations higher than 0.5 mEq/L on days 6 and 0 before calving were 3.6 times more likely to develop LDA after calving and for every 100 µmol/L increase in BHBA concentration above 1200 µmol/L from day 1 to 7 postpartum the odds of LDA were 8 times greater (LeBlanc et al., 2005).

Rumination Time and Activity as Tools to Monitor Transition Cow Health

Rumination is a cyclical process characterized by regurgitation, remastication, and reswallowing (Beauchemin, 1991). Rumination increases saliva secretion that aids in buffering the volatile fatty acids produced by microbial digestion (Beauchemin, 1991) and is essential in providing rumen bacteria greater access to feed particles during microbial fermentation (Russell and Rychlik, 2001). Rumination characteristics have been commonly used to quantify changes in diet composition and forage quality (Smith and Welch, 1976). More recently, however, rumination time (RT) has been used to access cow health and well-being (Radostits et al., 2007), predict the day of calving (Pahl et al., 2014; Schirmann et al.; 2013) and to assess the response of cows to disease (DeVries et al., 2009).

The typical method to monitor rumination is through visual observation (Krause et al., 1998), but indirect methods to measure RT based on rumination sounds (Heat Rumination Long Distance - HRLD, SCR Engineers Ltd, Netanya, Israel) have recently become commercially available in the USA. The system allows automatic measurement of RT and analysis of daily rumination pattern (Calamari et al., 2014). Mastication and rumination produce distinctive sounds that are recorded by a built-in microphone and the information can be summarized in 2 h-intervals and stored in the memory of a logger for

24 h (Schirmann et al., 2009). Recent work from Schirmann et al. (2009) demonstrated that the HRLD monitoring system provide estimates of RT that were similar to direct visual observation thus enabling its use for rumination monitoring on commercial farms.

Little research is available concerning the temporal relationship between intakes and RT (Schirmann et al., 2012). Schirmann et al. (2012) associated rumination time with DMI in prepartum dairy cows within 24 h in 2-h intervals and found no relationship between daily RT and DMI across cows and a weak negative relationship between daily RT and DMI within cows. In a 2 h difference between feeding and rumination, there was a strong negative association between rumination and DMI, indicating that cows spend little time ruminating during feeding periods. On the other hand, in a 4 h difference between feeding and rumination there was a shift from a negative to a positive relationship between RT and DMI, suggesting that RT lags intake by approximately 4 h (Schirmann et al., 2012).

Work from Soriani et al. (2012) and Calamari et al. (2014) have associated health disorders early postpartum with RT during the transition period. Soriani et al. (2012) observed that cows with reduced RT on day 10 and 2 before calving had greater NEFA and BHBA concentrations after calving compared with cows that had greater RT during the same period. Calamari et al. (2014) reported that the RT gradually decreased before parturition, reaching a minimum at calving. Additionally, cows that were diagnosed with at least one clinical disease postpartum had a lower RT between 3 and 6 DIM and their increase in RT after calving was slower compared with healthy cows (Calamari et al., 2014).

The HRLD system is also able to monitor activity through a neck-mounted monitoring device (Stevenson et al., 2014). Activity of a cow is defined as motion, movement and walking attitudes (Stevenson et al., 2014). The HRLD accelerometer is affixed to the cow's neck and it quantifies cow activity by the number of movements of the neck while cows are walking or in movement (Stevenson et al., 2014). An increase in walking activity was first associated with estrus (Roelofs, 2010); therefore, accelerometers were developed with the purpose to detect cows in heat. Other similar devices such as pedometers that are affixed to the cow's hind leg have quantified the number of steps the cow takes over a set of time (Stevenson et al., 2014). There is limited research evaluating the association between health disorders and changes in activity before such diseases are diagnosed and evaluating the use of activity as a diagnostic tool to detect a health disorder before it becomes clinically evident. Edwards and Tozer (2004) observed significant differences in pedometric activity between ill and healthy cows. The declines in activity of cows diagnosed with ketosis, left displaced abomasum, and digestive disorders were first observed 8, 9 and 8 d, respectively, earlier than when the diseases were diagnosed by trained employees.

Lying Behavior as an Indicator of Transition Cow Health

Lying behavior is an important measure of cow comfort (von Keyserlingk et al., 2009). Research into motivation of cows to lie down showed that it is a high priority behavior compared with feeding or social contact when these behaviors are restricted (Munksgaard et al., 2005). Lying behavior can be monitored using electronic tridimensional accelerometers (HOBO Pendant G Acceleration Data Loggers, Onset Computer Corp., Pocasset, MA). Accelerometers can be attached to the hind legs of the cow and can measure orientation of the leg, with the assumption that when the leg is horizontal, the animal is most likely to be lying down. Such devices have recently been shown to be accurate in determining time spent standing and lying as well as laterality of lying behavior and number of lying and standing bouts (Ledgerwood et al., 2010). Huzzey et al. (2005) described changes in standing and lying behaviors of dairy cows during the transition period and reported a decrease in daily lying time during the calving period (day -1 to day +1 relative to calving) with lying time reaching a nadir on the day of calving. Number of standing bouts increases on the day of calving compared with average daily standing bout during the 10 days prepartum and the 10 days postpartum (Huzzey et al., 2005). Changes in lying and standing behaviors have also been associated with poor health during the periparturient period and may be used to predict animals at risk for disease (Weary et al., 2009). It is assumed that illness causes lethargy and increased lying times are expected as an adaptive response to facilitate recovery (Johnson, 2002). Sepúlveda-Varas et al. (2014) reported that primiparous grazing cows that developed clinical diseases (e.g. retained placenta, milk fever, metritis and mastitis) spent more time lying down and tended to have longer lying bouts during day 3 and 4 relative to calving compared with healthy counterparts. Contrary, Proudfoot et al. (2009) observed that cows with dystocia had a higher number of standing bouts in the 24 h before calving compared with cows without dystocia. Jawor et al. (2012) observed that cows diagnosed with subclinical hypocalcemia at calving stood 2.6 h longer during the

24-h period before parturition and spent less time standing on day 1 after calving. Similarly, Cyples et al. (2012) demonstrated that cows challenged with *Escherichia coli intra-mammary* spent on average 10.6 h/d lying down on the day of challenge compared with 11.8 h/d day 2 prior the challenge.

Summary

Predisposition to health disorders immediately after parturition may be indicated by a reduced DMI prepartum. Thus, seems imperative to monitor individual intakes during the periparturient period to possibly identify cows at risk of developing diseases early postpartum. However, the technology required to record individual intakes is expensive and technically challenging for use on commercial dairy farms. Technology provided by SRC Dairy – HRLD has enabled indirect methods to measure feed intake, such as rumination time. Although, rumination time was not correlated to DMI over a 24 h period (Schirmann et al., 2012) changes in RT is expected when changes in DMI intake occurs, particularly around the time of calving (Schirmann et al., 2013). The system also allows monitoring the activity of the cow, through a neck-mounted sensor. Cows decrease activity as an adaptive response to facilitate recovery (Johnson, 2002) therefore, HRLD can provide an interesting measurement able to identify cows at risk of developing health disorders early postpartum. Accordingly, the objectives of the present study were to evaluate the association between health events and prepartum and postpartum daily RT and daily activity on transition cows and the correlation among metabolites, calcium, and haptoglobin and daily RT and daily activity.

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CHAPTER II

Characterization of peripartum rumination and activity of cows diagnosed with metabolic and uterine diseases.

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OVERVIEW

The objectives of the current observational study were to characterize the correlation among total serum Ca, non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHBA**) and haptoglobin concentrations and daily rumination time (**DRT**) and activity of periparturient cows, and to determine the association between periparturient events and peripartum DRT and activity. Holstein cows from one commercial dairy farm (nulliparous = 77, parous = 219) were enrolled into the study at approximately 21 d before expected calving date. Cows were fitted with individual Heat Rumination Long Distance system (**HRLD**, SCR Engineers Ltd., Netanya, Israel) from enrollment until approximately 21 ± 3 d postpartum. Blood samples collected weekly from enrollment to

21 d postpartum were used to determine concentrations of NEFA, BHBA, and haptoglobin. Blood samples collected within 72 h after calving were used to determine total serum Ca concentration. Sub-clinical ketosis was characterized by BHBA > 1,000 µmol/L in any sample and sub-clinical hypocalcemia was characterized by Ca < 8.55 ng/dL within 72 h after calving. Cows were examined 1, 7 ± 3 and 14 ± 3 d postpartum for diagnosis of retained fetal membrane and metritis. Total Ca (r = 0.15), NEFA (r = -0.27), and haptoglobin (r = -0.18) concentrations were weakly correlated with DRT. Concentration of BHBA (r = -0.14) was weakly correlated with activity. Postpartum DRT was reduced among cows that delivered twins compared with cows that delivered singletons (385.9 ± 17.1 vs 437.9 ± 4.8 min/d). Prepartum (430.8 ± 14. vs 465.8 ± 4.19 arbitrary unit) and postpartum (480.3 ± 19.4 vs. 536.5 ± 5.5 arbitrary unit) activity were reduced among cows that delivered twins compared with cows that delivered singletons. Delivery of stillborn calves was associated with reduced DRT prepartum (417.0 ± 23.4 vs. 478.0 ± 5.9 min/d) and postpartum (386.5 ± 19.3 vs 437.2 ± 4.8 min/d). On the other hand, cows delivering stillborn calves had increased activity prepartum compared with cows delivering live calves (499.3 ± 16.2 vs. 461.3 ± 4.1 arbitrary unit). Occurrence of retained fetal membrane tended to and was associated with reduced prepartum (444.3 ± 11.0 vs. 466.5 ± 4.3 arbitrary unit) and postpartum (488.2 ± 14.5 vs. 538.8 ± 5.7 arbitrary unit) activity, respectively. Cows diagnosed with metritis had reduced postpartum DRT (415.9 ± 10.1 vs 441.0 ± 5.2 min/d) and activity (512.5 ± 11.5 vs. 539.2 ± 6.0 arbitrary unit) as compared to cows not diagnosed with metritis. Postpartum activity was reduced among cows that were diagnosed with sub-clinical ketosis (502.20 ± 16.5 vs. 536.6 ± 6.2

arbitrary unit). Although differences in DRT and activity between populations of cows that developed periparturient diseases and healthy cows were observed, further studies are necessary to determine whether (and how) DRT and activity data may be used to precociously diagnose individuals that will develop such periparturient diseases.

INTRODUCTION

The peripartum period is very challenging to the dairy cow because of hormonal, metabolic and managerial changes. Dry matter intake, amount and daily variability, during the transition period is probably the most important factor affecting the health and performance of dairy cows (Grummer et al., 2004). Previous research has shown that cows that develop metritis postpartum have reduced prepartum feed intake, which is likely a predisposing factor for immunosuppression and greater susceptibility to uterine infections (Hammon et al., 2006; Huzzey et al., 2007). Dry matter intake and feeding and lying behaviors are also associated with metabolic diseases and lameness. Cows that developed sub-clinical hypocalcemia within 24 h after calving tended to have fewer daily visits to the water trough and feed bins but few changes in standing time and DMI were observed (Jawor et al., 2012). When hypocalcemia was induced in non-pregnant non-lactating cows, the DMI was reduced by approximately 45% within the period of induced hypocalcemia (Martinez et al., 2014). Goldhawk et al. (2009), using a BHBA threshold > 1,000 µmol/L for diagnosis of sub-clinical ketosis, demonstrated that cows diagnosed with sub-clinical ketosis had reduced DMI, reduced number of daily visits to the feeder and reduced time spent at the feeder. Edwards and Tozer (2004) demonstrated that the pattern of activity, measured by pedometry (Kibbutz Afikim, Israel), of cows that were diagnosed with displacement of abomasum and ketosis was different than the activity pattern of healthy cows. Therefore, it seems clear that the ability to monitor peripartum DMI, feeding behavior, and activity of individual cows and groups of cows could aid in the early identification of unhealthy cows and managerial deficiencies that predispose to such diseases.

Automated technologies for monitoring behavior of dairy cows have recently become more widely adopted in the USA. Recent experiments have demonstrated that the Heat Rumination Long Distance sensor system (**HRLD**, SCR Engineers Ltd., Netanya, Israel) is accurate in measuring rumination of different types of cows (pre-partum and post-partum) compared with visual observation (Schirmann et al., 2009). Although the HRLD system was not able to correctly predict individual cows DMI within a day and was actually negatively correlated with DMI within 2-h periods and with daily feeding time (Schirmann et al., 2012), changes in rumination over time are expected to reflect changes in DMI. Furthermore, these monitors could potentially be used as tools for early diagnosis of periparturient events (e.g. calving) and peripartum health disorders. Schirmann et al. (2013) demonstrated that daily rumination time (**DRT**) decreased by approximately 63 and 133 min during the 24 h before and 24 h after calving, respectively. Similarly, feeding time was decreased by approximately 66 and 82 min during the 24 h before and 24 h after calving, respectively (Schirmann et al., 2013).

The hypotheses of the current observational study were that peripartum DRT and activity are correlated with concentrations of metabolites (e.g. Ca, NEFA, and BHBA) and haptoglobin. Furthermore, we hypothesized that periparturient metabolic and infectious disorders are associated with DRT and activity. The objectives of the current study were to determine the correlation among concentrations of metabolites and haptoglobin and DRT and activity of periparturient cows, and to characterize the periparturient DRT and activity patterns of cows diagnosed with metabolic and uterine disorders as compared to healthy cows.

MATERIALS AND METHODS

Cows, Facilities, Management and Nutrition

The study was conducted in one dairy farm in Northwestern Wisconsin from July 2013 to April 2014 with Holstein cows enrolled from July 2013 to October 2013 and calving occurring from August 2013 to November 2013. Cows (nulliparous = 77, multiparous = 219) were enrolled into the study at 258.3 ± 0.2 d of gestation (mean ± SEM). During the prepartum period animals were separated by parity (nulliparous vs multiparous) and housed in one of three free-stall pens with three rows of stalls. The barns were naturally ventilated and had artificial lighting. The stocking density ranged from 85 to 110% of headlocks in the pen of nulliparous animals and from 60 to 90% in the pens of multiparous animals. As animals demonstrated signs of calving (discomfort, restlessness, tail twitching, and visualization of the allantoic sac through the vulva) they were moved to a loose housing pen with straw bedding.

During the immediate postpartum period (1 to 21 ± 3 DIM) primiparous and multiparous animals were commingled in 1 free-stall pen with three rows of stalls. The barns were naturally-ventilated and had artificial lighting. From 1 to 21 ± 3 DIM, pens were stocked at 80% and 95% of headlocks and stalls, respectively. From 21 ± 3 DIM until the end of lactation cows were housed in a cross-ventilated free-stall barn with stocking density varying between 110 and 120% of headlocks and between 119% and 130% of stalls.

From enrollment to calving nulliparous and multiparous animals were fed the same total mixed ration (TMR) but the TMR of multiparous animals contained anionic salts. From 1 DIM to the end of lactation primiparous and multiparous cows were fed the same TMR. During the prepartum period the TMR was offered once a day and during the postpartum period the TMR was offered once a day during the winter and twice a day during the summer (70% in the AM and 30% in the PM).

Rumination and Activity

Cows were fitted with individual rumination and activity sensors (HRLD, SCR Engineers Ltd., Netanya, Israel) from -21 to 21 ± 3 d relative to calving. Rumination was recorded in minutes/2 h intervals and total rumination minutes per day was used for statistical analysis unless otherwise stated. Activity was recorded every 2 h and total activity per day was used for statistical analysis unless otherwise stated.

Body Condition and Locomotion Score

At enrollment, 3 ± 3 and 24 ± 3 DIM all cows were scored for body condition (1 = emaciated and 5 = obese; 0.25 unit increment as described by Ferguson et al. (1994)

and locomotion (1 = normal locomotion and 5 = severely lame; as described by Sprecher et al. (1997). Cows with locomotion score $\geq$ 3 were considered lame.

Blood Sampling and Analysis of Metabolites, Haptoglobin, and Total Calcium

Blood samples were collected from all cows at -18 ± 3, -11 ± 3, -4 ± 3, 3 ± 3, 10 ± 3, and 17 ± 3 d relative to calving from the coccygeal vein/artery immediately after the morning feeding while cows were restrained in self-locking headlocks. Needles used were 22 gauge and 1 inch long and samples were collected into empty evacuated tubes and evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Tubes were placed in ice until centrifugation for plasma separation (3,000 rpm for 15 min at 4 ^{0}C). Plasma was aliquoted into microcentrifuge tubes and stored at -32 ^{0}C until analysis.

Samples collected weekly from -17 ± 3 to 17 ± 3 d relative to calving were analyzed for concentrations of NEFA using a colorimetric assay (Wako Chemicals USA, Richmond, VA; Ballou et al., 2009). Concentrations of BHBA were determined enzymatically (Ranbut, Randox Laboratories, Antrim, UK; Ballou et al., 2009) in samples collected weekly from 1 ± 1 to 17 ± 3 DIM. A plate reader (Spectramax 340; Molecular Devices, Sunnyvale, CA) was used to measure the absorbance for the colorimetric and enzymatic assays. Control serum (Randox Control Sera, Antrim, UK) was used for the NEFA and BHBA assays. The intra-assay CV were 6.1% and 9.8% for the NEFA and BHBA assays, respectively. The inter-assay CV were 8.9% and 11.8% for the NEFA and BHBA assays, respectively.

Haptoglobin concentration was determined in samples collected at -17 ± 3, -11 ± 3, -4 ± 3, 3 ± 3, 10 ± 3, and 17 ± 3 d relative to calving. A colorimetric procedure using a plate reader (Spectramax 340; Molecular Devices, Sunnyvale, CA) was used to measure the absorbance was used to determine haptoglobin concentration (Hulbert et al., 2011). The intra-assay and inter-assay CV were 4.1% and 9.8%, respectively.

Total serum Ca concentration was determined using a colorimetric assay described by Goff et al. (2012) and Oetzel et al. (1996). The inter-assay and intra-assay CV were 2% and 5%, respectively.

Clinical Examination and Definitions of Diseases

All cows were examined at 1, 7 ± 3 and 14 ± 3 DIM for diagnosis of retained fetal membrane and metritis. Retained fetal membrane was defined as retention of fetal membrane past 24 h postpartum. Metritis was defined as cows with watery, pink/brown, and fetid uterine discharge (LeBlanc, 2010). Cows that had at least one blood sample with BHBA concentration > 1,000 µmol/L were considered to have sub-clinical ketosis (Ospina et al., 2010). Cows with Ca concentration < 8.55 mg/dL in blood samples collected within 72 h after calving were considered to have sub-clinical hypocalcemia.

Milk Production

Cows were milked thrice daily and individual milk yield and milk conductivity data were recorded at each milking (AfiMilk, Kibbutz Afikim, Israel). Milk yield and conductivity data were collected daily in the first 21 DIM and weekly thereafter. For purposes of statistical analysis, daily milk yield from calving to 17 DIM and average milk yield in the first 90 DIM (**90dMilk**) were used.

Environment Data

Temperature and humidity data was collected using HOBO monitors (Make, model, company, city, state) installed in each of the barns in which pre-partum and post-partum cows were housed. Temperature and humidity index (**THI**) was calculated for every 2-h interval within a 24 h period (12 intervals) and daily for each barn. The percentage of 2-h intervals with THI > 68 was calculated for each day.

Statistical Analysis

This was an observational study. Animals were enrolled in weekly cohorts of 5 to 50 animals.

The correlation among daily milk yield (first 17 DIM) and concentrations of metabolites (NEFA and BHBA), total serum Ca, and haptoglobin and rumination and activity were evaluated using MedCalc Software (Ostend, Belgium). Partial correlation that included days relative to calving, parity, week of calving, BCS at enrollment, calf sex, and number of calves (singleton vs. twins) as explanatory variables were used to evaluate the correlation among daily milk yield (first 17 DIM) and concentrations of metabolites (NEFA and BHBA), total serum Ca, and haptoglobin and rumination and activity. Furthermore, average milk yield in the first 90 DIM was compared with prepartum and postpartum average DRT and activity.

Continuous data were analyzed by ANOVA for repeated measurements using the MIXED procedure of SAS version 9.2 (SAS/STAT®, SAS Inst. Inc., Cary, NC). The structure of covariance (auto-regressive, unstructured, or compound symmetry) was chosen according to the Bayesian akaike information criteria. Prepartum and postpartum behavior (DRT and activity) were analyzed separately because of the distinct patterns of DRT and activity during the prepartum and postpartum periods. Calving day (day 0) was included in the postpartum period. Univariate analyses were used to determine the association between animal characteristics (parity, calf sex and twin birth) and behavior (DRT, activity) and between health events (stillbirth, sub-clinical hypocalcemia, retained fetal membranes, metritis, and sub-clinical ketosis) and behavior (DRT and activity). The univariate analyses included DRT or activity as the dependent variable and animal characteristic or health event, day relative to calving, and the interaction between animal characteristics and day relative to calving or the interaction between health event and day relative to calving as independent variables. A multivariate analysis was used to identify the most important animal characteristics and health events associated with DRT and activity. The multivariate models included parity, calf sex, twin birth, stillbirth, sub-clinical hypocalcemia, retained fetal membranes, metritis, sub-clinical ketosis and day relative to calving as independent variables. Furthermore, the multivariate models included the interaction between each of the items (parity, calf sex, twin birth, stillbirth, sub-clinical hypocalcemia, retained fetal membranes, metritis, and sub-clinical ketosis) and days relative to calving as independent variables. Finally, pen in which cows were housed, week of calving, body condition and locomotion scores at enrollment, and number of days a cow stayed in the prepartum pen before calving were included in the multivariate models as independent variables. Independent variables with $P > 0.10$ were manually removed from the model until only independent variables with $P \leq 0.10$ remained in the model.

Correlations were considered as weak, moderate, and high when r < 0.3, r = 0.31-0.5, and r > 0.50, respectively. Statistical significance was defined as $P \leq 0.05$ and statistical tendencies as $0.05 < P \leq 0.10$. In the analysis of repeated measures, differences in means between two populations within a day were evaluated using the t-test.

RESULTS

The percentage of male calves and twin births were 54.4% (161/296) and 7.4% (22/296), respectively. The incidences of stillbirth, retained fetal membranes, metritis, sub-clinical hypocalcemia, and sub-clinical ketosis were 6.1% (18/296), 13.2% (39/296), 21.2% (62/293), 37.8% (94/249), and 12.7% (32/253), respectively. It is important to note that the weekly sampling/exam schedule adopted in the current experiment, despite being widely adopted by other research groups and the dairy industry, may result in some inaccuracies in the diagnosis of diseases and may not be sufficiently sensitive to identify sudden changes in concentrations of metabolites. The average daily THI was 58.6 ± 0.7 (range: 28.9 to 80.6) and the average daily percentage of 2-h intervals with THI > 68 was 22.8 ± 0.1% (range: 0 to 100%). Average milk yield in the first 90 DIM were 37.60 ± 0.63 and 47.51 ± 0.60 kg/d for primiparous and multiparous cows, respectively.

Association between Prepartum and Postpartum Daily Rumination Time and Activity

Prepartum DRT was strongly correlated with postpartum DRT according to the simple correlation (r = 0.60 (95% CI = 0.53, 0.67), $P < 0.01$) and partial correlation (r = 0.58, $P < 0.01$) analyses. Similarly, prepartum activity was strongly correlated with postpartum activity (correlation: r = 0.69 (95% CI = 0.62, 0.74), $P < 0.01$; partial correlation: r = 0.65, $P < 0.01$).

Association between Milk Yield and Daily Rumination Time and Activity

Average milk yield in the first 90 DIM was weakly correlated with average prepartum DRT (correlation: r = 0.27 (95% CI = 0.15, 0.37), $P < 0.01$; partial correlation: r = 0.14; $P = 0.02$). On the other hand, 90dMilk was moderately correlated with average postpartum DRT (correlation: r = 0.42 (95% CI = 0.32, 0.52), $P < 0.01$; partial correlation: r = 0.35; $P < 0.01$). Although there was no (r = -0.08 (95% CI = -0.19, 0.04), $P = 0.21$) correlation between 90dMilk and average prepartum activity, the partial correlation analysis demonstrated a tendency for 90dMilk and average prepartum activity to be weakly correlated (r = 0.11, $P = 0.06$). On the other hand, average postpartum activity was not correlated with 90dMilk (correlation: r = -0.10 (95% CI = -0.22, 0.02), $P = 0.11$; partial correlation: r = 0.05, $P = 0.39$).

Correlation among NEFA, BHBA, Ca, and Haptoglobin and Daily Rumination Time and Activity

There was a weak correlation between Ca concentration and DRT (correlation: r = 0.14 (95% CI = 0.02, 0.26), $P = 0.03$; partial correlation: r = 0.15, $P = 0.02$). There was no (r = 0.09 (95% CI = -0.04, 0.21), *P* = 0.17) correlation between Ca concentration and activity. Concentration of NEFA was weakly correlated with DRT (correlation: r = -0.27 (95% CI = -0.32, -0.22), *P* < 0.01; partial correlation: r = -0.27, *P* < 0.01). Although according to the correlation analysis there was a weak correlation between NEFA concentration and activity (r = 0.09 (95% CI = 0.04, 0.15), *P* < 0.01), there was no correlation between NEFA concentration and activity according to the partial correlation analysis (r = -0.002, *P* = 0.93). The correlation analysis indicated that there was a weak correlation between BHBA concentration and DRT (r = 0.08 (95% CI = 0.004, 0.15), *P* = 0.04) but according to the partial correlation analysis there was no correlation between BHBA concentration and DRT (r = 0.001; *P* = 0.98). There was a weak correlation between BHBA concentration and activity (correlation: r = -0.19 (95% CI = -0.26, -0.12), *P* < 0.01; partial correlation: r = -0.14, *P* < 0.01). Haptoglobin concentration was weakly correlated with DRT (correlation: r = -0.19 (95% CI = -0.25, -0.14), *P* < 0.01; partial correlation: r = -0.18; *P* < 0.01). Although according to the correlation analysis there was no correlation between haptoglobin concentration and activity (r = -0.03 (95% CI = -0.08, 0.03), *P* = 0.33), according to the partial correlation analysis haptoglobin concentration was weakly correlated to activity (r = -0.07, *P* = 0.02).

Associations between Health Disorders and Daily Rumination Time

Results of the univariate analysis referent to DRT during the prepartum and postpartum periods are described in Table 1. Although occurrence of twins was not (*P* = 0.67) associated with prepartum DRT, cows that delivered twins had (*P* < 0.01) reduced

DRT during the postpartum period (Figure 1). Animals that delivered stillborn calves had reduced DRT during the prepartum (P = 0.01) and postpartum (P = 0.01) periods (Figure 2). Prepartum DRT of cows diagnosed with sub-clinical hypocalcemia and healthy cows were not (P = 0.22) different (Figure 3). Although sub-clinical hypocalcemia was not (P = 0.87) associated with postpartum DRT, the interaction between occurrence of sub-clinical hypocalcemia and day relative to calving was (P < 0.01) associated with postpartum DRT. On the day of calving (P < 0.01) and 3 d after calving (P = 0.08) cows with sub-clinical hypocalcemia had and tended to have, respectively, reduced DRT than normocalcemic cows. Occurrence of retained fetal membranes was not (P = 0.72) associated with DRT during the prepartum period but the interaction between occurrence of retained fetal membranes and day relative to calving was (P = 0.05) associated with prepartum DRT (Figure 4). Similarly, retained fetal membrane was not (P = 0.14) associated with postpartum DRT but the interaction between occurrence of retained fetal membrane and day relative to calving was (P < 0.01) associated with DRT postpartum (Figure 4). From 2 to 8 d postpartum, cows with retained fetal membranes had (P < 0.05) reduced DRT as compared to healthy cows. No differences in prepartum DRT were observed between cows diagnosed with metritis and healthy cows (P = 0.47; Figure 5). Metritis (P = 0.03) and the interaction between metritis and day relative to calving (P = 0.02) were associated with postpartum DRT because from 2 to 9 d postpartum metritic cows had reduced DRT than healthy cows (P < 0.05) and on d 10 postpartum metritic cows tended (P = 0.09) to have reduced DRT compared with healthy cows (Figure 5). Although sub-clinical ketosis was not associated with prepartum (P = 0.13) and postpartum (P = 0.37) DRT, the interaction between occurrence of sub-clinical ketosis and day relative to calving was (P < 0.01) associated with postpartum DRT. From d 0 to 8 postpartum cows with sub-clinical ketosis had (P < 0.05) reduced DRT than healthy cows and on d 11 postpartum cows with sub-clinical ketosis tended (P = 0.10) to have reduced DRT than healthy cows (Figure 6).

Results from the multivariate analysis referent to DRT during the prepartum and postpartum periods are described in Table 2. Day relative to calving was (P < 0.01) associated with DRT because as cows approached calving there was a significant decrease in DRT with a NADIR on d 1. Although twin calving was not (P = 0.79) associated with prepartum DRT, cows delivering twins had (P = 0.04) reduced postpartum DRT as compared to cows delivering singletons. In the postpartum period the interaction between occurrence of twin calving and day relative to calving was (P < 0.01) associated with DRT because on d 3, 5, 12, 14, 16, and 17 cows delivering twins had (P < 0.05) reduced DRT and on d 4, 10, and 15 cows delivering twins tended (P < 0.10) to have to reduced DRT. Occurrence of stillbirth was not associated with prepartum (P = 0.44) and postpartum (P = 0.42) DRT. The interaction between occurrence of stillbirth and day relative to calving, however, was (P = 0.02) associated with prepartum DRT. Prepartum (P = 0.22) and postpartum (P = 0.84) DRT were not associated with sub-clinical hypocalcemia. Nevertheless, the interaction between occurrence of sub-clinical hypocalcemia and day relative to calving was associated with prepartum (P < 0.01) and postpartum (P < 0.01) DRT. Daily rumination time of cows with sub-clinical hypocalcemia was (P < 0.05) reduced on d -16, -13 and -11 and tended (P < 0.10) to be reduced on d -12. Cows with sub-clinical hypocalcemia had ($P < 0.01$) and tended (P = 0.10) to have reduced DRT on d 1 and 3 postpartum, respectively. No differences were observed in prepartum ($P = 0.61$) and postpartum ($P = 0.36$) DRT between cows that had retained fetal membrane and healthy cows. On the other hand, the interaction between occurrence of retained fetal membranes and day relative to calving was associated with prepartum ($P = 0.02$) DRT because cows with retained fetal membrane tended ($P = 0.07$) to have reduced DRT on d -1 prepartum compared with healthy cows. Similarly, the interaction between occurrence of retained fetal membranes and day relative to calving was ($P < 0.01$) associated with postpartum DRT because cows with retained fetal membranes had ($P < 0.05$) reduced DRT from d 2 to 7 and on d 17 and tended ($P = 0.06$) to have reduced DRT on d 8 as compared to healthy cows. Surprisingly, cows with retained fetal membranes tended ($P = 0.08$) to have greater DRT on d 16 than healthy cows. Occurrence of metritis was not associated with prepartum ($P = 0.22$) and postpartum ($P = 0.22$) DRT. Although sub-clinical ketosis was not associated with prepartum ($P = 0.29$) and postpartum ($P = 0.11$) DRT, the interaction between occurrence of sub-clinical ketosis and day relative to calving was ($P < 0.01$) associated with postpartum DRT. On d 0 cows with sub-clinical ketosis had ($P = 0.01$) greater DRT than normal cows but from d 8 to 17 postpartum DRT of cows with sub-clinical ketosis was (P < 0.05) reduced compared with healthy cows.

Associations between Health Disorders and Daily Activity

Results of the univariate analysis referent to activity during the prepartum and postpartum periods are described in Table 3. Cows that delivered twins had reduced activity during the prepartum ($P = 0.02$) and postpartum ($P < 0.01$) periods (Figure 7). Although animals that delivered stillborn calves had greater ($P = 0.02$) prepartum activity than those that delivered live calves, postpartum activity was not ($P = 0.80$) associated with stillbirth (Figure 8). The interaction between occurrence of stillbirth and day relative to calving was ($P = 0.03$) associated with activity. Sub-clinical hypocalcemia was not associated with prepartum ($P = 0.14$) and postpartum ($P = 0.91$) activity. Cows diagnosed with retained fetal membranes tended ($P = 0.06$) to have reduced activity prepartum and had ($P < 0.01$) reduced activity postpartum (Figure 9). The interaction between occurrence of retained fetal membrane and day relative to calving was ($P < 0.01$) associated with activity during the postpartum. Cows with retained fetal membranes had ($P < 0.05$) reduced activity from d 1 to 7 and on d 10 and 11 compared with healthy cows. Activity of cows with retained fetal membrane tended ($P < 0.10$) to be reduced on d 8 and 9 compared with healthy cows. No association between occurrence of metritis and prepartum activity was observed ($P = 0.68$) but cows that were diagnosed with metritis had reduced ($P = 0.04$) postpartum activity as compared to healthy cows (Figure 10). From d 2 to 5 cows with metritis had ($P < 0.05$) reduced activity and on d 1, 7, 10, 11, and 12 metritic cows tended ($P < 0.10$) to have reduced activity. Although occurrence of sub-clinical ketosis was not ($P = 0.96$) associated with prepartum activity, cows diagnosed with sub-clinical ketosis had ($P = 0.05$) reduced postpartum activity (Figure

11). Cows with sub-clinical ketosis had ($P < 0.05$) reduced activity from d 7 to 11 and on d 13, 15, 16, and 17 and tended ($P = 0.09$) to have reduced activity on d 12.

Results of the multivariate analysis referent to activity during the prepartum and postpartum periods are described in Table 4. Day relative to calving had ($P < 0.01$) a significant impact on activity because as cows approached calving there was a significant rise in activity with a peak of activity on d 1. Cows that delivered twins had ($P = 0.02$) reduced activity prepartum compared with animals that delivered singletons. The interaction between occurrence of twins and day relative to calving was ($P < 0.01$) associated with activity prepartum because on d -16, -15, -14, -12, and -11 cows delivering twins had ($P < 0.05$) reduced and on d -17 and -4 cows delivering twins tended ($P < 0.10$) to have reduced activity. There was no ($P = 0.74$) difference in activity postpartum between animals delivering singletons and twins. On the other hand, animals delivering stillborn calves had ($P < 0.01$) increased prepartum activity as compared to animals delivering live calves, but occurrence of stillbirth was not ($P = 0.17$) associated with postpartum activity. Occurrence of sub-clinical hypocalcemia was not associated with prepartum ($P = 0.34$) or postpartum ($P = 0.47$) activity. Occurrence of retained fetal membranes was not ($P = 0.31$) associated with prepartum activity but postpartum activity tended ($P = 0.08$) to be reduced among cows diagnosed with retained fetal membranes as compared with healthy cows. The nature of the interaction between occurrence of retained fetal membrane and activity was ($P < 0.01$) such that cows with retained fetal membrane had ($P < 0.01$) reduced activity from d 1 to 5 and tended ($P = 0.10$) to have reduced activity on d 6. Occurrence of metritis was not associated with prepartum ($P =

0.56) or postpartum (P = 0.24) activity. Similarly, occurrence of sub-clinical ketosis was not associated with prepartum (P = 0.28) or postpartum (P = 0.45) activity. The interaction between occurrence of sub-clinical ketosis and day relative to calving, however, was (P < 0.01) associated with postpartum activity: Cows with sub-clinical ketosis had (P = 0.03) reduced activity on d 10 and tended (P < 0.10) to have reduced activity on d 8, 15, and 16.

DISCUSSION

In the current observational study the DRT and activity patterns of periparturient animals presenting different health disorders were described. Although rumination time within a 2 h period or within a day may not accurately reflect total DMI within these periods (Schirmann et al., 2012), decreases in DRT overtime are observed when decreases in DMI are observed, particularly around the time of calving (Schirmann et al., 2013). Thus, variations in DRT may be used as a proxy for variations in DMI.

The strong correlation between prepartum and postpartum DRT reported herein is similar to the correlation between prepartum and postpartum DMI reported by several experiments (reviewed by Grummer et al., 2004). According to the review by Grummer et al. (2004), however, the correlation between prepartum and postpartum DMI seems to be more dependent on genetic potential of the animal than physical or chemical attributes of the diet. On the other hand, Mertens (1997) demonstrated that changes in processing of roughage that result in finer particles reduce the rate of mastication of dairy cows regardless of chemical composition of the diet. Experiments are necessary to determine how variability of particle size and physical effective fiber affect DRT of periparturient dairy cows. Interestingly, in the current experiment a strong correlation also was observed between prepartum and postpartum average activity. We are unaware of previous experiments that have demonstrated a similar correlation between prepartum and postpartum activity. Additional experiments are necessary to determine what animal, environmental and nutritional factors explain prepartum and postpartum DRT and activity.

Daily rumination time was weakly correlated with Ca concentration within 72 h after calving. Smooth muscle (e.g. rumen) and skeletal muscle contractions are dependent on availability of Ca (Murray et al., 2008). Among cows with sub-clinical hypocalcemia a reduced DRT was observed on the day of calving and 3 d later based on the univariate analysis. Soon thereafter, however, no differences between normocalcemic and cows with sub-clinical hypocalcemia were observed. Cows induced to develop hypocalcemia had reduced DMI (Martinez et al., 2014). Thus, it is likely that in the first hours following parturition the reduced availability of Ca and the likely reduced DMI of cows diagnosed with sub-clinical hypocalcemia led to reduced DRT. The weak correlation between Ca concentration and DRT and the small differences in mean DRT between cows with sub-clinical hypocalcemia and normocalcemic cows indicate that DRT on a single time point may not be accurate for diagnosis of sub-clinical hypocalcemia. It is not clear why there was no association between sub-clinical hypocalcemia and activity despite the skeletal muscle’s dependence on Ca for contraction. In an observational experiment, however, minor changes in standing time on the week before and on the week after calving were reported for cows diagnosed with hypocalcemia (Jawor et al., 2012).

There was a weak negative correlation between NEFA concentration and DRT and there was no correlation between BHBA concentration and DRT. This suggests that DRT is not representative of metabolic state within a day or that the frequency of sampling used in the current experiment may have been inadequate to identify subtle changes in metabolic status. Cows diagnosed with sub-clinical ketosis, however, had reduced DRT from calving to 8 d postpartum and had reduced activity from d 7 to 17 postpartum, except on d 14 postpartum, compared with healthy cows. Others have demonstrated significant changes in feeding behavior before and after diagnosis of ketosis (Goldhawk et al., 2009; González et al., 2008). Furthermore, Edward and Tozer (2004) demonstrated that cows diagnosed with ketosis and metabolic disorders had reduced steps/h during the postpartum period than healthy cows. The usefulness of DRT and activity data to aid on the diagnosis of sub-clinical ketosis is likely dependent on screening practices currently adopted by the herd.

The partial correlation analysis identified a weak negative correlation between haptoglobin concentration and DRT. When the prepartum and the postpartum data were analyzed separately, there was no correlation between prepartum haptoglobin concentration and prepartum DRT but there was a weak negative correlation between postpartum haptoglobin concentration and postpartum DRT (data not shown). We are unaware of experiments that have evaluated the association between haptoglobin concentration and DRT. In order to restore homeostasis and protect normal tissue, acute phase proteins such as haptoglobin are produced in response to tissue injury, infection, or inflammation (Bauman and Gauldie, 1994). In the current experiment, cows diagnosed with retained fetal membranes and metritis had haptoglobin concentrations 14 and 20% greater, respectively, than healthy cows during the postpartum period (data not shown). Therefore, it appears that the weak negative correlation between haptoglobin concentration and DRT during the postpartum period may be confounded by the occurrence of postpartum uterine disorders and other inflammatory processes.

Unexpectedly, cows that delivered twins had similar DRT during the prepartum period compared with cows that delivered singletons. The reduced postpartum DRT among cows that delivered twins compared with cows delivering singletons was somewhat expected because occurrence of twins increases the risk of uterine (e.g. retained fetal membrane and metritis) and metabolic diseases (Benzaquen et al., 2007; Dubuc et al., 2010). Silva-del-Río et al. (2010) demonstrated that despite the fact that cows that delivered twins had reduced energy balance during the prepartum period compared with cows that delivered singletons, only minor differences in DMI were observed during the prepartum period. It is possible that the small number of cows delivering twins in the current experiment was insufficient to detect statistical differences in prepartum DRT between cows delivering twins and singletons. Although according to the univariate analysis cows delivering twins had reduced activity during the prepartum and postpartum periods, only prepartum activity differed between cows delivering twins and singletons according to the multivariate analysis. This indicates that the reduced postpartum activity of cows that delivered twins may be confounded by infectious and metabolic diseases to which they are more predisposed to, compared with cows delivering singletons. The reduced activity of cows carrying twins during the prepartum period, on the other hand, may indicate discomfort.

Although the univariate analysis suggested that DRT differed between cows delivering stillborn and live calves, the lack of difference in DRT according to the multivariate analysis suggests differences in DRT between cows delivering stillborn and live calves may be a consequence of other diseases associated with stillbirth. On the other hand, according to the multivariate analysis, cows delivering stillborn calves had reduced activity during the prepartum period but not during the postpartum period. Proudfoot et al. (2009) demonstrated that DMI, feeding time, and number of standing bouts were reduced immediately before calving among cows presenting with dystocia. The increased prepartum activity observed among cows delivering twins and stillborn calves and the increased number of standing bouts of dystocic cows (Proudfoot et al., 2009) suggest that cows that have twins, cows that have stillborn calves, and cows that have dystocia may be restless due to discomfort before the onset of actual calving.

The interaction between retained fetal membrane and day relative to calving was associated with DRT during the prepartum and during the postpartum periods. Occurrence of metritis, however, was not associated with DRT. Previous experiments have demonstrated that cows diagnosed with metritis have reduced prepartum feed intake (Hammon et al., 2006; Huzzey et al., 2007). The reduced DMI is expected to cause immunosuppression (Hammon et al., 2006) through the increased severity and extent of negative energy balance. It is possible that no differences in DRT during the postpartum period were observed between healthy and metritic cows because occurrence of metritis is confounded by the occurrence of retained fetal membranes. In the current experiment, 66.7% of cows diagnosed with retained fetal membrane were also diagnosed with metritis, whereas only 14.2% of cows that did not have retained placenta were diagnosed with metritis. Because the aim of the current experiment was to demonstrate the association between health disorders commonly diagnosed on farm and either DRT or activity, then separating cows that presented with multiple diseases from those presenting only one disease did not seem appropriate. It is unclear why cows with retained fetal membranes but not metritic cows presented reduced activity during the postpartum period. Although study personnel diagnosed all the diseases reported herein, it is possible that farm personnel selectively retained more cows with retained fetal membrane in the hospital pen following calving, which was smaller than the pen of apparently healthy postpartum cows.

It is interesting to observe that regardless the type of health events diagnosed, all cows had a significant decrease in daily rumination time and a significant increase in activity within 24 to 48 h relative to calving. In the current experiment, animals were moved to a maternity pen at first sign of imminent calving but the exact time of calving was not recorded. Although it is possible that movement to a different pen may have affected DRT and activity, it is likely that the variability in DRT and activity on the day of calving is explained mostly by the calving itself. Schirmann et al. (2013) demonstrated that cows have a significant decrease in rumination starting approximately 48 h before calving reaching a nadir by 24 h after calving even when cows are not moved to a maternity pen as they present signs of calving.

CONCLUSIONS

The most important factors associated with DRT during the prepartum period were days relative to calving, stillbirth, sub-clinical hypocalcemia and retained fetal membranes. During the postpartum period, the most important factors associated with DRT were days relative to calving, twining, sub-clinical hypocalcemia and sub-clinical ketosis, and retained fetal membrane. Interestingly, during the prepartum period, only days relative to calving, twining and stillbirth were associated with activity, whereas days relative to calving, retained fetal membrane and sub-clinical ketosis were associated with postpartum activity. The relatively minor differences in postpartum DRT and activity between healthy and unhealthy cows suggest that monitoring such outcomes may not be sufficient to identify all unhealthy cows in a herd. Visual observation of the DRT data from the current study suggests that, for the diagnosis of infectious and metabolic diseases, the change in DRT and activity of a cow over time may be more important than the total DRT within a day. Further research is needed to evaluate this hypothesis.

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Table 1. Association among animal factors and health events and daily rumination time (DRT) prepartum and postpartum according to the univariate analysis.

Health events	Differences of least square means of DRT (min/d ± SEM)		*P* – value					
			Pre-partum			Post-partum		
	Pre-partum	Post-partum	Item	Day	Item x Day	Item	Day	Item x Day
Twins [φ]	9.6 ± 22.5	52.0 ± 17.8	0.67	0.23	0.86	< 0.01	< 0.01	0.01
Stillbirth [φ]	60.9 ± 24.1	50.7 ± 19.9	0.01	0.06	0.07	0.01	< 0.01	0.45
Sub-clinical hypocalcemia [φ]	16.5 ± 13.5	1.8 ± 10.8	0.22	0.03	0.11	0.87	< 0.01	< 0.01
Retained fetal membrane [φ]	-6.3 ± 17.2	20.7 ± 13.9	0.72	< 0.01	0.05	0.14	< 0.01	< 0.01
Metritis [φ]	10.3 ± 14.3	25.2 ± 11.3	0.47	0.01	0.46	0.03	< 0.01	0.02
Sub-clinical ketosis [φ]	-27.9 ± 18.3	13.5 ± 15.0	0.13	0.19	0.69	0.37	< 0.01	< 0.01

[φ] Cows that presented the event used as reference.

Table 2. Daily rumination time (DRT) according to animal characteristics and health events based on the multivariate analysis.

Items	DRT (min/d ± SEM)		*P* – value					
			Pre-partum			Post-partum		
	Pre-partum	Post-partum	Item	Day	Item x Day	Item	Day	Item x Day
Number of calves			0.79	0.66	0.86	0.04	< 0.01	< 0.01
Singleton	449.8 ± 23.7	406.7 ± 11.1						
Twins	441.8 ± 33.3	359.8 ± 21.7						
Stillbirth			0.44	0.66	0.02	0.42	< 0.01	0.39
No	443.9 ± 11.1	397.4 ± 16.0						
Yes	418.7 ± 30.0	417.9 ± 26.5						
Sub-clinical hypocalcemia			0.22	0.66	< 0.01	0.84	< 0.01	< 0.01
No	439.4 ± 18.0	384.4 ± 14.0						
Yes	423.2 ± 17.7	382.2 ± 14.2						
Retained fetal membrane			0.61	0.66	0.02	0.36	< 0.01	< 0.01
No	435.8 ± 15.8	390.9 ± 14.7						
Yes	426.8 ± 22.0	375.7 ± 16.3						
Metritis			0.22	0.66	0.32	0.22	< 0.01	0.39
No	448.5 ± 18.5	392.6 ± 15.2						
Yes	427.2 ± 18.7	375.6 ± 14.5						
Sub-clinical ketosis			0.29	0.66	0.89	0.11	< 0.01	< 0.01
No	436.4 ± 16.5	396.6 ± 11.3						
Yes	458.7 ± 25.5	369.9 ± 18.7						

Table 3. Association among animal factors and health events and daily activity prepartum and postpartum according to the univariate analysis.

Health events	Differences of least square means of activity (arbitrary unit ± SEM)		*P* – value					
			Pre-partum			Post-partum		
	Pre-partum	Post-partum	Item	Day	Item x Day	Item	Day	Item x Day
Twins [φ]	34.9 ± 15.4	56.2 ± 20.1	0.02	< 0.01	0.05	< 0.01	< 0.01	< 0.01
Stillbirth [φ]	-38.0 ± 16.7	5.9 ± 22.7	0.02	< 0.01	0.71	0.80	0.02	0.03
Sub-clinical hypocalcemia [φ]	-13.8 ± 9.2	-1.5 ± 12.3	0.14	< 0.01	0.22	0.91	< 0.01	0.68
Retained fetal membrane [φ]	22.2 ± 11.8	50.6 ± 15.5	0.06	< 0.01	0.90	< 0.01	< 0.01	< 0.01
Metritis [φ]	-4.1 ± 9.9	26.8 ± 12.9	0.68	< 0.01	0.92	0.04	< 0.01	0.02
Sub-clinical ketosis [φ]	0.7 ± 13.5	34.4 ± 17.6	0.96	< 0.01	0.11	0.05	< 0.01	< 0.01

[φ] Cows that presented the event used as reference.

Table 4. Daily activity according to animal characteristics and health events based on the multivariate analysis.

Items	Activity (arbitrary unit d ± SEM)		*P* – value					
			Pre-partum			Post-partum		
	Pre-partum	Post-partum	Item	Item	Item x Day	Item	Day	Item x Day
Number of calves			0.02	< 0.01	< 0.01	0.74	< 0.01	0.38
Singleton	501.5 ± 10.6	544.1 ± 19.8						
Twins	457.9 ± 18.6	534.3 ± 29.6						
Stillbirth			< 0.01	< 0.01	0.66	0.17	< 0.01	0.59
No	451.9 ± 10.2	523.8 ± 15.1						
Yes	507.6 ± 20.3	561.8 ± 29.3						
Sub-clinical hypocalcemia			0.34	< 0.01	0.24	0.47	< 0.01	0.80
No	478.9 ± 14.5	533.3 ± 22.1						
Yes	488.3 ± 14.3	542.7 ± 21.2						
Retained fetal membrane			0.31	< 0.01	0.93	0.08	< 0.01	< 0.01
No	489.0 ± 14.6	543.3 ± 14.1						
Yes	474.3 ± 16.3	510.9 ± 20.5						
Metritis			0.56	< 0.01	0.84	0.24	< 0.01	0.36
No	489.5 ± 18.8	551.6 ± 20.2						
Yes	496.9 ± 17.9	532.4 ± 20.7						
Sub-clinical ketosis			0.28	< 0.01	0.40	0.45	< 0.01	< 0.01
No	481.6 ± 13.6	534.8 ± 12.1						
Yes	497.3 ± 19.8	519.4 ± 22.5						

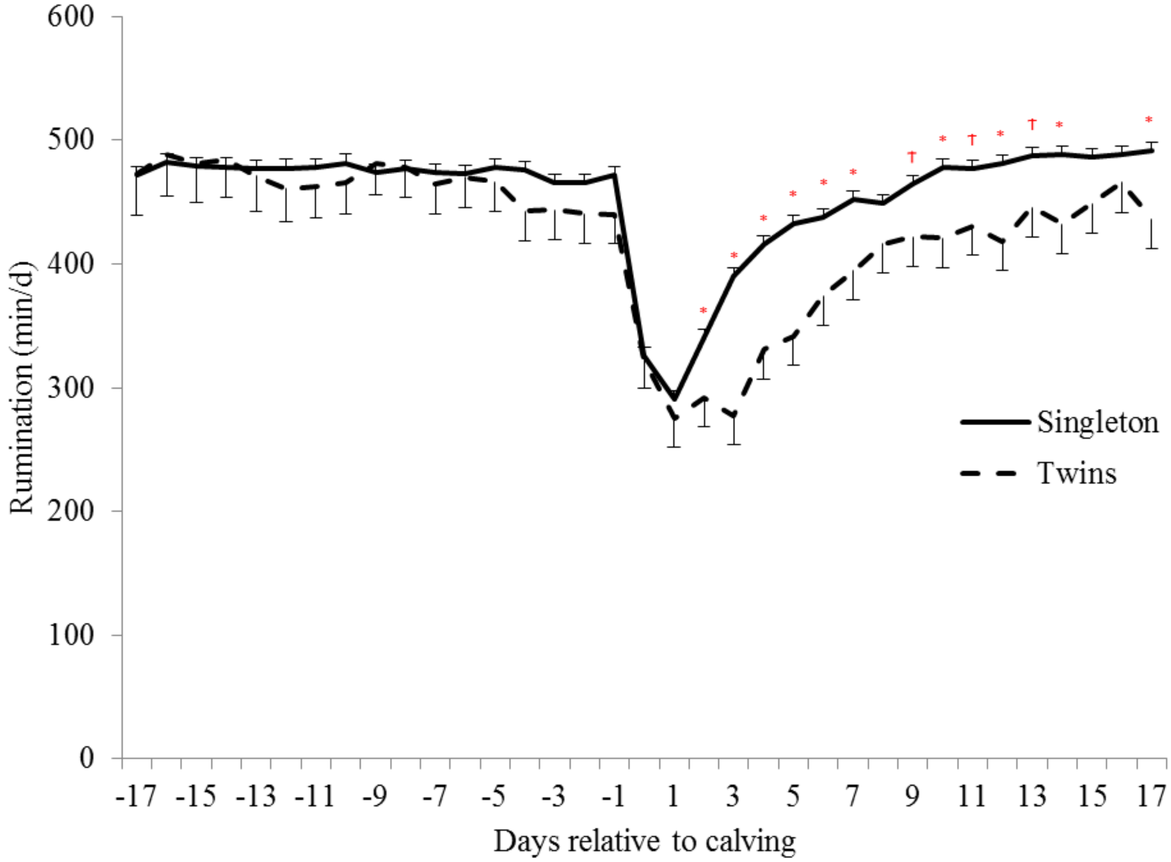

Figure 1. Association between occurrence of twins and daily rumination time. Prepartum: occurrence of twins – $P = 0.67$, day relative to calving – $P = 0.23$, interaction between occurrence of twins and day relative to calving – $P = 0.86$. Postpartum: occurrence of twins – $P < 0.01$, day relative to calving – $P < 0.01$, interaction between occurrence of twins and day relative to calving – $P = 0.01$. *Within day, means differ ($P < 0.05$). †Within day, means tended to differ ($P < 0.10$).

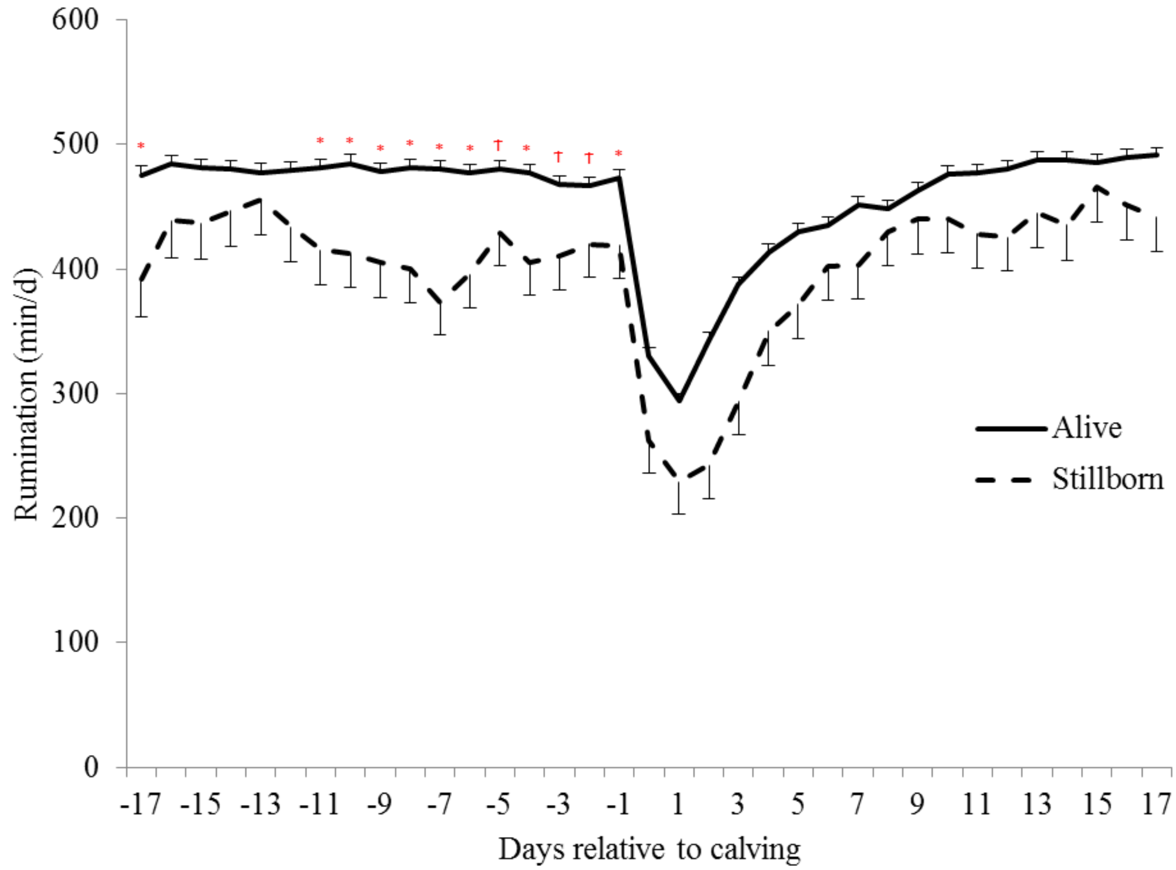

Figure 2. Association between occurrence of stillbirth and daily rumination time. Prepartum: occurrence of stillbirth – $P = 0.01$, day relative to calving – $P = 0.06$, interaction between occurrence of stillbirth and day relative to calving – $P = 0.07$. Postpartum: occurrence of stillbirth – $P = 0.01$, day relative to calving – $P < 0.01$, interaction between occurrence of stillbirth and day relative to calving – $P = 0.45$. *Within day, means differ ($P < 0.05$). †Within day, means tended to differ ($P < 0.10$).

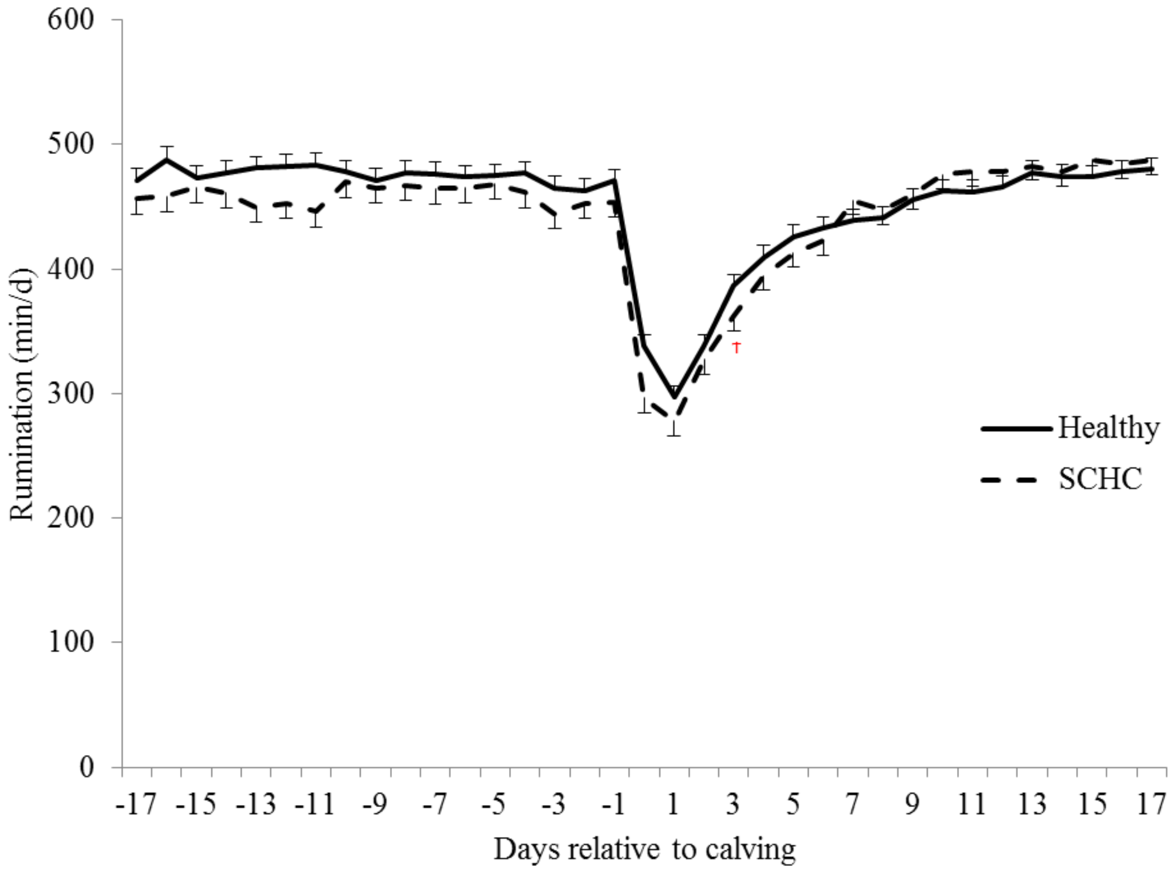

Figure 3. Association between occurrence of sub-clinical hypocalcemia (**SCHC**) and daily rumination time. Prepartum: occurrence of SCHC – P = 0.22, day relative to calving – P = 0.03, interaction between occurrence of SCHC and day relative to calving – P = 0.11. Postpartum: occurrence of SCHC – P = 0.87, day relative to calving – P < 0.01, interaction between occurrence of SCHC and day relative to calving – P < 0.01. [†]Within day, means tended to differ (P < 0.10).

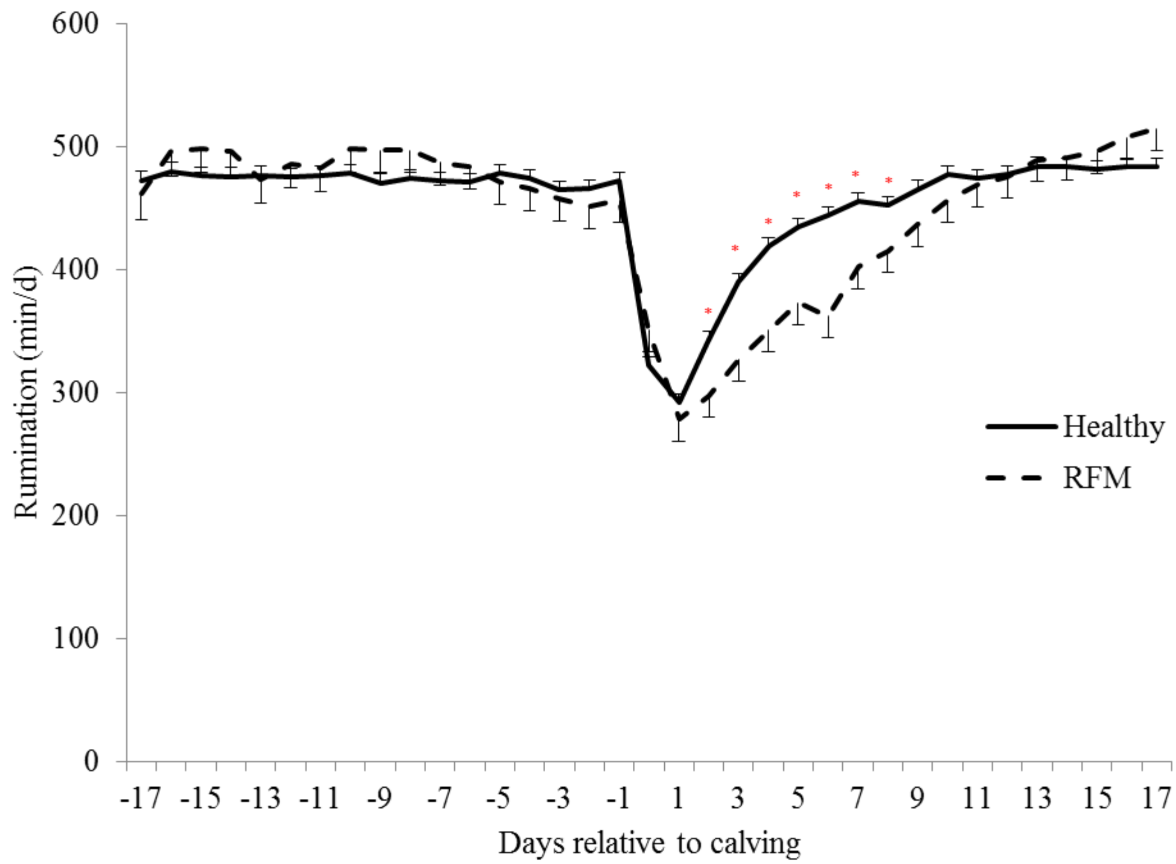

Figure 4. Association between occurrence of retained fetal membranes (**RFM**) and daily rumination time. Prepartum: occurrence of RFM – $P = 0.72$, day relative to calving – $P < 0.01$, interaction between occurrence of RFM and day relative to calving – $P = 0.05$. Postpartum: occurrence of RFM – $P = 0.14$, day relative to calving – $P < 0.01$, interaction between occurrence of RFM and day relative to calving – $P < 0.01$. *Within day, means differ ($P < 0.05$).

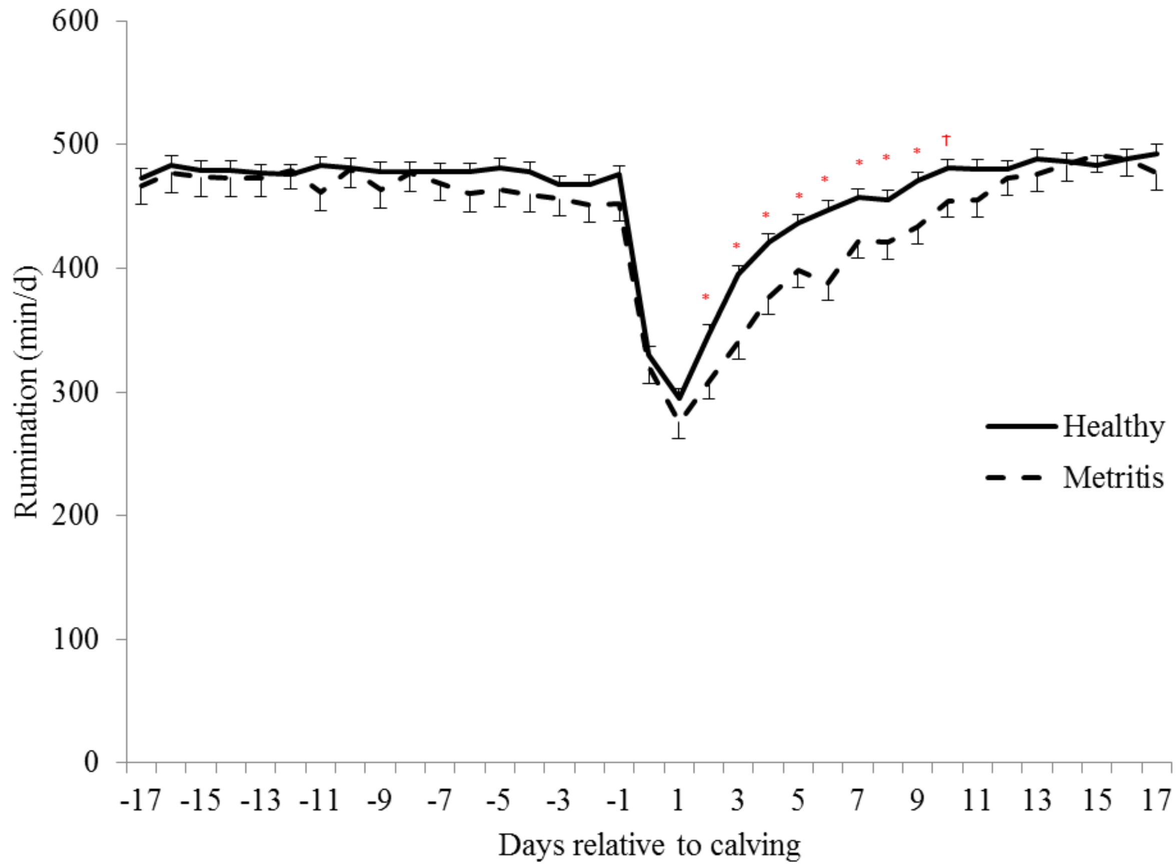

Figure 5. Association between occurrence of metritis and daily rumination time. Prepartum: occurrence of metritis – $P = 0.47$, day relative to calving – $P = 0.01$, interaction between occurrence of metritis and day relative to calving – $P = 0.46$. Postpartum: occurrence of metritis – $P = 0.03$, day relative to calving – $P < 0.01$, interaction between occurrence of metritis and day relative to calving – $P = 0.02$. *Within day, means differ ($P < 0.05$). †Within day, means tended to differ ($P < 0.10$).

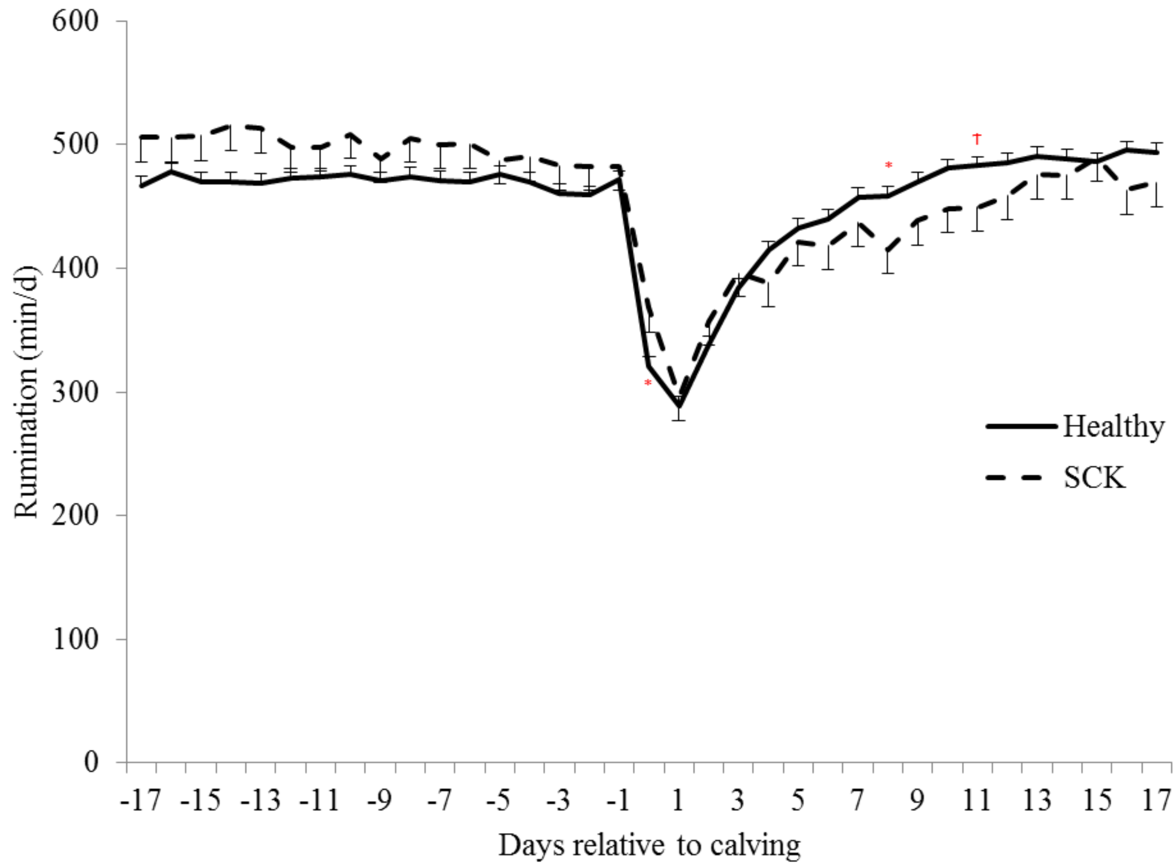

Figure 6. Association between occurrence of sub-clinical ketosis (**SCK**) and daily rumination time. Prepartum: occurrence of SCK – *P* = 0.13, day relative to calving – *P* = 0.19, interaction between occurrence of SCK and day relative to calving – *P* = 0.69. Postpartum: occurrence of SCK – *P* = 0.37, day relative to calving – *P* < 0.01, interaction between occurrence of SCK and day relative to calving – *P* < 0.01. [*]Within day, means differ (*P* < 0.05). [†]Within day, means tended to differ (*P* < 0.10).

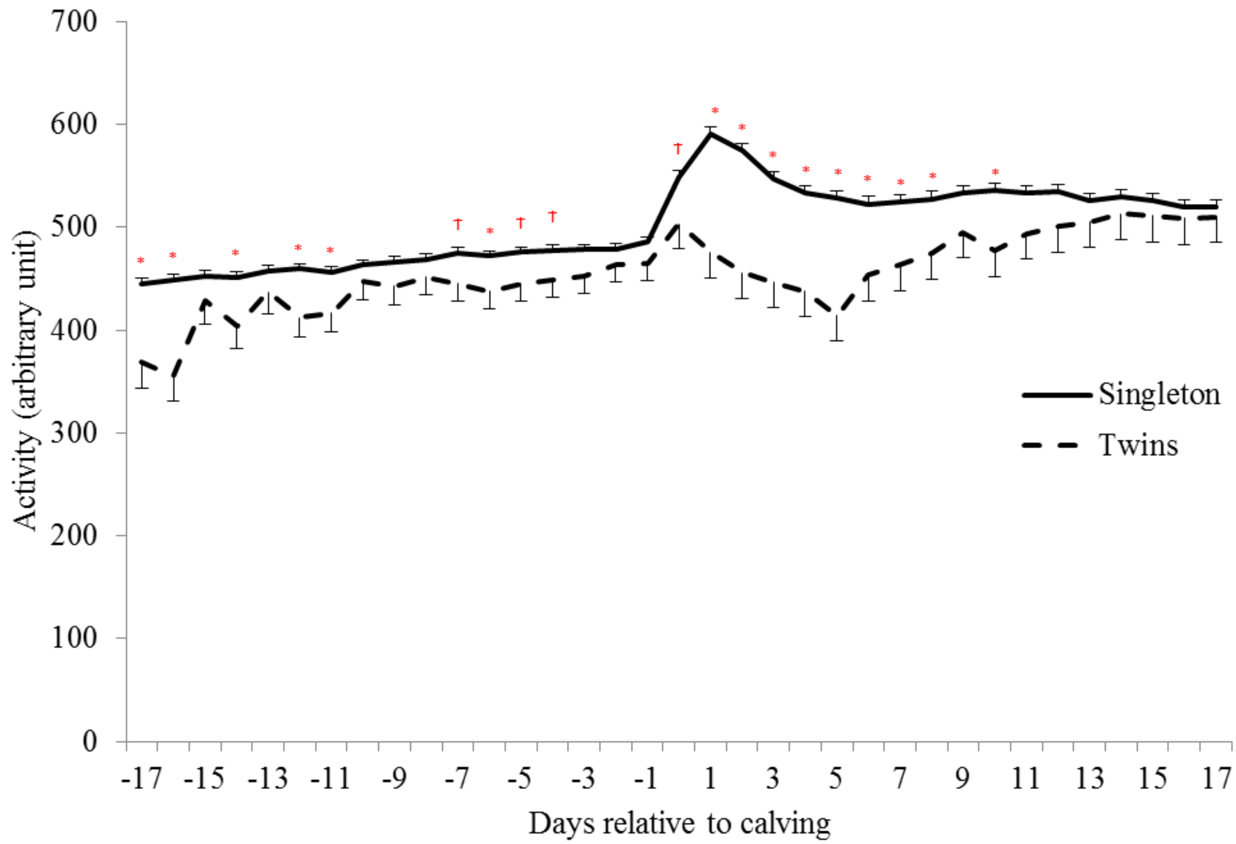

Figure 7. Association between occurrence of twins and daily activity. Prepartum: occurrence of twins – $P = 0.02$, day relative to calving – $P < 0.01$, interaction between occurrence of twins and day relative to calving – $P = 0.05$. Postpartum: occurrence of twins – $P < 0.01$, day relative to calving – $P < 0.01$, interaction between occurrence of twins and day relative to calving – $P < 0.01$. *Within day, means differ ($P < 0.05$). †Within day, means tended to differ ($P < 0.10$).

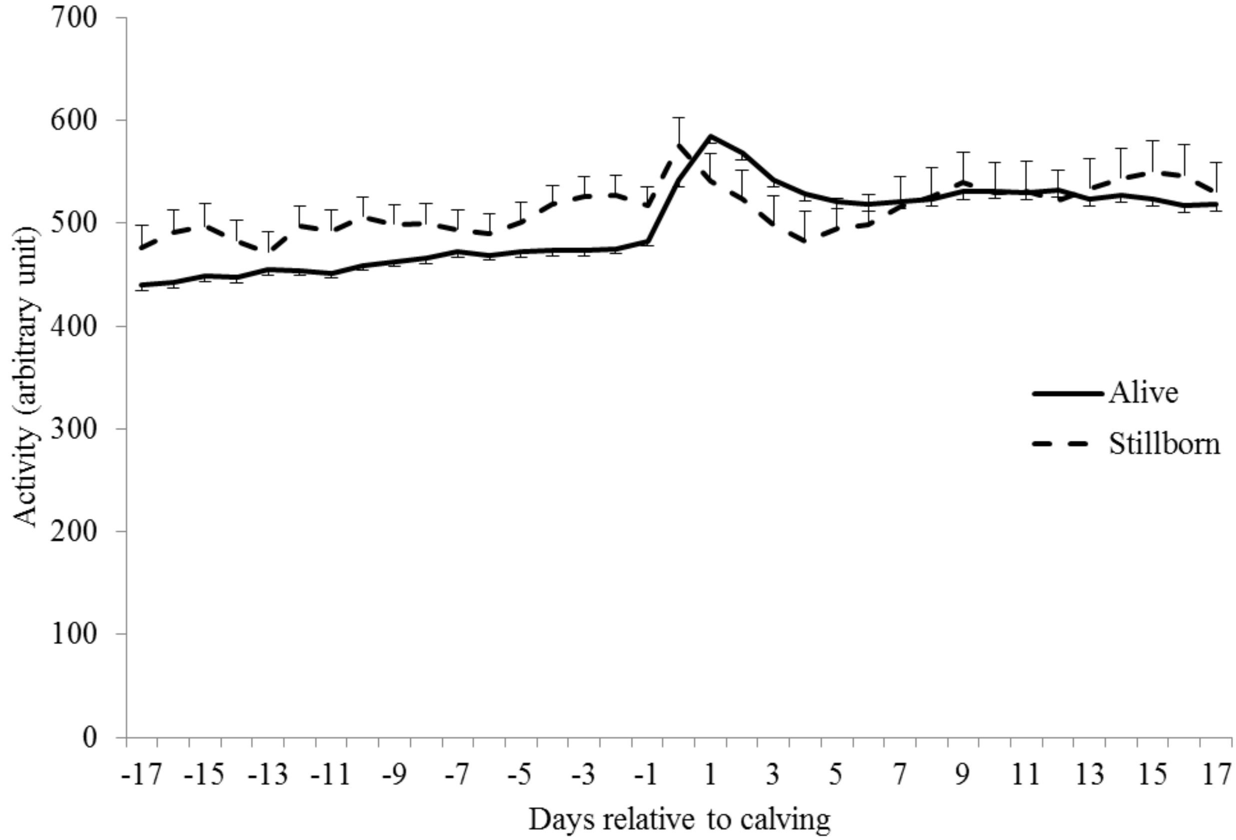

Figure 8. Association between occurrence of stillbirth and daily activity. Prepartum: occurrence of stillbirth – $P = 0.02$, day relative to calving – $P < 0.01$, interaction between occurrence of stillbirth and day relative to calving – $P = 0.71$. Postpartum: occurrence of stillbirth – $P = 0.80$, day relative to calving – $P = 0.02$, interaction between occurrence of stillbirth and day relative to calving – $P = 0.03$.

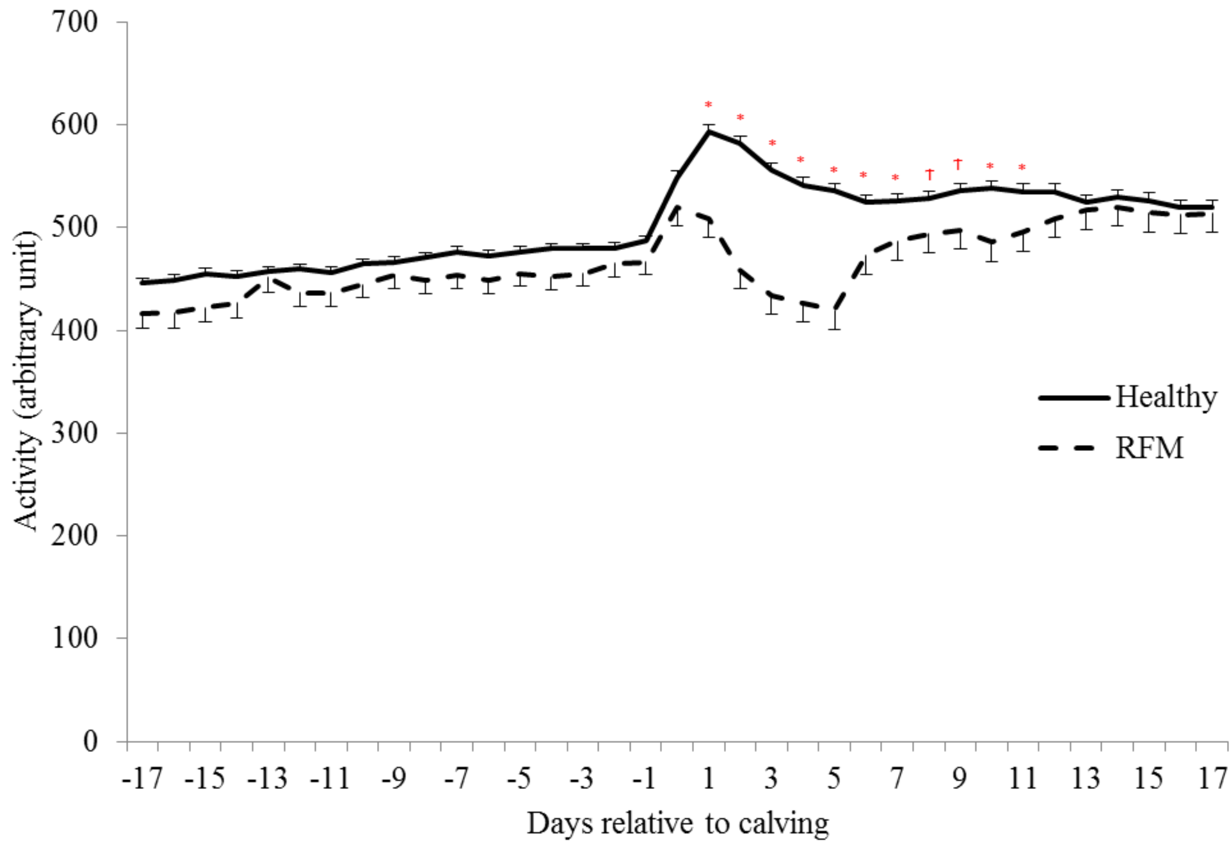

Figure 9. Association between occurrence of retained fetal membranes (**RFM**) and daily activity. Prepartum: occurrence of RFM – $P = 0.06$, day relative to calving – $P < 0.01$, interaction between occurrence of RFM and day relative to calving – $P = 0.90$. Postpartum: occurrence of RFM – $P < 0.01$, day relative to calving – $P < 0.01$, interaction between occurrence of RFM and day relative to calving – $P < 0.01$. *Within day, means differ ($P < 0.05$). †Within day, means tended to differ ($P < 0.10$).

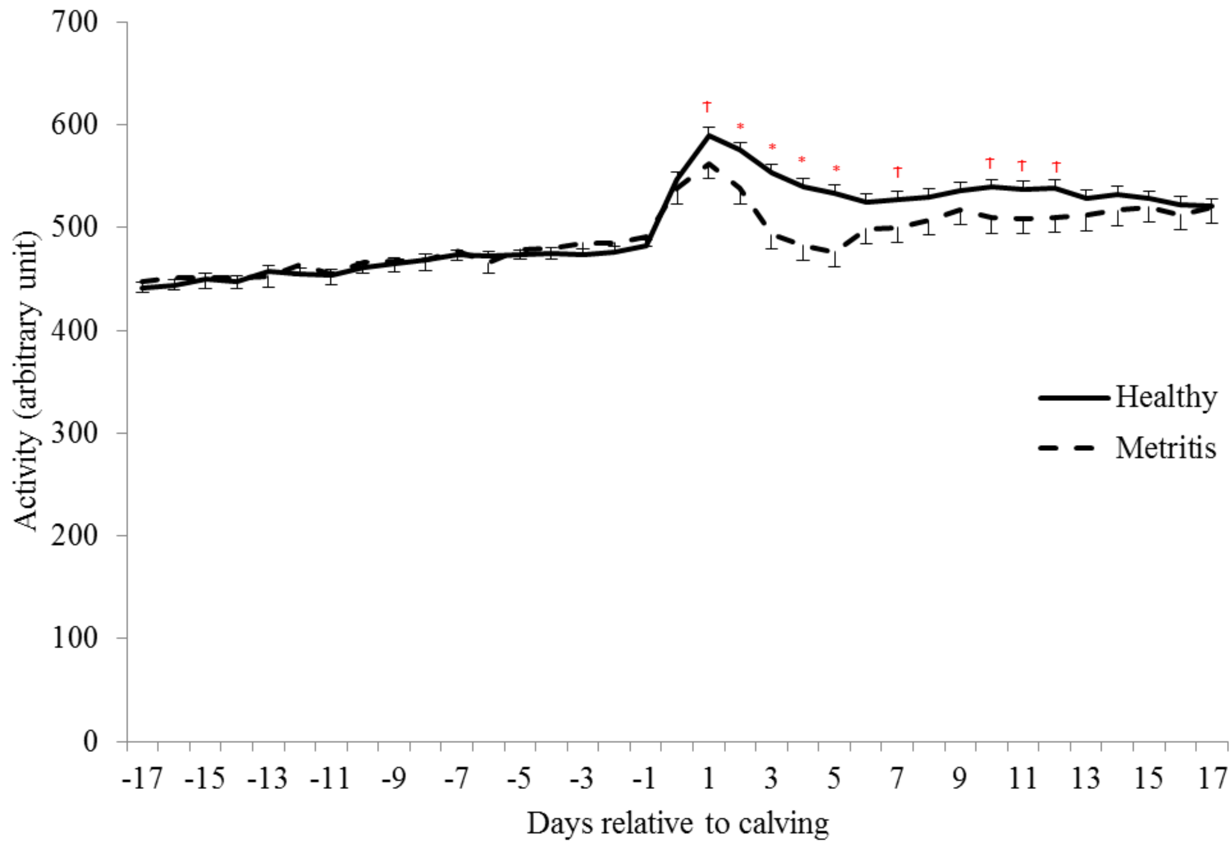

Figure 10. Association between occurrence of metritis and daily activity. Prepartum: occurrence of metritis – $P = 0.68$, day relative to calving – $P < 0.01$, interaction between occurrence of metritis and day relative to calving – $P = 0.92$. Postpartum: occurrence of metritis – $P = 0.04$, day relative to calving – $P < 0.01$, interaction between occurrence of metritis and day relative to calving – $P = 0.02$. *Within day, means differ ($P < 0.05$). †Within day, means tended to differ ($P < 0.10$).

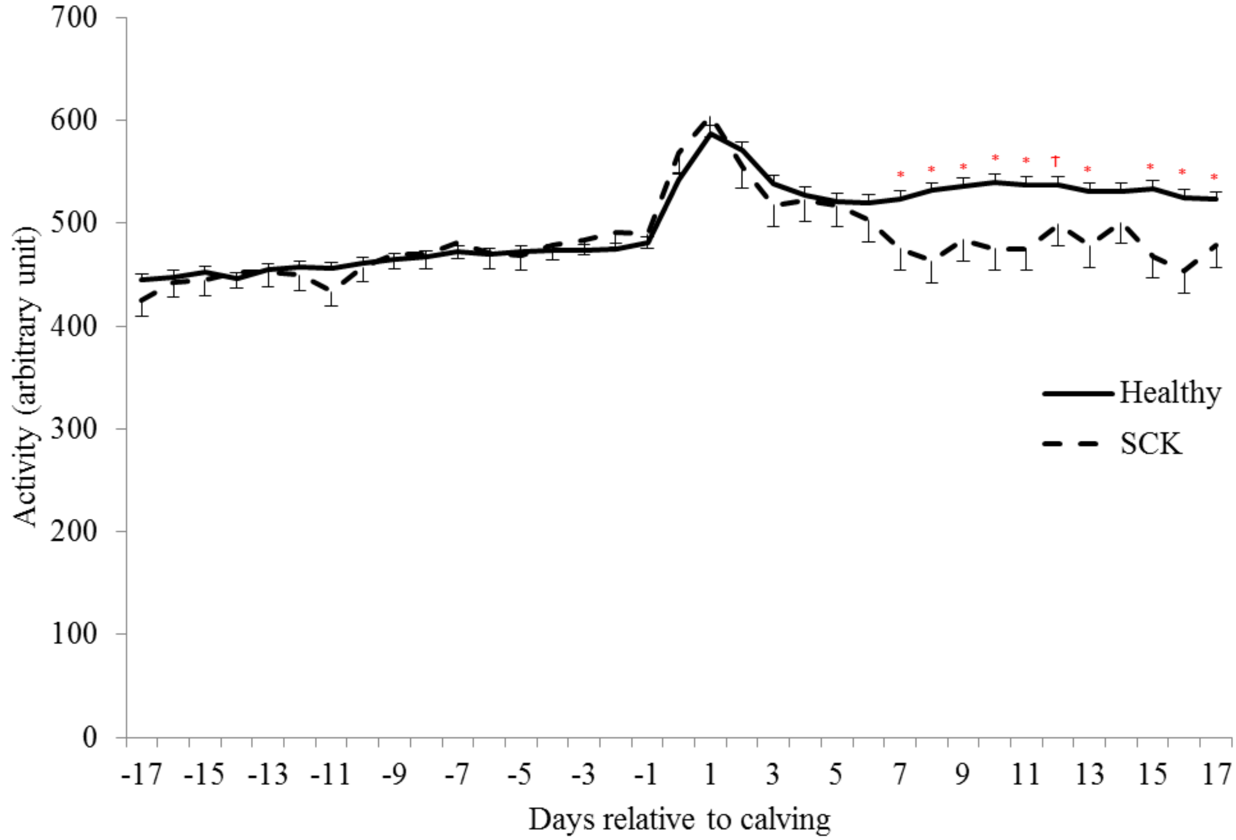

Figure 11. Association between occurrence of sub-clinical ketosis (**SCK**) and daily activity. Prepartum: occurrence of SCK – $P = 0.96$, day relative to calving – $P < 0.01$, interaction between occurrence of SCK and day relative to calving – $P = 0.11$. Postpartum: occurrence of SCK – $P = 0.05$, day relative to calving – $P < 0.01$, interaction between occurrence of SCK and day relative to calving – $P < 0.01$. *Within day, means differ ($P < 0.05$). †Within day, means tended to differ ($P < 0.10$).

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